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Research Paper

Exercise leads to the re-emergence of the cholinergic/nestin neuronal phenotype within the medial septum/diagonal band and subsequent rescue of both hippocampal ACh efflux and spatial behavior

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ABSTRACT

Exercise has been shown to improve cognitive functioning in a range of species, presumably through an increase in neurotrophins throughout the brain, but in particular the hippocampus. The current study assessed the ability of exercise to restore septohippocampal cholinergic functioning in the pyrithiamine-induced thiamine deficiency (PTD) rat model of the amnestic disorder Korsakoff Syndrome. After voluntary wheel running or sedentary control conditions (stationary wheel attached to the home cage), PTD and control rats were behaviorally tested with concurrent in vivo microdialysis, at one of two time points: 24-h or 2-weeks post-exercise. It was found that only after the 2-week adaption period did exercise lead to an interrelated sequence of events in PTD rats that included: (1) restored spatial working memory; (2) rescued behaviorally-stimulated hippocampal acetylcholine efflux; and (3) within the medial septum/diagonal band, the re-emergence of the cholinergic (choline acetyltransferase [ChAT+]) phenotype, with the greatest change occurring in the ChAT+/nestin+ neurons. Furthermore, in control rats, exercise followed by a 2-week adaption period improved hippocampal acetylcholine efflux and increased the number of neurons co-expressing the ChAT and nestin phenotype. These findings demonstrate a novel mechanism by which exercise can modulate the mature cholinergic/nestin neuronal phenotype leading to improved neurotransmitter function as well as enhanced learning and memory.

1. Introduction

Changes in cholinergic function are associated with the pathogenesis of memory and cognitive dysfunction across a range of neurological disorders. The anatomical organization of the cholinergic forebrain system positions acetylcholine (ACh) to modulate neuronal activity within, as well as across, the cortex and hippocampus (Hasselmo and Sarter, 2011; Woolf et al., 1984). The loss of cholinergic neurons in aging and disease reduces behaviorally relevant ACh efflux in both the hippocampus and cortex (see Pepeu and Giovannini, 2004). The loss of central cholinergic functioning in several neurological diseases has been linked to impaired neurotrophic signaling.

Cholinergic neurons are very responsive to changes in neurotrophin concentrations. Reductions in nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) lead to hypotrophy of cholinergic neurons, whereas sustained increases in these neurotrophins rescues degenerating forebrain cholinergic neurons (Burke et al., 1994; Dekker et al., 1994; Tuszynski et al., 1990). Furthermore, exogenous application of NGF and BDNF increase ACh release (Auld et al., 2001; Huh et al., 2008; Knipper et al., 1994) and recovers memory performance following the loss of basal forebrain neurons (Fischer et al., 1987; Frick et al., 1997; Higgins and Mufson, 1989; Markowska et al., 1996; Nagahara et al., 2009). Although these seminal works were conducted using exogenous delivery of neurotrophins, voluntary exercise robustly increases both BDNF and NGF for weeks within the hippocampus as well as the forebrain regions (Berchtold et al., 2010; Neeper et al., 1996).

Thus, it is somewhat surprising that changes in cholinergic phenotype expression and in vivo ACh efflux, relative to behavioral recovery, have not been examined following voluntary exercise. Cognitive recovery after cholinergic cell loss and following neurotrophin delivery requires weeks to emerge, suggesting that improved behavioral effects are mediated by structural changes in cholinergic neurons (Gustilo et al., 1999; Morse et al., 1993). Such remodeling could be mediated by the intermediate filament protein nestin, which is co-localized within 30–40% of cholinergic neurons in the medial septum/diagonal band ([MS/dB]; (Gu et al., 2002; Wang et al., 2006). This unique population of choline acetyltransferase (ChAT+)/nestin+ neurons has been documented in both the human and rat brain (Hendrickson et al., 2011). The distinctive role of these ChAT+/nestin+ neurons is unknown, but nestin may exert a cytoprotective function in the adult nervous system (Guo et al., 2010; Yu et al., 2011).

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Several human disorders of amnesia and dementia, as well as models of those disorders, have cholinergic cell loss as a key neuropathological feature (Schliebs and Arendt, 2011). Alcohol-related brain damage is a significant contributor to cognitive and memory decline. There is both a high prevalence of alcohol abuse (9%–22%) in patients with dementia as well as high rates of dementia (10%–24%) and cognitive decline (50%) in alcohol abusers (Ritchie and Villebrun, 2008). A history of alcohol abuse at middle age more than doubles the probability of diagnosis of a severe memory disorder as one ages (Kuzma et al., 2014). The toxic effects of alcohol, metabolic changes, as well as nutritional deficiency all contribute to alcohol-related brain damage. Thiamine deficiency is most commonly diagnosed in alcoholics, and if left untreated progresses into Korsakoff syndrome (KS), the most severe spectrum of memory dysfunction associated with alcoholism. We employed the pyrithiamine-induced thiamine deficiency (PTD) rat model of the amnestic disorder KS because of its high face and construct validity in replicating neuropathology and behavioral impairment (Savage et al., 2012). Although the hallmark neuropathology in both the PTD model, and KS, is neuronal loss in the anterior and midline thalamus as well as the mammillary bodies, there is a significant loss (30–40%) loss of MS/dB cholinergic neurons with concomitant reductions in cholinergic innervation of the hippocampus that leads to blunted hippocampal behaviorally-stimulated ACh efflux (Anzalone et al., 2010; Savage et al., 2007; Schliebs and Arendt, 2011). Furthermore, the PTD model is responsive to cholinergic modulation: increasing cholinergic tone within the septohippocampal circuit reduces or eliminates the amnestic profile of the PTD model (Roland et al., 2010; Roland et al., 2008). Thus, as in other models of memory dysfunction, the cholinergic system is critical in modulating the recovery of learning and memory.

Previous research has demonstrated that voluntary exercise is able to restore the spatial working memory deficit in the PTD model (Hall et al., 2014). In the current set of experiments, we test the premise that voluntary exercise has the capacity to rescue degenerating MS/dB cholinergic neurons and restore both behaviorally-stimulated hippocampal ACh efflux and spatial memory. Specifically, we hypothesize that the recovery of the ChAT+/nestin+ phenotype, in vivo ACh efflux and spatial working memory will require an extended period of neurotrophin exposure. The duration of 2-weeks for the length of exercise exposure was chosen because this period is sufficient to reverse hippocampal-based cognitive deficits in several other models of cognitive dysfunction (Hall et al., 2014; van Praag et al., 2005). Given that cholinergic remodeling takes time (Gustilo et al., 1999; Naumann et al., 1997), we assessed the recovery of septohippocampal functioning at two time periods: shortly after exercise (24-h post exercise) and after an extended adaption period (2-weeks post exercise). This design permits the detection of the critical period needed for exercise to modulate the cholinergic septohippocampal system.

2. Methods

2.1. Animals and treatment

Adult male Sprague–Dawley rats (N = 64; Harlan-Teklad Corp., IN), weighing between 300 and 350 g (9–11 weeks of age at the start of the experiment) served as subjects. Rats were pair-housed, placed in a temperature-controlled vivarium (20–22 °C), and maintained on a 12-h light/dark cycle with light onset at 07:00 h. Procedures were in full accordance with the Institutional Animal Care and Use Committee of Binghamton University and the National Institute of Health: Guide for the Care and Use of Laboratory Animals (2011).

Initially, rats were randomly assigned to one of two treatment conditions: (1) pyrithiamine-induced thiamine deficiency (PTD; n = 32) or (2) pair-fed controls (PF; n = 32). Fig. 1 displays the experimental timeline and design. Rats in the PTD condition received daily intraperitoneal (i.p.) pyrithiamine HBr injections (0.25 mg/kg; Sigma-Aldrich Corp., St. Louis, MO) in conjunction with ad libitum access to thiamine-deficient rat chow (Harlan-Teklad Laboratories, Indianapolis, IN). For the PF control condition, in order to replicate both dietary changes and injection procedures, rats were given a thiamine deficient chow equivalent to the amount consumed by animals in the PTD condition and were administered daily i.p. (0.4 mg/kg) injections of thiamine hydrochloride (Sigma-Aldrich Corp) to replace thiamine. Treatment continued for a period of 12–15 days until the onset of the prototypical neurological symptoms of thiamine deficiency (ataxia, loss of righting reflexes and ultimately tonic–clonic seizure activity). Subsequently, rats were closely monitored for seizure-like activity, and a thiamine bolus (100 mg/kg) was administered 4.5-h following onset of opisthotonus. The same dosage of thiamine was delivered 24-h later. Following treatment, PTD and PF rodents were placed back onto a normal diet consisting of Purina rat chow for a seven-day recovery period prior to surgery. During this time frame, rats recovered from the weight lost related to treatment.

2.2. Cannulation implantation surgery

Hippocampal cannulations were performed on all rats 7 days after PTD/PF treatment. Prior to surgery, administration of a ketamine cocktail (10 mL)/xylazine (1.43 mL) mixture at a dosage of 50 mg/kg (i.p.) was administered. Rodents were placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and the incisor bar set to 3.00 mm below the interaural line. Guide cannula (8 mm; Synaptech Technology Inc., Marquette, MI) were placed into the following ventral hippocampal coordinates [(AP) = −5.3 mm posterior to Bregma, (ML) = −5.1 mm lateral to the midline, (DV) = −4.2 mm] relative to Bregma (Paxinos and Watson, 2014). Dental acrylic cement with anchor bone screws secured guide cannula to the skull. Administration of 0.3 cm^3 of 0.05 mg/mL buprenorphine (buprenorphine hydrochloride, Hospira Inc., Lake Forest, IL) was administered pre- and post-operatively as an analgesic. Rodents were given a 7-day recovery period with ad libitum access to food prior to exercise exposure to ensure sufficient recuperatation.

2.3. Exercise paradigm

Following recovery from treatment (14 days post treatment, 7-days post surgery), PTD-treated and PF-treated rats were randomly assigned into one of two conditions: (a) a voluntary exercise condition (VEx; PTD = 16, PF = 16) or (b) a stationary condition (Stat; PTD = 16, PF = 16). The exercise wheel (35.56 cm diameter; turning resistance <6 g) was attached to a clear polycarbonate home cage (Lafayette Instruments, Lafayette, IN, 48.3 × 26.7 × 20.3 cm) for each pair of rats. To control for the environmental context, rats in the Stat condition had immobilized wheels attached to their cages. In the VEx condition wheels had a counter to measure daily wheel revolutions via a Dell Laptop equipped with AWM software (Lafayette Instruments). Throughout the duration of this procedure, all rats were slightly food restricted to 95% of free-feeding weight, to increase running distance (see Sherwin, 1998; Lee et al., 2002).

2.4. Behavioral testing

2.4.1. Spontaneous alternation with concurrent in vivo microdialysis

Rats were behaviorally tested either 24-h or 2-weeks following respective Stat/VEx conditions in order to determine the unique temporal profile of cholinergic recovery. Microdialysis procedure parameters were followed as previously described (see Savage et al., 2003). On the day of testing, the microdialysis probe (S-8020; 2 mm; Synaptech Inc.) was inserted into the hippocampal guide cannula, and the rat was placed into the opaque chamber (30 cm × 40 cm × 35 cm) to acclimate for a period of 60-min prior to maze testing. The probe was connected to a CMA microinfusion pump (CMA/400 pump; Holliston, MA) and artificial cerebrospinal fluid (7.4 pH solution: 127.6 mM NaCl, 0.9 mM NaH_2PO_4, 2 mM Na_2HPO_4, 4 mM KCl, 1.3 mM $CaCl_2$