

# Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolites in presenescent older rats

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**Abstract.** Endurance training is associated with higher binding of 3H-spiroperone to striatal D2 dopamine receptors of rats sacrificed 48 h following the last exercise bout (Gilliam et al. 1984). In the present study we investigated the effects of endurance training in presenescent older rats on the relationship between steady-state levels of DA and its metabolites in striatum versus the affinity and density of striatal D2 DA receptors. Citrate synthase activity of the gastrocnemius-plantaris muscle was  $29.06 \pm 2.27$   $\mu\text{mole/g}$  wet wt in 21-month-old trained rats versus  $22.88 \pm 1.13$   $\mu\text{mole/g}$  wet wt in 21-month-old untrained animals.

DOPAC levels and DOPAC/DA ratios were greater in the old controls. Endurance training was associated with lower DOPAC levels in the 21-month-old animals. Thus, endurance training may postpone selectively changes in DA metabolism over a portion of the lifespan.

As expected, the number of D2 DA binding sites was reduced with age (6 months  $B_{\text{max}}$ :  $429 \pm 21$  fmoles/mg protein; 21 months:  $355 \pm 20$ ) with no change in affinity. The  $B_{\text{max}}$  of old runners was significantly higher ( $457 \pm 38$  fmoles/mg protein) than that of old controls. Thus, endurance training appears to exert a protective effect on D2 dopamine receptors during the lifespan. Taken together, the present results suggest that there may be a possible reciprocal relationship between changes in DA metabolites and DA binding as a function of exercise in presenescent older rats, and that endurance training may decelerate the effects of age both on nigrostriatal dopamine neurons and on striatal D2 dopamine receptors during a portion of the lifespan.

**Key words:** Basal ganglia – D2 dopamine receptor – Dopamine metabolites – Endurance training – Aging

Millions of individuals devoted to cardiovascular conditioning believe that the quality of their lives will be improved by regular exercise (Black 1979; Dustman et al. 1984; Paffenbarger et al. 1986; MacRae et al. submitted). It has been suggested that the benefits of exercise occur through actions on many organ systems (Butler et al. 1982; Wilcox et al. 1982; Paffenbarger et al. 1983; Blair et al. 1984). For example, a beneficial association of exercise and enhanced cardiovascular functions has been demonstrated (Scheuer and Tipton 1977; Paffenbarger et al. 1983, 1986). The possible

trophic effects of exercise on brain functions and the role played by brain systems in the adaptive response to exercise may be amplified by aging. For example, the nigrostriatal dopamine system is known to deteriorate somewhat selectively with aging (Randall 1980; Severson and Finch 1980; Wilcox 1984; MacRae et al. submitted). Even in presenescent rodents, dopamine synthesis (Wilcox 1984) and the high-affinity agonist state of the D2 dopamine receptor (Severson and Randall 1985) are significantly lower than in young animals. Trophic effects of exercise might have a greater (and more beneficial) effect in an older, more compromised neurotransmitter system than in a young system that contains substantial redundancy (Samorajski et al. 1985). Indeed, trophic effects of exercise may be behaviorally significant only in an older brain that functions with a depleted striatal dopamine system.

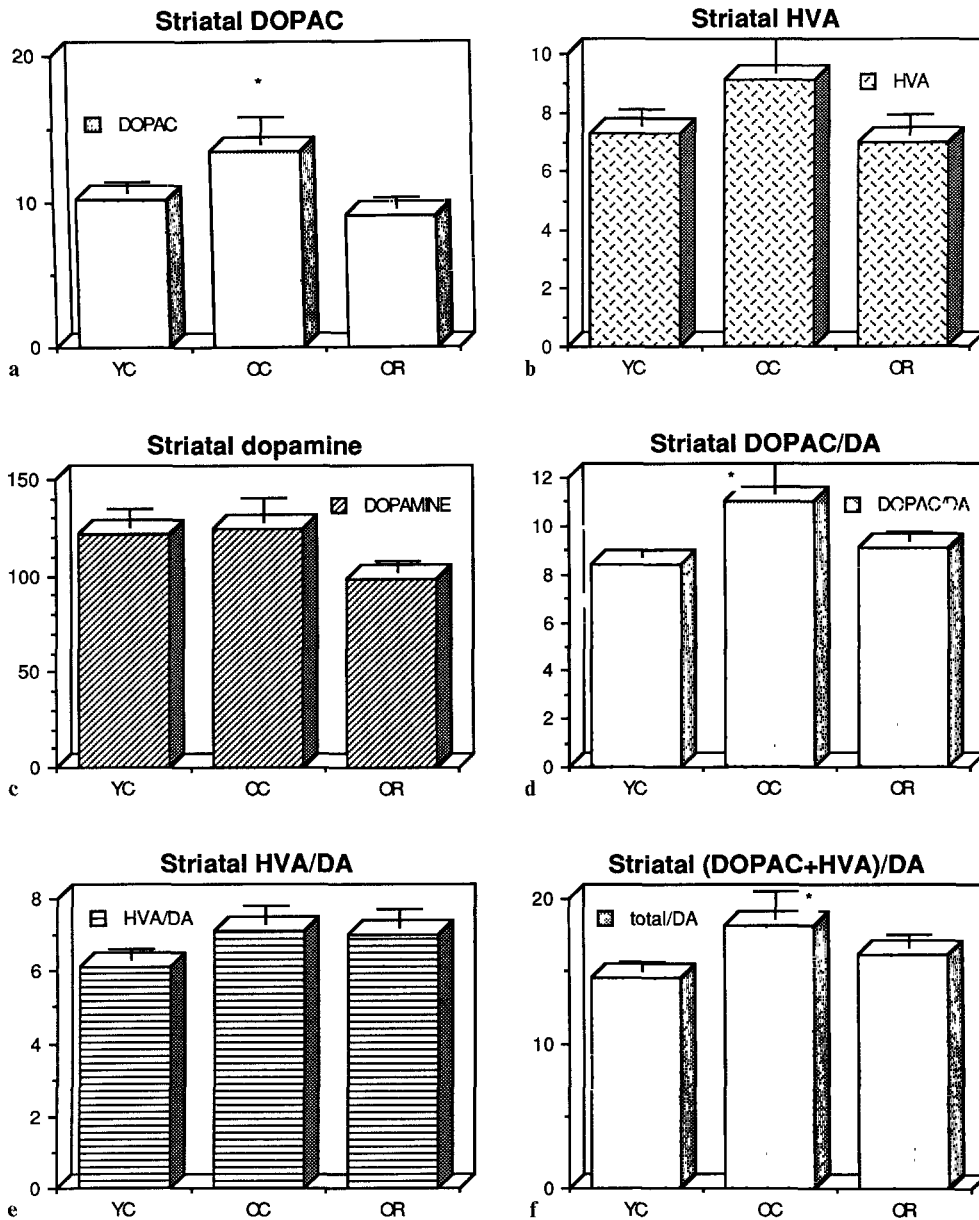
We have observed increased D2 dopamine receptor binding in striatum correlated with aerobic capacity, as shown by enhanced skeletal muscle oxidative activity following 12 weeks of treadmill conditioning (Gilliam et al. 1984). A recent paper using a complementary training technique has confirmed our original findings of changes in brain dopamine systems with exercise (de Castro and Duncan 1985). Furthermore, Heyes et al. (1985) have demonstrated that increased stimulation of brain dopamine receptors by drugs enhances the ability of rodents to run to exhaustion.

In the present study, we determined the effects of endurance training (12 weeks) on the relationships among steady state, in vivo levels of DA and its metabolites, the in vitro affinity, and density of striatal D2 DA receptors in presenescent rats (18 months at the initiation of training).

## Methods

**Subjects.** Male Sprague-Dawley rats were purchased from Harlan-Sprague-Dawley (Indianapolis, IN). Animals were held in the Animal Resources Center of the University of Texas at Austin until the appropriate age (3 or 18 months). Animals were single housed in clear Plexiglas cages with free access to food and water throughout the experiment. A 12-h light-dark cycle (lights on 7 a.m.–7 p.m.) was maintained during the experiments with training and sacrifice conducted during the period from 9 a.m. to noon.

**Exercise protocol.** Exercise was performed on a 20 lane motor driven rodent treadmill (Quinton) equipped with a metal



**Fig. 1a-f.** Endurance training effects on striatal dopamine metabolites in presenescent older Sprague-Dawley rats. *YC*=young (6 mo) sedentary control; *OR*=older (21 mo) runner; *OC*=older (21 mo) sedentary control. Panels *a-c*: data are mean  $\pm$  SEM  $\mu$ g metabolite/mg protein. \* $P$ <0.05, Newman-Keuls post-hoc test versus young control. Panel *d-f*: data are mean  $\pm$  SEM X 100 ratios of the indicated metabolite to dopamine. \* $P$ <0.05, Newman-Keuls post-hoc tests versus young control

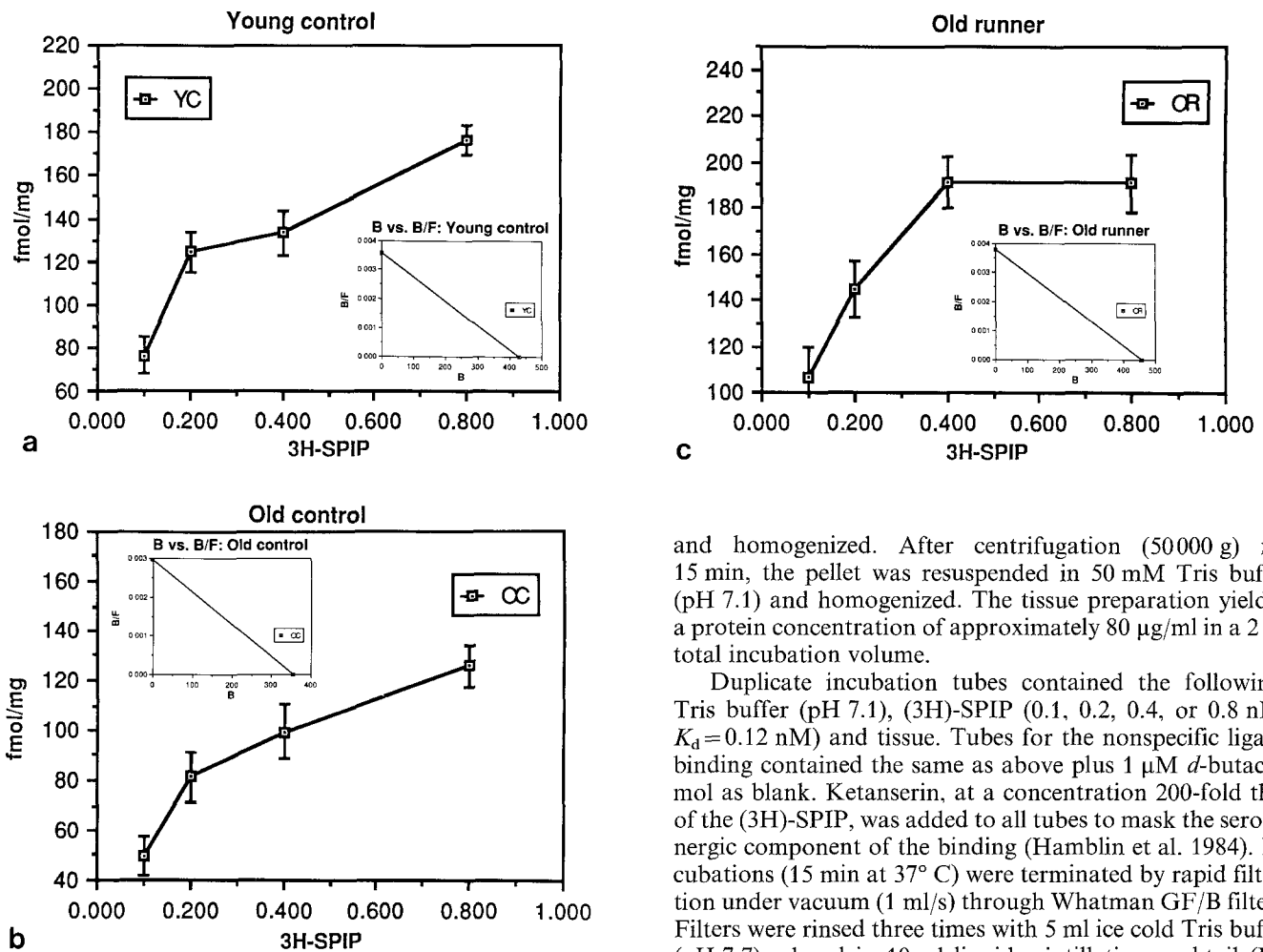
grid at the rear providing low amperage (1–2 mA) electrical shock when the animals failed to maintain the pace of the treadmill belt. All animals experienced a 2-week treadmill familiarization period during which the rats ran 5–10 min per day at a treadmill speed of 15 m/min for 5 days/week.

The untrained groups were maintained on a familiarization regime, running 5 min/day at a speed of 15–20 m/min for 2 days/week for the duration of the study. The trained groups underwent a progressive 5 day/week exercise program until the 12th week when they were running at a speed of 20 m/min for 60 min/day. Sacrifice for biochemical analyses occurred 24 h after the last testing session. All animals were sacrificed between 8 a.m. and noon.

**Gastrocnemius citrate synthase activity determination.** The hindlimb was removed at the hip immediately following

decapitation to avoid rapid post-decapitation contractions. Skin and subcutaneous adipose tissue were removed, and the gastrocnemius muscle was rapidly frozen in situ with aluminum tongs cooled with liquid nitrogen. Muscle was stored frozen at  $-80^{\circ}\text{C}$  until analyzed. Sacrifice and tissue sampling were identical for animals either running or sedentary immediately prior to sacrifice. Gastrocnemius homogenate citrate synthase activity was determined by the spectrophotometric technique described by Srere (1969). The reaction was followed on a Beckman DU-6 spectrophotometer with the temperature maintained at  $30^{\circ}\text{C}$  by a Haake water bath connected to flow-through cuvette holders.

**Materials.** (3H)-spiperone (3H-SPIP; 27.6 Ci/mole), was purchased from New England Nuclear (Boston, Massachusetts). Ketanserin was donated by Janssen (New Brunswick,



**Fig. 2a-c.** D2 dopamine receptor binding in the corpus striatum in presenescent, older sedentary and endurance trained Sprague-Dawley rats. Panels a-c: data are means  $\pm$  SEM specific D2 dopamine receptor binding in fmol/mg protein. \* $P < 0.05$ , Newman-Keuls post-hoc test versus old control. Insets: Scatchard transforms showing  $B_{max}$  (fmol/mg protein) as X-intercept

New Jersey). *d*-Butaclamol was purchased from Research Biochemical International (Wayland, MA). Dihydroxybenzylamine hydrobromide (DHBA) was purchased from Sigma.

**Brain tissue preparation.** Following sacrifice, the striata were rapidly dissected over ice, placed in 1000  $\mu$ l 50 mM Tris buffer (pH 7.7) and homogenized at the lowest possible speed with a Brinkman Polytron. A 200  $\mu$ l portion was placed in a microcentrifuge tube containing 160  $\mu$ l 10% trichloroacetic acid and 2 ng internal standard, DHBA. This was placed on dry ice and stored at  $-80^{\circ}$  C in preparation for determination of dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) concentrations. The remaining 800  $\mu$ l was stored at  $-80^{\circ}$  C for dopamine receptor binding.

**D2 dopamine receptor binding.** The homogenate was thawed on ice, diluted with 50 mM Tris buffer (pH 7.1) containing 120 mM NaCl, 5 mM KCl, 2 mM  $CaCl_2$  and 1 mM  $MgCl_2$ ,

and homogenized. After centrifugation (50000 g) for 15 min, the pellet was resuspended in 50 mM Tris buffer (pH 7.1) and homogenized. The tissue preparation yielded a protein concentration of approximately 80  $\mu$ g/ml in a 2 ml total incubation volume.

Duplicate incubation tubes contained the following: Tris buffer (pH 7.1), (3H)-SPIP (0.1, 0.2, 0.4, or 0.8 nM;  $K_d = 0.12$  nM) and tissue. Tubes for the nonspecific ligand binding contained the same as above plus 1  $\mu$ M *d*-butaclamol as blank. Ketanserin, at a concentration 200-fold that of the (3H)-SPIP, was added to all tubes to mask the serotonergic component of the binding (Hamblin et al. 1984). Incubations (15 min at  $37^{\circ}$  C) were terminated by rapid filtration under vacuum (1 ml/s) through Whatman GF/B filters. Filters were rinsed three times with 5 ml ice cold Tris buffer (pH 7.7), placed in 10 ml liquid scintillation cocktail (HP Ready-Solv; Beckman) and counted 24 h after by liquid scintillation spectrometry (efficiency = 45%). Saturation binding data were analyzed for each animal (Scatchard 1949; Limbird 1985). Homogenate protein content was determined by colorimetric assay (Lowry et al. 1951).

**Dopamine and metabolite levels.** The homogenate was thawed on ice, centrifuged at 20000 g for 30 min, and the supernatant filtered (Bioanalytical Systems MF-1, 0.45 micron regenerated cellulose filters). Filtrate (60  $\mu$ l) was injected for chromatographic analysis of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). The chromatographic system consisted of a Beckman model 112A pump, model 210 injector valve (20  $\mu$ l loop) and a reverse phase 3 micron ultrasphere C18 analytic column (0.46  $\times$  15 cm) protected by a 4 cm prefilter containing 10 micron packing. Column eluant was monitored by a Bioanalytical Systems amperometric detector model LC4A with detector potential set at +0.65 V using a glassy carbon working electrode. The LC4A detector (sensitivity set at 1 nA) was connected to a Shimadzu CR1A data processor for initial peak processing. The mobile phase consisted of 0.1 M  $NaH_2PO_4$ , 250 mg/l sodium ethylenediamine tetraacetic acid ( $Na_2EDTA$ ), 60 mg/l sodium octyl sulfate (SOS; ion pairing reagent) and 2% reagent grade methanol. With a 1.2 ml/min flow rate typical retention times were: DHBA = 3.9 min, DOPAC = 4.7 min, DA = 7.7 min, and HVA = 12.6 min. Protein content was determined by the method of Lowry et al. (1951).

*Statistical analysis.* Data from the citrate synthase activity, D2 dopamine binding, and dopamine metabolite levels determinations were analyzed by ANOVA followed by post hoc Newman-Keuls tests (Wilcoxon et al. 1979; Winer 1971).

## Results

Citrate synthase activity of the gastrocnemius-plantaris muscle of the 21-month trained animals was  $29.1 \pm 2.3$   $\mu\text{mole/g}$  wet wt versus  $22.9 \pm 1.1$  for the presenescent untrained rats. Thus, skeletal muscle oxidative capacity was enhanced by 27% in the older animals by the endurance training regime.

Twenty-one-month old sedentary rats had higher DOPAC levels ( $13.5 \pm 1.9$   $\mu\text{g/mg}$  protein versus  $9.1 \pm 0.7$   $\mu\text{g/mg}$  protein) and DOPAC/DA ratios ( $0.110 \pm 0.017$  versus  $0.091 \pm 0.004$ ) than both age-matched endurance trained animals and young sedentary rats (Fig. 1). Levels of HVA and DA were not altered either by age alone or by a combination of age and endurance training. However, HVA/DA ratios were elevated to the same extent in aged sedentary and endurance trained rats relative to young control animals (young control:  $0.061 \pm 0.003$ ; old runner:  $0.07 \pm 0.005$ ; old control:  $0.071 \pm 0.005$ ).

As expected from the literature (cf. reviews in Wilcoxon 1984; Severson and Randall 1985; Rogers and Bloom 1985) the number of D2 DA binding sites in striatum ( $B_{\text{max}}$ ) was lower in the older control animals (6 compared with 21 months:  $429 \pm 21$  versus  $355 \pm 20$  fmol/mg protein; Fig. 2). The  $B_{\text{max}}$  in old runners ( $457 \pm 38$  fmol/mg protein) was significantly higher than that of the old sedentary animals. There was no change in the affinity of binding ( $K_d$ ) in any group from 0.12 nM.

## Discussion

The relationships between chronic endurance training and central nervous system transmitter functions have not been examined in detail. Single bouts of exercise alter levels of norepinephrine (NE; decreases: Gordon et al. 1966; increases: Brown and Van Huss 1973) and serotonin (increases: Barchas and Freedman 1963). Enhanced NE synthesis (Gordon et al. 1966) has similarly been reported. More recently, Semenova et al. (1981) and Lukaszyk et al. (1983) observed that 30 min exercise in untrained animals increased NE levels in cortex and hippocampus while reducing levels in the hypothalamus; serotonin levels decreased in striatum, cortex and midbrain; DA levels decreased in hypothalamus, striatum, cortex and midbrain. In cerebrum, levels of NE were increased as were levels of serotonin in midbrain following 8 weeks of continuous treadmill running (Brown et al. 1979). Similarly, recent studies have suggested that the response to cholinergic blocking drugs is altered by acute and chronic exercise (McMaster and Carney 1985a, b). Information suggesting a mechanism by which exercise might modulate DA receptor function is sparse. In what we believe to have been the first study in which the effect of chronic endurance training on brain catecholamine receptors was examined, we reported that specific binding of 3H-spiperone to striatal DA receptors was enhanced (Gilliam et al. 1984).

The major finding of the present study is that a 5 day/week endurance training regime, at approximately 80% of the maximal oxygen consumption, over a 3-month period

stabilizes striatal DOPAC levels, DOPAC/DA ratios, and the number of D2 BA receptors in presenescent older rats sacrificed 24 h after the last endurance training session. Whereas aging (to 21 months) is associated with elevated DA metabolism and lowered D2 DA receptor density, endurance trained rats demonstrate DOPAC levels and D2 DA receptor numbers equivalent to those of young adult animals. Our studies suggest that changes in DA metabolism and in D2 DA receptors that normally occur with aging appear to be damped in 21-month-old rats trained and sacrificed according to the protocol of the present study. In the present experiments, both metabolites and receptor binding were assessed within the same brain region of the same animal in response to exercise. In addition, specific resolution of D2 dopamine receptors was achieved by masking a possible serotonergic component of the 3H-SPIP binding. In this way endurance training effects on striatal DA synapses could be estimated with an enhanced likelihood of establishing neurochemical correlates of functional significance to the organism.

In the long run, viable approaches to the study of exercise effects on brain transmitters will require experiments with numerous controls both for the training effect and for the neurochemical measures of interest. It will also be important to include evaluations of transmitter interactions. This becomes most feasible when both synaptic content and receptor binding of each transmitter are measured. Such experiments can extend present knowledge by showing exercise effects as putative patterns among components of functional sensorimotor circuits. The effect of exercise on neurotransmitter systems appears to be an important line of investigation which may become increasingly relevant in the study of changes in reaction time during aging and in alterations in the response to dietary and pharmacological interventions in the older population.

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## References

- Barchas JD, Freedman DX (1963) Brain amines: response to physiological stress. *Biochem Pharmacol* 12:1232-1235
- Black HR (1979) Nonpharmacologic therapy for hypertension. *Am J Med* 66:837
- Blair SN, Goodyear NN, Gibbons LW (1984) Physical fitness and incidence of hypertension in healthy normotensive men and women. *JAMA* 252:487
- Brown BS, Van Huss W (1973) Exercise and rat brain catecholamine. *J Appl Physiol* 34(5):664-669
- Brown BS, Payne T, Kim C, Moore G, Krebs P, Martin W (1979) Chronic response of rat brain norepinephrine and serotonin levels to endurance training. *J Appl Physiol: Respirat Environ Exercise Physiol* 46(1):19-23
- Butler J, O'Brien M, O'Malley K, Kelly JG (1982) Relationship of  $\beta$ -adrenoceptor density to fitness in athletes. *Nature* 298:60-62
- deCastro JM, Duncan G (1985) Operantly conditioned running: effects on brain catecholamine concentrations and receptor densities in the rat. *Pharmacol Biochem Behav* 23:495-500
- Dustman RE, Ruhling RO, Russell EM, Shearer DE, Bonekat W, Shigeoka JW, Wood JS, Bradford DC (1984) Aerobic exer-

- cise training and improved neuropsychological function of older individuals. *Neurobiol Aging* 5:35-42
- Gilliam PE, Spirduso WW, Martin TP, Walters TJ, Wilcox RE, Farrar RP (1984) The effects of exercise training on 3H-spiroperone binding in rat striatum. *Pharmacol Biochem Behav* 20:863-867
- Gordon R, Spector S, Sjoerdsma A, Udenfriend S (1966) Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and exposure to cold. *J Pharmacol Exp Ther* 153:440-447
- Hamblin MW, Leff SE, Creese I (1984) Interactions of agonists with D2 dopamine receptors: evidence for a single receptor population existing in multiple agonist affinity states in rat striatal membranes. *Biochem Pharmacol* 33:877-887
- Heyes MP, Garnett ES, Coates G (1985) Central dopaminergic activity influences rats ability to exercise. *Life Sci* 36:671-677
- Limbird LL (1985) Cell surface receptors: a short course on theory and methods. Boston, Nijhoff, pp 1-196
- Lowry OH, Rosebrough I, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-273
- Lukaszyk A, Buczko W, Wisniewski K (1983) The effect of strenuous exercise on the reactivity of the central dopaminergic system in the rat. *Pol J Pharmacol Pharm* 35:29-36
- McMaster SB, Carney JM (1985a) Exercise-induced changes in scheduled controlled behavior. *Physiol Behav* 35:337-341
- McMaster SB, Carney JM (1985b) Changes in drug sensitivity following acute and chronic exercise. *Pharmacol Biochem Behav* 23:191-194
- Paffenbarger RS Jr, Wing AL, Hyde RT, Jung DL (1983) Physical activity and incidence of hypertension in college alumni. *Am J Epidemiol* 117:245-257
- Paffenbarger RS Jr, Hyde RT, Wing AL, Hsieh CC (1986) Physical activity, all-cause mortality, and longevity of college alumni. *New Engl J Med* 314:605-613
- Randall PK (1980) Functional aging of the nigrostriatal system. *Peptides [Suppl 1]* 1:177-184
- Rogers J, Bloom FE (1985) Neurotransmitter metabolism and function in the aging central nervous system. In: Finch CE, Schneider EL (eds) *Handbook of the biology of aging* (2nd edition). New York, Van Nostrand Reinhold, pp 645-691
- Samorajski T, Delaney C, Durham L, Ordy JM, Johnson JA, Dunlap WP (1985) Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57/BL6J mice. *Neurobiol. Aging* 6:17-24
- Scatchard G (1949) The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* 51:660-672
- Scheuer J, Tipton CM (1977) Cardiovascular adaptations to physical training. *Annu Rev Physiol* 39:221
- Semenova TP, Ivanov VA, Tretyak TM (1981) Brain levels of noradrenaline, dopamine and serotonin in rats with different levels of motor activity. *Neurosci Behav Physiol* 2:153-155
- Severson JA, Finch CE (1980) Reduced dopaminergic binding during aging in the rodent striatum. *Brain Res* 192:147-162
- Severson JA, Randall PK (1985) D2 Dopamine receptors in aging mouse striatum: determination of high- and low-affinity agonist binding sites. *J Pharmacol Exp Ther* 233:361-368
- Srere PA (1969) Citrate synthase. In: Lowenstein JM (ed) *Methods in enzymology* 13. New York, Academic, pp 3-5
- Wilcox RE (1984) Changes in biogenic amines and their metabolites with aging and alcoholism. In: Hartford JT, Samorajski T (eds) *Alcoholism in the elderly*. New York, Raven, pp 85-115
- Wilcox RE, Hightower WH, Smith RV (1979) Post-hoc data analysis in biomedical research. *Am Lab* 11:32-45
- Wilcox RG, Bennett T, Brown AM (1982) Is exercise good for high blood pressure? *Br Med J* 285:767
- Winer BJ (1971) *Statistical principles in experimental design*. New York, McGraw-Hill

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