

Research report

Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus

Daniel P. McCloskey^a, David S. Adamo^a, Brenda J. Anderson^{a,b,*}

^aDepartment of Psychology, SUNY Stony Brook, Stony Brook, NY 11794-2500, USA

^bProgram in Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794-2500, USA

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Abstract

Acute bouts of exercise have been shown to produce transient increases in regional cerebral glucose utilization, oxygen uptake, and cerebral blood flow in motor cortex, striatum, and hippocampus. The purpose of this study was to determine whether or not chronic exercise will cause long-term metabolic plasticity in brain structures activated during physical activity. The activity of cytochrome oxidase (COX), is coupled to the production of ATP, and reflects long-term plasticity in metabolic capacity. The present study examined whether or not 6 months of voluntary exercise would increase COX activity in the striatum, sensorimotor cortex, and three hippocampal subfields. Five-month-old, female Long–Evans hooded rats were randomly assigned to a control or exercise condition. Exercising rats had running wheels attached to their home cages. After the training period, fresh brains were rapidly frozen and sectioned with a cryostat. COX activity was measured using COX histochemical methods and optical densitometry. Rats in the exercise condition had significantly higher optical density in the hindlimb and forelimb motor cortices (18%, $P < 0.01$) and dorsolateral caudate putamen (17%, $P < 0.01$), but not in the ventrolateral caudate putamen or any subfield of the hippocampus. Although exercise is believed to increase neuronal activity in the hippocampus, motor cortex and striatum, only limb representations in the motor cortex and striatum increase bioenergetic capacity after regular exercise. © 2001 Elsevier Science B.V. All rights reserved.

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Topic: Neural plasticity

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1. Introduction

Numerous studies suggest that exercise improves behavior, and therefore influences brain function. Participation in physical fitness programs may improve mental abilities in aged adults [14,16,27]. Active adults have shorter discrimination and reaction times than sedentary adults [57]. There is a positive relationship between activity levels and both cerebral blood flow and cognitive ability in the elderly [47]. Although human studies are vulnerable to confounding factors, data from rodents support the hypothesis that exercise may improve cognition and performance. In rats, exercise improves spatial learning [2,17,19,60], reduces escape latency [56] and

reduces age-related declines in spontaneous activity [52]. These studies raise the question of how and where exercise influences the brain.

Candidate structures that undergo exercise-related plasticity are likely to be active during physical activity. Acute metabolic mapping studies suggest that the hippocampus, motor cortex, and striatum have higher frequencies of neural activity during exercise. Single bouts of exercise in rats have been shown to produce transient increases in local cerebral glucose utilization (LCGU) in the striatum and motor cortex [62]. Likewise, LCGU increases in the hippocampus [62], a structure associated with spatial learning [4,33]. Movement is associated with increases in regional cerebral blood flow (rCBF) in the motor cortex and striatum in humans [39,48], and in the sensorimotor cortex and cerebellum in dogs [25]. Extracellular lactate, believed to be shuttled from glial cells to neurons during periods of high neuronal activity [32], increases in the rat

*Corresponding author. Tel.: +1-631-632-7821; fax: +1-631-632-7876.

E-mail address: banderson@notes.cc.sunysb.edu (B.J. Anderson).

striatum and hippocampus during physical movement by as much as 15% [12]. In addition to exercise-related increases in hippocampal LCGU and extracellular lactate, it has been shown that hippocampal CA1 pyramidal cells that represent the running position have a discharge rate that correlates with running speed [10], and the frequency of the hippocampal theta rhythm is related, in part, to the speed of locomotion [53]. Taken together these data demonstrate that metabolism increases during exercise in the hippocampus, striatum and motor cortex.

In addition to transient fluctuations in metabolism, exercise is also known to cause neurochemical and structural plasticity in the hippocampus and striatum. Exercise-related plasticity in areas of the hippocampus include increases in muscarinic receptor binding [18], NGF and BDNF mRNA [35,36], bFGF mRNA [22], cell proliferation and survival [60]. Exercise effects in the striatum include increases in DOPAC and D2 receptor binding [31]. Cells in both the dorsolateral striatum and hippocampal CA3 regions of exercising gerbils are partially protected from ischemia-related damage [58]. Although acute metabolic mapping studies identify neural structures active during exercise, neural plasticity suggests that exercise exerts an influence over these structures beyond any single episode of exercise. If exercise causes an increase in the level of sustained neural activity, either directly or through a form of plasticity, then the striatum, motor cortex and hippocampus should exhibit a long-lasting increase in the capacity to meet the additional metabolic demand.

Alterations in behavior and sustained levels of neural activity are known to alter cytochrome oxidase (COX) activity [23,64]. COX is an essential mitochondrial enzyme in the electron transport chain. Its activity is coupled to the production of ATP [30], which is primarily used to restore and maintain resting ionic gradients [55]. Because metabolic activity is tightly coupled to neuronal activity, COX is an indicator of regional functional activity in the brain. Unlike transient fluctuations in blood flow and glucose uptake, upregulation of COX takes place over longer periods of time. Therefore, increases in COX over control values reflect an increase in the capacity for ATP production.

The regions of interest for this study were the hippocampus, striatum and motor cortex. The motor regions were further restricted. Limb representations of the motor cortex [34], and limb representation in the striatum (i.e. dorsolateral caudate putamen) [15,9] were chosen. To establish that any changes were restricted to regions believed to be active during exercise, COX reactivity was also measured in a control region. Tongue, lip, and jaw representations are not expected to be active during exercise, and are located in the ventrolateral caudate putamen [15]. Therefore, the ventrolateral caudate putamen was chosen to serve as a control region. We hypothesized that exercise-related increases in COX reactivity should occur in the hippocampus and in limb representations of the motor

cortex and striatum, but not in face representations found in the ventrolateral striatum. Coronal sections were histochemically reacted for COX. Optical density for the COX reaction product was measured.

2. Materials and methods

2.1. Exercise training

Twenty female Long–Evans hooded rats (Charles River) were group housed until 5 months-of-age. Three litters were then divided into two conditions: a Voluntary Exercise condition (VX, $n=10$) and an Inactive Control condition (IC, $n=10$). Rats in both groups were housed individually in standard tub cages. VX animals had running wheels (34 cm diameter \times 10.5 cm width) attached to their cages. Wheel rotations were recorded electronically with custom software. Animals remained in their respective conditions for 6 months. Water and food consumption were monitored weekly. VX animals received food ad libitum. IC animals had food consumption yoked to VX levels to prevent overeating and to maintain equal body weights across groups.

2.2. Tissue processing

After completion of exercise training, all animals were anesthetized with 100 mg/kg sodium pentobarbital and decapitated. Brains were removed and rapidly frozen in isopentane at -40°C . All brain tissue was stored at -75°C . Whole brains were cut into 40 μm coronal sections using a cryostat maintained at -14°C . Three sets of alternate coronal sections were mounted on subbed slides and stored at -75°C .

Brains from five extra control animals were homogenized together using a mortar and pestle. The homogenate paste was frozen and stored in Eppendorf tubes at -75°C . Homogenate sections 10-, 20-, 40-, and 80- μm -thick were slide mounted and stored with the tissue sections.

2.3. Staining and histochemistry

One of the three sets of slides with alternate sections was Nissl stained with Cresyl Violet. A second set of slides was used for COX histochemistry. Sections were reacted with the histochemical method of Wong–Riley [63], but modified to increase stain intensity [51]. Tissue sections and homogenized standards were immersed in 0.1 M phosphate buffer (4% sucrose, and 0.5% glutaraldehyde) for 5 min. Then, they were immersed in 0.1 M phosphate buffer (4% sucrose) four times for 5 min each. The tissue sections and homogenates were then preincubated in Tris buffer (0.05 M, pH 7.6; 0.5% dimethylsulfoxide (DMSO), 10% sucrose and 0.023% cobalt chloride) for 10 min. A phosphate buffer rinse was then followed by

incubation for 1 h in the COX histochemical solution (50 mg diaminobenzidine, 7.5 mg cytochrome c, 2 mg catalase, 0.25 ml DMSO and 5 g sucrose in 0.1 M phosphate buffer per 100 ml solution). The solution was saturated with O_2 and maintained at 37°C. After incubation, the slides were transferred into phosphate buffer with 10% formalin and 4% sucrose for 30 min. Slides were then dehydrated and coverslipped.

Three COX incubations were conducted on separate days. Each incubation contained slides from all animals and homogenate standards that were 10-, 20-, 40- and 80- μ m-thick. The homogenate standards were used, as described by Gonzalez-Lima and Garrosa, to normalize values across incubations [24], and to ensure that optical density is linearly related to amount of COX enzyme [24]. Homogenates were also run in a separate incubation to determine the linearity of the reaction as a function of incubation time. Four sets of homogenate sections, 40- μ m-thick, were reacted for 15, 30, 45 or 60 min.

2.4. Regions of interest

Measurements from four coronal planes were taken for each of the 10 regions of interest within three primary structures, striatum, motor cortex, and hippocampus. Motor cortical measures from both layers 2 to 3 and 5 to 6 were taken from coronal planes that were -0.3, -0.8, -1.8, and -2.3 mm from bregma. When stimulated, motor cortical representations that lie within and near these planes have been shown to produce contractions of the leg muscles required for running [34]. Within each coronal plane, the regions of interest were divided into six sampling areas. Two to three sample areas were chosen for measurement by the roll of a die.

Similarly, the same coronal planes used for the motor cortex were used for measuring COX reactivity in the dorsolateral caudate putamen, a region that has greater deoxyglucose uptake during stimulation of the forelimb areas of the motor cortex [9], and receives anatomical projections from forelimb and hindlimb areas of the motor cortex [15]. The ventrolateral caudate putamen served as a control region because it receives input from the tongue, lip, and jaw representations in the motor cortex [15]. These representations should not be active during running. Measurements in the control region were taken from six coronal planes (-0.3, -0.8, -1.8, -2.3, -2.9, and -3.1 mm from bregma). For both caudate putamen regions, measures were taken from two to three randomly chosen sample areas per coronal plane. Within those areas, measures were taken between bundles of white matter yielding a measure of COX activity in the neuropil.

Exercise has been shown to influence all hippocampal subfields (dentate gyrus [60], CA3 [58], and CA1 [35,36]), therefore we measured COX levels from four coronal planes through the dorsal hippocampus (-1.8, -2.3, -2.9,

and -3.1 mm from bregma). Within each plane, measures were taken from two to three randomly chosen areas for each of the cell layer and apical dendrite containing neuropil of CA3 and CA1. Likewise, measures were taken from two to three randomly chosen sampling areas from both the cell layer and molecular layer in the dentate gyrus. For each region of interest, the values from the two to three samples in each plane were averaged. A grand mean for each region of interest was then computed from the four averages taken from each of the four coronal planes.

2.5. Image processing and data analysis

Reacted tissue sections were viewed under a Nikon Microphot-FX light microscope. Images of the regions of interest were digitally recorded using a Sony XC-70 CCD camera mounted on the microscope. Using NIH imaging software (v. 1.57), images were calibrated to a known optical density scale (Kodak Step Tablet #2). Mean optical density (MOD) measures were corrected for shading error. It has previously been shown that optical density of the histochemical reaction product is closely correlated with COX activity measured spectrophotometrically [11].

A two-way analysis of variance (group \times litter) was used to analyze heart weight data. To demonstrate that MOD was linearly related to amount of COX, a regression analysis was used to test the relationship between MOD and homogenate section thickness. We also tested for a linear relationship between MOD and length of time in the incubation solution. Group effects for each region of interest were tested with a two-way (group \times litter) analysis of covariance using MOD from the ventrolateral caudate as the covariate. Using the control region as a covariate for experimental effects on the regions of interest reduces the contribution of intrinsic differences in COX activity across animals to error term variability, and therefore increases the power for detecting treatment effects.

3. Results

3.1. Physical activity condition

One inactive control animal developed a tumor in the last month of the training period and was excluded from the analyses. Animals maintained comparable weights throughout the course of the experiment. Voluntary exercise animals produced 5438 (\pm 122 S.E.M.) running wheel rotations/day (about 6147 m/day). A two-way analysis of variance (group \times litter) revealed significant main effects for group and litter on heart weight [Group: $F_{1,18}$ =9.26, P <0.05; Litter: $F_{2,18}$ =4.59, P <0.05]. Hearts from the VX animals weighed 19% more, on average, than hearts from the IC animals.

3.2. Linearity of optical density measures

MOD was shown to be a linear function of the amount of COX by demonstrating that the relationship between MOD and section thickness was linear. Regression analysis of the MOD from 10-, 20-, 40- and 80- μm -thick homogenate sections revealed a significant linear trend [$r=0.996$, $P<0.01$; see Fig. 1a]. MOD was shown to have a linear relationship with amount of COX reactivity, by demonstrating that MOD varied linearly with incubation time. Regression analysis of the MOD for the 15, 30, 45 and 60 min incubation times for 40- μm -thick homogenates revealed a significant linear trend [$r=0.913$, $P<0.01$; Fig. 1b].

3.3. Optical densitometry

COX activity in the neuropil of the ventrolateral caudate putamen did not differ across groups [$F_{1,18}=0.332$, $P>0.10$; see Fig. 2]. For subsequent analyses, COX reactivity in the ventrolateral caudate putamen was used as a covariate. A test for an interaction between the covariate and exercise condition in subsequent regions of interest

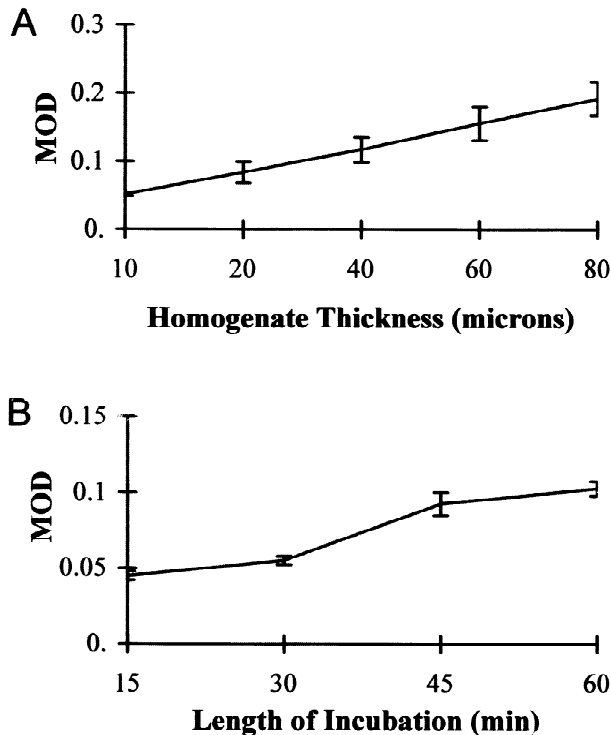


Fig. 1. Mean optical density (MOD) of homogenized brain tissue standards as a function of homogenate thickness (A: $r=0.99$, $P<0.05$) and, as a function of time in incubation solution at 37°C (B: $r=0.99$, $P<0.05$). Taken together these linear functions suggest that mean optical density has a linear relationship with cytochrome oxidase activity. The regression equation was found for the MOD of the 10, 20, 40, and 80 μm homogenate sections from the three incubations and subsequent analyses were adjusted toward the regression line to standardize MOD across incubations.

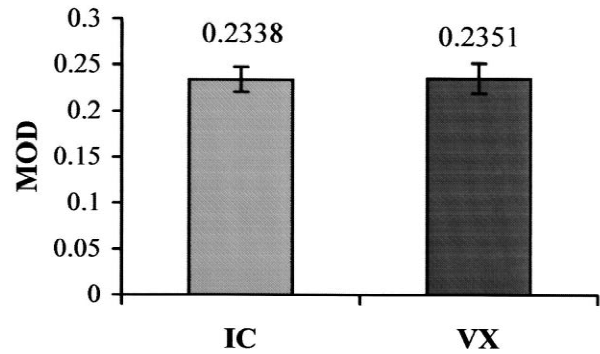


Fig. 2. Mean optical density (MOD) for ventrolateral caudate putamen in voluntary exercise (VX) and inactive control (IC) rats. Ventrolateral caudate putamen receives projections from tongue, lip, and jaw regions of the motor cortex, and therefore should not be influenced by exercise. Exercise had no significant effect on the MOD in this region.

satisfied the assumption of homogeneity of regression [for all regions: $F<1$, $P>0.10$].

3.3.1. Caudate putamen

Two-way ANCOVAs with experimental condition and litter as the between subjects factors, and the control brain region as the covariate, were run for each region of interest. In the neuropil of the dorsolateral caudate, exercise produced a significant 17% increase in MOD [$F_{1,18}=10.5$, $P<0.01$; see Fig. 3]. A significant effect of litter was found on MOD in this area [$F_{2,18}=3.9$, $P<0.05$], but no significant condition by litter interaction was found.

3.3.2. Motor cortex

In the hindlimb and forelimb areas of the motor cortex, exercise produced a significant 18% increase in MOD [$F_{1,18}=12.209$, $P<0.01$]. When analyzed by layer, both layers 2/3, and 5/6 had higher MOD in the exercise condition relative to controls [layers 2/3: 20%, $F_{1,18}=10.7$, $P<0.01$; layers 5/6: 14%, $F_{1,18}=7.97$, $P<0.05$; see Fig. 4]. There were no significant litter effects or

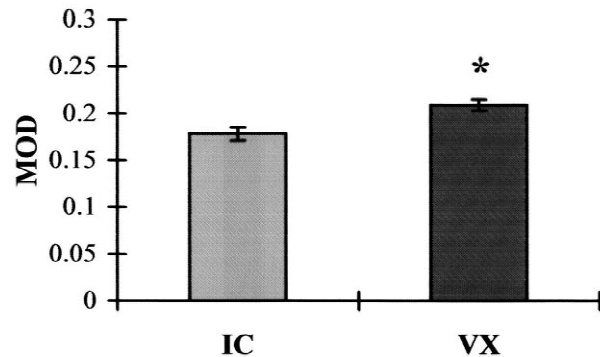


Fig. 3. Mean optical density (MOD) for dorsolateral caudate putamen in voluntary exercise (VX) and inactive control (IC) rats. Dorsolateral caudate putamen receives projections from the forelimb and hindlimb areas of the rat motor cortex. Exercise significantly increased MOD in this region. * $P<0.05$.

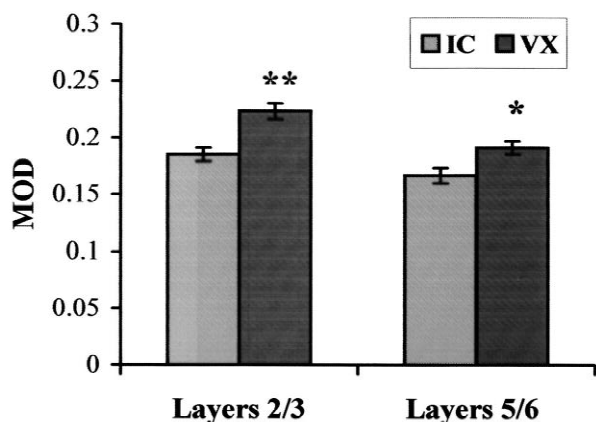


Fig. 4. Mean optical density (MOD) for motor cortex in voluntary exercise (VX) and inactive control (IC) rats. Samples from layers 2/3 (intracortical projections) and 5/6 (extracortical projections) were analyzed in the motor cortical areas that produce forelimb and hindlimb muscle contractions when microstimulated. Exercise significantly increased MOD in layers 2/3 and 5/6 of this region. ** $P < 0.01$, * $P < 0.05$.

condition \times litter interactions for layers 2/3, layers 5/6 or an average of the two layers.

3.3.3. Hippocampus

There was no significant effect of exercise on MOD in

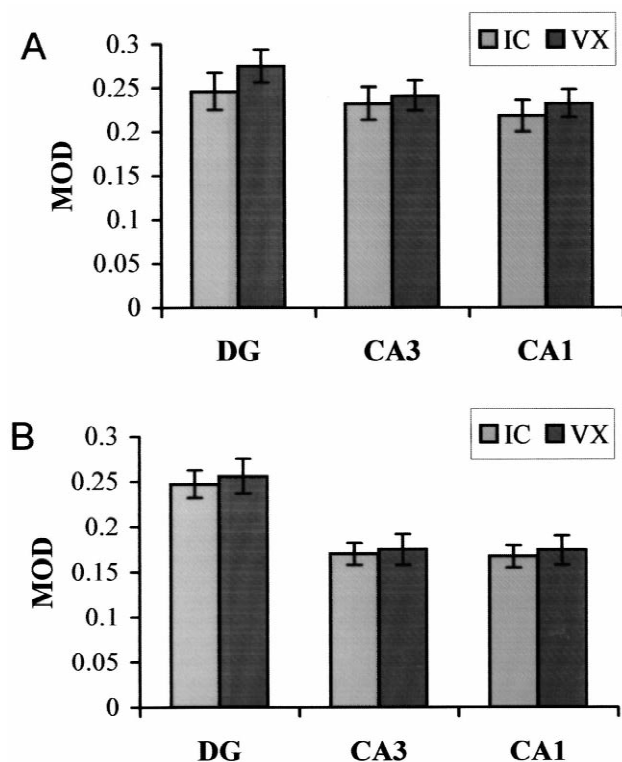


Fig. 5. Mean optical density (MOD) for hippocampus in voluntary exercise (VX) and inactive control (IC) rats. Exercise did not significantly influence MOD in the cell layer (A) of any subfields of the hippocampus (i.e. area CA1, CA3, and dentate gyrus (DG)). Exercise did not influence MOD in the apical dendrite-containing neuropil of CA1 and CA3 or the molecular layer of the dentate gyrus (B).

the hippocampus [6%, $F_{1,18} = 0.281$, $P > 0.05$]. There were no significant differences between the exercise group and the control group in any cell layers of any subfield of the hippocampus [CA3: 4%, $F_{1,18} = 0.111$, $P > 0.05$; CA1: 6%, $F_{1,18} = 0.279$, $P > 0.05$; dentate gyrus, 12%, $F_{1,18} = 0.951$, $P > 0.05$; see Fig. 5A]. Nor were there any group differences in MOD for the neuropil containing apical dendrites of any hippocampal subfield [CA3: 3%, $F_{1,18} = 0.085$, $P > 0.05$; CA1: 4%, $F_{1,18} = 0.158$, $P > 0.05$; dentate gyrus molecular layer: 4%, $F_{1,18} = 0.140$, $P > 0.05$; see Fig. 5B]. There were no significant main effects for litter or condition \times litter interactions in any subfield of the hippocampus for either dendrite containing neuropil or cell layer.

4. Discussion

Wong–Riley has demonstrated that COX activity and reactivity is reduced in central sensory structures after complete cessation of visual and auditory input, and can be used as a metabolic marker for neuronal activity [63]. COX has since been used to map sites of plasticity related to behavioral tasks. Learning-related modifications in COX activity have been seen in sensory systems [43,44], the caudoventral caudate putamen, and in the perirhinal cortex [23]. Learning-related plasticity is seen as early as the first sensory relay in the central nervous system, thus suggesting that sensory systems, under normal physiological conditions, are capable of plasticity rather than being ‘hard-wired’ [23]. The present results demonstrate that changes in behavioral output induce plasticity in central COX reactivity. The increase in COX reactivity in the present study were not found in face representations of the striatum or in the hippocampus, but were found in the limb representations of the motor cortex and striatum.

In the present study, 6 months of voluntary wheel running increased COX activity in forelimb and hindlimb representations of the motor cortex of 11-month-old rats. Significant effects were seen in both layers 2/3 and 5/6. The difference between the exercise and control condition was larger in layers of the motor cortex that send and receive intracortical projections (2/3) than layers that send extracortical projections (5/6). Greater COX activity may make both intra- and extracortical projection layers of the motor cortex capable of higher rates of neuronal activity. The increase in the capacity for oxidative metabolism may be required for higher frequency neural activity associated with faster running speeds, or longer durations of high neuronal activity associated with increased endurance.

The dorsolateral caudate putamen, known to receive input from forelimb and hindlimb representations of the motor cortex of rats [9,15] had greater COX activity in the exercise group relative to the control group. However, there were no increases in COX activity in the ventrolateral caudate putamen following 6 months of voluntary exercise. This region is believed to receive primary input

from the tongue, lip and jaw representations in the rat motor cortex [15] and therefore is unlikely to be active during exercise. Previous studies showing that rCBF and LCGU increase in striatum during exercise did not differentiate between these representational areas [12,62]. Furthermore, exercise has been shown to increase D2 receptor density in striatal homogenates [31], but no work has been done to precisely localize sites of change within the striatum. The current study indicates that the capacity for oxidative metabolism is differentially upregulated across striatal subdivisions rather than being a generalized effect within the striatum. The data raise the issue of whether or not previously reported exercise-related effects in the striatum might also be restricted to limb representations.

We found no evidence for significant adaptation in COX activity after 6 months of exercise in any neuropil or cell layer of any hippocampal subfield, despite the known relationship between CA1 hippocampal pyramidal cell discharge rate and running speed [10]. Our findings are surprising given that exercise increases extracellular lactate concentration in the hippocampus [12], and LCGU in the CA1 subfield of the hippocampus [62]. The failure to find any exercise-related increases in any area of the hippocampus was further surprising because exercise has been shown to upregulate hippocampal mRNA expression for neurotrophins [35,36] and angiogenic factors [22] and increase hippocampal muscarinic receptor density [18] and dentate gyrus cell proliferation and survival [60].

The hippocampus is associated with spatial learning, which is known to be influenced by alterations in metabolism [21,28,46,50]. Impairments in spatial learning have been shown to correspond to reductions in COX activity after permanent bilateral artery ligation [13]. The reductions in COX activity preceded hippocampal cell loss. Likewise, inhibition of COX activity with sodium azide can impair spatial learning [5]. In the present study daily running rates were similar to those of rats showing improved acquisition of spatial learning after only 7 weeks of exercise [2]. Therefore the total amount of running in the present study far exceeded the amount of exercise sufficient to produce faster acquisition of spatial learning. Despite this, no group differences in COX activity were seen in any region of the hippocampus after 6 months of exercise. Although the data suggest that spatial learning ability after exercise is not supported by significant increases in oxidative metabolism, a more direct test will be necessary to explore this relationship.

Exercise has been shown to provide positive benefits for health, leading to longer survival [26] and reduced all-cause mortality [6,41]. Perhaps the increase in COX activity associated with exercise contributes to these global beneficial effects of exercise. COX activity is coupled to the production of ATP [30]. Therefore, reductions in COX increase the likelihood of producing radical oxygen species [54], which in turn can cause mitochondrial DNA mutations and a further production of radical oxygen species

[40]. Such a vicious cycle then leads to energy crisis. Radical oxygen species, and their consequent damage, have been implicated in aging [29], Alzheimer's disease [3] and Parkinson's disease [3], all of which are associated with reduced COX activity [7,8,37,38,49,61]. Alzheimer's disease and Parkinson's disease exhibit mutations in mitochondrial DNA that encode subunits of COX [40,42]. Pharmacological strategies being explored for Alzheimer's disease and Parkinson's disease include the administration of enzymes that increase the capacity for electron transport and reduce free radical damage [1,40]. Perhaps the exercise-related increases in COX in limb representations can counter age-related decreases in COX activity, and reduce ROS production and consequent damage to mitochondrial DNA. Exercise-related upregulation of COX activity should also leave tissue less vulnerable to damage during periods of high energy demand (e.g., ischemia). Exercise has been shown to reduce oxidative damage in rats [45]. Exercise has also been shown to reduce cell loss after ischemia in both hippocampal CA3 and dorsolateral striatum [58]. The present findings raise the question of whether or not exercise-related neuroprotection would occur in the dorsolateral striatum of the rat, and if so, whether or not it could be related to exercise-induced increases in dorsolateral striatal COX activity. Future studies will investigate whether or not exercise protects these regions in the rat during periods of extreme metabolic demand, and whether or not the protection extends to other damaging factors.

The present results demonstrate that changes in behavioral output can induce plasticity in central COX reactivity. Similar to exercise-related changes in COX activity in striate muscle [20] and heart [59], exercise increases COX reactivity in limb representations of the caudate putamen and sensorimotor cortex. Metabolic plasticity was not found in face representations of the ventrolateral caudate putamen, but was found in limb representations in the dorsolateral caudate putamen. The limited distribution of the effects reported here raises the possibility that previous reports of exercise-related plasticity in the striatum may be restricted to limb representations. These results raise the question of whether or not areas with greater COX reactivity after exercise are those likely to exhibit exercise-related retardation in aging, and whether or not they are protected during periods of high metabolic demand.

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