

Wheel Running Alters Serotonin (5-HT) Transporter, 5-HT_{1A}, 5-HT_{1B}, and Alpha_{1b}-Adrenergic Receptor mRNA in the Rat Raphe Nuclei

Benjamin N. Greenwood, Teresa E. Foley, Heidi E.W. Day, Daniel Burhans, Leah Brooks, Serge Campeau, and Monika Fleshner

Background: Altered serotonergic (5-HT) neurotransmission is implicated in the antidepressant and anxiolytic properties of physical activity. In the current study, we investigated whether physical activity alters factors involved in the regulation of central 5-HT neural activity.

Methods: *In situ* hybridization was used to quantify levels of 5-HT transporter (5-HTT), 5-HT_{1A}, 5-HT_{1B}, and α_{1b} -adrenergic receptor (α_{1b} ADR) messenger ribonucleic acids (mRNAs) in the dorsal (DRN) and median raphe (MR) nuclei of male Fischer rats after either sedentary housing or 3 days, 3 weeks, or 6 weeks of wheel running.

Results: Wheel running produced a rapid and lasting reduction of 5-HT_{1B} mRNA in the ventral DRN. Three weeks of wheel running decreased 5-HTT mRNA in the DRN and MR and increased α_{1b} ADR mRNA in the DRN. After 6 weeks of wheel running, 5-HTT mRNA remained reduced, but α_{1b} ADR mRNA returned to sedentary levels. Serotonin_{1A} mRNA was increased in the MR and certain DRN subregions after 6 weeks only.

Conclusions: Data suggest that the central 5-HT system is sensitive to wheel running in a time-dependent manner. The observed changes in mRNA regulation in a subset of raphe nuclei might contribute to the stress resistance produced by wheel running and the antidepressant and anxiolytic effects of physical activity.

Key Words: Exercise, depression, anxiety, learned helplessness, serotonin, 5-HT autoreceptors

The ability of physical exercise to reduce the incidence and severity of human depression and anxiety is well accepted and has been extensively reviewed (Brosse et al 2002; Dunn et al 2001; Dunn and Dishman 1991; Fox 1999; Lawlor and Hopker 2001; Martinsen 1990a, 1990b; Martinsen and Morgan, 1997; Morgan 1985; Mutrie 2000; Paluska and Schwenk 2000; Salmon 2001; Scully et al 1998; Suh et al 2002). Results of rodent studies similarly indicate that voluntary wheel running provides antidepressant and anxiolytic effects in several animal models of depression and anxiety, including the forced-swim test (Solberg et al 1999), chronic mild stress (Solberg et al 1999), and behavioral depression/learned helplessness (Dishman et al 1997; Greenwood et al 2003a; Moraska and Fleshner 2001). Although the antidepressant and anxiolytic properties of physical activity are clear, underlying mechanisms remain unresolved.

Many factors could contribute to the beneficial effects of physical activity on mental health; however, given the important role of serotonin (5-hydroxytryptamine, 5-HT) in the etiology and treatment of affective disorders (Anderson and Mortimore 1999; Blier and de Montigny 1999; Den Boer et al 2000; Graeff et al 1996; Ninan 1999; Owens and Nemeroff 1994), it seems likely that central 5-HT systems are involved in the antidepressant and anxiolytic properties of exercise (Chaouloff 1989; Dey 1994; Dunn and Dishman 1991; Greenwood et al 2003a; Ransford 1982). We have recently reported that, compared with the

sedentary condition, 6 weeks of wheel running attenuates the activity (measured by c-Fos immunoreactivity) of 5-HT neurons in the rat dorsal raphe nucleus (DRN), during exposure to uncontrollable stress (Greenwood et al 2003a). The observation that wheel running alters 5-HT neural responses to stress is especially important considering that 1) stress is one of the foremost causal factors in the etiology of depression and anxiety (D'Aquila et al 1994; Kendler et al 1999); and 2) 5-HT plays a critical role in the development and expression of stress-induced depressive and anxiolytic behaviors (Borsini 1995; Gingrich and Hen 2001; Graeff et al 1996; Lucki 1998; Maier and Watkins 1998; Neumaier et al 2002; Petty et al 1997). These data suggest that wheel running might produce its antidepressant and anxiolytic properties by affecting factors capable of influencing the activity of 5-HT neurons.

Nearly every region of the brain receives 5-HT innervation from 5-HT neurons originating from the DRN or median raphe nucleus (MR) (Jacobs and Azmitia 1992; Molliver 1987). Raphe 5-HT neural activity is modulated by a wide spectrum of factors, including 5-HT itself (Adell et al 2002). The serotonin transporter (5-HTT) (Tao et al 2000) and 5-HT_{1A} and 5-HT_{1B} autoreceptors (Davidson and Stamford 1995; Hervas et al 1998; Hopwood and Stamford 2001; Sprouse and Aghajanian 1987) each play important roles in the regulation of extracellular 5-HT in the raphe and 5-HT release throughout the brain (Adell et al 2002). The 5-HTT is responsible for the reuptake of 5-HT back into presynaptic neurons and is active both within the raphe nuclei and in projection sites (Horschitz et al 2001). Serotonin_{1A} and 5-HT_{1B} autoreceptors are located on the soma/dendrites and terminals, respectively, of 5-HT neurons (Riad et al 2000) and, upon stimulation, inhibit the synthesis and release of 5-HT (Adell et al 2001; Stamford et al 2000). Human and animal studies have implicated 5-HTT and 5-HT_{1A} and 5-HT_{1B} autoreceptors in depression (Drevets et al 1999; Lemonde et al 2003; Owens and Nemeroff 1998), anxiety (Holmes et al 2003; Lin and Parsons 2002; Zhuang et al 1999), and the action of antidepressant/anxiolytic drugs (Anthony et al 2000; Blier and Ward 2003; Holmes et al 2002; Schloss and Williams 1998).

From the Departments of Integrative Physiology (BNG, TEF, DB, LB, MF) and Psychology (HEWD, SC) and the Center for Neuroscience (BNG, TEF, HEWD, SC, MF), University of Colorado, Boulder, Colorado.

Address reprint requests to Monika Fleshner, Ph.D., University of Colorado-Boulder, Center for Neuroscience, Department of Integrative Physiology, Campus Box 354, Boulder, CO 80309-0354; E-mail: fleshner@colorado.edu.

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In addition to 5-HT, raphe 5-HT neural activity is also sensitive to norepinephrine, another neurotransmitter classically associated with depression (Anand and Charney 2000; Delgado and Moreno 2000; Nutt 1997), anxiety (Gorman et al 2002; Ressler and Nemeroff 2000), and the antidepressant/anxiolytic effects of physical activity (Dishman 1997b; Dishman et al 1997, 2000; Dunn 1996; Ransford 1982). Specifically, norepinephrine provides tonic excitation of raphe 5-HT neurons through an α_1 -adrenergic receptor (α_1 ADR) mechanism (Aghajanian 1985; Baraban and Aghajanian 1980a, 1980b; Pudovkina et al 2003; Yoshimura et al 1985). Although the effects of antidepressant/anxiolytic medications on the expression or function of raphe α_1 ADRs are unknown, blockade of α_1 ADRs in the DRN prevents the development of learned helplessness (Grahn et al 2002), implicating raphe α_1 ADRs in stress-related mood disorders.

It is clear that alterations in raphe 5-HTT, 5-HT_{1A}, 5-HT_{1B}, and/or α_1 ADRs could contribute to the antidepressant/anxiolytic effects of physical activity by altering central 5-HT neural transmission, although effects of physical activity on these parameters remain largely unexplored. We have recently reported that 6 weeks of wheel running increases 5-HT_{1A} messenger ribonucleic acid (mRNA) in the DRN and prevents classic learned helplessness behaviors produced by exposure to uncontrollable stress (Greenwood et al 2003a). Interestingly, the behavioral consequences of uncontrollable stress are sensitive to the duration of prior wheel running, whereby 6 weeks of wheel running prevents learned helplessness, but 3 weeks of wheel running does not (Greenwood et al 2003a, in press). The purpose of the current study was to determine the effects of wheel running, after durations known to be either insufficient (3 weeks) or sufficient (6 weeks) to prevent learned helplessness, on 5-HTT, 5-HT_{1A}, 5-HT_{1B}, and α_{1B} ADR mRNA levels in the DRN and MR. The effects of acute wheel running (3 days) on these parameters were also examined. We chose to focus on the α_{1B} ADR subtype because α_{1B} ADR mRNA is highly expressed throughout the raphe nuclei (Day et al 1997), and virtually all 5-HT neurons of the DRN express α_{1B} ADR mRNA (Day et al 2004).

Methods and Materials

Animals

A total of 37 adult male Fischer F344 rats weighing 208.8 ± 16.4 g at the beginning of the experiment were housed in a temperature- (22°C) and humidity-controlled environment and were maintained on a 12:12 hour light/dark cycle (lights on 6 AM–6 PM). Animals acclimatized to these housing conditions for 1 week prior to experimental manipulation. All animals were individually housed in Nalgene Plexiglas cages (45 × 25.2 × 14.7 cm) with attached running wheels. Wheels were rendered immobile during the acclimation period for the physically active animals and during the duration of the experiments for sedentary rats. Care was taken to minimize animal discomfort during all procedures. All experimental protocols were approved by the University of Colorado Animal Care and Use Committee. All rats had ad libitum access to food and water and were weighed weekly.

Activity

Animals were randomly assigned to either remain sedentary with locked running wheels (Sedentary; $n = 12$) or were allowed voluntary access to running wheels for either 3 days (3-day run; $n = 8$), 3 weeks (3-week run; $n = 8$), or 6 weeks (6-week run; $n = 9$). At the start of a running cycle, the wheels in the cages of

physically active rats were unlocked, and these rats were allowed voluntary access to their wheels. Daily wheel revolutions were recorded with Vital View software (Mini Mitter, Bend, Oregon), and distance was calculated by multiplying wheel circumference (1.081 m) by the number of revolutions.

In Situ Hybridization

Between 8 AM and 11 AM, rats were killed by decapitation after their assigned durations of wheel running or sedentary housing. To control for potential effects of aging, a subgroup ($n = 4$) of sedentary rats was killed at each time point. Brains were removed, frozen rapidly in isopentane and dry ice (−40 to −50°C), and stored at −80°C until sliced into 10- μ m coronal sections on a cryostat. Dorsal raphe nucleus and MR slices were thaw-mounted directly onto polylysine-coated slides and stored at −80°C until processed for single-labeled radioactive in situ hybridization as described elsewhere (Day and Akil 1996; Greenwood et al 2003c). Briefly, sections were fixed in 4% paraformaldehyde for 1 hour, acetylated in .1 mol/L triethanolamine containing .25% acetic anhydride (10 min), and dehydrated in graded alcohol. Complementary (c)RNA riboprobes (courtesy of Dr. Stanley Watson, University of Michigan, Ann Arbor) complementary to 5-HTT (490 mer: 828–1318), 5-HT_{1A} (910 mer: 333–1243), 5-HT_{1B} (860 mer: 210–1070), or α_{1B} ADR (766 mer: 144–910) were prepared from cDNA subclones in transcription vectors and labeled with [³⁵S]uridine triphosphate (Amersham-PharmaciaBiotech, Piscataway, New Jersey), according to standard transcription methods. Riboprobes were diluted in 50% hybridization buffer containing 50% formamide, 10% dextran sulfate, 2× saline sodium citrate, 50 μ mol/L phosphate-buffered saline (pH = 7.4), 1× Denhardt's solution, and .1 mg/mL yeast transfer RNA. Brain sections representing the rostral to caudal extent of the DRN and MR were hybridized with the probe overnight (55°C). The next day, sections were washed in 2× saline sodium citrate, treated with RNase A (200 μ g/mL) for 1 hour at 37°C, and washed to a final stringency of .1× saline sodium citrate at 65°C for 1 hour. Dehydrated, air-dried sections were exposed to x-ray film (Biomax-MR; Eastman Kodak, Rochester, New York) for 1 to 3 weeks. For each probe, slides (each containing four brain sections) from all rats were processed in a single experiment to allow for direct comparisons. Control experiments with “sense” probes indicated that the labeling observed with the “antisense” probes was specific (data not shown).

Image Analysis

Levels of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, or α_{1B} ADR mRNAs were analyzed by computer-assisted optical densitometry. Brain section images were captured digitally (CCD camera, model XC-77; Sony, Tokyo, Japan), and the relative optical density of the x-ray film was determined with Scion image version 4.0 (Scion, Frederick, Maryland). A macro was written that enabled signal above background to be automatically determined. For each section, a background sample was taken over an area of white matter, and the signal threshold was calculated as mean gray value of background +3.5 SD. The section was automatically density-sliced at this value, so that only pixels with gray values above these criteria were included in the analysis. Results are expressed as mean integrated density, which reflects both the signal intensity and the number of pixels above assigned background (mean signal above background × number of pixels above background). Care was taken to ensure that equivalent areas were analyzed between animals. Quantification of 5-HTT,

5-HT_{1A}, 5-HT_{1B}, or α_{1b} ADR mRNAs in the DRN occurred at rostral (–7.40 mm to –7.64 mm posterior to bregma; Paxinos and Watson 1998), mid (–7.80 mm to –8.00 mm) and caudal (–8.3 mm to –8.5 mm) levels. Each subject's mean integrated density of a particular cRNA probe at a given level represents the average of three DRN slices chosen for analysis at that approximate level. For analysis of each cRNA probe in the MR, four sections (from approximately –8.0 mm to –8.3 mm posterior to bregma; Paxinos and Watson 1998) were chosen from each subject for analysis. The integrated density obtained for each of the four slices were then averaged to give each subject's mean integrated density of a particular cRNA probe in the MR.

Statistical Analysis

Body weights were analyzed with two repeated measures analysis of variance (ANOVA), one for the first 3 weeks of the study (which included sedentary, 3-week run, and 6-week run groups) and one for the last 3 weeks (which included remaining sedentary and 6-week run rats only). Repeated measures ANOVA was also used to analyze the average weekly distance run by the 3-week and 6-week runners. Group differences in DRN and MR 5-HTT, 5-HT_{1A}, 5-HT_{1B}, or α_{1b} ADR mRNA were analyzed with one-way ANOVA. One-way ANOVA was also used to analyze group differences in the entire DRN (mean of DRN subregions), to determine the effect of wheel running on levels of a particular mRNA in the whole DRN. Fisher protected least significant difference post hoc analysis was performed when required. No differences due to age were revealed by ANOVA for any outcome measure between sedentary rats sacrificed after 3 days, 3 weeks, or 6 weeks of sedentary housing (data not shown). Therefore, the values of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, or α_{1b} ADR mRNAs in the sedentary group represent the average of sedentary rats killed after each time point. To determine the relationship between wheel running and expression of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, or α_{1b} ADR mRNA in the DRN, regression analysis was performed by simple regression, on average distance run and the last night's distance run, to 5-HTT, 5-HT_{1A}, 5-HT_{1B}, or α_{1b} ADR mRNA levels in the rostral, mid, and caudal DRN and MR. Alpha was set at .05 for each analysis.

Results

Activity and Body Weight

Weekly body weight change and running distance are shown in Figure 1. Consistent with prior reports (Campisi et al 2003), body weight (Figure 1A) increased steadily over 6 weeks, and 3-week and 6-week runners gained less weight (90.9% and 90.0% of sedentary mean, respectively), compared with sedentary counterparts. Repeated measures ANOVA revealed significant main effects of time [$F(3,66) = 458.443, p < .0001$] and activity [$F(2,22) = 3.45, p = .05$] and a reliable interaction between time and activity conditions [$F(6,66) = 7.745, p < .0001$] on body weight during the first 3 weeks of the study. Both 3-week and 6-week runners weighed less than sedentary rats by the end of the first week of voluntary wheel access ($p = .02$ and $p = .05$, respectively), and this pattern remained for the duration of the study. Importantly, at no point were the body weights of 3- and 6-week runners significantly different from each other. Analysis of variance also revealed significant main effects of time [$F(2,22) = 27.24, p < .0001$] and activity [$F(1,11) = 12.27, p = .005$], but not a reliable time \times activity interaction, on body weight during the last 3 weeks of the study.

Three-week and 6-week runners ran an average of $12.1 \pm .7$

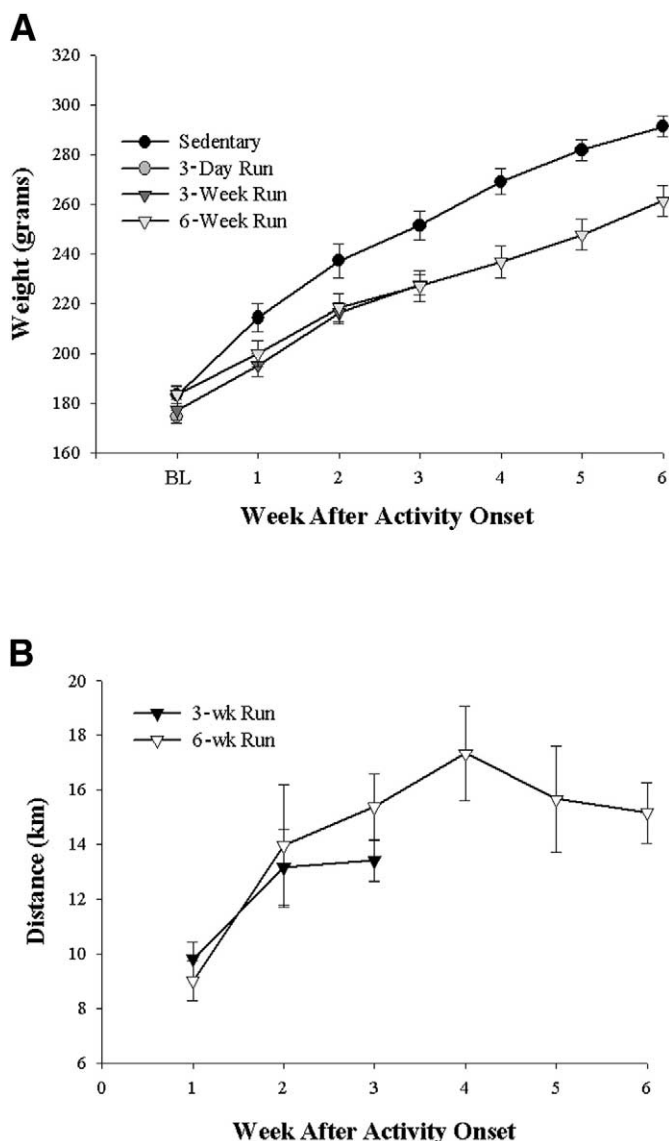


Figure 1. Adult male Fischer rats remained sedentary or were allowed voluntary access to running wheels for 3 days (3-Day Run), 3 weeks (3-Week Run), or 6 week (6-Week Run). **(A)** Mean weekly body weight change (in grams) of sedentary and physically active rats. **(B)** Mean distance (in kilometers) run each week by the rats in the 3-week and 6-week running groups. Values represent group means \pm SEM. BL, baseline.

km and 14.4 ± 1.3 km per week, respectively (Figure 1B). The average weekly running distance of 3-week and 6-week runners increased steadily during each group's first 3 weeks of wheel access. Repeated measures ANOVA revealed a significant main effect of time [$F(2,30) = 11.37, p = .0002$] on running distance during the first 3 weeks of the study. Neither the main effect of group nor the time \times group interaction reached statistical significance, indicating that the 3-week and 6-week running groups ran equal distances during the first 3 weeks. As previously reported in Fischer rats (Campisi et al 2003; Greenwood et al 2003b), the average weekly distance run by the 6-week runners reached a plateau after the first 3 weeks of wheel access.

5-HTT mRNA

For anatomical reference, 5-HTT mRNA expression in the rostral to caudal DRN and MR is shown in Figure 2. Compared

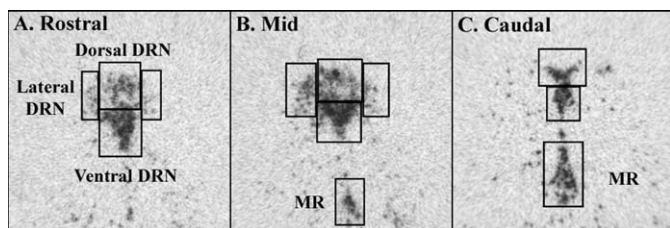


Figure 2. Representative autoradiographic coronal sections through the rostral (A), mid (B), and caudal (C) dorsal raphe nucleus (DRN) and median raphe nucleus (MR) of a sedentary rat processed with in situ hybridization for serotonin transporter messenger ribonucleic acid (mRNA). Outlined with boxes are the MR and the dorsal, ventral, and lateral subregions of the DRN in which quantification of mRNA occurred.

with sedentary levels, 3 weeks and 6 weeks, but not 3 days, of wheel running resulted in a reduction in 5-HTT mRNA in the DRN and MR. Analysis of variance revealed reliable main effects of activity in the rostral [$F(3,28) = 2.83, p = .05$], mid [$F(3,29) = 3.0, p = .04$], and caudal [$F(3,32) = 3.67, p = .02$] aspects of the dorsal DRN (Figure 3A); the rostral [$F(3,29) = 7.15, p = .001$] and mid [$F(3,30) = 3.3, p = .03$], but not caudal, aspects of the ventral DRN (Figure 3B); the rostral [$F(3,29) = 3.6, p = .02$] and mid [$F(3,30) = 2.9, p = .05$] aspects of the lateral DRN (Figure 3C); and the MR [$F(3,32) = 4.12, p = .01$] (Figure 3E). As shown in Figure 3D, there was also a significant main effect of activity on 5-HTT mRNA levels in the mean DRN (average of all DRN subregions) [$F(3,29) = 3.49, p = .02$]. Figure 4A contains representative autoradiographs illustrating group differences in the relative levels of 5-HTT mRNA in the rostral DRN.

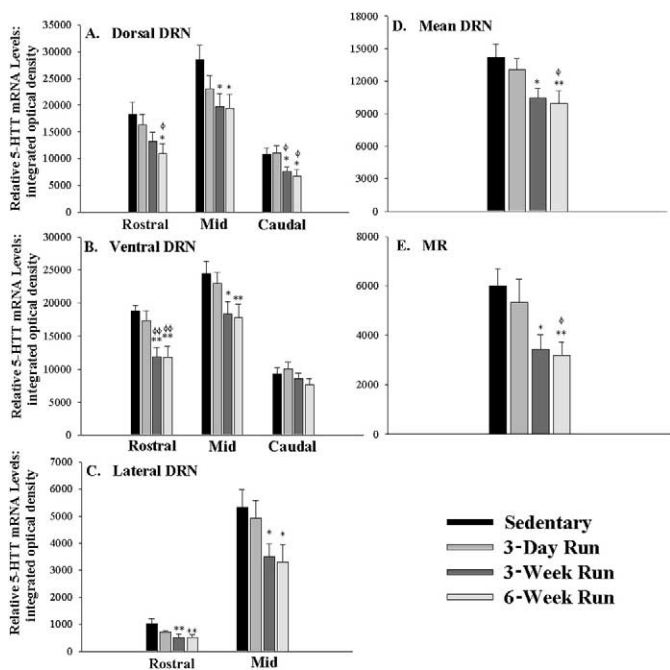


Figure 3. Expression of serotonin transporter (5-HTT) mRNA in the dorsal (A), ventral (B), lateral (C), and total (D) dorsal raphe nucleus (DRN) and median raphe nucleus (MR) (E) of sedentary rats and rats allowed 3 days (3-Day Run), 3 weeks (3-Week Run), and 6 weeks (6-Week Run) of voluntary access to running wheels. Values represent mean integrated density \pm SEM. Fisher protected least significant difference: * $p < .05$, ** $p < .01$ with respect to Sedentary; $^{\phi}p < .05$, $^{\phi\phi}p < .01$ with respect to 3-Day Run.

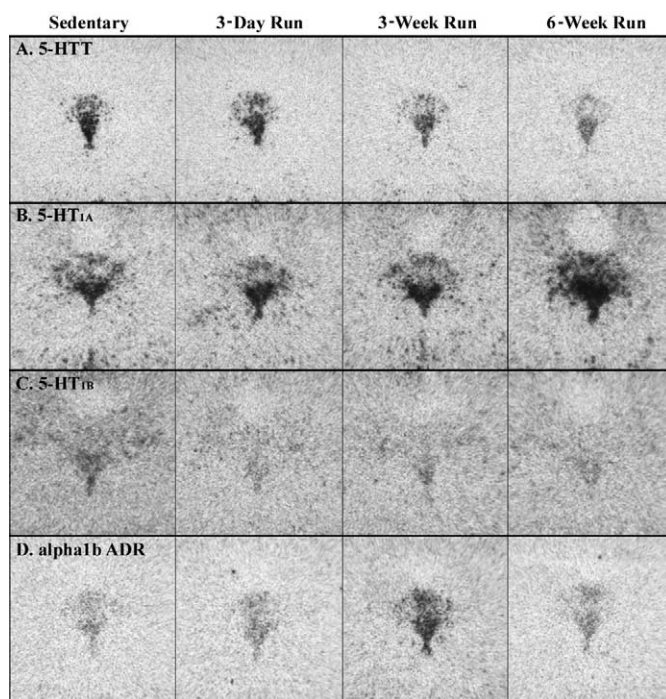


Figure 4. Representative autoradiographic coronal sections through the rostral dorsal raphe nucleus (DRN) showing the relative levels of serotonin transporter (5-HTT) messenger ribonucleic acid (mRNA) (A), 5-HT_{1A} mRNA (B), 5-HT_{1B} (C), or α_{1b} -adrenergic receptor (α_{1b} ADR) mRNA (D) labeled with in situ hybridization in sedentary rats (left column) and rats allowed 3 days (3-Day Run; second column), 3 weeks (3-Week Run; third column), or 6 weeks (6-Week Run; right column) of voluntary access to running wheels.

5-HT_{1A} mRNA

Contrary to the effect of wheel running on 5-HTT mRNA, 5-HT_{1A} mRNA was increased in the DRN and MR after 6 weeks, but not 3 days or 3 weeks, of wheel access. Analysis of variance revealed significant differences between groups when all DRN subregions were averaged together [$F(3,31) = 2.79; p = .05$] (Figure 5D); however, analysis of individual DRN subregions revealed that the greatest effect of wheel running occurred in specific aspects of the DRN. Although the main effect of activity on 5-HT_{1A} mRNA levels did not quite reach statistical significance in the rostral aspect of the dorsal DRN [$F(3,30) = 2.03, p = .13$] (Figure 5A), ANOVA revealed a reliable main effect of activity in the mid aspect of the dorsal DRN [$F(3,32) = 5.36, p = .004$] (Figure 5A). Wheel running did not affect levels of 5-HT_{1A} mRNA anywhere in the ventral DRN (Figure 5B), although the main effect of activity was significant in the mid aspect of the lateral DRN [$F(3,32) = 3.48, p = .02$] (Figure 5C) and the MR [$F(3,30) = 3.7, p = .02$] (Figure 5E). In no other DRN subregion did wheel running alter 5-HT_{1A} mRNA levels. Representative autoradiographs illustrating the relative levels of 5-HT_{1A} mRNA in the mid DRN between groups are shown in Figure 4B.

5-HT_{1B} mRNA

Wheel running affected 5-HT_{1B} mRNA levels in select subregions of the DRN similarly to 5-HT_{1A} mRNA levels, although unlike 5-HTT and 5-HT_{1A} mRNA, 5-HT_{1B} mRNA was rapidly altered by wheel running. Serotonin_{1B} mRNA levels were reduced below sedentary values after only 3 days of wheel running and remained reduced in 3-week and 6-week runners. The main effects of activity on 5-HT_{1B} mRNA levels were not significant

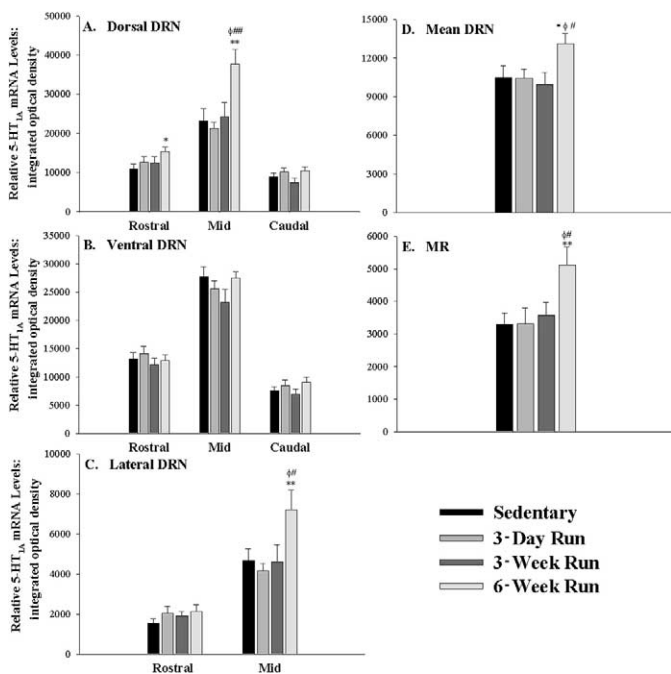


Figure 5. Expression of serotonin (5-HT)_{1A} messenger ribonucleic acid (mRNA) in the dorsal (A), ventral (B), lateral (C), and mean (D) dorsal raphe nucleus (DRN) and median raphe nucleus (MR) (E) of sedentary rats and rats allowed 3 days (3-Day Run), 3 weeks (3-Week Run), and 6 weeks (6-Week Run) of voluntary access to running wheels. Values represent mean integrated density \pm SEM. Fisher protected least significant difference: * $p < .05$, ** $p < .01$ with respect to Sedentary; † $p < .01$ with respect to 3-Day Run; # $p < .05$, ## $p < .01$ with respect to 3-Week Run.

anywhere in the dorsal (Figure 6A) or lateral (Figure 6C) DRN; however, ANOVA revealed significant main effects of activity in the rostral [$F(3,26) = 2.84$, $p = .05$] and mid [$F(3,25) = 2.9$, $p = .05$], but not caudal, aspects of the ventral DRN (Figure 6B). Wheel running did not significantly reduce 5-HT_{1B} mRNA levels in the mean DRN (Figure 6D) or MR (Figure 6E), although 5-HT_{1B} mRNA levels followed a similar pattern to that observed in the ventral DRN. Figure 4C contains representative autoradiographs illustrating group differences in relative levels of 5-HT_{1B} mRNA in the rostral DRN.

α_{1B} ADR mRNA

Compared with sedentary values, wheel running increased α_{1B} ADR mRNA levels in the DRN, although the increase was transient, appearing after 3 weeks of wheel access but absent again after 6 weeks. Analysis of variance revealed reliable main effects of activity on α_{1B} ADR mRNA levels in the rostral [$F(3,24) = 7.28$, $p = .001$] and mid [$F(3,24) = 3.54$, $p = .03$], but not caudal, aspects of the dorsal DRN (Figure 7A); the rostral [$F(3,25) = 2.9$, $p = .05$] and caudal [$F(3,24) = 3.12$, $p = .04$], but not mid, aspects of the ventral DRN (Figure 7B); and the rostral [$F(3,24) = 4.7$, $p = .01$], but not mid, aspects of the lateral DRN (Figure 7C). There was a significant effect of activity on α_{1B} ADR mRNA levels in the entire DRN [$F(3,20) = 4.07$, $p = .02$] (Figure 7D). Wheel running did not alter α_{1B} ADR mRNA levels in the MR (Figure 7E). Representative autoradiographs illustrating the relative levels of α_{1B} ADR mRNA in the rostral DRN between groups are shown in Figure 4D.

No reliable correlations within running groups were revealed with simple regression analysis between the average distance run, or the distance run on the last night before sacrifice, and

levels of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, or α_{1B} ADR mRNA in any subregion of the DRN or MR.

Discussion

Wheel running alters the levels of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, and α_{1B} ADR mRNA in the DRN and 5-HTT and 5-HT_{1A} mRNA in the MR. Although mRNA levels reported in this study are only indirect indications of protein expression or function, mRNA is a good indicator of changes in gene expression that could occur after an increase in physical activity status. Therefore, these data suggest that wheel running alters the mRNA expression, and possibly protein synthesis and function, of several factors involved in regulation of DRN and MR 5-HT neural activity and central 5-HT neurotransmission. Because of the important role of 5-HT in depression and anxiety, the modulation of raphe 5-HT mRNA levels observed in the current study might be associated with the antidepressant and/or anxiolytic effects of physical activity.

The general distribution patterns observed here are largely in agreement with previous studies reporting the localization of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, and α_{1B} ADR in the MR and their widespread distribution throughout all subdivisions and at all levels of the DRN (Day et al 1997, 2004; Doucet et al 1995; McLaughlin et al 1996; Rattray et al 1999; Wright et al 1995). Although a small percentage of 5-HT_{1A} and α_{1B} ADR mRNA are expressed in non-5-HT (i.e., 5-HTT mRNA negative) cells of the raphe nuclei (Day et al 2004), colocalization studies indicate that the majority of 5-HTT, 5-HT_{1A}, 5-HT_{1B}, and α_{1B} ADR mRNA are located within 5-HT neurons; moreover, an estimated 100% of the 5-HT cells in the rodent DRN contain 5-HTT, 5-HT_{1A}, 5-HT_{1B}, and α_{1B} ADR mRNA (Bonaventure et al 1998; Day et al 2004; Rattray et al 1999).

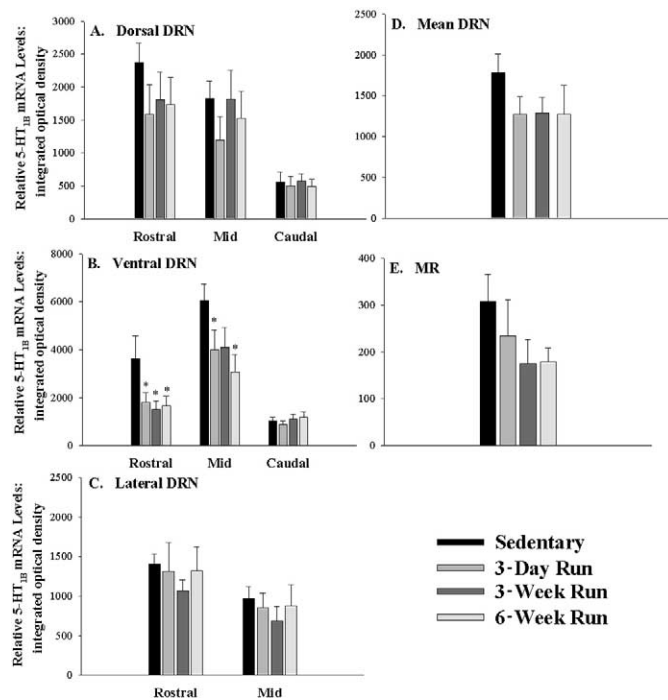


Figure 6. Expression of serotonin (5-HT)_{1B} messenger ribonucleic acid (mRNA) in the dorsal (A), ventral (B), lateral (C), and mean (D) dorsal raphe nucleus (DRN) and median raphe nucleus (MR) (E) of sedentary rats and rats allowed 3 days (3-Day Run), 3 weeks (3-Week Run), and 6 weeks (6-Week Run) of voluntary access to running wheels. Values represent mean integrated density \pm SEM. Fisher protected least significant difference: * $p < .05$ with respect to Sedentary.

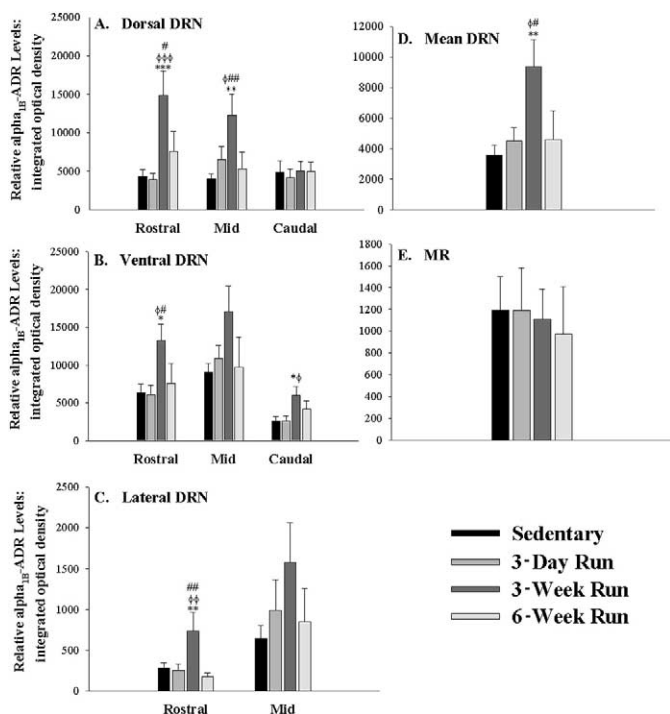


Figure 7. Expression of α_{1B} -adrenergic receptor (α_{1B} ADR) messenger ribonucleic acid in the dorsal (A), ventral (B), lateral (C), and mean (D) dorsal raphe nucleus (DRN) and median raphe nucleus (MR) (E) of sedentary rats and rats allowed 3 days (3-Day Run), 3 weeks (3-Week Run), and 6 weeks (6-Week Run) of voluntary access to running wheels. Values represent mean integrated density \pm SEM. Fisher protected least significant difference: * p < .05, ** p < .01, *** p < .001 with respect to Sedentary; ϕ p < .05, $\phi\phi$ p < .01, $\phi\phi\phi$ p < .001 with respect to 3-Day Run; # p < .05, ## p < .01 with respect to 6-Week Run.

Therefore, the changes in mRNA expression in the DRN and MR of physically active rats observed in the current study are likely occurring predominantly in 5-HT neurons.

Although 3 days of wheel running was not sufficient to affect 5-HTT mRNA, 5-HTT mRNA levels after 3 weeks of wheel running were lower than sedentary values throughout the DRN and MR and were maximally reduced after 6 weeks of wheel running. The effect of wheel running on 5-HTT mRNA is similar to some reported effects of chronic selective-5-HT-reuptake inhibitors, which decrease raphe 5-HTT mRNA (Le Poul et al 2000; Lesch et al 1993; Swan et al 1997) and forebrain 5-HTT ligand binding (Kovachich et al 1992; Kugaya et al 2003; Watanabe et al 1993), although the effects of selective-5-HT-reuptake inhibitors on 5-HTT are far from resolved (Burnet et al 1994; Koed and Linnet 1997; Lopez et al 1994).

Although 5-HTT mRNA is restricted to the cell bodies of origin located in the raphe nuclei, 5-HTT protein is functional both within the raphe nuclei and in axon terminal regions of 5-HT neurons projecting to distal sites (Tao et al 2000). Therefore, changes in 5-HTT mRNA levels could represent altered transporter activity within the DRN and MR and/or in any region of the brain receiving 5-HT projections from the DRN or MR (see Table 1). The regional specificity of the effect of wheel running on 5-HTT is important because a decrease in transporter activity could potentially produce opposing effects on central 5-HT neurotransmission, depending on where in the brain the decrease occurs. For example, an increase in extracellular 5-HT in

the raphe nuclei induced by a reduction in 5-HT uptake capability could inhibit raphe 5-HT neural activity and 5-HT release in terminal regions by increasing 5-HT_{1A} autoreceptor-mediated inhibition of 5-HT cell firing (Rutter et al 1995). Also, 5-HT_{1A} autoreceptor-mediated inhibition of 5-HT neural activity could be even further exacerbated in physically active rats that might have an increase in 5-HT_{1A} autoreceptors in the DRN. In contrast, a decrease in 5-HT uptake capability in other brain regions could result in an increase in 5-HT neurotransmission in those regions that is similar to the effects of 5-HTT blockade (Rutter and Auerbach 1993). Future studies will be needed to clarify the neuroanatomical specificity of the effects of wheel running on 5-HTT and the functional outcome of such changes on 5-HT neurotransmission.

Six weeks of wheel running increased levels of 5-HT_{1A} mRNA in the dorsal aspect of the rostral-mid DRN, the lateral aspect of the mid DRN, and the MR. The regional selectivity of the effect of 6 weeks of wheel running on 5-HT_{1A} mRNA in the DRN is similar to that previously reported, with the exception of that for the lateral-mid DRN in which an increase in 5-HT_{1A} mRNA was not previously observed (Greenwood et al 2003a). In our previous study, however, we did not differentiate between the rostral and mid or lateral DRN. Thus, examination of the lateral DRN with finer anatomical precision in the current study could account for this discrepancy, illustrating the importance of independent examination of unique DRN subregions.

It is especially interesting that wheel running increased 5-HT_{1A} mRNA in the raphe nuclei. An increase in 5-HT_{1A} autoreceptor function could presumably decrease 5-HT neurotransmission by enhancing autoinhibition of 5-HT neurons. This effect is opposite to that potentially produced by other changes observed in the raphe nuclei of physically active rats, such as a decrease in 5-HTT and 5-HT_{1B} autoreceptors; that could lead to an increase in 5-HT neurotransmission. Furthermore, in contrast to the effects of wheel running, other antidepressant treatments decrease raphe 5-HT_{1A} mRNA (Le Poul et al 2000) and desensitize raphe 5-HT_{1A} autoreceptors (Blier et al 1998; Elena Castro et al 2003; Mochizuki et al 2002), effects that might help reverse the deficit in 5-HT neurotransmission thought to occur in depression. It is possible that alterations in 5-HT_{1A} autoreceptors might be one, but not the only, component involved in the complex etiology and treatment of depression. Indeed, both increased (Stockmeier et al 1998) and decreased (Arango et al 2001; Drevets et al 1999, 2000) density of 5-HT_{1A} autoreceptor binding have been observed in the raphe nuclei of suicides or depressed subjects. Regardless of the role of 5-HT_{1A} autoreceptors in affective disorders, the 5-HT_{1A} autoreceptor is only one factor involved in regulation of central 5-HT neurotransmission, and the contribution that the increase in 5-HT_{1A} mRNA has on overall 5-HT transmission in the brains of physically active rats remains unknown.

In contrast to the effects of wheel running on 5-HT_{1A} autoreceptor mRNA, wheel running rapidly decreased 5-HT_{1B} autoreceptor mRNA in the DRN. Although the decrease in 5-HT_{1B} mRNA was only statistically reliable in the ventral aspect of the rostral-mid DRN, a similar pattern was also present in the whole DRN and the MR. The group differences in the dorsal DRN and MR likely failed to reach statistical significance owing to the relatively lower levels of 5-HT_{1B} mRNA in these regions compared with the ventral portion of the DRN.

The observed decrease in DRN 5-HT_{1B} mRNA is similar to previous studies reporting reduced 5-HT_{1B} autoreceptor mRNA in the DRN (Anthony et al 2000; Neumaier et al 1996) and presynaptic 5-HT_{1B} autoreceptor sensitivity in the hypothalamus

Table 1. Main Serotonergic Projection Sites of the Dorsal and Median Raphe Nuclei

Region and Subregion	Serotonergic Projections Sites	References
Dorsal Raphe Nucleus		
Rostral	Caudate putamen, amygdala, striatum, medial thalamus, substantia nigra, olfactory cortex, septum	Imai et al 1986; Steinbusch et al 1981; van der Kooy and Hattori 1980
Mid	Caudate putamen, central amygdala, lateral parabrachial nucleus, dorsal cap of the paraventricular hypothalamic nucleus, dorsal hippocampus, medial septum	Lowry 2002; van der Kooy and Hattori 1980
Caudal	Hippocampus, locus coeruleus	Imai et al 1986; Lowry 2002
Dorsal	Caudate putamen, lateral and medial hypothalamus, frontal cortex, thoracic spinal cord	Bowker et al 1982; Kazakov et al 1993; Lowry 2002; Tohyama et al 1979
Ventral	Cortex, caudate putamen	Kazakov et al 1993; Lowry 2002
Lateral	Lateral and medial hypothalamus	Kazakov et al 1993
Unspecified	Dorsal striatum, globus pallidus, mid-posterior dorsal hippocampus, basolateral amygdala, rostral ventrolateral medulla, frontal cortex, intergeniculate leaflet, bed nucleus of the stria terminalis	Hensler et al 1994; Jacobs and Azmitia 1992; Lowry 2002; McQuade and Sharp 1997
Median Raphe Nucleus		
	Suprachiasmatic nucleus, ventral posterior hypothalamus, anterior hypothalamus, ventrolateral dorsomedial hypothalamus, medial mammillary nucleus, hippocampus, caudate putamen, amygdala, medial septum, locus coeruleus	Hensler et al 1994; Imai et al 1986; Jacobs and Azmitia 1992; Lowry 2002; McQuade and Sharp 1997

and hippocampus (Dremencov et al 2000; Gur et al 2002; Newman et al 2000) after chronic selective-5-HT-reuptake inhibitors. A decreased ability to synthesize terminal 5-HT_{1B} autoreceptors, similar to reductions in 5-HTT in terminal regions of the raphe nuclei, could lead to an enhancement of basal 5-HT neurotransmission in projection regions of the ventral DRN in physically active rats (Table 1). Interestingly, several weeks of treadmill training has also been reported to reduce postsynaptic 5-HT_{1B} mRNA in the cerebellum and frontal cortex (Chennaoui et al 2001) and desensitize 5-HT_{1B} receptors in the substantia nigra (Chennaoui et al 2000; Seguin et al 1998). These data suggest that physical activity might reduce the expression and/or activity of both terminal 5-HT_{1B} autoreceptors as well as postsynaptic 5-HT_{1B} heteroreceptors.

Recent work by Neumaier et al suggests that increased DRN 5-HT_{1B} levels are associated with both increased anxiety in rats that have been exposed to stress (Clark et al 2002; Neumaier et al 1997) and reduced anxiety (Kaiyala et al 2003) or stress resistance (Neumaier et al 2002) in stress-naïve rats. Wheel running, however, in the absence of stress, produces anxiolysis (Dishman 1997b) and stress resistance (Dishman et al 1997; Greenwood et al 2003a), yet is accompanied by a decrease in raphe 5-HT_{1B} mRNA. It is now clear that the involvement of the 5-HT_{1B} receptor in depression and anxiety is complex and could depend on the stress status, the genetic background, and the physical activity status of the animal.

Changes in 5-HTT, 5-HT_{1A}, and 5-HT_{1B} mRNA levels occurred in specific DRN subregions and at specific levels. Although the effect of wheel running on overall 5-HT neurotransmission remains unknown, one functional consequence of the regional specificity of the effects of wheel running could be selective changes in 5-HT neurotransmission in brain areas receiving 5-HT projections from those areas of the DRN or MR with altered 5-HTT, 5-HT_{1A}, or 5-HT_{1B} mRNA levels. For example, wheel running reduced 5-HT_{1B} mRNA levels in specifically the ventral aspect of the rostral to mid DRN, but not the MR. This suggests that wheel running might alter terminal 5-HT_{1B} autoreceptor regulation in those brain regions receiving

projections from the ventral DRN, such as the caudate putamen and cortex (Kazakov et al 1993; Lowry 2002), whereas terminal 5-HT_{1B} autoreceptors in other regions innervated predominantly by the dorsal aspect of the DRN or the MR, such as the hippocampus (Lowry 2002), will not be affected. Table 1 lists the main 5-HT projections from the DRN and MR so that similar anatomical comparisons can be made for the other mRNA changes observed. Refer to Lowry (2002) for a detailed description of 5-HT projections from the raphe nuclei.

The level of α_{1B} ADR mRNA throughout the DRN was increased after 3 weeks of wheel running, but this increase was not present in the MR and was not sustained after 6 weeks of wheel running. The current study is the first of which we are aware to investigate the effects of physical activity on central α_{1B} ADRs. The transient effect of wheel running on α_{1B} ADR mRNA is intriguing and could possibly be induced by some unidentified reaction of central norepinephrine systems to wheel running that either diminishes over time or is compensated for by changes in receptor expression. Indeed, central norepinephrine systems are activated by acute exercise (Meeusen et al 1997) and are altered by habitual physical activity (Brown et al 1979; Chaouloff 1989; Da Costa Gomez et al 1996; Dishman 1997a, 1997b; Dishman et al 1997, 2000; Dunn 1996; Greenwood et al 2003a; Lambert 1998; Ransford 1982; Soares et al 1999).

We have recently observed that the behavioral effects of wheel running, like the time-dependent therapeutic properties of antidepressant drugs (Quitkin et al 1996), are sensitive to the duration of activity whereby 6 weeks, but not 3 weeks, of wheel running prevents the shuttle-box-escape deficit and exaggerated-fear conditioning associated with learned helplessness (Greenwood et al, in press). This time-dependent effect presents a useful tool to help determine how changes in the brain that might occur after different durations of wheel running contribute to the stress-protective effects of exercise. Although many factors could be involved, of the changes reported here, only the increase in raphe 5-HT_{1A} mRNA after 6 weeks of wheel running occurs in a time course consistent with the behavioral effects of wheel

running in the learned helplessness model. Growing evidence suggests that learned helplessness depends on hyperactivity and sensitization of DRN 5-HT neurons produced by uncontrollable stress (Grahn 1999; Maier and Watkins 1998; Maier et al 1993, 1994, 1995a, 1995b; Maswood et al 1998; Takase et al 2004). Therefore, the increase in 5-HT_{1A} autoreceptor mRNA produced by 6 weeks of wheel running could contribute to the prevention of learned helplessness by constraining DRN 5-HT neural activity and 5-HT_{1A} autoreceptor down-regulation during uncontrollable stress (Greenwood et al 2003a).

The current results suggest that wheel running modulates mRNA for several factors involved in regulation of DRN and MR neural activity and central 5-HT neurotransmission. Although only correlative, these data suggest that increased 5-HT_{1A} mRNA might be more directly related to the protective effect of wheel running against learned helplessness than other changes that occur before 6 weeks of wheel access. These data further support a role for 5-HT in the stress resistance produced by wheel running and the antidepressant and anxiolytic properties of exercise.

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