



HHS Public Access

Author manuscript

Neuroscience. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Neuroscience. 2015 October 1; 305: 248–256. doi:10.1016/j.neuroscience.2015.07.058.

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 n-Methyl-d-Aspartate glutamate receptor subunits (GluNs) 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 sixteen-week-old male Wistar rats were housed in home cages with free access to running wheels and running output was monitored for four weeks. Wheel access was terminated and tissue 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, and an opposing effect in the ventral hippocampus 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 dorsal hippocampus, without producing alterations in the ventral hippocampus 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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Conflict of Interest

The authors claim no conflict of interest.

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Keywords

exercise; hippocampus; NMDA receptor; aging; dorsal; ventral

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; Colcombe et al., 2006; Pajonk et al., 2010; Varma et al., 2014) 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 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 and Gil-Mohapel, 2014) with striking increases in cell proliferation and associated cognitive performance (van Praag et al., 1999; van Praag et al., 2005; Van der Borght et al., 2007; Wu et al., 2008; Siette et al., 2013; Speisman et al., 2013; Gibbons et al., 2014; Merkle 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 an exercise wheel. However, the functional molecular components of hippocampal cognition, glutamatergic receptors, have been less investigated in both voluntary exercise and environmental enrichment. With regards 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; Kobil 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-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) 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 review see (Hartley et al., 2014)) and temporal memory (for 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 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 effected similarly subsequent to physical activity.

Materials and Methods

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), 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 x 9.7 cm wide with magnetic switches connected to a PC for monitoring). The total number of revolutions was recorded in 10 minute bins and summed for each 24 h period for four (VitalView, Minimitter Inc.).

Tissue Collection

Following cessation of voluntary exercise (or age-matched for sedentary controls), within one hour of removal from running wheel cages, rats 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 500µm thick sections and stored at -80°C until further processing (Figure 3Ai and 3Bi).

Western Blot Analysis

Procedures optimized for measuring neuronal levels of both phosphoproteins and total proteins was performed as previously described (Kim et al., 2014; Galinato et al., 2015; Navarro and Mandyam, 2015; Staples et al., 2015). Tissue was homogenized on ice by sonication in buffer (320 mM sucrose, 5 mM HEPES, 1 mM EGTA, 1 mM EDTA, 1% SDS, with Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktails II and III diluted 1:100; Sigma), heated at 100 degrees C for five minutes, and stored at -80 degrees C until determination of protein concentration by a detergent-compatible Lowry method (Bio-Rad, Hercules, CA). Samples were mixed (1:1) with a Laemmli sample buffer containing β-mercaptoethanol. Each sample containing protein from one animal was run (20 µg per lane) on 8% SDS-PAGE gels (Bio-Rad) and transferred to polyvinylidene fluoride membranes (PVDF pore size 0.2 µm). Blots were blocked with 2.5% (for phosphoproteins) or 5% milk (w/v) in TBST (25 mM Tris-HCl (pH 7.4), 150 mM NaCl and 0.1% Tween 20 (v/v)) for one hour at room temperature and were incubated with the primary antibody for 16–20 h at 4 °C. Primary antibodies and dilutions are listed in Table 1. Blots were then washed three times for 5 min in TBST, and then incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat antibody to rabbit in TBST (for dilutions see Table 1). After another three washes for 5 min with TBST, immunoreactivity was detected using SuperSignal West Dura chemiluminescence detection reagent (Thermo Scientific) and collected using HyBlot CL Autoradiography film (Denville Scientific) and a Kodak film processor. Blots were then stained Coomassie Blue to normalize to the quantity of protein loaded in each lane (Welinder and Ekblad, 2011). Densitometry was performed using ImageStudio software (Li-Cor Biosciences). X-ray films were digitally scanned at 600dpi resolution, then bands of interest were selected in identically sized selection boxes within

the imaging program which included a 3 pixel extended rectangle for assessment of the background signal. The average signal of the pixels in the “background” region (between the exterior border of the region of interest selection box and the additional 3 pixel border) was then subtracted from the signal value calculated for the band of interest. This was repeated for the coomassie stained lane corresponding to the band of interest, and the signal value of the band of interest following subtraction of the background calculation was then expressed as a ratio of the corresponding coomassie signal (following background subtraction). This ratio of expression for each band was then expressed as a percent of the age- and hippocampal subregion specific sedentary controls on the same blot.

Statistical analysis

Body weight was monitored weekly for all subjects beginning at onset of voluntary exercise (age-matched for sedentary controls). Weight gained during the duration of the experiment was analyzed by a Two-Way ANOVA with age and physical activity the between-subject independent variables and weight gained the dependent variable.

Running output was monitored in one hour bins and analyzed as daily output over a period of 4 weeks. Daily running activity was statistically assessed utilizing a mixed-model Two-Way ANOVA with age-group as the between-subject and day of activity as the within-subject independent factors and number of wheel revolutions the dependent factors. Post-hoc comparisons (Fisher’s LSD) were performed comparing young adult and mature adult wheel activity on each day, as the daily average activity output within each age group to the day one average activity. Change in running activity was assessed as the percent change of wheel revolutions between the first three and last three activity days and was statistically analyzed by two-sided t-test. For circadian activity analysis, wheel revolutions per hour were analyzed on the first and last day of running activity for each age as Day 1 vs Day 28 activity within age groups (performed as a Repeated Measures Two-Way ANOVA with activity day and hour of day as the independent factors and wheel revolutions as the dependent factor).

The effects of exercise (analyzed within age groups and hippocampal subregions) on GluN subunit expression were analyzed using two-sided unpaired t-tests. A Pearson correlation was performed between running output on the last day of wheel access, the independent variable, and relative GluN subunit expression, the dependent variable, within each age and hippocampal subregion.

Data presented are expressed as mean \pm SEM. Values of $p < 0.05$ were considered statistically significant. Graphs and statistical analysis were generated using GraphPad Prism 6.0 software.

Results

Weight Gain during Voluntary Running in Young and Mature Adult Rats

Rats were weighed once weekly during the exercise period (or time matched for sedentary controls); the average amount of weight gained by each group is presented in Figure 1. Two-way ANOVA analysis with age and activity as the independent factors and weight change as

the dependent factor demonstrated a significant main effect of age [$F_{(1, 23)}=205.71$, $p<0.0001$] and a significant main effect of activity [$F_{(1, 23)}=18.60$, $p=0.0003$], with both factors resulting in reduced weight gains. The interaction of the two factors (age x activity) was not significant [$F_{(1, 23)}=3.08$, $p=0.0926$]; therefore, post-hoc analysis was not conducted for this comparison.

Running Output in Young and Mature Adult Rats

Running output as revolutions per hour and day was monitored for the duration of the exercise period (Figure 2). Mixed model two-way ANOVA analysis, with age as the between-subjects independent factor, day of running the within-subject independent factor, and average wheel revolutions per day as the dependent factor, demonstrated a significant main effect of age [$F_{(1, 13)}=19.58$, $p=0.0007$], significant main effect of time [$F_{(27, 351)}=5.83$, $p<0.0001$], and a significant interaction of the two factors (age x time) [$F_{(27, 351)}=3.66$, $p<0.0001$]. Subsequent post-hoc comparisons demonstrated the young adult runners demonstrating greater activity levels than their mature adult counterparts ($p<0.05$; days 4–28; Figure 2A). Additionally, compared to day 1, the amount of running measured in young adult rats was significantly increased by day 4 ($p<0.05$) and remained higher for the duration of the activity period (p values); this pattern of increase in activity was not observed in the mature adult runners. Young adult runners exhibited a nearly 500% increase in activity (calculated as [(Average running days 26, 27, and 28) - (Average running days 1, 2, and 3)] / (Average running days 1, 2, and 3) * 100) while mature adult runners exhibited a significantly smaller increase (141.5% increase over the average of day 1–3) over the duration of the running period ($p=0.05$; Figure 2B).

Circadian Patterns of Running Output in Young and Mature Adult Runners

Further analyses were performed with regards to the pattern of running during the first and last 24-hour period in young adult and mature adult runners (Figures 2C and 2D). Repeated measures ANOVA in the young adult animals demonstrated a main effect of hour of day [$F_{(23, 184)}=6.428$, $p<0.0001$], a main effect of running day [$F_{(1, 8)}=12.67$, $p=0.0074$], and a significant interaction of the two factors (hour x day) [$F_{(23, 184)}=6.717$, $p<0.0001$]. Post-hoc analysis revealed no significant difference in running behavior at any time of day on the first day of running activity, but increased running during the dark phase of the 12h/12h light cycle as compared to the light phase on the last day of running activity (Figure 2C). Similarly, repeated measures ANOVA in the mature adult animals demonstrated a main effect of hour of day [$F_{(23, 138)}=5.050$, $p<0.0001$], a main effect of running day [$F_{(1, 6)}=8.241$, $p=0.0284$], and a significant interaction of the two factors (hour x day) [$F_{(23, 138)}=6.717$, $p<0.0001$] and post-hoc analysis demonstrated significant preference for running during the dark cycle on the last day of activity (Figure 2D). Repeated measure Two-Way ANOVA of running output on the final day of activity between young and mature adult rats demonstrated a significant main effect of age [$F_{(1, 14)}=8.295$, $p=0.012$], hour of day [$F_{(23, 322)}=6.783$, $p<0.0001$], and a significant interaction of the two factors (age x hour) [$F_{(23, 322)}=3.451$, $p<0.0001$]. Post-hoc analysis revealed significant increases in running behavior in young animals during the dark phase (hours 6–12) as compared to mature adult runners.

GluN Expression Following Voluntary Exercise in Young and Mature Adult Rats

Animals were euthanized following the completion of the running period, and their hippocampal tissue was processed for western blot analysis (Figure 3Ai and Bi). The expression of select GluN subunits, in relation to age-matched sedentary controls, is presented in Figure 3. Two-sided unpaired t-tests for each GluN subunit and phosphorylation site revealed significant differences between sedentary and exercising young adults in GluN1 DH (increase; $t=3.313$, $df=13$, $p=0.0056$) and VH (decrease; $t=4.182$, $df=13$, $p=0.0011$), GluN2A in the DH (increase; $t=3.685$, $df=13$, $p=0.0027$), and the ratio of 2A/2B in DH ($t=2.685$, $df=13$, $p=0.0187$).

Similar to young adult animals, mature adult rats were euthanized following the completion of the running period, and their hippocampal tissue was processed for western blot analysis as previously described. Two-sided unpaired t-tests for each GluN subunit and phosphorylation site revealed a significant reduction in GluN2A in the DH ($t=2.365$, $df=10$, $p=0.0369$). However, there was no other significant difference in expression of other GluN subunits in the DH or VH of mature running animals as compared to age- and region-matched sedentary controls.

Relationship between GluN Expression and running output in young and mature adult rats

Pearson's correlations of various metrics of running output and relative subunit expression revealed a significant negative correlation between running activity and GluN2A expression in the VH of young adults in relation to the last day of running ($r = -0.76$, $R^2 = 0.58$, $p = 0.03$) and total running ($r = -0.75$, $R^2 = 0.56$, $p = 0.03$), while running activity in mature adults was positively associated with GluN2A expression in the VH (last week of running: $r = 0.86$, $R^2 = 0.73$, $p = 0.03$; last day of running: $r = 0.88$, $R^2 = 0.78$, $p = 0.02$) (Table 2). Other relationships were not statistically significant.

Discussion

The primary goal of this work was to identify the role of exercise in modulation of GluN expression in distinct hippocampal subregions in young adult animals and determine if this modulation differs in mature adult animals. By allowing rats to begin running at two different stages of adulthood, we are able to report two major findings: 1) there are striking behavioral (running output) differences between young adult and mature adult runners, 2) running-induced changes in GluN subunit expression was subregionally distinct within the hippocampus in young adult and mature adult runners.

Broadly speaking, young adult animals escalated their running activity and showed significantly more running output than mature adult animals, and this was reflective of the magnitude of GluN expression change in the DH. From a translational perspective, this difference in running behavior is potentially an important observation, as much of our understanding of how running influences molecular and cellular components of learning is derived from analysis of young adult and aged animals. Specifically, we found that young adult animals, over the 28 day wheel access period, increased their activity while mature adult animals maintained a low-level of voluntary exercise. This discrepancy has been

observed previously in middle-aged and aged animals (6 month and 24 months of age), with the animals of advanced age running 1/10th the distance per day of the younger animals (McCullough et al., 2013). Similarly, it is observed that rats run significantly more at a younger age (4 months old) than they did as older rats (6–10 months of age) when allowed to freely exercise (Gulve et al., 1993). Taken together, the current findings in young adult and mature adult rats add to the previous studies conducted in middle-aged and aged rats that running output decreases with age, and this effect is observed at a time point during adult development, much earlier than previously investigated. A minor limitation in the current study is the lack of behavioral explanation for the age-based discrepancy in running behavior during adulthood. For example, peripheral physiological constraints such as reduced musculature efficiency (Hepple et al., 2003), increased propensity towards fatigue (Norton et al., 2001), and reduced blood oxygenation and utilization (Poole and Ferreira, 2007) could contribute to the effects.

With regards to the microanalysis of wheel running in young and mature adult animals, we found that rats in both groups on day one of wheel access do not have a preference for the day or night cycle for running activity. By day 2 of wheel access, both young adult and mature adult runners had established a preference for physical activity during the dark phase of the 24 hour cycle, suggesting that the temporal pattern of diurnal wheel running activity was similar in both groups (Klante et al., 1999).

Secondly, our findings demonstrate that young adult rats were more sensitive to the modulating effects of running on GluN expression when compared to mature adults. This finding is important because, the most widely investigated subunits of the GluN receptors are GluN2A and GluN2B due in large part to their extensive distribution in the adult hippocampus and their pivotal roles in hippocampal dependent learning and memory (Paoletti et al., 2013). With regards to hippocampal LTP, the GluN2A subunit is thought to be responsible for a larger influx of calcium upon receptor activation (potentially facilitating the induction of LTP) while GluN2B containing receptors are believed to moderate the intracellular signaling cascades (potentially facilitating the persistence of LTP) (Erreger et al., 2005). Therefore, the ratio of GluN2A to GluN2B expression can be indicative of the propensity towards LTP induction (Yashiro and Philpot, 2008). Further, the phosphorylation of these subunits (GluN2A at tyrosine 1325 and GluN2B at tyrosine 1472), both translationally modified by Src or Fyn kinase (Wang et al., 2014), can impact the functionality of the receptor. Increased phosphorylation of GluN2A at tyrosine 1325 is implicated in increasing the calcium conductance of GluN2A-containing receptors (Taniguchi et al., 2009) while phosphorylation of GluN2B at tyrosine 1472 implies increases in GluN2B-containing receptors at the synaptic membrane (Goebel-Goody et al., 2009). Taken together, the expression patterns of GluN subunits can be indicative of the total number of receptors, cellular localization, or functionality, of the receptors. Our findings demonstrate that there was a distinct difference in GluN modulation due to wheel running by age during adulthood in the DH, with young adult animals having increases in GluN1, GluN2A, and the ratio of GluN2A to GluN2B subunit expression, while mature adult animals exhibit a reduction of GluN2A in response to running. The changes in GluN subunit expression in DH of young adult runners are likely to be associated with previously reported increased cognitive capacities in the hippocampus (O'Callaghan et al., 2007; Griffin et al.,

2009; Berchtold et al., 2010; Li et al., 2013; Siette et al., 2013; Speisman et al., 2013). In the VH, GluN expression was also differentially modulated in young adult runners (reduced expression of GluN1 and 2B) compared with mature adult runners (no change), providing further evidence that the effects of running on GluN subunit expression are affected by developmental effects during adulthood. Furthermore, linear regression analysis indicated that exercise output predicts GluN2A expression in the VH in young and mature adults, where higher output predicted lower GluN2A expression in young adults, whereas higher output predicted higher GluN2A expression in mature adults. These results suggest that while adaptations in GluN2A receptor expression are produced by long-term running activity, they are significantly varied in young adult versus mature adult animals. Taken together, given that the hippocampus subregions are associated with distinct functional roles, with the DH being implicated in cognitive functions (such as spatial navigation and contextual memories) and the VH responsible for the emotional regulation (negative affect and anxiety-like responses; (for review see (Fanselow and Dong (2010))), it will be important to determine the functional significance and direct linkage of the current findings on the varied GluN subunit expression in the DH and VH in young adult and mature adult runners.

These findings, while intriguing, must be presented with a caveat. We must acknowledge that this enhanced GluN sensitivity to exercise observed in young adult animals is likely due, at least in part, to the reduction in the amount of activity measured in the mature adult animals. There is possibly a threshold of minimal physical activity which must be achieved in order for the GluN modulating effects of exercise to be observable, and the mature adult animals did not breach this threshold. Therefore, future studies to address this would require a forced exercise paradigm, which was avoided in the present work to avoid the potential for stress effects subsequent to forced activity. Additionally, we examined hippocampal homogenate from the entirety of the DH and the VH. As the hippocampus is further subdivided into 3 functionally distinct regions (the cornu amonis 3 [CA3], cornu amonis 1 [CA1], and the dentate gyrus [DG]), there may be a loss of effect by not evaluating the regions separately.

In summary, our findings here reveal developmental effects of age during adulthood- and region- specific modulation of hippocampal GluN receptors following extended access to voluntary wheel running. Future studies stemming from this work should include investigations into the emotional and behavioral correlates of these findings to better attribute the molecular changes in receptor expression to a quantifiable and translational output.

Acknowledgments

The authors would like to thank Ms. McKenzie Fannon for her careful and thoughtful review and critique of this manuscript, Eva Zamora-Martinez and Jaqueline Quigly for assistance with animal handling, Atoosa Ghofranian and Jacob Garrett for assistance with tissue processing, as well as acknowledge NIH, AA020098, AA06420 and DA034140 (to CDM), and T32AA00747 (to MCS) for funding this work. This is manuscript number 29085 from The Scripps Research Institute.

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Highlights

- Young adult runners escalate running activity
- Age is a factor in the hippocampal GluN response to voluntary wheel running
- GluN response is subregionally distinct within the hippocampus

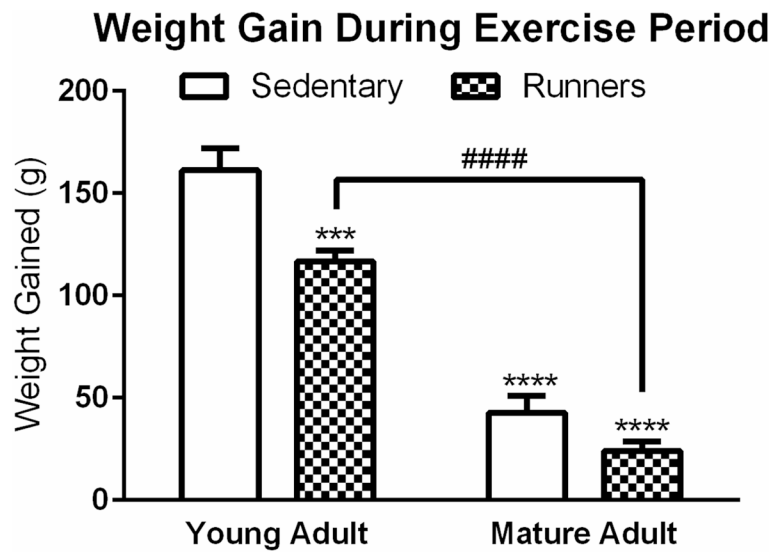


Figure 1. Weight Gain during the Exercise Period

The weight gained by each animal was monitored during the exercise period (Weight on final day of running – weight on first day of running). Running and age were significant factors in the amount of weight gained by each animal. Asterisk denotes a significant difference between sedentary young adult rats and other groups, hatch denotes a significant difference between young adult and mature adult runners. ***p 0.001, ****p 0.0001, ####p 0.0001.

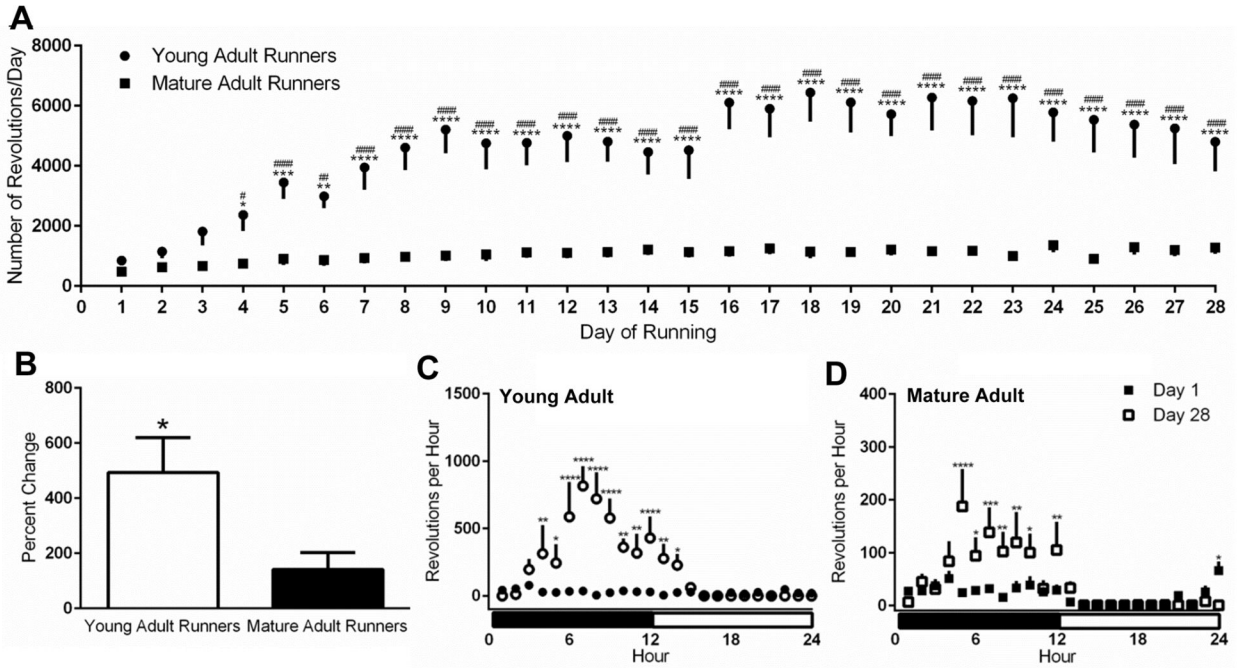


Figure 2. Running Activity in Young Adult and Mature Adult Rats

A) Daily running activity measured as number of wheel revolutions per day for young adult and mature adult rats. Asterisk denotes a significant difference between young adult and mature adult animals on a given running day, hatch denotes a significant difference of running activity between a given day of activity and the first day of wheel access. B) Percent change in running activity in young adult and mature adult rats from the first day of running to the last day of running. C) Daily wheel running patterns of young adult rats on day one (filled circle) and day 28 (open circle) of wheel access. Asterisk denotes a significant difference in activity between groups at a given time of day. D) Daily wheel running patterns of mature adult rats on day one (filled square) and day 28 (open square) of wheel access. Asterisk denotes a significant difference in activity between groups at a given time of day. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$

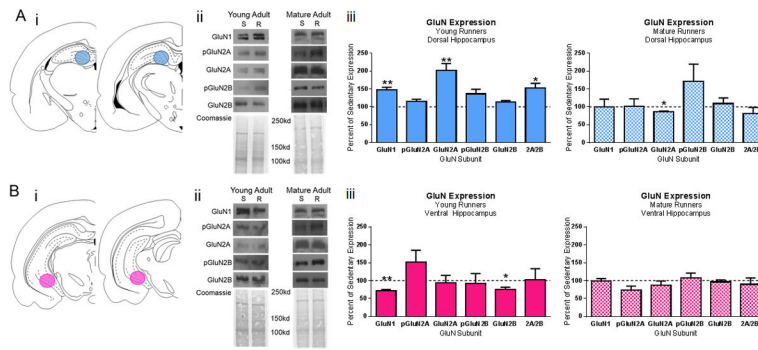


Figure 3. GluN Expression in the Dorsal and Ventral Hippocampus of Young Adult and Mature Adult Runners

Dorsal (A) and ventral (B) hippocampus tissue was collected from young and mature adult runners. i) Schematic representations of dorsal (AP -3.14 mm to -4.30 mm from bregma) and ventral hippocampus (AP -5.3 mm to -6.1 mm from bregma) sections adapted from (Paxinos and Watson, 2007). Tissue punches were collected from $500\ \mu\text{m}$ thick sections of young adult and mature adult sedentary and running rats. Blue circles represent site of dorsal tissue collection and pink circles represent site of ventral tissue collection via tissue punch. ii) Representative western blots and associated coomassie staining in sedentary (S) and running (R) animals. iii) Summarized data for GluN subunit expression as percent of sedentary age-match controls. Asterisks denote significant differences between age groups of runners within hippocampal subregion. *p 0.05, **p 0.01

Table 1

Primary and Secondary Antibody Dilutions for Western Blot Analysis of GluN Subunits.

Protein of Interest	Reported kD	Blocking	1* Vendor	1* Catalog Number	1* Host	1* Dilution	2* Vendor	2* Catalog Number	2* Host	2* Dilution	Observed kD
GluN1	130	5% Milk	Santa Cruz Biotechnology	1467	Goat	1:100	Santa Cruz Biotechnology	2020	Donkey	1:1000	125
pGluN2A	180	2.5% Milk	PhosphoSolutions	P1514-1325	Rabbit	1:200	Bio-Rad	170-6515	Goat	1:2000	200
GluN2A	178	5% Milk	Santa Cruz Biotechnology	9056	Rabbit	1:200	Bio-Rad	170-6515	Goat	1:2000	200
pGluN2B	190	2.5% Milk	Cell Signaling	4208S	Rabbit	1:200	Bio-Rad	170-6515	Goat	1:2000	200
GluN2B	178	5% Milk	Santa Cruz Biotechnology	9057	Rabbit	1:200	Bio-Rad	170-6515	Goat	1:2000	200

Table 2

Running Activity and GluN Expression in Young Adult and Mature Adult Dorsal and Ventral Hippocampus.

	Young Adult	Mature Adult
	GluN2A	GluN2A
Total Running (Sum)	DH $R^2=0.23$, $p=0.22$ VH $R^2=0.56$, $r = -0.76$; $p = 0.03^*$	DH $R^2<0.01$, $p = 0.97$ VH $R^2=0.59$, $p = 0.07$
Percent Change in Running	DH $R^2=0.18$, $p = 0.30$ VH $R^2=0.23$, $p = 0.22$	DH $R^2=0.25$, $p = 0.31$ VH $R^2=0.14$, $p = 0.47$
Last Week of Running (Sum)	DH $R^2=0.40$, $p=0.09$ VH $R^2=0.51$, $r = -0.71$; $p = 0.05$	DH $R^2 = 0.03$, $p = 0.74$ VH $R^2=0.73$, $r = 0.86$; $p = 0.03^*$
Last Day of Running	DH $R^2=0.40$, $p = 0.09$ VH $R^2=0.59$, $r = -0.75$; $p = 0.03^*$	DH $R^2=0.08$, $p = 0.58$ VH $R^2=0.78$, $r = 0.88$; $p = 0.02^*$

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