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The Effects of Exercise Training on [^{3}H]-Spiperone Binding in Rat Striatum

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GILLIAM, P E , W W. SPIRDUSO, T. P MARTIN, T. J. WALTERS, R. E WILCOX AND R. P. FARRAR. *The effects of exercise training on [^{3}H]-spiperone binding in rat striatum* PHARMACOL BIOCHEM BEHAV **20**(6) 863–867, 1984.—Thirty male Sprague-Dawley rats 100 days of age were divided into three groups interval trained, endurance trained, and pair-weighted controls. Both trained groups ran up to one hour per day, 6 days per week for 12 weeks. The interval trained group ran up to 20 repeat intervals at 54 meters per minute for 30 seconds, while the endurance trained group ran at 27 meters per minute for 60 minutes. The animals were sacrificed, and the effects of aerobic training were documented by measuring cytochrome oxidase activity in the mixed quadriceps muscles.The cytochrome oxidase activity of the interval and endurance trained groups increased 49%, and 31% respectively, above the control group. [^{3}H]-spiperone was used to label dopamine receptors in the striatum The endurance group was not significantly different from the interval group in [^{3}H]-spiperone receptor binding, so the two exercise groups were combined to form one group of runners. The runners had significantly higher [^{3}H]-spiperone receptor binding than the controls, $F(1,26)=4.87$, $p<0.05$. The mean and standard error for receptor binding was 89±13 fmoles/mg protein for the runners and 60±5 fmoles/mg protein for the controls

Exercise Striatum Dopamine Spiperone binding Oxidative capacity

SKELETAL muscle physiological adaptations to chronic endurance exercise are well documented [11]. In contrast, little is known of the effects of chronic endurance exercise upon the central nervous system, in particular exercise effects on brain neurotransmitters. A few investigators have reported brain neurotransmitter changes in response to chronic exercise. Brown and Van Huss [7] found higher steady state whole brain norepinephrine concentrations in male Sprague-Dawley rats after 8 weeks of an interval training program. They later extended their findings by examining norepinephrine and serotonin in three brain areas (cerebral cortex, cerebellum, and remainder of the brain) of endurance trained and sedentary female rats [6]. Both norepinephrine and serotonin levels were significantly higher in most brain areas of the exercised rats.

These studies relating chronic exercise to brain neurotransmitter levels are provocative but not conclusive inasmuch as they have measured neurotransmitter concentrations. Neurotransmitter concentrations fluctuate greatly, are easily affected by the method of sacrifice and the time between dissection and freezing, and give little indication of the dynamics of the transmitter system. Receptor binding is somewhat more indicative of transmitter dynamics. It is particularly critical for neuron function since the neurotransmitter receptor binding is the first step in the postsynaptic response to an electrochemical signal [20].

The vast heterogeneity of the brain with respect to behavioral and neurotransmitter functions suggests that it is important to examine region specific transmitter functions associated with movement control. The dopaminergic nigrostriatal system has been strongly implicated in the initiation and control of movement [2, 10, 16]. For example, Wolf *et al.* [27] and Spirduso *et al.* [25] reported higher [^{3}H]-spiperone binding in the striatum of rats that were faster in speed of movement initiation than in their slower counterparts.

Inasmuch as Spirduso and Farrar [24] demonstrated that regular endurance exercise in aging rats attenuated the decline in movement initiation seen with non-exercised old rats, and since movement initiation has been shown to be related to nigrostriatal dopamine function, it is reasonable to propose that a systematic exercise training program may either directly or indirectly produce changes in nigrostriatal dopamine function.

Due to the fact that training protocols vary so much from laboratory to laboratory, validation of the training adaptations achieved need to be documented in order for valid comparisons and inferences to be made. Recently, Davies *et al.* [8] found that muscle cytochrome oxidase activity was highly correlated (r=.92) with endurance capacity, and both parameters increased with exercise training. Some marker of endurance capacity, such as cytochrome oxidase activity, should be used in any experiment in which exercise is an independent variable.

Since intensity, duration and type of training required to evoke brain neurotransmitter changes have been different in the few studies on the topic [6,7], two different chronic exercise training protocols were used. In this study the effects of interval and endurance exercise training on DA re-

TABLE 1
BODY WEIGHTS OF YOUNG MALE SPRAGUE-DAWLEY RATS AT 100 AND 180 DAYS OF AGE

Groups	Body Weights Before Training 100 days of age	Body Weights At Sacrifice 180 days of age
Controls	375 8 ± 24	547 3 ± 42
Interval Trained	384.3 ± 40	549.7 ± 33
Endurance Trained	367.9 ± 20	531 4 ± 27

Values are mean ± standard deviations
No significant differences among any groups

ceptor binding (as labeled by [^{3}H]-spiperone) in the striatum of male Sprague-Dawley rats were examined. Cytochrome oxidase activity in the mixed quadriceps muscles was employed as a marker of endurance capacity

METHOD

Treatment of Animals and Training Protocols

Thirty male Sprague-Dawley rats, 100 days of age, were randomly assigned to one of three groups: endurance trained, interval trained, or pair-weighted control. Each group consisted of 10 animals All animals were individually housed in a constant temperature and humidity room on a 6:00 a.m./6 00 p.m. light/dark cycle for the duration of the study The animals in the endurance and interval trained groups were given ad lib access to water and standard rat chow. The controls were given free access to water but food intake was restricted such that they gained body weight at the same rate as the two experimental groups. Body weights were determined twice weekly and the food restricted accordingly. The three groups did not differ significantly in body weights before or after the exercise protocol (see Table 1).

The exercised groups were trained on a motor driven treadmill (Quinton) Training consisted of either a low intensity endurance protocol or a high intensity interval protocol. Both training procedures were performed 6 days/week for 12 weeks.

Endurance Training Protocol

Endurance training consisted of daily running at a speed of 27 m/min and 0% grade, a workload of approximately 80% of their maximum oxygen consumption [22]. Animals trained under this regime initially maintained this pace for 20 min/day. Each subsequent day two additonal minutes were added to the training time until 60 minutes of continuous running were attained. This level was continued throughout the remainder of the study.

Interval Training Protocol

Interval training consisted of daily running under one of two protocols. Each protocol was preceded by a 3 minute warm-up period of running at 16.2 m/min. Protocol A required running at 54 m/min for 30 seconds followed by 60 seconds of running at a speed of 16.2 m/min. This sequence was initially repeated three times and progressively increased to 20 repetitions by the fourth week. On alternate days the animals were trained under Protocol B, which consisted of three minutes of running at 40.5 m/min followed by one minute of running at 16.2 m/min. Initially, two repetitions were performed and progressively increased until by the fourth week six repetitions were completed. Both protocols required the rats to work at or above 95% of their maximum oxygen consumption [22].

These training protocols were designed such that the interval trained group was performing at a higher intensity during the sprints than the endurance trained. Estimations based on the data of Shepherd and Gollnick [22], place the rate of oxygen consumption during the endurance training protocol at 7 6 ml O_2/100 g/min. Interval training, however, during the sprints, required 9 0 ml O_2/100 g/min under Protocol B and 10.4 ml O_2/100 g/min under Protocol A. The rationale for two different training procedures for the interval trained group was to avoid possible fatigue associated problems that may result from chronic high intensity interval training. Protocol B, of the interval training regime, allowed the animals to run at a higher intensity than those in the endurance trained group while allowing the animals to recover from the previous training under the higher speed Protocol A.

Muscle Tissue Preparation

Following the twelfth week of training one animal from each of the two experimental groups was rested for 48 hours before sacrifice. The rats were sacrificed between 7 00 and 7:30 a.m. The remaining animals maintained their training program up to the 48 hours prior to sacrifice. The quadriceps muscles were rapidly removed, gleaned of fat, weighed, and stored at −80°C until assayed On the day of the assay the quadriceps were diced while frozen and placed into ice cold 175 mM KCl, 10 mM reduced glutathione, and 2 mM EDTA, 1 g/20 ml The tissue was homogenized three times for 5 seconds at a variac setting of 50 with a Brinkman Polytron PT20. The generator and tissue were cooled in an ice bath between homogenizations. The homogenate was centrifuged at 700×g and the supernatant decanted and frozen in liquid and thawed three times.

Cytochrome Oxidase Activity

Cytochrome oxidase activity was measured polargraphically using a Yellow Springs Instruments oxygen electrode Model 5331 in a water-jacketed reaction vessel maintained at 37°C The medium was that of Abu-Errish and Sanadi [1] which consisted of 20 mM potassium phosphate, 0.1 mM EDTA, (pH 7 25), 10 mM ascorbate, 250 μM cytochrome C and 2.4 mM TMPD (N,N,N,N, tetramethyl-P-phenylenediamine) The cytochrome oxidase activity of the three groups, expressed as μmoles O_2/g wet weight of muscle, was compared by analysis of variance and Newman-Keuls post-hoc tests [26] Protein was determined by the method of Bradford [5].

[^{3}H]-Spiperone Receptor Binding

Following sacrifice, the striatum was rapidly dissected over ice and stored at −80°C for assay In vitro striatal [^{3}H]-spiperone receptor binding was carried out as previously described [19, 25, 27]. Briefly, the striatal tissue was thawed on ice and homogenized in 50 mM Tris pH 7.7 buffer containing 0 1% ascorbic acid using a Brinkman Polytron. The homogenates were centrifuged (50,000×g) for 10 minutes, the supernatant was discarded and the pellet resuspended in the pH

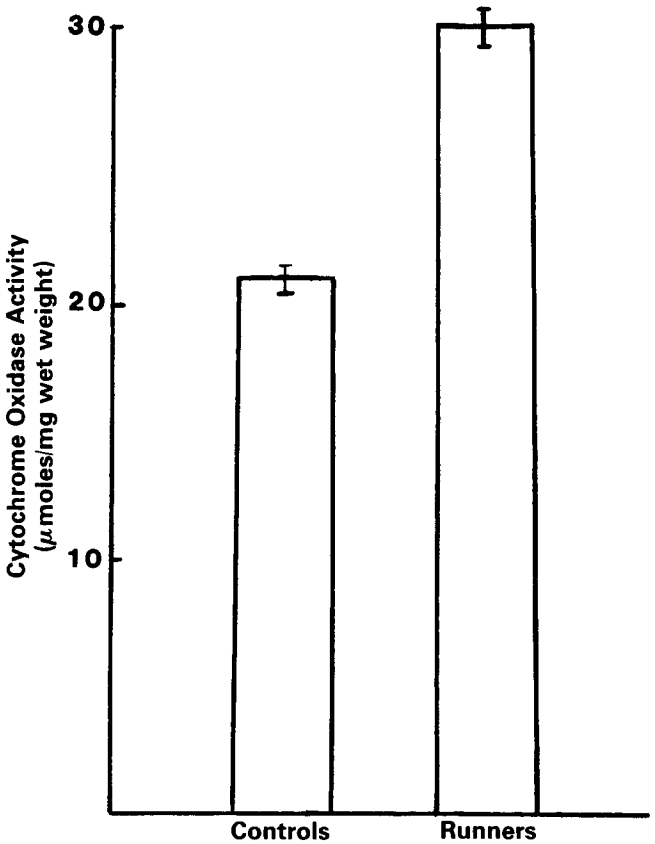

FIG 1 The cytochrome oxidase activity of the quadriceps muscles of young male Sprague-Dawley rats expressed as μmoles oxygen per gram of wet weight The two groups are significantly different at $p<0.01$ Error bars are standard errors

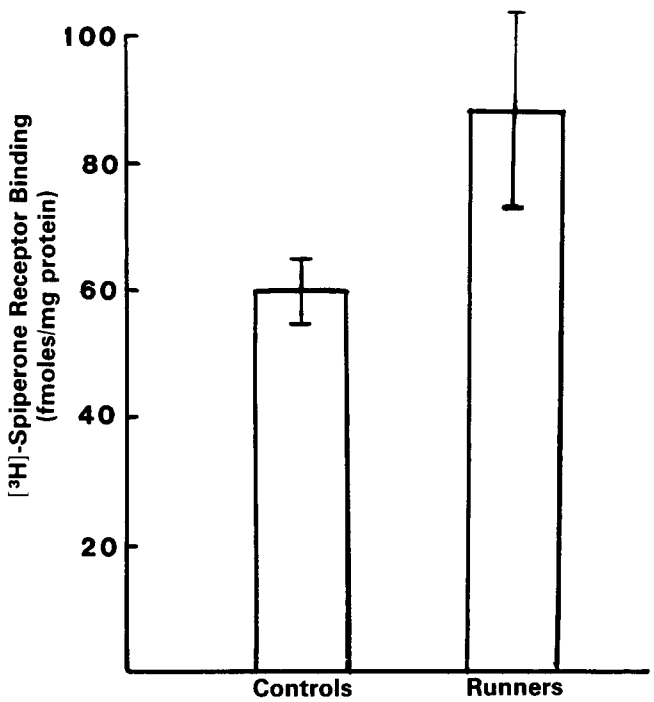

FIG 2 The [3H]-spiperone binding in the striatum of young male Sprague-Dawley rats expressed as fmoles per milligrams of protein. The two groups are significantly different at $p<0.05$ Error bars are standard errors

7.7 Tris buffer. This procedure was repeated. After discarding the supernatant a second time, the pellet was resuspended in a 50 mM Tris buffer (pH 7.1) containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM CaC12, 1 mM MgCl2 and 10 μM pargyline. The homogenate was again centrifuged (50,000×g) for 10 minutes, the supernatant discarded and the pellet resuspended in the pH 7.1 Tris buffer for preincubation (10 min at 37°C). This tissue preparation yielded a protein concentration of approximately 200 μg per incubation tube. Triplicate incubation tubes for total ligand binding contained Tris buffer (pH 7.1), ascorbic acid and concentrations of the ligand of 0.20 nM (Kd 0.23 nM; Gilliam and Wilcox, unpublished observations). Triplicate tubes for nonspecific ligand binding contained the same as above plus 1 μM d-butaclamol as the blank in each tube. Under these conditions striatal [3H]-spiperone binding is primarily due to dopaminergic receptors [13]. After a 15 minute incubation at 37°C, the contents of each tube were rapidly filtered under vacuum through Whatman GF/B microfiber filters, using Milipore filtration manifolds and the filters rinsed 3 times with ice cold Tris buffer (pH 7.7). The filters were placed in 10 ml liquid scintillation cocktail (HP Ready-Solv Beckman) and counted 24 hours later by liquid scintillation spectrometry, Beckman LS 9000, efficiency=40%. Fmoles/mg protein of each group were compared by analysis of variance and Newman-Keuls post-hoc tests [26]. Protein was determined by the method of Lowry *et al.* [15]

RESULTS

Muscle Cytochrome Oxidase Activity

Cytochrome oxidase activity of the quadriceps muscles, a marker of the effects of the exercise training, revealed that the exercised animals were indeed well-trained, $F(2,26)=55.43$, $p<0.01$ The interval trained group (mean=31.06±0.7 μmoles O_2/g wet weight) increased 49% above the controls (21.2±0.6) while the endurance trained group (27.7±0.9) increased 31% above the controls. Both trained groups were significantly different from the control group at the $p<0.01$ level.

Since the two experimental groups were not different on [3H]-spiperone binding they were combined into one running group. The running group showed a 40% increase in cytochrome oxidase activity above controls (Fig 1). The cytochrome oxidase activity for the running group was 29.7±0.8 μmoles O_2/g wet weight and for the control group was 21.2±0.6 μmoles O_2/g wet weight.

[3H]-Spiperone Receptor Binding

Since the endurance and interval groups were not significantly different from each other they were pooled to form one running group [26]. The running group was significantly higher in [3H]-spiperone receptor binding than the control, $F(1,26)=4.87$, $p<0.05$; Fig. 2.

The [3H]-spiperone receptor binding for the running group was 89±13 fmoles/mg protein and 60±5 for the control group.

DISCUSSION

This report has shown that animals, exercised on a moderate to high intensity endurance running protocol, are significantly different from sedentary controls in at least one nigrostriatal dopamine marker, receptor binding. This study is the first report in which the training protocol was well controlled, and shown to have produced a significant change in oxidative capacity, along with changes in brain neurotransmitter function. The few investigators who have reported brain neurotransmitter changes with exercise, either have had poorly controlled exercise protocols [7], or have not used any marker of training to show that the animals were indeed well trained [6,7]. While a shock control group was not included in the present study, a series of reports (reviewed by Bannon and Roth [3]) suggest that striatal dopamine markers are unaffected by the low level, infrequent foot shocks employed in this report

Though not many researchers have examined the effects of chronic exercise training on brain function, several have examined the effects of acute bouts of exercise on brain neurotransmitters. Gordon *et al.* [9] found an increased conversion of whole brain tyrosine to norepinephrine in rats after an acute bout of running (one hour) in a large motor driven rotating drum (7 rpm). The experimenters concluded that under normal circumstances, the level of brain norepinephrine is maintained during exercise because of an enhanced rate of norepinephrine synthesis. However, they did not notice an increased conversion of tyrosine-C14 to dopamine. This may have been due to the fact that they examined whole brain regions, rather than a specific region, striatum, which is associated with the control of movement and contains 80% of all of the dopamine in the brain [14]. Barchas and Freedman [4] examined the response of brain levels of norepinephrine and serotonin to an exhaustive bout of exercise. They found a 15% increase in serotonin levels, and a 20% decrease in norepinephrine levels after an exhaustive swim of four to six hours. After a run to exhaustion (approximately three hours), the rats had a 10% increase in serotonin and a 10% decrease in norepinephrine.

Semenova *et al.* [21] hypothesized that differences in motor activity of rats might be related to differences in the brain levels of norepinephrine, dopamine, and serotonin. Their results indicate that hypoactive rats are characterized by higher functional activity of the serotonergic system of the brain, whereas hyperactive animals exhibited higher activity of their catecholaminergic system. Therefore, dopamine, norepinephrine, and serotonin levels may not only respond to acute bouts of exercise, but may be responsible for activity levels within a species

A maintenance of dopamine binding accompanying exercise, might have accounted for maintained speed of movement initiation found by Spirduso and Farrar [24] in old exercised rats compared to old sedentary rats. A relationship between striatal dopamine function and movement initiation has been demonstrated in normal rats by Wolf *et al.* [27] and Spirduso *et al.* [25] They reported that animals that were fast reactors to a stimulus, had higher [^{3}H]-spiperone receptor binding than the slow reactors, both between strains, and within the same strain of rat. Also, significantly lower levels of dopamine, and dopamine receptor binding, have been consistently associated with the motor disorder, Parkinson's disease [12,18]

While it is not possible to determine nigrostriatal dopamine function in humans directly, the observation that well trained humans have faster speeds of movement initiation than their sedentary counterparts (see Spirduso [23] for review), might be attributable in part to the retention of nigrostriatal dopamine receptors in the exercised individuals Marshall and Berrios [17] have shown that injections of the dopamine receptor stimulant apomorphine can apparently reverse some movement dysfunctions in old rats. Exercise has been shown to modify various parameters of serotonin, norepinephrine, and dopamine function. Therefore, it is possible that chronic exercise has the potential to maintain the integrity of some motor functions normally lost with aging. Exercise could maintain relationships between release and binding in several brain regions, and among the various transmitter systems associated with motor behavior

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