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# DEVELOPMENTAL EFFECTS OF WHEEL RUNNING ON HIPPOCAMPAL GLUTAMATE RECEPTOR EXPRESSION IN YOUNG AND MATURE ADULT RATS

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**Abstract—Recent evidence suggests that the behavioral benefits associated with voluntary wheel running in rodents may be due to modulation of glutamatergic transmission in the hippocampus, a brain region implicated in learning and memory. However, the expression of the glutamatergic ionotropic N-methyl-d-aspartate receptor (GluN) in the hippocampus in response to chronic sustained voluntary wheel running has not yet been investigated. Further, the developmental effects during young and mature adulthood on wheel running output and GluN expression in hippocampal subregions has not been determined, and therefore is the main focus of this investigation. Eight-week-old and 16-week-old male Wistar rats were housed in home cages with free access to running wheels and running output was monitored for 4 weeks. Wheel access was terminated and tissues from the dorsal and ventral hippocampi were processed for Western blot analysis of GluN subunit expression. Young adult runners demonstrated an escalation in running output but this behavior was not evident in mature adult runners. In parallel, young adult runners demonstrated a significant increase in total GluN (1 and 2A) subunit expression in the dorsal hippocampus (DH), and an opposing effect in the ventral hippocampus (VH) compared to age-matched sedentary controls; these changes in total protein expression were not associated with significant alterations in the phosphorylation of the GluN subunits. In contrast, mature adult runners demonstrated a reduction in total GluN2A expression in the DH, without producing alterations in the VH compared to age-matched sedentary controls. In conclusion, differential running activity-mediated modulation of GluN subunit expression in the hippocampal subregions was revealed to be associated with developmental effects on running activity, which may contribute to altered hippocampal synaptic activity and behavioral outcomes in young and mature adult subjects.** 

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*Abbreviations:* BDNF, brain-derived neurotrophic factor; DH, dorsal hippocampus; GluN, glutamatergic ionotropic N-methyl-d-aspartate receptor; LTP, long-term potentiation; VH, ventral hippocampus.

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## INTRODUCTION

Physical activity has long been touted as a critical component of a long and high-quality life. Individuals who routinely exercise (via sustained physical activity) benefit from a longer life expectancy (Haapanen-Niemi et al., 2000; Savela et al., 2010; Autenrieth et al., 2011; Wen et al., 2011; Lee et al., 2014) and show improvements in cognitive function (for reviews, see Hillman et al., 2008; Erickson and Kramer, 2009; Smith et al., 2013). Specifically, studies have highlighted increases in hippocampal volume (Colcombe and Kramer, 2003; Colcombe et al., 2003, 2006; Pajonk et al., 2010; Varma et al., 2015) and hippocampal functioning (Colcombe and Kramer, 2003; Pajonk et al., 2010; Chang et al., 2012; Loprinzi and Kane, 2015) in both young adult and mature adult populations. Further, these findings in humans have been replicated in a large volume of preclinical studies, supporting the connection between physical activity and cognitive capacities, and prompting inquiry into its molecular and cellular underpinnings (for a review of the overlapping studies, see Voss et al., 2013). The most often cited molecular mechanism in the hippocampus underlying the structural and functional improvement in exercising animals is adult neurogenesis and expression of brain-derived neurotrophic factor (BDNF) (van Praag et al., 2005; Yau et al., 2014) with striking increases in cell proliferation and associated cognitive performance (van Praag et al., 1999, 2005; Van der Borght et al., 2007; Wu et al., 2008; Siette et al., 2013; Speisman et al., 2013; Gibbons et al., 2014; Merkley et al., 2014). Interestingly, these pro-neurogenic effects are also observed in models of environmental enrichment (reviewed in Bekinschtein et al., 2011), a housing condition which often included access to a running wheel. However, the functional molecular components of hippocampal cognition, glutamatergic receptors, have been less investigated in both voluntary exercise and environmental enrichment paradigms. With regard to environmental enrichment, there is an increase in glutamatergic receptor expression (Tang et al., 2001; Andin et al., 2007), but evidence suggests that voluntary exercise is the strongest contributing factor to the molecular changes observed in environmentally enriched animals (Ehninger and Kempermann, 2003; Kobilo et al., 2011). While one study in mice implicated the glutamatergic

signaling system as the potential source of physical activity's positive modulation of hippocampal volume and function (Biedermann et al., 2012), the mechanisms associated with neuronal plasticity underlying the enhanced hippocampal function in the context of sustained physical activity have not been explicitly determined. Furthermore, the effects of exercise on the expression of plasticity-associated proteins in the hippocampus during young and mature adulthood have not been examined.

One molecular component that is associated with neuronal synaptic plasticity and is critical to hippocampal function is the glutamatergic ionotropic N-methyl-D-aspartate receptor (GluN). In the hippocampus, GluN receptor activation is essential for long-term potentiation (LTP) (Bashir et al., 1993; Shipton and Paulsen, 2014), the primary cellular property believed to underlie hippocampal learning and memory (Bliss and Collingridge, 1993). GluNs are heteromeric tetramers which are comprised of two GluN1 obligatory subunits and any combination of two GluN2A-D or GluN3A-B (Paoletti et al., 2013). As an obligatory subunit, GluN1 can serve as a suitable indicator of total GluN expression. The composition of the remaining subunits and their phosphorylation states can imply specific membrane localization and functionality, and thereby facilitate learning and memory (Paoletti et al., 2013). There has been considerable inquiry into how exercise via voluntary wheel running can independently influence GluN expression and function in the hippocampus. For example, prolonged wheel running (with resistance) in 12-week-old rats has been implicated in increasing GluN subunit GluN2A and GluN2B mRNA expression (Molteni et al., 2002) and receptor functionality (Farmer et al., 2004; Dietrich et al., 2005; Vasuta et al., 2007). However, it is unknown whether such effects on GluN subunits by exercise are higher during young adulthood (6–10 weeks of age; a developmental timeframe associated with higher neurogenesis, higher density of α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) receptors glutamatergic ionotropic α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid receptors (GluAs) and BDNF protein levels in the hippocampus) compared with mature adulthood (16–20 weeks of age; Gulve et al., 1993; Coutinho et al., 2006; Lang et al., 2009; Carreton et al., 2012). Furthermore, this molecular-level regulation, evaluated thus far nearly exclusively in the dorsal hippocampus (DH) of young adult rodents, could be one potential mechanistic underpinning of exercise's role in the enhancement of hippocampal-sensitive cognition (Voss et al., 2013). Given the mechanistic distinction and role of DH in spatial memory (for a review see Hartley et al., 2014) and temporal memory (for a review see Eichenbaum, 2014) and ventral hippocampus (VH) in emotional regulation and anxiety-like behaviors ((Moser et al., 1995; Kjelstrup et al., 2002; Bannerman et al., 2004; Pothuizen et al., 2004; Pentkowski et al., 2006); for a review see Miller and Hen, 2015), it is essential to understand the effects of exercise on these anatomically defined regions of the hippocampus.

The current study therefore investigated the effect of prolonged voluntary exercise on glutamatergic receptor expression in distinct hippocampal subregions in animals of two disparate ages during adulthood. We hypothesize that, expression of GluNs will be increased in young adult runners and that this activity-related effect will be attenuated in mature adult runners compared with their age-matched sedentary controls. Additionally, we speculate that alterations in GluN expression would be greater in the DH due to the critical role of GluNs in DH cognitive function. However, as the role of GluNs in VH emotional functionality has not been extensively evaluated, it is possible that the two distinct regions be affected similarly subsequent to physical activity.

## EXPERIMENTAL PROCEDURES

Experimental procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85–23, revised 1996) and approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

### Animals

Adult male Wistar rats (Charles River, Hollister, CA), were housed in a temperature-controlled (22 °C) vivarium on a 12-h/12-h light/dark cycle (lights on at 8:00 P.M.) with *ad libitum* access to food and water. At either 8 weeks of age (young adult) or 16 weeks (mature adults), rats assigned to the voluntary exercise group were moved into individual housing with *ad libitum* access to a running wheel (Nalgene activity wheels 34.5-cm diameter × 9.7-cm width with magnetic switches connected to a PC for monitoring). The total number of revolutions was recorded in 10-min bins and summed for each 24-h period for four weeks (VitalView, Starr Life Sciences Corp., Oakmont, PA).

### Tissue collection

Following cessation of wheel running, within one hour of removal from running wheel cages, rats (wheel running and age matched sedentary controls) were briefly anesthetized with isoflourane, then rapidly decapitated and the brain was immediately removed. The brain was cut along the mid-sagittal axis and right hemisphere and was quickly frozen in dry ice-cooled isopentane and stored at −80 °C until further processing. Dorsal (−3.14 to −4.30 mm from bregma) and ventral (−5.30 to −6.1 mm from bregma as identified in Paxinos and Watson, 2007) hippocampal tissue punches were collected from 500-μm thick sections and stored at −80 °C until further processing (Fig. 3Ai, Bi).

### Western blot analysis

Procedures optimized for measuring levels of both phosphoproteins and total proteins was performed as previously described (Kim et al., 2015; Galinato et al.,