Physical Activity Attenuates Intermittent Hypoxia-induced Spatial Learning Deficits and Oxidative Stress

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Rationale: Exposure to intermittent hypoxia (IH), such as occurs in sleep-disordered breathing, is associated with substantial cognitive impairments, oxidative stress and inflammation, and increased neuronal cell losses in brain regions underlying learning and memory in rats. Physical activity (PA) is now recognized as neuroprotective in models of neuronal injury and degeneration.

Objectives: To examine whether PA will ameliorate IH-induced deficits.

Methods: Young adult Sprague-Dawley rats were randomly assigned to one of four treatment groups including normal activity (NA) or PA for 3 months and then subjected to either normoxia (RA) or exposure to IH during the light phase during the last 14 days.

Measurements and Main Results: Significant impairments in IH-exposed rats emerged on both latency and pathlength to locate the hidden platform in a water maze and decreased spatial bias during the probe trials. These impairments were not observed in PA-IH rats. In addition, the PA-IH group, relative to NA-IH, conferred greater resistance to both lipid peroxidation and 8-hydroxy-2'-deoxyguanosine (DNA damage) in both the cortex and hippocampus. In support of a neuroprotective effect from PA, PA-IH versus NA-IH rats showed greater AKT activation and neuronal insulin growth factor-1 in these regions.

Conclusions: Behavioral modifications such as increased physical activity are associated with decreased susceptibility to IH-induced spatial task deficits and lead to reduced oxidative stress, possibly through improved preservation of insulin growth factor-1-Akt neuronal signaling. Considering the many advantages of PA, interventional strategies targeting behavioral modifications leading to increased PA should be pursued in patients with sleep-disordered breathing.

Keywords: sleep apnea; oxidative stress; neurotrophic factors

Obstructive sleep apnea (OSA) is an increasingly prevalent condition in modern society that occurs in persons of all ages, ethnicities, and sexes, with 2 to 5% of the general population being affected (1). OSA is primarily characterized by repetitive episodes of complete or partial upper airway obstruction occurring during sleep that in turn induce intermittent hypoxia, hypercapnia, and sleep fragmentation. OSA is associated with an increased risk for development of metabolic, cardiovascular, and neurobehavioral morbidities in untreated patients (2). Recent animal studies have demonstrated that chronic exposure to the hypoxia/reoxygenation patterns observed in patients with

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Intermittent hypoxia (IH) during sleep, which mimics sleep apnea in a rodent, induces significant neurocognitive deficits. The potential effects of lifestyle issues such as physical activity remain unexplored.

What This Study Adds to the Field

Nonstrenuous physical activity attenuated such IH-induced adverse effects, possibly via reduced oxidative stress, and preserved insulin growth factor 1 expression and AKT activity. These findings have clear translational implications and should foster campaigns aiming to increase physical activity habits among patients with sleep apnea.

sleep apnea mimics many of the behavioral and cognitive consequences of OSA in humans, namely learning and memory deficits and hypersomnolence (3–5). It is now well accepted that oxidative stress and inflammation play a significant role in OSA-associated pathologies, particularly in the context of neuronal cell losses in humans (6, 7), and behavioral and pathophysiological responses to intermittent hypoxia in rodents (8–17).

Oxidative stress, a common hallmark of neurodegenerative and age-related diseases, arises when the balance of reactive oxygen species (ROS) and endogenous antioxidant mechanisms becomes altered, such that increased damage to lipids, proteins, and DNA/RNA occurs, ultimately resulting in altered cellular and molecular function and subsequent dysfunction (18, 19). An increasing body of evidence suggests that moderate exercise and regular physical activity (PA) are highly beneficial to neural function under conditions associated with increased oxidative stress, such as aging or other specific disease states. In humans, PA improved mental function and was beneficial in the prevention of both age- and disease-related cognitive decline (20–24). In rodents, moderate PA was protective against excitotoxic, ischemic, and oxidative neuronal injury and degeneration (25–27). It has now become clear that the protective effects of increased moderate PA appear to be mediated via reduction in oxidative stress in the brain as well as through increased levels of neurotrophic factors involved in brain function, synaptic plasticity, and cell survival, such as insulin growth factor 1 (IGF-1), and increased activity of AKT kinase (25–27). Furthermore, evidence suggests that enriched environments, which include an increase in physical activity, may be capable of promoting tolerance to the adverse behavioral consequences of IH exposures (28). Therefore, we examined whether application of a PA regimen to young adult rats would attenuate IH-induced behavioral impairments and oxidative stress damage in brain regions underlying learning and memory.

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METHODS

Animals

A total of 124 adult male Sprague-Dawley rats (200–250 g) were obtained from a commercial supplier (Charles River, Portage, MI). All animals were group housed in accordance with institutional guidelines and were then randomly assigned to one of the experimental groups as described below. All efforts were made to minimize suffering and the number of animals used.

PA

PA was induced in a motorized forced exercise/walking wheel system (Lafayette Instruments, Lafayette, IN) that allowed for multiple animals to be run in parallel. Animals were familiarized to the apparatus for 1 week to reduce novelty stress, and then were divided into physically active (PA, $n = 24$) and non-physically active (NA, $n = 24$) groups. The PA groups were exposed to the walking wheeled system for 1 hour per day at 15 m/min, usually between 5:00 and 7:00 P.M., whereas the NA control group were placed in the wheeled system and remained in the immobile apparatus (sham training) for the same period of time.

IH Exposures

Animals were housed in eight identical commercially designed chambers (30×20×20 in) that can accommodate six rats each and are operated under a 12-hour light–dark cycle (Oxycycler model A44XO; Reming Bioinstruments, Redfield, NY). Gas was circulated around each of the chambers, attached tubing, and other units at 60 L/min (i.e., one complete change per 30 s). The O₂ concentration was continuously measured by an O₂ analyzer and was changed throughout the 12 hours of light time (6:00 A.M. to 6:00 P.M.) by a computerized system controlling the gas valve outlets, such that the moment-to-moment desired oxygen concentration of the chamber was programmed and adjusted automatically. Deviations from the desired concentration were met by addition of N₂ or room air (RA) through solenoid valves. For the remaining 12 hours of nighttime, oxygen concentrations were kept at 21%. A custom oxygen concentration profile for the 12 daylight hours was originally extrapolated from the saturation recordings of adult patients with moderate to severe obstructive sleep apnea and has been extensively used in all previous IH experiments in our laboratory (3). This specific and validated profile (3) consists of 90 seconds of 10% O₂ alternating with 90 seconds of RA for 12 hours during the light phase, and typically results in nadir PaO₂ of 37 to 42 mm Hg and oxyhemoglobin saturations ranging from 68 to 76%. Ambient CO₂ in the chamber was periodically monitored and maintained at 0.03% by circulating the gas through soda lime. The gas was also circulated through a molecular sieve (Type 3A; Fisons, East Grinstead, UK) to remove ammonia. Humidity was measured, and was maintained at 40 to 50% by circulating the gas through a freezer and silica gel. Ambient temperature was kept at 22 to 24°C. After 12 weeks of either NA or PA, rats were started on the IH protocol for 14 days, after which water maze experiments were initiated and IH exposures continued until completion of all maze procedures.

Morris Water Maze

The Morris water maze was configured to test cognitive performance that reflects spatial reference learning and memory. The maze consisted of a white circular pool, 1.8 m in diameter and 0.6 m in height, filled to a level of 35 cm with water maintained at a temperature of 27°C. Pool water was made opaque by addition of 150 to 250 ml of nontoxic white tempera paint. A Plexiglas escape platform (10 cm in diameter) was positioned 2 cm below the water surface and could be placed at various locations throughout the pool. The platform was retracted manually during probe trials. A circular white curtain, extending from the ceiling to the floor, surrounded the pool. For spatial tasks that depend on allocentric strategies, extra-maze cues were hung from the curtain at fixed locations. For cued tasks, the platform was marked by a black cylinder, elevated 10 cm above the water surface. Training regimens and maze configurations varied for each task as described below. Maze performance was recorded by a video camera suspended above the maze and interfaced with a video tracking system (HVS Imaging, Hampton, UK). Albino rats were tattooed with a black

mark to allow video tracking. Rats were given 4 training days with four training trials per day for task acquisition; 24 hours later they were tested with the hidden platform for four probe trials.

Lipid Peroxidation Assay

An MDA-586 kit (OxisResearch, Portland OR) was used to measure the relative malondialdehyde (MDA) production, a commonly used indicator of lipid peroxidation (29), in rat brain cortex according to the manufacturer's instructions. Briefly, after anesthesia with pentobarbital (50 mg/kg intraperitoneally), experimental animals were perfused with 0.9% saline buffer for 5 minutes and the cortex was dissected, snap frozen in liquid nitrogen, and stored at –80°C until assay the following day. Cortical tissues were homogenized in 20 mM phosphate buffer (pH 7.4) containing 0.5 mM butylated hydroxytoluene to prevent sample oxidation. After protein concentration measurement, equal amounts of proteins (2.0–2.5 mg protein from each sample) were used in triplicate to react with chromogenic reagents at 45°C in 500 μ L buffer for 1 to 2 hours. The samples were then centrifuged and clear supernatants measured at 586 nm. The level of MDA production was then calculated with the standard curve obtained from the kit according to the manufacturer's instructions (OxisResearch).

Immunohistochemistry

Rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and perfused transcardially with 200 ml phosphate-buffered saline (PBS) at ambient temperature and then with 2.5% paraformaldehyde in cold PBS containing 5% sucrose, pH 7.4. The brains were extracted immediately from the skull after perfusion and placed overnight in a fixative containing 1% paraformaldehyde in PBS and 30% sucrose at 4°C. Postfixed brains were sectioned on a freezing microtome. Coronal sections (30–(40 μ m) were then washed extensively in PBS and incubated in 0.4% Triton X-100 in PBS containing 1.5% normal goat serum (Vector Laboratories, Burlingame, CA) for 1 hour. Nonspecific binding sites were blocked with 10% rabbit serum for 1 hour at 37°C. Cells were incubated with primary anti-8OH-dG antibody (1:100) (1F711; Pharmingen San Diego, CA) at 4°C overnight. Anti-rabbit anti-mouse IgG conjugated to biotin and ABC reagents and avidin conjugated to horseradish peroxidase were used. To localize peroxidase, cells were treated with diaminobenzidine for 10 minutes. Sections were also processed with IGF-1 antibodies and sections were then double labeled with either anti-Neu-N (1:1,000) or flial fibrillary acidic protein (GFAP) (1:1,000) antibodies. To this effect, coronal sections were initially incubated in 0.3% H₂O₂ for 30 minutes, washed in PBS, and blocked with a PBS/0.4% Triton X-100/0.5% tyramide signal amplification (TSA) blocking reagent containing 10% normal goat serum (Vector Laboratories) for 1 hour. Sections were then serially incubated with IGF-1 antibodies (Santa Cruz Biotechnology, Cat # sc-74116, 1:100; or 2.5 μ g/ml from R&D Systems Minneapolis, MN) at 4°C for 48 hours, followed by a biotinylated anti-rabbit antibody (Vectastain Elite ABC kit, Vector Laboratories Burlingame, CA; 1:600) in a PBS/0.5% TSA blocking reagent/10% goat serum dilution, and finally with streptavidin–horseradish peroxidase diluted 1:100 in PBS/0.5% TSA blocking reagent followed by tetramethylrhodamine tyramide diluted 1:50 for 2 minutes (Perkin Elmer Life Sciences Waltham, MA). Sections were subsequently incubated with serum raised against the neuronal marker Neu-N or the glial marker GFAP (Chemicon, Temecula, CA; 1:1,000), followed by a biotinylated anti-mouse antibody (Vectastain Elite ABC kit, 1:200) and by fluorescein tyramide reagent (1:50) for 3 minutes (Perkin Elmer Life Sciences). Sections were then washed in PBS and mounted onto glass slides. In general, 10 to 20 sections per animal containing the CA1 region of the hippocampus were visualized using both fluorescent and confocal microscopes by an investigator blinded to the exposures. In addition, hippocampal tissue lysates were prepared and IGF-1-like immunoreactivity was assessed using a high-sensitivity ELISA assay (Uscn Life Science Inc. Wuhan, PR China; cat # E0050Ra), which has a linear range of 15.6 to 1,000 pg/ml and a sensitivity of 7.9 pg/ml.

Western Blotting

Brain tissue was rapidly harvested under pentobarbital anesthesia, and CA1 hippocampal samples were dissected at 4°C, snap frozen in liquid nitrogen, and kept at –80°C until analysis. The samples were homog-

enized in a lysis buffer (50 mM TRIS, pH 7.5, 0.4% NP-40, 10% glycerol, 150 mM NaCl, 10 mg/ml aprotinin, 20 mg/ml leupeptin, 10 mM ethylenediaminetetraacetic acid, 1 mM sodium orthovanadate, 100 mM sodium fluoride), and the protein concentration was determined using the Bradford method (Bio-Rad DC Hercules, CA). Samples (40 μ g protein) were resolved on 12% sodium dodecyl sulfate-polyacrylamide gels using electrophoresis (Novex/Invitrogen, Carlsbad, CA) for 120 minutes at 120 V, and electroblotted onto 0.2- μ m nitrocellulose membranes for 90 minutes at 30 V. Membranes were blocked with 5% nonfat dry milk in TRIS-buffered saline plus 0.05% Tween 20 (TBS-T) and were then incubated overnight at 4°C with primary antibodies recognizing phosphorylated (P)-AKT and AKT (Cell Signaling Technology, Beverly, MA; 1:1,000), and later with anti- β -actin for confirmation of equivalent protein loading (1:10,000; Sigma, St. Louis, MO) both diluted in 5% milk. Membranes were washed with TBS-T and incubated with either horseradish peroxidase-linked anti-rabbit or anti-mouse (for P-AKT, AKT, and β -actin; all in 1:10,000, from Cell Signaling Technology). Proteins were visualized by enhanced chemiluminescence (Amersham, Piscataway, NJ). The intensities of the bands corresponding to the protein of interest were quantified using scanning densitometry and compared using *t* tests or analysis of variance (ANOVA) as appropriate. The calculated PAKT/AKT ratio was considered as the phosphorylation ratio and compared among experimental groups. In addition, we verified AKT activity in hippocampal whole tissue lysates using a commercially available ELISA assay (Stressgen's StressXpress nonradioactive Akt/PKB Kinase Activity Assay, Ann Arbor, MI; cat # EKS-400).

Statistical Analyses

Both untransformed and normalized data were analyzed with the SPSS statistical software package (SPSS Inc., Chicago, IL). After initial one- or two-way ANOVAs, the data were analyzed using Fisher least significant difference or Student-Newman-Keuls *post hoc* tests. Mean escape latencies, swim distances, and swim speeds were analyzed by repeated-measures ANOVA and used to measure performance in the water maze. Student-Newman-Keuls *post hoc* tests were used as appropriate. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Animals maintained on a 6-week PA regimen showed significant reductions in body weight in comparison to NA controls before IH exposure (Figure 1, *P* < 0.001).

On a standard place discrimination task, NA-IH exhibited longer latencies and pathlengths to locate the hidden platform compared with PA-IH, NA-RA, and PA-RA animals (*n* = 18 per experimental condition; Figure 2A). ANOVA revealed significances by trial block interactions for both latency ($F_{3,44} = 4.940$, *P* < 0.001) and pathlength ($F_{3,44} = 5.247$, *P* < 0.001). *Post hoc* analyses showed that NA-IH rats were significantly impaired on both latency and pathlength with respect to all other groups on trial block four (*P* < 0.01). No significant group differences in swimming speed or performance during the cued task occurred among the four groups, indicating that the observed differences were not due to sensorimotor or motivational differences among the groups (Figure 2B). However, probe trials further showed reduced spatial bias in NA-IH rats as indicated by shorter pathlengths in the target quadrant (Figure 2C).

In a separate group of rats (*n* = 6 per time point), IH exposure was associated with significant increases in MDA production in the cortex of rats exposed to IH (*P* < 0.03 vs. RA; Figure 3). MDA production in PA-IH rats exposed to 21 days of IH was not significantly different from either control rats (not significant vs. RA, data not shown), indicating that PA reduced IH-induced cortical tissue lipid peroxidation (Figure 3). In addition, marked increases in 8-OHdG immunoreactivity were apparent only in NA-IH-exposed rats and not in PA-IH-

Figure 1. Mean changes in body weights of physically active (PA) and non-physically active (NA) rats (*n* = 24 per group, **P* < 0.01).

exposed animals (Figure 4A). Indeed, cell counts per field showed 4.7 ± 2.1 positively labeled cells in NA-IH in the hippocampus compared with 0.8 ± 0.7 in PA-IH-exposed rats (*P* < 0.001; *n* = 6 per experimental group with 10 sections counted per animal; Figure 4B).

We extended the latter observations and found significant decreases in the PAKT/AKT ratio for the NA-IH and PA-IH groups. However, PA was associated with higher PAKT/AKT ratios compared with NA (*P* < 0.03; Figures 5A and 5B), reflecting an overall effect of PA on the PAKT/AKT ratios. The Western blot findings were further confirmed using an AKT activity assay, whereby the overall AKT activity after IH in PA-exposed rats remained significantly higher than in NA-IH animals (Figure 5C; *P* < 0.01).

To further assess whether the protective effect of PA may be related to induction of IGF-1 pathways by regular physical activity (24, 30, 31), immunohistochemistry of the hippocampus for IGF-1 was conducted and showed increased expression of IGF-1 after PA-IH compared with IH-NA rats (Figure 6A). Of note, IGF-1 expression was circumscribed to neurons (i.e., NeuN-labeled cells), and not in glia (i.e., GFAP-labeled cells; Figure 6B). Hippocampal lysates were subjected to IGF-1 ELISA and showed increased concentrations in PA-RA (197.5 ± 27.5 pg/mg tissue) compared with NA-RA (98.1 ± 18.5 pg/mg tissue; *P* < 0.04; *n* = 5 per group). Similar PA-IH showed overall preservation of control IGF-1 tissue levels (102.7 ± 25.9 pg/mg tissue; *n* = 5), but NA-IH showed marked reductions in tissue IGF-1 levels (23.4 ± 18.9 pg/mg tissue; *n* = 6; *P* value < 0.02 vs. PA-IH).

DISCUSSION

Sleep apnea has increasingly been recognized as a cause of neurocognitive dysfunction in a significant proportion of both adults and children suffering from this condition (32–34). During sleep, patients with OSA undergo repeated periods of IH, which consist of alternations between hypoxia and reoxygenation, leading to the production of increased levels of ROS that contribute to tissue injury (35, 36). Although additional factors, such as hypercapnia, increased intrathoracic pressure swings, and sleep fragmentation, undoubtedly play an important role in the pathophysiology of the morbid consequences of OSA, it is becoming apparent that the episodic hypoxemia encountered by patients with sleep-disordered breathing imposes serious consequences on neuronal function (8–17).

The beneficial effects of regular physical activity and other forms of exercise on hippocampal function and overall brain

Figure 2. (A) Mean latencies (s) and pathlengths (cm) to locate the target platform during place training in physically active (PA) and non-physically active (NA) rats either exposed to intermittent hypoxia (IH) or maintained in room air (RA) ($n = 24$ per group, $*P < 0.05$). (B) Mean latencies (s) to locate the target platform during cued training in PA and NA rats either exposed to IH or maintained in RA ($n = 24$ per group). (C) Mean probe trial pathlength in the target quadrant after completion of water maze testing in PA and NA rats either exposed to IH or maintained in RA ($n = 24$ per group, $*P < 0.01$).

health have been repeatedly and rather conclusively demonstrated in recent years. The benefits of exercise have been best delineated for learning and memory functions, protection from neurodegeneration, and alleviation of depression, particularly in elderly populations. Exercise increases synaptic plasticity by directly modifying synaptic structure and also through potentiation of synaptic strength, and by enhancing the underlying support systems that mediate plasticity, including neurogenesis, metabolism, and vascular function (37–38). In the context of our present study, we found no evidence that less strenuous regular physical activity, such as that used herein, was indeed associated with obvious improvements in acquisition or retention of a spatial memory task in otherwise healthy young adult rats (i.e., no statistically significant differences in PA-RA compared with NA-RA groups). However, in the presence of IH, the beneficial effects of PA emerged.

Because hypoxia/reoxygenation events mimicking those that occur in patients with sleep apnea have shown activation and propagation of large-scale oxidative and inflammatory processes at both the molecular and cellular level in neurons exposed to IH, it is possible that regular PA may counteract some of these mechanisms, which have been further implicated in the adverse structural and functional consequences of OSA within the central nervous system. Indeed, the present study shows for the first time that moderate daily physical activity is capable of ameliorating behavioral dysfunction and reducing oxidative stress after IH exposures during sleep. Thus, physical activity emerges as an effective nonpharmacological intervention aiming to preserve neuronal structure and function in the context of OSA. Furthermore, these novel observations on the potential benefit of regular physical activity in patients with OSA also emphasize the possibility that differences in the global physical activity of patients with OSA (41–45), which is

capable of promoting nervous-system plasticity, may underlie important components of the variance in end-organ morbidity associated with the disorder.

Free radicals, such as ROS and reactive nitrogen species (RNS), are highly reactive molecules that occur naturally as products of normal cellular oxidative processes. In normal physiological conditions, these reactive species can act as transient signaling molecules. However, in the context of specific disease states, such as OSA, both ROS and RNS can be excessively generated and react with cellular constituents to induce cellular damage, disruption of function, and/or degen-

Figure 3. Relative fold increase in malondialdehyde (MDA) production, an indicator of lipid peroxidation, in cerebral cortex of physically active (PA) and non-physically active (NA) rats exposed to IH ($n = 6$ per group, $*P < 0.05$, PA vs. NA). MDA ratios of IH:RA in PA and NA groups are shown.

Figure 4. (A) Hippocampal and cortical sections illustrating 8-OHdG labeling, a marker of DNA oxidative damage, in physically active (PA) and non-physically active (NA) rats exposed to intermittent hypoxia (IH). (B) Mean counts of 8-OHdG positively labeled cells in hippocampus of PA and NA rats exposed to IH or room air (RA) ($n = 6$ per experimental group; 10 sections were counted by blinded investigator per animal). Arrows indicate examples of positively labeled cells. Cx = cortex; HPC = hippocampus.

eration due to the increased oxidative and nitrosative loads (46). In addition to its role of tissue injury these ROS may also act as signaling molecules that activate antiapoptotic pathways. For example, ROS amplify the phosphorylation of Akt and inhibit apoptosis (47). In the present study, we found only modest increases in the ratio of phosphorylated Akt to total Akt in animals maintained on a chronic PA routine, and such findings were further corroborated in Akt activity assays. The role for Akt and its downstream substrates has now been repeatedly substantiated in the promotion of both neuronal survival and vascular remodeling during apoptotic injury. However, because we only assessed Akt changes at one late time point, we should note that physical activity-induced changes in cell survival factors, such as AKT, often display biphasic responses, which gradually return to baseline with more chronic exposure (48), and that such temporal patterns may account for the relatively modest changes observed herein. Furthermore, our findings may also represent an overall decreased cellular stress in PA animals, because the expression of AKT is substantially up-regulated during conditions of cellular stress (49). Of note, we have previously shown that exposure to IH profiles similar to those used herein are associated with substantial decreases in Akt

expression in the hippocampus (50). The role(s) of the Akt pathway in brain function have emerged as pivotal in IGF and cytokine signaling in neurons, modulating multiple cellular processes, including apoptosis, growth, proliferation, migration, and metabolism (51). In addition, Akt may serve to activate nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase in a cascading feedback loop, illustrating that regulation of Akt-dependent pathways is a complex interplay of negative and positive influences that may be ultimately acting through common effector mechanisms (52–54). Therefore, although our findings of increased Akt activity during IH after physical activity seem to indicate a protective effect, additional studies will be needed to determine the exact role of AKT-mediated pathways in this context.

In humans, intermittent hypoxia and subsequent increases in oxidative stress have indeed been implicated in the adverse cardiovascular and metabolic effects of OSA, as well as in some of the neurodegenerative changes observed in these patients (6, 7). Animal studies have identified a comparable constellation of increased oxidative stress and inflammatory markers, all of which have been increasingly implicated in the cellular damage and consequent behavioral impairments induced

Figure 5. (Top panel) Western blot of phospho-AKT and total AKT in hippocampal lysates from rats exposed to physical activity (PA) or no physical activity (NA) and to intermittent hypoxia (IH) or room air (RA). (Left bottom panel) Mean ratio of phospho-AKT to total AKT in lysates of the hippocampal CA1 region of PA and NA rats exposed to RA or IH, ($n = 6$ per group). (Right bottom panel) Akt activity in lysates of the hippocampal CA1 region of PA and NA rats exposed to RA or IH ($n = 6$ per group).

by chronic exposures to repeated periods of hypoxia and reoxygenation during sleep (8–17). Interestingly, a vast body of evidence has corroborated the role of perturbations in free radical biology and inflammatory signaling in the pathogenesis of multiple neurodegenerative disorders, suggesting that such changes represent a common pathway ultimately leading to degenerative cellular losses over time (55, 56). Regular physical activity is a highly effective nonpharmacological approach to the improvement of mental function and reduction of oxidative stress-induced tissue damage (57). For example, regular physical activity improves visuospatial task performance in elderly humans and reduces age-related oxidative stress (57). Similarly, regular physical activity improved the performance on learning and memory tasks in rodents and was also associated with reduced levels of membrane lipid peroxidation and oxidative DNA damage at both the systemic and neuronal levels (58). Collectively, the available evidence from both basic and clinical studies strongly supports the hypothesis that moderate physical activity, such as that in the present study, can indeed promote the ability to cope with oxidative stress. Consistent with previous findings from our laboratory, we found that exposures to IH were associated with enhanced lipid peroxidation in the rat cortex (8, 9) and that physical activity markedly attenuated this phenomenon. Furthermore, IH also induced evidence of oxidative DNA damage in the hippocampus and cortex, which was also markedly reduced by the 6-week PA regimen. These findings suggest that decreases in oxidative stress underlie, at least in part, the beneficial effects of physical activity in animals chronically exposed to episodic hypoxia during sleep.

Although the mechanism(s) underlying the neuroprotective effects of our PA regimen remain to be elucidated, recent findings indicate that NADPH oxidase, a key enzyme in the production of free radical species, may be involved in both the pathophysiological consequences of IH exposures and the beneficial effects of physical activity. Indeed, chronic exposures to cyclical hypoxia and normoxia, which intend to emulate the gas exchange patterns of patients with OSA, will induce the neuronal NADPH oxidase system, and genetic ablation of key units of this enzyme are highly protective during IH, suggesting that activation of NADPH oxidase is partly responsible for the increased neuronal inflammation and oxidative stress observed in animal models of sleep apnea (13, 16). In addition, genetic polymorphisms in the NADPH oxidase enzyme were recently found to

contribute to the decreases in systemic markers of lipid peroxidation in response to a chronic, moderate physical activity program in humans (59), suggesting that a PA regimen may directly decrease the oxidative load associated with chronic IH exposures via direct effect on NADPH oxidase-dependent mechanisms. However, it needs to be stressed that physical activity can also exert positive effects on endogenous antioxidant enzymes, such as catalase superoxide dismutases, and the glutathiones (60), and that therefore its beneficial effect may reflect activities on both the pro- and antioxidant arms. Clearly, the interactions between regular physical activity and the oxidant-antioxidant pathways need to be further explored in the context of chronic IH.

PA may also alter potential adaptive and regenerative mechanisms that confer resistance to IH-induced neurocognitive susceptibility (57). For example, although exposures to low oxygen environments are capable of inducing pathological changes, exposure to hypoxia can also provoke cardiovascular, respiratory, and neuronal adaptations aiming to preserve oxygen delivery to brain tissues and provide neuroprotection (61). However, although most of those adaptations in the context of sustained hypoxia have been attributed to up-regulation of hypoxia-inducible factor 1 α , dichotomous effects of chronic IH have been reported on the various members of the hypoxia-inducible factor family (62–65). Consequently, many of the pathophysiological changes occurring in IH are either not observed or are attenuated when a similar level of hypoxia is administered in a sustained fashion (10). Physical activity could also augment the adaptive mechanisms that are ineffectively regulated in the context of IH, because physical activity is associated with enhanced cerebral blood flow and angiogenesis, the latter ultimately aiding in the maintenance of energy homeostasis, enhancement of metabolic waste removal, and conferring of resistance to injury (66). Additionally, increased production of neurotrophic factors, such as brain-derived neurotrophic factor and nerve growth factor, and enhancement of neurogenesis have been implicated in the beneficial effects of physical activity in the brain (27, 56) and could be operative in the context of IH, particularly when considering the time-dependent activation of neuronal progenitors during chronic exposures to IH during sleep (67). Indeed, changes in neuronal precursor generation, cell survival and incorporation, and increased cell density have all been reported after both voluntary

Figure 6. (A) Representative double-labeled immunohistochemical staining of hippocampal sections for insulin growth factor (IGF)-1 (red) and Neu-N (green) in non-physically active (NA; upper panel) and physically active (PA; lower panel) rats exposed to intermittent hypoxia (IH). (B) Upper panels: Higher magnification of a representative double-labeled immunohistochemical staining of hippocampal sections for IGF-1 (red) and NeuN (green) in PA and NA rats exposed to IH. Bottom panel: Representative double-labeled immunohistochemical staining of hippocampal sections for IGF-1 (red) and glial fibrillary acidic protein (GFAP) (green), showing that IGF-1 is preferentially expressed in neurons and not in glia. Similar findings were consistently found in four rats in each experimental group.

exercise and the imposed moderate physical activity used in our study (68). One recent addition to the armamentarium of beneficial effects induced by physical activity on neuronal function is IGF-1 (24, 30, 31), and our findings further support the possibility that PA-induced preservation of neuronal IGF-1 expression during IH may underlie components of the beneficial effect of exercise on IH-induced cognitive deficits. Indeed, regular physical activity was associated with overall enhancements in IGF-1 tissue levels, such that the IH-induced reductions in IGF-1 led to preservation of normal levels in PA-IH but markedly low concentrations in NA-IH conditions. Of note, we have recently reported on the relative resistance to neurocognitive deficits among children with OSA who are able mount a systemic increase in circulating IGF-1 plasma levels compared with the cognitive deficits found among those children with OSA of similar magnitude in whom IGF-1 plasma concentrations were low (69).

In conclusion, our findings clearly illustrate that regular nonstrenuous physical activity confers protection from the adverse functional consequences of IH exposures on learning and memory in rodents and is associated with a decrease in markers for oxidative stress and preservation of Akt activity and IGF-1 expression in the brain. However, further studies are needed to assess the impact of PA regimens on adaptive and regenerative mechanisms within the context of intermittent hypoxia, as well as to elucidate the specific oxidative and growth factor signaling mechanisms activated by PA. Notwithstanding such considerations, the present findings provide initial

support to the concept that behavioral interventions that promote physical activity and reduce oxidative stress may be especially beneficial in symptomatic patients afflicted with this disorder.

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