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Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats

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ABSTRACT

We tested whether daily exercise modulates immune and neuroimmune cytokines, hippocampus-dependent behavior and hippocampal neurogenesis in aging male F344 rats (18mo upon arrival). Twelve weeks after conditioned running or control group assignment, the rats were trained and tested in a rapid water maze followed by an inhibitory avoidance task. The rats were BrdU-injected beginning 12 days after behavioral testing and killed 3 weeks later to quantify cytokines and neurogenesis. Daily exercise increased neurogenesis and improved immediate and 24 h water maze discrimination index (DI) scores and 24 h inhibitory avoidance retention latencies. Daily exercise decreased cortical VEGF, hippocampal IL-1β and serum MCP-1, GRO-KC and leptin levels but increased hippocampal GRO-KC and IL-18 concentrations. Serum leptin concentration correlated negatively with new neuron number and both DI scores while hippocampal IL-1β concentration correlated negatively with memory scores in both tasks. Cortical VEGF, serum GRO-KC and serum MCP-1 levels correlated negatively with immediate DI score and we found novel positive correlations between hippocampal IL-18 and GRO-KC levels and new neuron number. Pathway analyses revealed distinct serum, hippocampal and cortical compartment cytokine relationships. Our results suggest that daily exercise potentially improves cognition in aging rats by modulating hippocampal neurogenesis and immune and neuroimmune cytokine signaling. Our correlational data begin to provide a framework for systematically manipulating these immune and neuroimmune signaling molecules to test their effects on cognition and neurogenesis across lifespan in future experiments.
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1. Introduction

Developing novel strategies to protect cognition in our burgeoning elderly population is critical for managing the burden and cost of its care. Hippocampal neurogenesis is a form of plasticity that declines significantly with age in rodents (Bizon et al., 2004; Dupret et al., 2008; Kuhn et al., 1996), dogs (Siwak-Tapp et al., 2007) and non-human primates (Aizawa et al., 2009; Gould et al., 1999b) primarily because the neural progenitor cell (NPC) precursors of new neurons and glia become increasingly quiescent with age (Cameron and McKay, 1999). The abundance of neurons added daily to the young mammalian hippocampus (Cameron and McKay, 2001) suggests that neurogenesis contributes to hippocampal integrity and indeed, measures of neurogenesis and ability in hippocampus-dependent tasks generally relate in young mammals (Deng et al., 2010; but see Epp et al., 2011; Gould et al., 1999a). Measures of neurogenesis have been related to measures of performance in hippocampus-dependent tasks among aged dogs (Siwak-Tapp et al., 2007), aged non-human primates (Aizawa et al., 2009) and when an experimental manipulation introduces enough variability into both measures to detect the relationship in aged rats (Bizon et al., 2004; Dupret et al., 2008; Kempermann et al., 2002; Speisman et al., 2012). Combined, these data suggest that protecting hippocampal neurogenesis from the effects of age may also protect some forms of cognition.

Experimental manipulations that produce neuroimmune responses can impair hippocampal neurogenesis and cognition. For example, systemic or central bacterial lipopolysaccharide (LPS) injections activate microglia, potently block neuronal differentiation (Ekdahl et al., 2003; Monje et al., 2003) and disrupt the integration of young neurons into existing hippocampal circuitry (Belarbi et al., 2012). Of the cytokines known to be stimulated by LPS (see Erickson and Banks, 2011), only a handful have been shown to affect *in vivo* or *in vitro* neurogenesis (Ben-Hur et al., 2003; Buckwalter et al., 2006; Grotendorst et al., 1989; Liu et al., 2009; Lum et al., 2009; Monje et al., 2003; Qin et al., 2008; Turrin et al., 2001; Vallieres et al., 2002; Villeda et al., 2011). In humans, experimental

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LPS impairs verbal and non-verbal memory (Reichenberg et al., 2001), but confirming its effects on neurogenesis awaits technology that permits the visualization of neurogenesis in the living brain. However little, if any, evidence of hippocampal neurogenesis is detected in the post-mortem tissue of patients who exhibited profound memory loss after γ-irradiation therapy, which also stimulates neuroimmune signaling (Coras et al., 2010; Correa et al., 2004; Crossen et al., 1994; Monje et al., 2007). The deleterious effects of LPS and γ-irradiation on hippocampal neurogenesis in rodents can be blocked by non-steroidal anti-inflammatory treatment (Monje et al., 2003; Rola et al., 2008; Tan et al., 2011), confirming a role for downstream immune and/or neuroimmune signaling cascades in mediating the effects of these treatments on neurogenesis.

In aged rodents, systemic or central LPS administration stimulates exaggerated microglial responses, cytokine levels and memory impairment (Barrientos et al., 2006; Chen et al., 2008; Godbout et al., 2005; Xu et al., 2010). In fact, the transcription of neuroimmune molecules is upregulated categorically with age but most robustly in aged rodents that exhibit impaired performances across hippocampus-dependent tasks (Blalock et al., 2003; Kohman et al., 2011a). Whole brain preparations have revealed that the concentrations of some cytokines that increase with age in rodents also associate negatively with measures of long-term potentiation and spatial ability (Felzien et al., 2001; Griffin et al., 2006; Prechel et al., 1996; Ye and Johnson, 1999). In aged and aging humans, increased circulating immune cytokine concentrations have been linked to cognitive impairments (Gimeno et al., 2008; Krabbe et al., 2009, 2004; Magaki et al., 2007; Rachal Pugh et al., 2001; Rafnsson et al., 2007; Weaver et al., 2002). In a recent study, Villeda and colleagues elegantly narrowed a list of 17 potential circulating cytokines (of 66 examined) down to 6 that related to age-impaired in neurogenesis and cognition. They then showed that increased circulating eotaxin concentrations alone compromise neurogenesis, synaptic plasticity and memory across hippocampus-dependent tasks (Villeda et al., 2011). These data highlight that the systematic testing of circulating and central cytokine biomarker correlates of neurogenesis and cognition can reveal mechanistic candidates. Importantly, these candidates can include hypoactive or senescent immune and neuroimmune cytokine signaling, particularly in aged rats (Conde and Streit, 2006; Ziv et al., 2006).

Elderly humans who exercise regularly exhibit better scores on cognitive tests and have larger hippocampal volumes relative to sedentary elderly humans (Christensen and Mackinnon, 1993; Churchill et al., 2002; Colcombe and Kramer, 2003; Erickson et al., 2010). Young and aged rodents that exercise daily on a running wheel exhibit enhanced measures of plasticity that include neurogenesis and long-term potentiation and better performances on hippocampus-dependent tasks (Brown et al., 2003; Creer et al., 2010; Kronenberg et al., 2003; Kumar et al., 2012; Lambert et al., 2005; Lugert et al., 2010; Madronal et al., 2010; Steiner et al., 2008; Suh et al., 2007; van Praag et al., 1999; van Praag et al., 2002, 2005). In young rats that run voluntarily, increased levels of neurogenesis are associated with reduced hippocampal IL-1β levels (Chennaoui et al., 2008; Farmer et al., 2004; Leasure and Decker, 2009; Stranahan et al., 2006), suggesting that physical activity may stimulate plasticity and improve cognition by modulating neuroimmune signaling pathways. There is even evidence in aged mice that cognition and immune system signaling can be modulated by physical exercise (Kohman et al., 2011a, 2011b). Therefore, we tested the effects of conditioned wheel running on the rapid acquisition and retention of a water maze hidden platform location, inhibitory avoidance acquisition and retention, hippocampal neurogenesis and 24 immune and neuroimmune cytokine concentrations in aging F344 rats. We expected that conditioned runners would exhibit better learning and memory indices and have higher rates of neurogenesis than control rats. We also expected that conditioned runners might have altered levels of immune and/or neuroimmune cytokines that may relate to measures of hippocampal integrity and/or hippocampal neurogenesis.

2. Methods

2.1. Subjects

All rat subjects were treated in accordance with University of Florida and federal policies regarding the humane care and use of laboratory animals. Upon arrival, sexually naïve male Fischer 344 rats (18 mo; $n = 12$) purchased from the National Institute of Aging colony at Harlan Sprague Dawley Laboratories (Indianapolis, IA) were housed individually in corn cob bedding-lined hanging shoebox cages located in a colony room maintained on a 12:12 h light: dark cycle at 24 ± 1 °C. The rats were given access to Harlan Teklad Rodent Diet #8604 and water *ad libitum*. All rats were weighed weekly and checked daily to ensure that they did not exhibit age-related health problems including (but not limited to) poor grooming, reduced food and water intake, excessive porphyrin secretion or weight loss.

One week after arrival, the rats were assigned randomly to the conditioned runner or control group ($n = 6$ per group). Control rats were maintained individually in standard laboratory cages with access to food and water *ad libitum* for the 18 weeks-long duration of the experiment while runners were conditioned to run for food to prevent the well-documented decreases in running behavior exhibited by aged rats across weeks of an experiment (Cui et al., 2009; Holloszy et al., 1985; Kumar et al., 2012). Therefore, runners were housed individually in a chamber containing a running wheel (model H10-38R, Coulbourn Instruments, Allentown, PA) on which they could run for unlimited food (Kumar et al., 2012). A Graphic State Notation computer program (Version 3.02, Coulbourn Instruments, Allentown, PA) recorded wheel rotations and was programmed to deliver 45 mg food pellets (Harlan Teklad Rodent Diet #8604) based upon wheel rotations. The frequency of 45 mg food pellet delivery was decreased from 1 pellet per rotation at the beginning of conditioning to 1 pellet per 3–4 m by ~4 weeks. By the 8th week of conditioning, all runners consistently ran ~ 4 km per week. If a conditioned runner lost more than 10% of the weight expected based on their pre-conditioning baseline and the weight changes of the control rats, the number of wheel rotations required for food delivery was reduced. Note that the body masses of conditioned runners (418.52 ± 5.12 g) were similar to controls (414.26 ± 5.26 g) at the beginning of the experiment ($t_{(10)} = -0.45$; $p = 0.66$) and tended to be smaller (357.97 ± 12.79 and 417.50 ± 33.41 g, respectively) at the end of the experiment ($t_{(10)} = 1.97$; $p = 0.08$). The experiment timeline is depicted in Fig. 1.

2.2. Water maze training and testing

Each rat was trained and tested in a black water maze tank (1.7 m diameter) housed in a well-lit room. The tank was filled with water (27 ± 2 °C) to a depth of 8 cm below the tank rim. A Columbus Instruments tracking system (Columbus, OH) was used to record latencies (s), pathlengths (cm), % time spent in the outer annulus of the maze and platform crossings. Rats were initially habituated to the pool on three trials during which they were released from different pool locations and allowed to climb onto a visible platform. Rats were dried with towels and warm air between blocks and before being returned to their home cages.

2.2.1. Visible platform training

Beginning the 13th week of the experiment, the rats were trained in 5 blocks of 3 60 s visible platform trials (15 min inter-block