Reversibility of exercise-induced dendritic attenuation in brain cardiorespiratory and locomotor areas following exercise detraining

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Nelson, Amanda J., and Gary A. Iwamoto. Reversibility of exercise-induced dendritic attenuation in brain cardiorespiratory and locomotor areas following exercise detraining. J Appl Physiol 101: 1243–1251, 2006. First published June 22, 2006; doi:10.1152/japplphysiol.00483.2006.—It has been shown previously that dendritic branching in cardiorespiratory and locomotor brain areas can be attenuated with exercise training (ET). It was not known whether this process was reversible. Twenty-three (n = 23) male Sprague-Dawley rats were individually caged and divided into two groups: untrained (UN; n = 11) and detrained (DTR; n = 12). DTR were provided with a running wheel at 21 days of age and exercised spontaneously. After 120 days (70 days of ET followed by 50 days of detraining), ET indexes were obtained, including maximal oxygen uptake, percent body fat, resting heart rate, and heart weight-to-body weight ratios. The brain was processed according to a modified Golgi-Cox procedure. Impregnated neurons from the periaqueductal gray (PAG), posterior hypothalamic area (PH), nucleus of the tractus solitarius (NTS), and cuneiform nucleus (CIN) were examined in coronal sections. Neurons were traced using a camera lucida technique and analyzed using the Sholl concentric ring analysis of dendritic branching. r-Tests compared the mean number of intersections per neuron by grouping inner rings, outer rings, and total number of intersections per animal. There were no significant differences between UN and DTR in PH, PAG, CIN, and NTS in the inner rings, outer rings, and total number of intersections per animal. A separate group of animals was used to show that a training effect in the CIN and NTS was present at 56 days of ET. Our results show that dendritic attenuation resulting from 70 days of ET in PH, PAG, CIN, and NTS was completely reversed with 50 days of detraining.

Golgi staining; dendritic branching; locomotion; neuroplasticity

IT IS WELL KNOWN THAT REGULAR exercise training (ET) results in a number of physiological adaptations that enhance physical performance, including a constellation of changes in the exercising periphery (i.e., skeletal and cardiac muscle structure and function) and dramatic shifts in cardiorespiratory control functions (9). The converse of ET is the principle of training reversibility or detraining, which has been defined as the “cessation or marked reduction of ET leading to partial or complete reversal of these training-induced adaptations” (47).

Although the effects of detraining differ depending on the duration of training reduction or cessation, short-term detraining (<4 wk) has been shown to induce a number of cardiorespiratory, metabolic, and muscular adaptations (26). In fact, by the second week of exercise detraining, autonomic adjustments are made, including a loss of resting bradycardia (26). In addition, mean blood pressure increases (19) and maximal oxygen uptake (V O2 max) decreases (21, 45) within 4 wk of detraining.

A previous study from our laboratory (48) demonstrated the first evidence of structural neuroplasticity in the cardiorespiratory and locomotor centers (CRLC) of the brain accompanying the effects of 85 or 120 days of ET. This included several areas associated with locomotor or cardiorespiratory function, the latter including blood pressure, heart rate, and cardiac contracility during exercise. The posterior hypothalamus (PH) and cuneiform nucleus (CIN; CnF in Paxinos and Watson, Ref. 50) are both associated with locomotor and cardiorespiratory function. The periaqueductal gray (PAG; CG in Paxinos and Watson, Ref. 50) and nucleus tractus solitarius (NTS; SoIM in Paxinos and Watson, Ref. 50) are also associated with cardiorespiratory function. The dendritic fields of neurons in all of these areas, PH, CIN, PAG, and NTS, were profoundly attenuated with voluntary wheel running (48).

Several studies suggest that structural neuroplastic changes induced by chronic stimuli, such as enriched environments or chronic restraint stress, are reversible (37, 38, 53). Kleim and colleagues (37) utilized an acrobatic environmental condition vs. simple motor activity condition (often referred to as AC/ MC) model to demonstrate that motor skill learning, not motor activity alone, results in decreased Purkinje cell density within the cerebellar cortex. However, when the acrobatic condition training was discontinued, these neuroplastic effects of the acrobatic or complex environment could be reversed or detrained (37). Radley and colleagues (53) observed a significant reduction in branch number on pyramidal neurons in the medial prefrontal cortex following 3 wk of repeated restraint stress, which underwent a complete reversal by the end of 6 wk.

In the present study, we used a Golgi-Cox staining procedure to examine the dendritic branching pattern of four areas of the brain associated with cardiorespiratory or locomotor activity, which had previously been shown to demonstrate ET-induced neuroplastic changes. Given the extensive evidence that detraining effects on humans could be demonstrated within...
several weeks, we hypothesized that 50 days of detraining would be sufficient to reverse the ET-induced attenuation of dendrites within the CRLC of the brain we previously reported (48).

METHODS

All animal use was approved by the University of Illinois’ Institutional Animal Care and Use Committee under protocol no. 03151.

Animal preparation. Twenty-three (n = 23) male Sprague-Dawley rats (Sasco-Charles River) were kept in individual cages and randomly divided into two groups and kept for 120 days, untrained (UN; n = 11) and detrained (DTR; n = 12). An additional group of animals (n = 20) was randomly divided into two groups and kept for only 56 days, UN (n = 10) and trained (TR; n = 10). Both groups, 120 days and 56 days, began as 21-day-old weanlings. Males were selected for this investigation because they demonstrate body composition changes similar to humans (18) and obviously do not present variations with hormonal levels due to estrous cycling. Animals were maintained in a temperature-controlled environment, fed ad libitum, and kept on a 12:12-h light-dark cycle. Humidity varies in our facility with the ambient conditions with the exception that conditions of low humidity (winter months) are corrected with humidifying devices. DTR and TR rats were provided with a running wheel of 106 cm in circumference (Nalgene) placed inside their cage. These rats were allowed to exercise spontaneously. The total number of wheel rotations was recorded daily for the duration of the experiment. The total distance run by each animal per week was then calculated from the total number of wheel rotations. DTR rats were provided with a running wheel from day 1 to day 70. The wheels were then removed from their cages, and the DTR rats were then housed in standard cages until they were euthanized at day 120. TR rats were provided with a running wheel inside their cages until they were euthanized at day 56. The UN rats (56 and 120 day) were not provided with a running wheel. It is important to note that the TR rats were to be utilized for another experiment in which blood samples were to be collected at the peak of running activity. Thus we were not attempting to equalize the wheel running between groups. The 56-day rats were utilized simply to determine whether it was possible to obtain training effects within the time specified for DTR wheel running. Furthermore, the 56-day UN and TR animals were conditioned at a different time than the 120-day UN and DTR animals (56 day: April to May; 120 day: July to October). This was because we were limited in the number of exercise wheels available. Due to the number of differences in the treatment of the groups, direct comparisons between the 56- and 120-day rats were not attempted.

Treadmill familiarization. Since the target day for euthanasia was 56 or 120 days, the animals were accordingly schedule for familiarization trials and oxygen consumption (V\textsubscript{O2}) testing. The treadmill familiarization occurred 4–6 days before V\textsubscript{O2} testing. In turn, the V\textsubscript{O2} testing was carried out 1 wk before euthanasia. All animals were given two familiarization trials on the treadmill apparatus (10) to adapt them to the testing environment. Each familiarization trial lasted 7 min, and the trials were conducted on nonconsecutive days. All familiarization trials were conducted at 0% grade. The speed during the first familiarization trial increased progressively from 10 to 15 m/min throughout the 7-min duration. The speed during the second familiarization trial increased progressively from 15 to 20 m/min.

V\textsubscript{O2 max} testing protocol. V\textsubscript{O2 max} was determined for all rats according to previously established methods (31) 1 wk before euthanasia. This method uses a metabolic chamber designed to fit into a stall of a four-lane rodent treadmill and utilizes the techniques described by Brooks and White (10) for determining V\textsubscript{O2} and carbon dioxide production.

No electrical stimulation was used during familiarization trials or V\textsubscript{O2 max} testing to induce exercise. This was partly because we did not wish to induce stress, which has been shown to cause neuroplastic changes (43). When necessary, a gentle nudge with a piece of a common windshield snow brush (connected to an external lever) was applied to induce exercise (animals apparently wished to avoid the texture of the brush).

V\textsubscript{O2 max} was determined by having each rat perform a maximal exercise test. Briefly, this test (31) began with a 3-min warm-up at a treadmill grade and speed of 0% and 15 m/min, respectively. The treadmill speed and/or grade was increased every 3 min. V\textsubscript{O2 max} was defined as the point at which the V\textsubscript{O2} did not increase with further increases in workload or when the rat was unable or unwilling to continue running. Confirmation that V\textsubscript{O2 max} was truly attained in each animal was demonstrated by having each rat perform a subsequent maximal exercise test after 48 h of recovery from the initial test. With the second test, each rat was given a 3-min warm-up at a treadmill grade and speed of 0% and 15 m/min. The treadmill grade and speed were then increased to the highest workload each animal was able to sustain during the initial maximal test. V\textsubscript{O2} and carbon dioxide production were recorded. The treadmill speed was then increased by 5 m/min, and V\textsubscript{O2} and carbon dioxide production were recorded. If the measured V\textsubscript{O2} was similar between the two workloads, the animal was considered to be at V\textsubscript{O2 max}, and the exercise test was terminated. Following each V\textsubscript{O2 max} test, the chamber was flushed and recalibrated for the next test.

Gross anatomical and physiological adaptations to detraining. After 70 days of ET, the running wheels were removed from the DTR animals. Fifty days later, after the V\textsubscript{O2 max} data were acquired, the UN and DTR 120-day animals were sedated briefly by halothane inhalation. Immediately after the animals became manageable, they were anesthetized with a combination of alpha-chloralose (65 mg/kg) and urethane (800 mg/kg) administered intraperitoneally. The resting heart rate of each animal was measured using the amplified EKG signal and processed using a Gould Biotach. Each animal was weighed. Body composition analysis using dual-energy X-ray absorptiometry (Hologic 4500A) was performed on each animal. The 120-day animals were killed with an overdose of pentobarbital sodium. Their hearts were removed and weighed. The entire brain was removed and used for analysis of neuroplastic adaptations.

After acquiring the V\textsubscript{O2 max} data, as previously described, the 56-day animals were transferred to the experiment room and decapitated. Large samples of blood were collected from these same animals for a separate analysis of hormones. Decapitation has been judged as the best means of sampling because it is the fastest and most humane means of obtaining relatively large blood samples for hormone assays (14). Their hearts were removed and weighed. The entire brain was removed and used for analysis of neuroplastic adaptations. Please note that since the 56-day animals were also used for a hormone assay we could not anesthetize them for body composition analysis because recovery would have involved an extensive period of inactivity as well as stress induced by anesthetic procedures, which would have invalidated the hormonal assay (corticosterone).

Tissue preparation. After data regarding training effects were acquired, the entire brain was removed and immersed in Golgi-Cox impregnation solution from both the 56- and 120-day animals (28, 29). The brains were stored individually in glass jars and kept in a light-safe box. Forty-eight hours later, the Golgi-Cox solution was renewed and the brains were returned to the light-safe box for 2 wk.

The tissue was frozen, and 200-μm coronal sections of the entire brain were obtained from a sliding microtome (54). The coronal sections were mounted on 2% gelatin-coated slides and pressed to the slides using moistened bipulorous paper. Each slide was kept in a humidity chamber for a minimum of 4 h.

Slides were placed in glass staining dishes and processed according to Gibb and Kolb’s (28) Golgi-Cox procedure. Briefly, the slides were placed through distilled H2O (dH2O; 1 × 1 min), ammonium hydroxide in the dark (1 × 30 min), dH2O (1 × 1 min), Kodak Fix diluted 1:1 with dH2O (1 × 30 min), dH2O (1 × 1 min), 70% ethanol (1 × 1 min), 95% ethanol (1 × 1 min), 100% ethanol (2 × 5 min), and
Visualization of neurons. Not all animals processed for Golgi staining yielded useable tissue in every area of interest because of inadvertent mechanical damage either during dissection or tissue sectioning. Therefore, tissue from 28 animals (56 day: UN = 7, DTR = 7; 120 day: UN = 7; TR = 7) were used to evaluate each area. We examined Golgi-impregnated neurons from four areas of the brain, including PAG, PH, NTS, and CfN. The areas were selected from sections containing these regions. The target sections included +4.84, +4.70, +0.28, and −4.30 in reference to the interaural line, according to Paxinos and Watson (50). Although all four areas were examined in the 120-day animals, two areas (CfN and NTS) were investigated in the 56-day group to show that neuroplastic adaptations due to ET could exist at that time point. Figure 1 illustrates the CRLC examined in this study. Each area was further delineated by superimposing a 5 × 5 rectangular grid over known anatomical landmarks using an eyepiece reticle.

Selected sections of the neuraxis were analyzed quantitatively. Golgi-Cox stained neurons from each of the investigated areas were traced at ×400 total magnification using an Olympus BH2 or an American Optical Series 10 microscope equipped with a drawing tube. The neurons traced were chosen by an independent investigator blind to the experimental conditions to minimize any bias. Neurons were only used in these analyses if the whole neuron and its processes were visible and traceable without intervening obstructions.

Localization and rationale of CRLC and control areas investigated. The cardiovascular controlling network within the PAG is organized in columns (3). According to Lovick (41), the dorsal column of the PAG is involved in pressor and the ventrolateral column mediates depressor responses. Stimulation of the dorsomedial and dorsolateral columns produces an increase in blood pressure and causes defensive behavior in rats (6). The dorsolateral column originates in the caudal PAG at the level of the dorsal raphe and extends to the rostral PAG at the level of Edinger Westphal nuclei (6). We chose a 1.00 × 1.50-mm sampling area in the caudal-most PAG within the dorsomedial and dorsolateral columns for analysis located on a target level corresponding to section +0.28 (referred to the interaural line) of the Paxinos and Watson rat brain atlas (50).

The PH has been identified as a pressor and locomotor region and is located within the anatomical boundaries of the fornix, mammillothalamic tract, and the third ventricle (56, 64, 66). Electrical stimulation of this region of the brain has been shown to elevate sympathetic nerve activity and evoke increases in blood pressure and heart

![Fig. 1. Localization of cardiorespiratory and locomotor centers (CRLC) (posterior hypothalamic area (PH), periaqueductal gray (CG), cuneiform nucleus (CnF), and nucleus of the tractus solitarius (SoLM)) areas investigated. Figure, including abbreviations, was adapted from Paxinos and Watson (50). 3V, third ventricle; 12, nucleus of hypoglossal nerve; 12n, hypoglossal nerve; Amb, nucleus ambiguus; Arc, arcuate nucleus; CG, central gray; cic, commissure inferior colliculus; CnF, cuneiform nucleus; cp, cerebral peduncle; Cu, cuneate nucleus; DCIC, dorsal cortex of inferior colliculus; DGL, dorsal lateral geniculate nucleus; DLL, dorsal nucleus lateral lemniscus; DTg, dorsal tegmental nucleus, pericentral; Ecu, external cuneate nucleus; f, fornix; F, nucleus of the fields of Forel; fr, fasciculus retroflexus; ic, internal capsule; IGL, intergeniculate leaf; InsF, infundibular stem; IOA, inferior olive subunit A of medial nucleus; IOB, inferior olive subunit B of medial nucleus; IOC, inferior olive subunit C of medial nucleus; IOD, inferior olive dorsal nucleus; LDtg, lateral dorsal tegmental nucleus; LH, lateral hypothalamic area; LPBS, lateral parabrachial nucleus, superior; LRt, lateral reticular nucleus; LVO, lateroventral periolivary nucleus; Mep, middle cerebellar peduncle; ml, medial lemniscus; MIF, medial longitudinal fasciculus; MM, medial mammillary nucleus; MRe, mammillary recess of the 3rd ventricle; MSO, medial superior olive; mt, mammillothalamic tract; MV, medioventral periolivary nucleus; PF, parafascicular thalamic nucleus; PH, posterior hypothalamic area; PMD, pre-mammillary nucleus, dorsal; Po, posterior thalamic nucleus; PnO, pontine reticular nucleus, oral part; PVP, paraventricular thalamic nucleus, posterior; py, pyramidal tract; RPa, nucleus raphe pontis; RPO, rostral preolivary nucleus; RV, rostral ventrolateral medulla; scp, superior cerebellar peduncle; Sot, nucleus of solitary tract; Sol, nucleus of solitary tract, lateral; SolM, nucleus of solitary tract, medial; SPO, superior paraventricular nucleus; sumx, supramammillary decussation; SuM, supramammillary nucleus; tz, trapezoid body; Tz, nucleus trapezoid body; VPM, ventral posteromedial thalamic nucleus; ZID, zona incerta, dorsal; ZIV, zona incerta, ventral.](https://jap.physiology.org/doi/10.1152/jappl.01003.2006)
rate response (5, 24, 65). The PH also contains the hypothalamic locomotor region, which evokes coordinated locomotion when electrically stimulated or exposed to GABA antagonists (24, 65). Paxinos and Watson’s (50) depiction of this area includes the PH. A target level corresponding to sections +4.70 to +4.84 (referred to the interaural line) of their rat brain atlas was chosen for the purposes of this study. This area corresponds to the caudal aspect of the hypothalamic locomotor region and has been shown to demonstrate increased Fos labeling in response to exercise (33). At these levels, the PH becomes more extensive as one moves in a rostral direction. A 1.50 × 1.50-mm sampling area was superimposed over the PH and is illustrated in Fig. 1.

The NTS plays a vital role in coordinating autonomic function by relaying visceral afferent input, which controls heart rate and blood pressure (4, 51). Hindlimb somatic input, including that of muscle afferents, has a major contribution to this area (63). The NTS is located in the dorsal medial medulla and was targeted at a level corresponding to section −4.30 (referred to the interaural line) as shown in Fig. 1, which is adapted from Paxinos and Watson (50). A single 0.50 × 3.00-mm sampling area was superimposed bilaterally over the medial NTS for sampling.

The CIN is a readily discerned part of the mesencephalic locomotor region (61) and was targeted at a level corresponding to section +0.28 (referred to the interaural line) as shown in Fig. 1. A 0.50 × 1.00-mm sampling area was placed bilaterally over the area of interest for cell selection.

**Analysis of neurons.** After the neurons were traced, the maximum and minimum dimensions of each soma were recorded. The average of these measures was taken as the average soma “diameter” after the method of Burke et al. (11). Each neuron was then analyzed using the Sholl analysis (60) of dendritic branching, which assumes that dendritic arborization is an indirect measure of available postsynaptic space. Intersections between dendrites and each concentric ring were then counted. The location and number of intersections were then plotted, as shown in Fig. 2, and used for statistical comparisons.

**Data analysis.** All data are reported as means ± SE. Individual means were compared between groups using a Student’s t-test for each of the training indexes under investigation. For a group difference to be statistically significant, \( P < 0.05 \) had to be achieved. When the training effect was evaluated, the one-tailed approach was used if the training adaptation had been previously documented (58).

Although descriptive comparisons were made between groups using the traditional Sholl analysis graphs of concentric ring intersections, \( t \)-tests were conducted for comparisons of the mean number of intersections per neuron between UN and DTR 120-day animals and between UN and TR 56-day animals by grouping rings 1–6 (inner rings) and rings ≥7 (outer rings) (35, 48). The inner rings encompass 1–120 μm, and the outer rings encompass ≥121 μm. \( t \)-Tests were also performed for comparisons of the total number of intersections per animal between UN and DTR 120-day animals and between UN and TR 56-day animals in each CRLC investigated, which is representative of the dendritic field. The relative size of the cell body was determined by averaging the long and short axes of each soma, and \( t \)-tests were performed to compare soma size between groups. As noted above, the UN and TR 56-day animals and UN and DTR 120-day animals were conditioned at different times. Thus, statistical comparisons between the 56- and 120-day groups of animals were not conducted. All analyses were conducted using the GBSTAT software package.

**RESULTS**

**Wheel running and environment.** The 120-day DTR rats \((n = 12)\) ran 4,873 ± 543 m/wk, and 56-day TR rats \((n = 10)\) ran 7,466 ± 1,246 m/wk. Although temperature and photoperiod were controlled in the animal quarters, our facilities only correct humidity if it is unusually low. Thus significant differences were observed in the average relative humidity during the wheel running between 56-day (63.9 ± 2.0%) and 120-day (76.0 ± 1.2%) experiments, according to figures compiled by the Champaign/
Urbana University of Illinois Willard Airport for the US National Weather Service and Federal Aviation Administration. Representative records taken from the individual room where the animals exercised from the past year (2005–2006) also reflected these differences. Humidity within the animal quarters for the equivalent period that the 56-day experiment was conducted was significantly different from the 120-day experiment (56 day: 44.4 ± 0.8%; 120 day: 49.7 ± 0.6%).

### Absolute body and heart mass and heart weight-to-body weight ratio.

There were no significant differences in absolute body mass, heart weight, or heart weight-to-body weight ratio between 120-day UN and DTR animals (Table 1).

Mean body weight differed significantly between 56-day UN (348 ± 9 g) and TR animals (310 ± 8 g). Heart weight-to-body weight ratio is often used to assess training effects in animal models (2, 13) and was also significantly different between 56-day UN (0.34 ± 0.01) and TR animals (0.40 ± 0.01) in the present study.

### Body composition.

Body weight ranged from 310 to 539 g for the 120-day UN and DTR animals in the present study, which was well above weight minimums (>200 g) needed for dual-energy X-ray absorptiometry scanning to be feasible (7). There were no significant differences in body fat percentage between UN and DTR rats (UN: 9 ± 1%; DTR: 7 ± 1%).

### VO\(_2\) max.

VO\(_2\) max values were obtained at average speeds of 30 ± 2 m/min for UN and at 39 ± 2 m/min for DTR rats in the 120-day group. However, there was no significant difference in VO\(_2\) max between UN and DTR 120-day animals (Table 1).

VO\(_2\) max values were obtained at average speeds of 38 ± 2 m/min for UN and at 48 ± 2 m/min for TR rats in the 56-day group. VO\(_2\) max was significantly higher for TR (76 ± 3 ml·kg\(^{-1}\)·min\(^{-1}\)) compared with UN (65 ± 3 ml·kg\(^{-1}\)·min\(^{-1}\)) in the 56-day group.

### Heart rate training effect.

After anesthetization, the resting heart rate of the UN and DTR animals was obtained by using an ECG. There were no significant differences in resting heart rate between the 120-day UN and DTR animals (Table 1).

### Analysis of neurons.

The 120-day data are depicted in Fig. 2, which shows in traditional Sholl analysis graphs the mean number of intersections per neuron between each ring in a series of concentric rings of all four anatomical areas studied. Note that the same general trend was illustrated for each of the CRLC investigated in that the mean number of intersections per neuron decreased as the distance from the cell body increased. There were no observable differences in the number of intersections per neuron when UN and DTR animals were compared at each concentric ring.

Statistical comparisons were made on data incorporating populations of concentric rings. Figure 3 illustrates the data in the 120-day UN and DTR animals. There were no significant differences in the mean number of intersections per animal in the 120-day animals between UN (n = 7) and DTR (n = 7) by grouping rings 1–6 (inner rings) and rings 7–12 (outer rings) in the NTS (A), PH (B), PAG (C), and CIN (D). There were no significant differences between groups.

### Table 1. Gross anatomical and physiological adaptations to 70 days of training followed by 50 days of detraining.

<table>
<thead>
<tr>
<th></th>
<th>Untrained (n = 11)</th>
<th>Detrained (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>430±17</td>
<td>411±17</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>134±2</td>
<td>112±3</td>
</tr>
<tr>
<td>Heart weight-to-body weight ratio</td>
<td>0.32±0.01</td>
<td>0.33±0.01</td>
</tr>
<tr>
<td>%Fat-free mass</td>
<td>91±1</td>
<td>93±1</td>
</tr>
<tr>
<td>Resting heart rate, beats/min</td>
<td>379±5</td>
<td>374±12</td>
</tr>
<tr>
<td>Maximal oxygen consumption, ml·kg(^{-1})·min(^{-1})</td>
<td>55±2</td>
<td>60±3</td>
</tr>
</tbody>
</table>

Values are means±SE. There were no significant differences between groups in 120-day animals in any of these parameters.

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**Fig. 3.** Comparison of the mean number of intersections per animal in the 120-day animals between UN (n = 7) and DTR (n = 7) by grouping rings 1–6 (inner rings) and rings 7–12 (outer rings) in the NTS (A), PH (B), PAG (C), and CIN (D). There were no significant differences between groups.
differences between groups in the PH, PAG, CfN, and NTS when the inner rings were compared (six rings closest to the cell body). There were also no significant differences between groups in the PH, PAG, CfN, and NTS when the outer rings were compared (7th ring distant from the cell body). Figure 4 shows the total number of intersections per animal and represents the total dendritic field for cells in the 120-day UN and DTR animals. There were no significant differences between DTR and UN in the PH, PAG, CfN, and NTS.

Figure 5, A and B, illustrates the Sholl analysis of concentric rings in the CfN and NTS, respectively, for the 56-day group. Figure 5, C and D, show that significant differences were found between UN and TR in the 56-day group in the CfN and NTS, respectively, when the inner rings were compared and also when the outer rings were compared. There were also significant differences between groups in the CfN (UN: 626.24 ± 6.72; TR: 388.29 ± 23.69) and NTS (UN: 552.43 ± 10.72; TR: 388.14 ± 20.15) at 56 days when the total number of intersections per animal were compared.

**Soma size.** The average of the long and short axes of the cell bodies was not significantly different between groups in the 120-day group, as shown in Table 2. The average of the long and short axes of the cell bodies was significantly different between groups in the 56-day group in the CfN (UN: 26.45 ± 0.48; TR: 24.65 ± 0.57), which is consistent with our laboratory’s previous findings (48). However, there was no significant difference between groups for the 56-day group in the NTS (UN: 26.09 ± 0.37; TR: 23.57 ± 0.33).

Table 2. Average of long and short axes of 20 cell bodies per animal from each of the cardiorespiratory and locomotor centers areas examined in the 120-day animals

<table>
<thead>
<tr>
<th>Area</th>
<th>Untrained (n = 7)</th>
<th>Detrained (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior hypothalamic area, μm</td>
<td>20.81±0.46</td>
<td>21.73±0.95</td>
</tr>
<tr>
<td>Periaqueductal gray, μm</td>
<td>21.97±0.42</td>
<td>21.47±0.43</td>
</tr>
<tr>
<td>Cuneiform nucleus, μm</td>
<td>24.66±0.48</td>
<td>23.64±0.55</td>
</tr>
<tr>
<td>Nucleus tractus solitarius, μm</td>
<td>24.62±0.32</td>
<td>24.95±0.67</td>
</tr>
</tbody>
</table>

Values are means ± SE. There were no significant differences between untrained and detrained animals.

Fig. 4. Comparison of the total number of intersections per animal between UN (n = 7) and DTR (n = 7) of all CRLC areas investigated in the 120-day animals. There were no significant differences between groups.

Fig. 5. Sholl analysis of the number of Golgi-stained dendritic intersections per concentric ring in the 56-day animals (UN: n = 7; TR: n = 7) in the CfN (A) and NTS (B). Comparison of the mean number of intersections per animal in the 56-day animals between UN (n = 7) and TR (n = 7) by grouping rings 1–6 (inner rings) and rings ≥7 (outer rings) in the CfN (C) and NTS (D). **Significance at P < 0.01.
DISCUSSION

In the present study, we found that 70 days of voluntary wheel running followed by 50 days of detraining (120-day DTR) results in PH, PAG, NTS, and CfN dendritic profiles that are similar to control (120-day UN) animals. This was accompanied by clear evidence of detraining in the 120-day DTR animals in all anatomical and physiological indexes investigated (i.e., body composition, aerobic capacity, resting heart rate).

We have recently demonstrated that 85 or 120 days of voluntary wheel running results in attenuated dendritic cortisolizations in the PH, PAG, NTS, and CfN (48). In the present study, it was found that 56 days of ET were sufficient to induce anatomical/physiological adaptations and resulted in significant dendritic attenuation in the CfN and NTS. Since the 56-day animals were trained at a different time than the 120-day animals, direct comparisons are probably not valid. Even if one assumes that the animals themselves are similar, one must consider the effect of seasonal (circannual) influences. Wheel-running activity in rodents has not been studied with the seasonal variables photoperiod and temperature controlled. One would anticipate that these factors would be extremely important in the natural state. It has been indicated that serotonin binding and thyroid function exhibit seasonal rhythms, which seem to be independent of photoperiod and temperature (1, 67). These factors might play a role in determining voluntary activity. Furthermore, in the present case, although the effects of photoperiod and temperature were constant for both groups, we were not able to control the effects of humidity. The humidity is important because it contributes to thermoregulation in the form of evaporative cooling. Rats lick their fur to accomplish this (27). One might assume that the condition of elevated humidity would make this measure less effective and affect overall comfort of the animal with respect to exercise.

However, these data show that it is likely that changes also occurred in the 120-day DTR after a similar period of training. It is noteworthy that a training effect with dendritic alterations in the CRLC of the brain may occur over a much shorter timescale than 85 or 120 days of ET as reported in our laboratory’s previous work (48). Thus it is also reasonable to expect that, if the 120-day DTR animals had continued wheel running past 70 days, the appearance of the dendrites would have been similar to that in our previous report of attenuated dendrites in the CRLC due to ET.

The time frame of detraining was meant to approximate that used in human detraining studies. It is well known that the exercise-gained cardiorespiratory and metabolic adaptations acquired over several months can be lost within a few weeks (36, 47, 55). This includes decreased $V_{O_2\text{max}}$ (19, 20, 45), increased mean blood pressure (9, 42), and decreased ventricular mass (9, 42, 47). In fact, a follow-up of the Dallas Bed Rest and Training Study (57) concluded that short-term physical inactivity of 3 wk caused a greater deterioration in cardiovascular capacity than 30 yr of aging (44).

There are relatively few studies of detraining using animal models. However, Evangelista et al. (26) showed similar adaptations after the cessation of exercise. Following a 10-wk treadmill training program, Evangelista and colleagues observed almost complete regression of resting heart rate in rats within 2 wk of detraining and a 12% reduction in peak $V_{O_2\text{max}}$ within 1 wk of detraining (26).

The present study suggests that dendritic attenuation resulting from 70 days of ET in the PH, PAG, NTS, and CfN undergoes a complete reversal by the end of 50 days of detraining. Apical dendrites in the medial prefrontal cortex undergo similar changes due to 3 wk of repeated restraint stress followed by 3 wk of recovery (53). For the purposes of this study, the 70-day TR/50-day DTR protocol was chosen to maximize the likelihood of observing the reversal phenomenon in the CRLC investigated and to match the 120-day time period used in our laboratory’s previous neuroplasticity work (48).

Although it is difficult to assign a functional role for these changes in terms of locomotion, certainly one may speculate on the possible functional significance of these areas on the cardiovascular and respiratory systems. These areas may be identified with sympatoexcitatory. Electrical (5, 17) and chemical (24, 65) stimulation of the PH has been shown to increase blood pressure and heart rate. Similarly, electrical (23) and chemical (12) stimulation of the PAG results in cardiovascular changes, including increased blood pressure. The region of the CfN has been shown to be at least an electrical stimulus site for the defense reaction, which includes increases in blood pressure (32, 39). At least some of these sites are known to modulate parasympathetic outflow. For example, the PH is known to inhibit the depressor effects and bradycardia associated with the baroreflex (17). Finally, our own research using Fos labeling has shown that these areas are activated during normal exercise (34). Furthermore, ET reduces the extent of this labeling (33).

If one assumes that PH, CfN, and PAG participate in sympatoexcitation, the findings from the present study correlate with a DTR-induced reversal of the well-known decrease in sympathetic outflow at a given absolute workload with ET (8). The sympathetic component of the baroreflex is attenuated in normotensive (15) and spontaneously hypertensive (40) rats. This may also imply that sympathetic outflow could be increased at a given workload or an increase in the sympathetic component of the baroreflex after detraining. To our knowledge, there have been no studies to verify these ideas.

Although there appears to be some debate in the literature over whether resting sympathetic outflow is attenuated (30) or unchanged (25, 49) with endurance training, it is possible that the present findings may be reflective of a subtle decrease in sympathetic tone at rest. Taylor and colleagues (62) have identified a sympathetic restraint of respiratory sinus arrhythmia in the human, which if affected might be the only outward manifestation.

Deconditioning may occur in response to several commonly encountered conditions, including hypodynamic environments (bed rest) and hypogravic environments (i.e., spaceflight). Data from spaceflight and bed rest experiments have provided evidence for elevated sympathetic nerve activity and circulating catecholamines (59), which may be linked to alterations within sympatoexcitatory sites. Fifteen days of head-down bed rest has been shown to reduce cardiac vagal activity in healthy men (22). Convertino and Fritsch (16) showed an attenuation of human carotid-cardiac vagal baroreflex responses after physical detraining. The hindlimb-unloaded rat was developed by the National Aeronautics and Space Administration as a land-based model to mimic the physiological effects of spaceflight.
and bed rest (46). Hindlimb unloading has been shown to attenuate baroreflex-mediated sympathoexcitation (46). The aforementioned inhibitory effects of such sympathoexcitatory sites (such as PH) on parasympathetic function may be the linkage to these changes. Certainly, this may be through properties conferred by dendritic plasticity in CRLC of the brain.

Interpretation of the findings of neuroplastic changes of the NTS is complicated by the heterogeneity of the area. However, the recent paper by Potts and Waldrop (52) has shown that somatically activated NTS cells are largely unresponsive to baroreceptor stimulation. Clearly, correlation of the neuroplastic effects with either of these cell groups would be an important demonstration.

Although thus open to many interpretations, these data clearly indicate that, in addition to more readily measured parameters of cardiorespiratory and locomotor control, the CNS itself undergoes intrinsic changes, which parallel changes in the exercise trained or, in this case, untrained state of the organism.

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