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Research report

The consequences of uncontrollable stress are sensitive to duration of prior wheel running

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Abstract

The behavioral consequences of uncontrollable stress, or learned helplessness (LH) behaviors, are thought to involve hyperactivity of serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN). Other brain regions implicated in LH and capable of affecting 5-HT systems, such as the bed nucleus of the stria terminalis (BNST), amygdala, and habenula, could contribute to DRN 5-HT hyperactivity during uncontrollable stress. Six weeks of wheel running prevents LH and attenuates uncontrollable stress-induced c-Fos expression in DRN 5-HT neurons, although the duration of wheel running necessary for these effects is unknown. In the current study, 6 but not 3, weeks of wheel running blocked the shuttle box escape deficit and exaggerated fear produced by uncontrollable tail shock in sedentary rats. Corresponding to the duration-dependent effects of wheel running on LH behaviors, 6 weeks of wheel running was required to attenuate uncontrollable stress-induced 5-HT neural activity, indexed by c-Fos protein expression, in the DRN and c-Fos expression in the lateral ventral region of the BNST. Wheel running, regardless of duration, did not affect c-Fos expression anywhere in the amygdala or habenula. These data indicate that the behavioral effects of uncontrollable stress are sensitive to the duration of prior physical activity and are consistent with the hypothesis that attenuation of DRN 5-HT activity contributes to the prevention of LH by wheel running. The potential role of the BNST in the prevention of LH by wheel running is discussed.
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1. Introduction

Exposure to uncontrollable, but not controllable, stressors results in a sequellae of physiological and behavioral changes, termed behavioral depression [92] or learned helplessness (LH; [62]). LH behaviors include impaired escape performance [77,84] and exaggerated fear conditioning [61]. Interestingly, as well as being prevented or reversed by antidepressant and anxiolytic drugs [64,66,69,72], LH behaviors are sensitive to the physical activity status of the organism. Six or more weeks of voluntary access to running wheels prior to exposure to uncontrollable shocks protects rats from developing LH [23,42,74].

Recent work exploring the mechanisms by which wheel running prevents LH have focused on serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN; [42]) because the DRN and 5-HT play a clear role in (a) stress-related affective disorders such as depression and anxiety [5,37,76,78], (b) the pharmacological action of antidepressant and anxiolytic drugs [9,10,22,85], and (c) the modulation of the behavioral effects of uncontrollable stressors [14,29,30,63,65,75,81]. Specifically, we have proposed that

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attenuation of 5-HT neural activity in the DRN during uncontrollable stress is involved in the protection against LH provided by wheel running [42]. A growing body of literature indicating that LH is induced by hyperactivation and sensitization of 5-HT neurons located in the DRN supports this hypothesis. For example, exposure to uncontrollable, compared to controllable, tail shocks result in greater c-Fos induction in DRN 5-HT neurons [39,89], and exaggerated 5-HT efflux within the DRN [71] and in DRN projection sites [2,3,8,80] during stress and later behavioral testing. Moreover, manipulations that decrease 5-HT neural activity in the DRN during uncontrollable stress eliminate LH behaviors [66,68]. Finally, compared to the sedentary condition, 6 weeks of wheel running attenuates uncontrollable tail shock-induced c-Fos expression in 5-HT-immunoreactive neurons of the DRN [42].

Although increased 5-HT_{1A} autoreceptor-mediated inhibition of DRN 5-HT neurons has been suggested to contribute to the attenuation of DRN 5-HT neural activity produced by wheel running [42,44], it is possible that other regions afferent to the DRN could play a role. Structures that contain corticotrophin releasing hormone (CRH) or excitatory amino acids (EAAs), such as glutamate or aspartate, are likely candidates because both CRH [59] and NMDA agonists [90] can modulate the activity of DRN 5-HT neurons. Moreover, intra-DRN microinjections of CRH-R2 [46] or EAA [40] antagonists prevent typical consequences of uncontrollable stress. The bed nucleus of the stria terminalis (BNST) and the central amygdala (CeA) project to the DRN and these projections could contain either CRH and/or EAAs [1,17,24,52,57,73,82]. Additionally, the lateral habenula (LHb) projects to the DRN and these projections are thought to contain aspartate [1,52,53,57]. Indeed, electrical stimulation of the LHb excites DRN neurons [32,94] and lesions of the LHb eliminate stress-induced increases in 5-HT in the DRN [4].

Pharmacological and behavioral data are consistent with roles for these structures in modulation of stress and fear. In fact, it is generally accepted that the BNST and amygdala (AMG) are critical in anxiety and fear, respectively [18,56,91]. Activation of the BNST produces neuroendocrine [25,27] and behavioral [13] responses that resemble those produced by stress; and inactivation of the BNST decreases neuroendocrine and behavioral responses to stress [41]. Similarly, lesions of the CeA or basolateral AMG (BLA) reduce or eliminate fear [7,50,65]. It is thought that information about environmental signals are associated with aversive unconditioned stimuli in the BLA [15,49], whereas the CeA provides output to autonomic brainstem nuclei, the hypothalamus, and the ventral periaqueductal grey (PAG), among other regions, to orchestrate freezing and autonomic responses that make up the fear response [19,55]. Indeed, stimulation of the CeA produces behavioral, cardiovascular, and neuroendocrine components of fear [6,26,50]. In general, the anxiogenic and fear responses elicited by BNST and AMG stimulation are what could be expected from stimulation of the DRN [36–38]. Most importantly, lesions of the BNST [47] or LHb [4] eliminate typical behavioral consequences of uncontrollable stress.

Because lesions or manipulations that reduce the electrical activity of the BNST, AMG, or LHb eliminate behavioral stress responses, a decrease in cellular activation of these regions during uncontrollable stress could contribute to the prevention of LH by wheel running. Furthermore, attenuation of stress-induced cellular activation in these structures could contribute to the attenuation of DRN 5-HT neural responses during uncontrollable stress observed in physically active rats [42]. The current studies, therefore, examined the effects of 3 weeks and 6 weeks of wheel running on LH behaviors and uncontrollable stress-induced c-Fos immunoreactivity (IR) in the BNST, AMG, LHb, and 5-HT-immunoreactive neurons of the DRN. We hypothesize that the potential time-dependent effect of wheel running on LH behaviors will correspond to time-dependent effects of wheel running on attenuation of stress-induced c-Fos expression in the BNST, AMG, LHb, and 5-HT neurons of the DRN.

2. Material and methods

2.1. Animals

Adult, male Fischer F344 rats weighing 161 ± 26 g at time of arrival were used in all experiments. Rats were housed in a temperature (22 °C) and humidity-controlled environment and were maintained on a 12:12 h light/dark cycle (lights on 0600–1800). Animals were allowed to acclimate to these housing conditions for 2 weeks before any experimental manipulation. Animals were individually housed in Nalgene Plexiglas cages (45 × 25.2 × 14.7 cm) with attached running wheels. Wheels were rendered immobile with a metal stake during the acclimation period for the physically active animals and during the duration of the experiments for all 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.

2.2. Activity

Animals were randomly assigned to either remain sedentary with locked running wheels (Sedentary) or were allowed voluntary access to running wheels for either 3 (3-week Run) or 6 (6-week Run) weeks. Three-week runners lived with locked wheels (Mini Mitter, Bend, OR) during the first 3 weeks of the studies during which time 6-week runners had voluntary access to their wheels. After the first 3 weeks, wheels in the cages of the 3 week runners were unlocked and these rats were also allowed to run voluntarily on their wheels until the end of the studies. In order to not

disturb the rats the day their wheels were unlocked, 3-week runners were weighed 2 days after their wheels were unlocked at the beginning of week 3. Daily wheel revolutions were recorded digitally using Mini Mitter Vital View Data Acquisition software and distance was calculated by multiplying wheel circumference (1.081 m) by the number of wheel revolutions.

2.3. Uncontrollable stress protocol

Following the assigned duration of sedentary living or wheel access, sedentary and physically active rats were randomly assigned to either receive uncontrollable tail shock stress (Stressed) or to remain in their home cages (No Stress). 3-week Run non-stressed rats were not included in immunohistochemical studies in order to minimize the number of animals used and because previous work has revealed that basal c-Fos levels in the DRN is not changed with wheel running in the absence of stress [42]. Stressed rats were restrained in Plexiglass tubes (23.4 cm long and 7.0 cm in diameter) and received 100, 5 s tail shocks (1.5 mA) on a 1-min variable-interval schedule. Following stressor termination, all rats were returned to their home cages, thus physically active rats exposed to tail shock were allowed access to their running wheels following stressor exposure. All rats were stressed during their inactive (light) cycle, between 0800 and 1000. This tail shock protocol was used in these experiments because tail shock is a consistent, quantifiable stressor that is known to produce LH [67] and we have previously demonstrated that 6 weeks of wheel running alters many behavioral and physiological responses to uncontrollable tail shock [33,34,42,43,74].

2.4. Behavioral testing

Both conditioned fear and shuttle box escape learning were tested 24 h following stress or control treatment by an experimenter blind to the treatment condition of the animals, as previously described [42,65]. Each rat (n = 8/group) was scored every 8 s as either freezing or not freezing for the first 10 min after placement in the shuttle box (46 × 20.7 × 20 cm). In order to be scored as freezing, all 4 paws had to be on the shuttle box grid floor, and there had to be an absence of all movement except for that required for respiration. Following this initial observation period, rats received 2, 0.6 mA foot shocks that could be terminated by crossing to the other side of the shuttle box (fixed ratio-1 (FR-1) trials). Shocks terminated automatically if escape did not occur by 30 s. In these cases, a 30-s escape latency was assigned. Prior uncontrollable stress does not alter FR-1 shuttle box escape latencies [65]; therefore, all animals were exposed to shocks of equal duration.

Following the two FR-1 trials, rats were observed again for 20 min and scored for freezing as before. Previous work has indicated that this freezing is a measure of fear conditioned to the contextual cues of the shuttle box [31]. The post FR-1 observation period was followed by 3 more FR-1 escape trials and then by 25, FR-2 escape trials. FR-2 trials differed from FR-1 trials in that rats were required to cross to the other side of the shuttle box and then back to terminate foot shock. Shocks occurred with an average intertrial interval of 60 s and each shock was terminated after 30 s if an escape response had not occurred. A single test session lasted approximately 50 min.

2.5. Immunohistochemistry

Rats (n = 8/group) were deeply anesthetized with sodium pentobarbital (Nembutal) approximately 90 min following tail shock termination, the time at which c-Fos protein can be optimally detected in the DRN following 100 inescapable tail shocks [39]. Rats were perfused transcardially with 100 ml of cold physiological saline, followed by 400–500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Extracted brains were post-fixed in 4% paraformaldehyde for 1 h and then transferred to PB, containing 0.1% sodium azide and 30% sucrose. Whole brains were stored at 4 °C until sectioning. Following rapid freezing in isopentane and dry ice (−40 to −50 °C), 35 μm coronal sections were cut on a cryostat (CM 1850, Leica Microsystems, Nussloch, Germany) at −20 °C and placed in PB containing 0.1% sodium azide at 4 °C until staining. All tissue was processed simultaneously during staining to allow direct comparisons between groups to be made.

2.5.1. c-Fos labeling

Immunohistochemistry for c-Fos protein was performed as previously described [42,43]. Briefly, 35-μm brain sections representing the rostral to caudal extent of either the DRN, BNST, AMG, or LHb were rinsed in 0.01 M phosphate-buffered saline (PBS) followed by a 30-min incubation in 0.3% hydrogen peroxide. Sections were incubated at room temperature (RT) for 12 h in blocking solution containing 0.1% sodium azide, 0.5% Triton X-100, 5% normal goat serum, and polyclonal rabbit anti-c-Fos IgG (Santa Cruz Antibodies, Ca) at a dilution of 1:15,000. This incubation was followed by another series of washes in PBS after which the sections were incubated at RT for 2 h in blocking solution containing a 1:200 dilution of biotinylated goat anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA). Sections were then incubated with avidin–biotin–horseradish peroxidase complexes (ABC; Vecastain Elite ABC kit, Vector Laboratories, Burlingame, CA) in PBS containing 0.5% Triton X-100 for 2 h. After washes with PB, sections were placed in a solution containing 3,3′ -diaminobenzidene tetrahydrochloride (DAB), ammonium chloride, cobalt chloride, nickel ammonium sulfate, and glucose oxidase in PB for 10 min. The peroxidase reaction was started by addition of glucose solution and reacted for 15–20 min, yielding a dark brown/black reaction product. The reaction was stopped by rinses in PBS.

2.5.2. c-Fos/5-HT double labeling in the DRN

Sections of the DRN were reacted for 5-HT IR immediately following c-Fos staining as previously described [39,42]. Following PBS rinses, DRN sections were incubated in blocking solution for 30 min. Tissue was then incubated in a 1:10,000 dilution of 5-HT antibody (DiaSorin, Stillwater, MN) for 48 h at 4 °C. Sections were then placed in goat anti-rabbit IgG (1:200; Jackson ImmunoResearch) for 2 h, followed by a 2-h incubation in PBS containing a 1:500 dilution of peroxidase anti-peroxidase (Sigma, St. Louis, MO). Tissue was rinsed in PB and reacted with DAB and glucose oxidase. The peroxidase reaction was initiated by addition of glucose and allowed to proceed for approximately 15 min, yielding a light brown reaction product. The reaction was stopped by PBS washes.

Controls for the cross-reactivity of c-Fos and 5-HT antisera consisted of processing additional sections except without addition of the primary antibody. Sections processed this way demonstrated a lack of labeling. Stained sections were mounted onto gelatin coated, glass slides and air-dried overnight. Slide-mounted sections were dehydrated in a series of alcohols, rinsed in Histoclear, and coverslipped with Depex.

2.6. Image analysis

An observer blind to the treatment condition of the subjects analyzed all brain sections. In order to determine if physical activity status affected stress-induced activation of unique populations of 5-HT neurons within the DRN, the DRN was divided into dorsal, ventral, and lateral portions based on the rat brain atlas by Paxinos and Watson [79]. Each of these regions was analyzed at 3 levels corresponding to the rostral (−7.64 mm posterior to bregma; [79], mid (−8.00 mm posterior to bregma), and caudal (−8.30 mm posterior to bregma) regions of the DRN, with the exception of the lateral wings, which are not present at the caudal level of analysis (for anatomical visualization of DRN regions analyzed see Ref. [42]). Accordingly, 6 double labeled sections of the DRN, 1 pair each corresponding to either the rostral, mid, or caudal DRN were selected from each subject for analysis. Each DRN section was assessed for the number of single c-Fos positive nuclei, the number of single 5-HT-positive cells, and the number of double-labeled cells. Small, dark brown/black particles were counted as single c-Fos-stained nuclei. Larger, light brown, or blue particles without a darker nucleus were counted as single 5-HT-stained cells and larger, lighter brown particles that contained a darker nucleus were counted as double-labeled c-Fos/5-HT cells. Results are expressed as the mean number of single- or double-labeled cells. Each subject's values of single c-Fos, single 5-HT, and double-labeled cells at each rostrocaudal level of the DRN were obtained by averaging the sections chosen for analysis at that level.

The lateral portions of the dorsal (BNSTld) and ventral (BNSTlv) regions of the BNST were chosen for analysis. As described previously [24], the BNSTld contains the oval region of the BNST and our analyses of the BNSTlv included the nearby fusiform region of the BNST [79]. The BNSTld and BNSTlv are located −0.26 mm to −0.40 mm from Bregma according to the rat brain atlas by Paxinos and Watson. We analyzed the BLA and the CeA, separately, from −1.60 mm to −2.56 mm from Bregma [79]. Our analysis of the CeA included the medial, capsular, and lateral divisions of the CeA as described by Paxinos and Watson [79]. Analysis of the LHb occurred from −2.56 mm to −3.8 mm from Bregma [79].

Three sections from each sub-region of the BNST, AMG, and LHb were selected from each subject for analysis, thus, the value for each subject is the average of the 3 chosen sections. Total c-Fos immunoreactivity was quantified using the National Institute of Health (version 1.6) image analysis system as previously described [43]. These results are expressed as number of c-Fos particles per mm^2 of brain area measured. Mean areas measured were 0.17 mm^2, 0.18 mm^2, 0.13 mm^2, 0.20 mm^2, and 0.07 mm^2 for the BNSTld, BNSTlv, BLA, CeA, and LHb, respectively.

2.7. Statistical analysis

Repeated measures analysis of variance (ANOVA) were used to determine group differences in body weight and in running distances during the final 3 weeks of wheel running. Escape latencies were collapsed into 5 blocks of 5 trials and analyzed with two-way (stress × activity), repeated measures ANOVA, followed by a Newman–Keuls analysis. Freezing scores were collapsed into 10, 2 min blocks and also analyzed with two-way (stress × activity), repeated measures ANOVA followed by a Newman–Keuls analysis. Group differences in total and single c-Fos protein, single 5-HT, and double c-Fos/5-HT in each brain region were analyzed by one-way ANOVA followed by Fisher's protected least significant difference (PLSD) post hoc analysis when required. To determine the relationship between distance run and LH behaviors or c-Fos expression in the DRN and BNST, regression analysis was performed by simple regression on both total and average weekly distance run to escape latency, freezing score, double c-Fos/5-HT cells in the DRN, and c-Fos IR in the BNST. Alpha was set at 0.05 for each analysis. Actual group sizes varied (from 6 to 8/group) within and between brain regions due to disruptions in tissue integrity incurred during brain removal, slicing, processing, etc.

3. Results

3.1. Activity and body weights

Weekly body weight change and running distance are shown in Fig. 1. Body weight (Fig. 1A) increased steadily

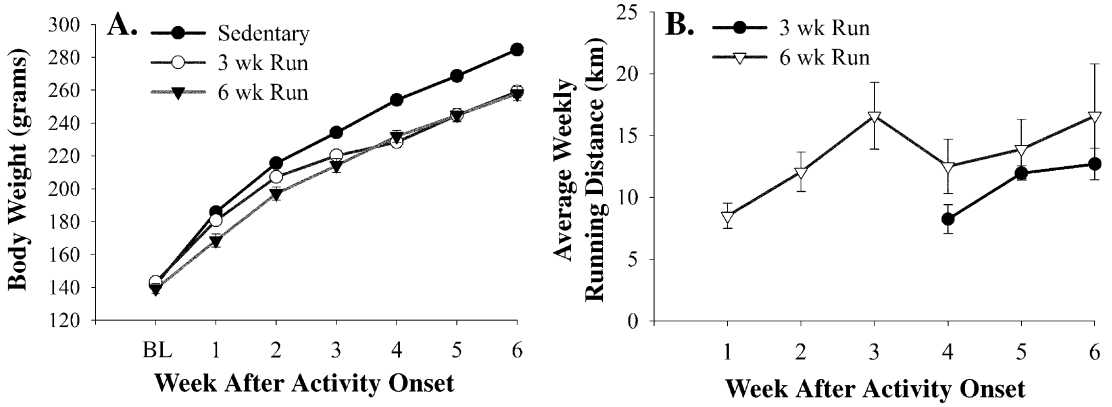

Fig. 1. Adult male Fischer rats were allowed voluntary access to running wheels for either three (3-weeks Run) or six (6-weeks Run) weeks or remained sedentary. (A) Mean weekly body weight change (grams) of physically active and sedentary rats. (B) The mean distance (kilometers) ran each week by the physically active rats. Values represent group means ± SEM. *BL*, Baseline.

over 6 weeks and 3-week and 6-week runners gained significantly less weight compared to sedentary counterparts. Repeated measures ANOVA revealed significant main effects of time ($F(6, 270) = 1916.82$, $P < 0.0001$) and activity ($F(2, 270) = 10.95$, $P < 0.02$), and a reliable interaction between time and activity conditions ($F(12, 270) = 11.77$, $P < 0.0001$). 6-week runners weighed less than sedentary rats by the end of the first week of voluntary wheel access ($P = 0.0003$) and remained lighter until the end of the study ($P < 0.0001$ at all remaining time points). Mean body weights of 3-week runners were equal to that of sedentary rats while wheels remained locked during the first 3 weeks of the study. The significant ($P = 0.008$) decrease in body weight of the 3-week runners, relative to sedentary counterparts, on week 3 reflects a decrease in weight gain due to the 2 days of voluntary running which occurred prior to weighing the rats on week 3. For the remainder of the study, 3-week runners weighed less than sedentary rats ($P < 0.0001$) and body weights of 3-week runners were not statistically different from those of 6-week runners.

Three-week and 6-week runners ran an average of 10.9 ± 0.6 km and 13.3 ± 2.5 km per week, respectively (Fig. 1B). Repeated measures ANOVA revealed a significant main effect of time ($F(2, 24) = 4.89$, $P = 0.02$) on running distance during the 6-week running group's last 3 weeks, and the 3-week running group's only 3 weeks, of running. Neither the main effect of group, nor the time-by-group interaction reached statistical significance. The average weekly running distance of 3-week and 6-week runners increased steadily during each group's first 3 weeks of wheel access. Following 3 weeks of wheel access, the average weekly running distance of 6-week runners reached a plateau; therefore, 3-week and 6-week runners ran equivalent distances the week prior to testing (12.7 ± 1.3 km and 16.6 ± 4.5 km, respectively; $P = 0.42$). Both body weight changes and running distances for Fischer rats used in LH behavioral studies were similar to those reported in Fig. 1 (data not shown).

3.2. Time course effect of wheel running on LH behaviors

To determine the effect of different durations of wheel running on LH behaviors, rats either remained sedentary or were allowed 3 weeks or 6 weeks of voluntary access to running wheels. Sedentary, 3-week runners, and 6-week runners were then either exposed to uncontrollable tail shock or remained in their home cages. Twenty four hours later, rats were tested in shuttle boxes for escape performance and freezing.

Escape latencies are shown in Fig. 2A. Sedentary rats exposed to uncontrollable stress failed to learn to escape from escapable foot shock and 6 weeks of prior wheel running prevented this deficit in shuttle box escape. In contrast, 3 weeks of wheel running was insufficient to prevent the shuttle box escape deficit. No differences in escape latencies during the FR-1 trial were found, however, ANOVA revealed reliable main effects of stress ($F(1, 42) = 42.98$, $P < 0.0001$) and activity ($F(2, 42) = 4.04$, $P = 0.02$), and a significant interaction between stress and activity conditions ($F(2, 42) = 7.3$, $P = 0.002$) on latency to escape foot shock during the FR-2 trials. Although the interaction between escape trials and activity condition did not reach statistical significance ($F(8, 168) = 1.77$, $P = 0.08$), reliable interactions were found between escape trials and stress treatment ($F(4, 168) = 13.27$, $P < 0.0001$) and escape trials, stress treatment, and activity condition ($F(8, 168) = 2.54$, $P = 0.01$). Newman–Keuls analysis revealed that both sedentary rats and 3-week runners exposed to uncontrollable stress displayed significantly longer FR-2 escape latencies compared to rats in all other groups during all 5 blocks of FR-2 trials with the exception of the first block of FR-2 trails during which 3-week runners exposed to stress displayed escape latencies similar to rats in other groups. Escape latencies of 3-week and 6-week runners not exposed to stress were not statistically different from those of sedentary non-stressed rats. At no point during escape testing did the escape latencies of 6-week runners exposed to uncontrollable stress differ from those of animals not exposed to stress.

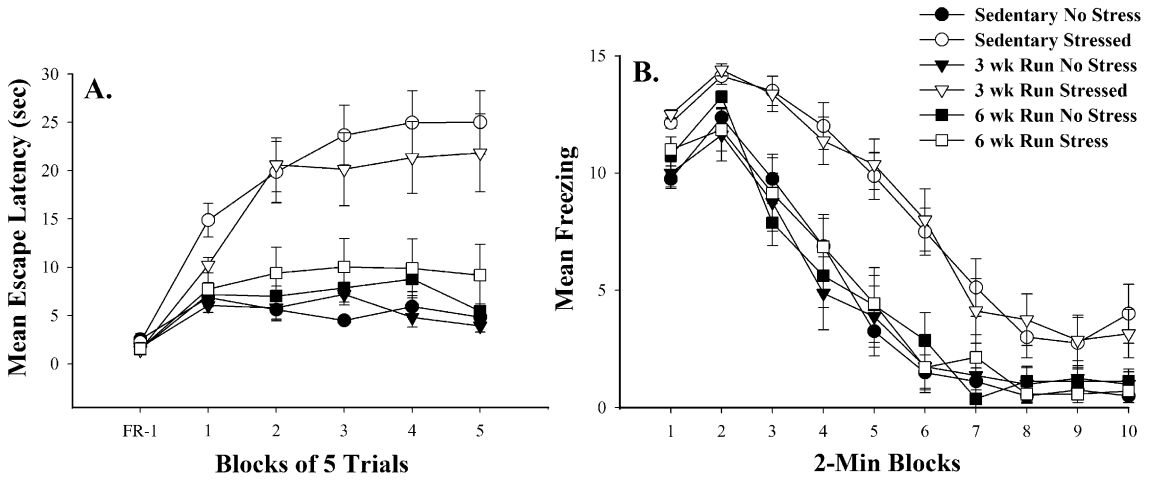

Fig. 2. Sedentary rats and rats allowed voluntary access to running wheels for either three (3-weeks Run) or six (6-weeks Run) weeks were exposed to 100 uncontrollable tail shocks (Stressed) or remained in their home cages (No Stress). Shuttle box escape latencies and freezing behavior were measured sequentially 24 h later. (A) Mean shuttle box escape latencies for one block of five FR-1 trials (FR-1) and five blocks of five FR-2 trials (1–5). (B) The mean number of 8 s observation periods during which freezing occurred across blocks of 2 min after two shocks in the shuttle box. Values represent group means ± SEM.

A similar pattern was present with regard to conditioned fear (Fig. 2B). 6 weeks, but not 3 weeks, of prior running wheel access significantly blocked the effect of uncontrollable stress on freezing behavior. By itself, physical activity had no effect on fear conditioning. ANOVA revealed reliable main effects of stress treatment ($F(2, 41) = 4.5$, $P = 0.02$) and activity ($F(1, 41) = 25.78$, $P < 0.0001$) and a significant interaction between stress and activity conditions ($F(2, 41) = 6.05$, $P = 0.005$) on time spent freezing in the shuttle box. There was also a reliable interaction between 2 min blocks of freezing and activity ($F(9, 369) = 4.65$, $P < 0.0001$) but not between 2 min blocks of freezing and stress treatment, or 2 min blocks of freezing, stress treatment, and activity. Newman–Keuls analysis revealed that sedentary rats and 3-week runners exposed to uncontrollable stress spent an equal amount of time freezing and froze more than rats in all other groups during freezing trials. Freezing scores in all other groups were not statistically different from each other.

No reliable correlations within running groups were revealed by simple regression analysis between the total or average distance run and FR-2 escape latencies, nor between the total or average distance run and average freezing time.

3.3. c-Fos expression in the DRN

Prior work indicates that LH behaviors are dependent upon hyperactivity of DRN 5-HT neurons during exposure to uncontrollable stress. 6 weeks of wheel running prevents LH and attenuates uncontrollable stress-induced activity of DRN 5-HT neurons [42]. To determine if 3 weeks of wheel running is sufficient to reduce c-Fos expression in DRN 5-HT neurons during uncontrollable stress, stress-induced activity of 5-HT cells of the DRN was compared between sedentary rats and rats allowed access to running wheels for 3 and 6 weeks using double immunohistochemistry for the immediate early gene product, c-Fos, and 5-HT.

Table 1 shows the mean number of single 5-HT-positive cells and single (5-HT-negative) c-Fos-positive nuclei in each area of the DRN examined in sedentary, 3-week run, and 6-week run rats. As expected, neither stress nor activity treatment affected the number of 5-HT-positive cells anywhere in the DRN. Exposure to stress did, however, significantly increase the number of single c-Fos-positive particles in every region of the DRN examined, and 3 weeks, but not 6 weeks, of wheel running attenuated stress-induced c-Fos in non-5HT cells of the ventral aspect of the mid DRN. Neither 3 weeks nor 6 weeks of wheel running altered single c-Fos immunoreactivity in any other DRN region.

Fig. 3 contains photomicrographs showing double c-Fos/5-HT IR in the DRN. The mean number of double c-Fos/5-HT positive cells in the dorsal aspect of the DRN is shown in Fig. 4A. ANOVA revealed a reliable main effect of group in the rostral ($F(4, 26) = 12.22$, $P < 0.0001$), mid ($F(41, 25) = 45.78$, $P < 0.0001$), and caudal ($F(4, 24) = 29.06$, $P < 0.0001$) aspects of the dorsal DRN. Post hoc analysis revealed that exposure to uncontrollable stress elevated c-Fos above controls throughout the rostrocaudal extent of the dorsal DRN in all stressed groups ($P < 0.01$ in all comparisons). 3 weeks of wheel running resulted in a trend towards a reduction in the number of stress-induced double-labeled cells in the rostral ($P = 0.08$) and mid ($P = 0.10$) aspects of the dorsal DRN compared to sedentary stressed rats, however, consistent with our previous report [42], 6 weeks of wheel running was sufficient to cause a significant attenuation of stress-induced c-Fos in 5-HT cells of the rostral ($P = 0.02$) and mid ($P = 0.02$) dorsal DRN. Wheel running, in the absence of stress, had no effect on the number of double labeled cells in any region of the dorsal DRN examined.

Table 1
Mean and standard error of the mean (SEM) of 5-HT-positive cells and single c-Fos-positive nuclei in different regions of the DRN of sedentary rats and rats allowed either 3 or 6 weeks of voluntary access to running wheels following exposure to uncontrollable stress or control treatment

	Brain region	Sedentary control (n = 6)		6-week Run control (n = 6)		Sedentary stressed (n = 6)		3-week Run stressed (n = 7)		6-week Run stressed (n = 6)	
No. of single 5-HT positive cells	Dorsal DRN										
	Rostral	78.9	9.25	72.0	4.0	81.2	11.06	92.7	5.0	66.83	6.63
	Mid	101.5	8.55	90.83	7.67	94.38	3.74	102.6	3.9	96.37	10.9
	Caudal	71.0	5.0	69.7	5.48	61.17	4.04	63.64	5.67	63.4	4.36
	Ventral DRN										
	Rostral	61.4	4.3	65.5	5.43	53.75	11.7	62.9	5.34	58.83	8.08
	Mid	83.1	7.19	77.17	6.2	98.75	3.8	94.17	4.0	98.4	9.62
	Caudal	57.2	2.03	52.17	2.45	44.33	6.5	54.28	3.8	49.0	2.76
	Lateral DRN										
	Rostral	16.7	1.4	19.0	2.9	23.0	4.6	23.8	2.21	17.83	4.57
	Mid	112.9	5.82	106.33	8.32	104.62	1.31	134.2	12.32	94.75	18.5
No. of single c-Fos positive cells	Dorsal DRN										
	Rostral†	8.92	5.4	0.67	0.42	70.8***	7.9	67.8***	5.28	83.5***	7.9
	Mid†	6.14	4.06	4.33	2.64	44.1***	4.5	32.7***	3.28	42.7***	5.44
	Caudal †	1.6	0.7	1.7	0.54	28.4***	5.4	18.8**	2.6	29.5***	7.17
	Ventral DRN										
	Rostral †	4.17	2.5	1.0	0.52	24.6**	5.73	20.57**	3.76	22.92***	3.2
	Mid†	0.92	0.6	0.67	0.5	25.4***	4.23	12.71**‡§	1.97	24.7***	4.02
	Caudal†	0.8	0.58	1.5	0.8	13.5**	3.0	8.0**	1.63	15.3***	2.73
	Lateral DRN										
	Rostral†	24.17	15.7	0.67	0.7	150.0***	17.1	128.1***	14.03	142.0***	8.8
	Mid†	7.6	4.22	1.33	0.9	129.6***	8.56	102.2***	11.2	137.4***	13.5

† One-way ANOVA: $P < 0.001$.
‡ Fisher's protected least significant difference: $P < 0.05$ vs. sedentary stressed.
** Fisher's protected least significant difference: $P < 0.001$ vs. control.
*** Fisher's protected least significant difference: $P < 0.0001$ vs. control.
§ Fisher's protected least significant difference: $P < 0.05$ vs. 6-week run stressed.

Fig. 4B shows the mean number of double c-Fos/5-HT positive cells in the ventral aspect of the DRN. ANOVA revealed a reliable main effect of group in the rostral ($F(4, 26) = 12.53$, $P < 0.0001$), mid ($F(4, 25) = 23.91$, $P < 0.0001$), and caudal ($F(4, 24) = 8.71$, $P = 0.0002$) aspects of the ventral DRN. Post hoc comparisons revealed that, like the dorsal DRN, tail shock exposure increased the number of double labeled cells throughout the rostrocaudal extent of the ventral DRN ($P < 0.01$ in all comparisons). 6 weeks, but not 3 weeks, of wheel running reduced the effect of stress in the mid ($P = 0.03$), but not the rostral or caudal, ventral DRN. Again, wheel running, in the absence of stress, did not alter the number of double labeled cells in any region of the ventral DRN examined.

The mean values of double c-Fos/5-HT-positive cells in the lateral aspect of the rostral and mid DRN are depicted in Fig. 4C. Levels of c-Fos in the caudal aspect of the lateral DRN are not given because lateral wings are not present in the DRN at the caudal level of analysis. ANOVA revealed a reliable main effect of group in the

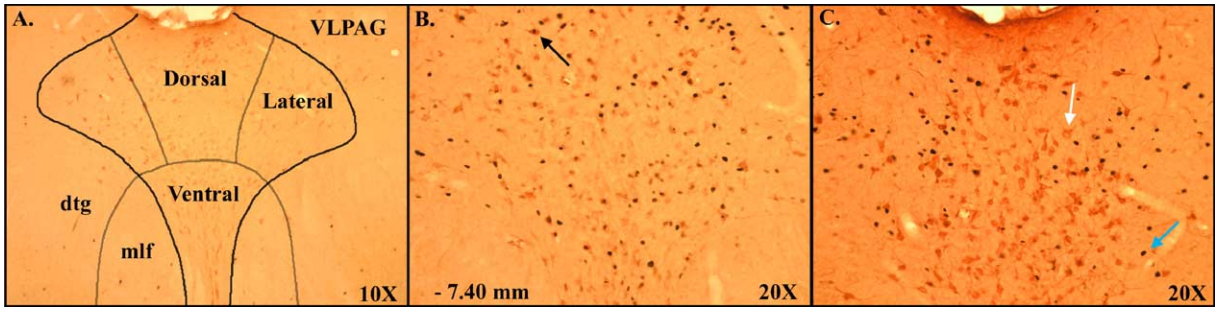

Fig. 3. Representative photomicrographs showing c-Fos (black) and serotonin (red) double immunolabeling in coronal sections through the rostral dorsal raphe nucleus. (A) Sedentary control. (B) Dorsal aspect of the dorsal raphe nucleus of a sedentary rat exposed to uncontrollable tail shock. (C) Dorsal aspect of the dorsal raphe nucleus of a 6-week runner exposed to uncontrollable tail shock. Black arrow points at a double-labeled cell. Blue arrow points at a single c-Fos-labeled cell. White arrow points at a single serotonin labeled cell. *VLPAG*, ventral lateral periaqueductal grey; *dtg*, dorsal tegmental bundle; *mlf*, medial longitudinal fasciculus; *dorsal*, dorsal aspect of the dorsal raphe nucleus; *lateral*, lateral aspect of the dorsal raphe nucleus; *ventral*, ventral aspect of the dorsal raphe nucleus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

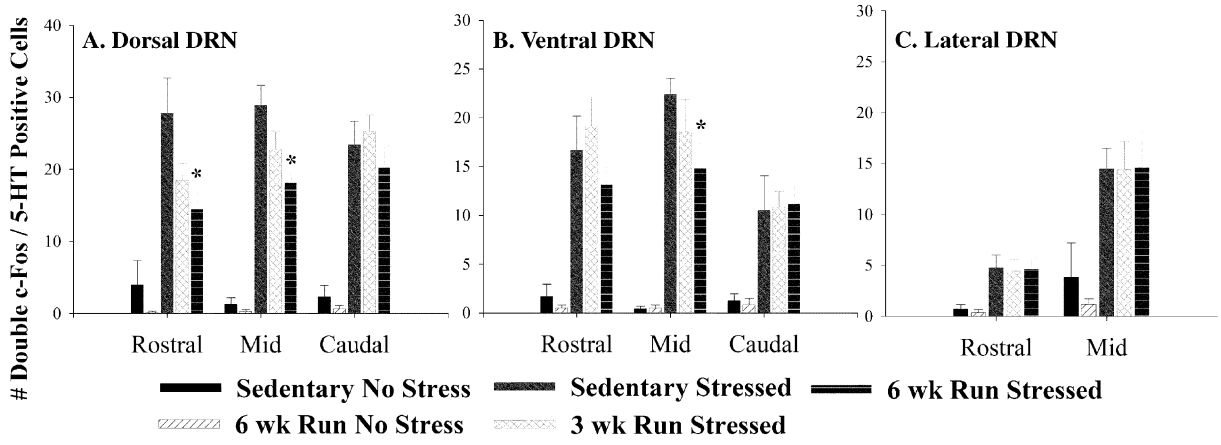

Fig. 4. Uncontrollable stress-induced activation of serotonergic (5-HT) neurons in the dorsal (A), ventral (B), and lateral (C) aspects of the rat dorsal raphe nucleus (DRN) is represented by the number of double c-Fos/5-HT-labeled cells. Sedentary rats and rats allowed either three (3-week Run) or six (6-week Run) weeks of voluntary access to running wheels were exposed to uncontrollable shock (Stressed) or remained in their home cages (No Stress). Ninety minutes following the final tail shock, brains were removed and processed for c-Fos and 5-HT immunoreactivity. Values represent mean number of double labeled cells ± SEM. Fisher's-PLSD: *$P < 0.05$ with respect to sedentary stressed group.

rostral ($F(4, 23) = 6.24$, $P = 0.001$) and mid ($F(4, 24) = 5.48$, $P = 0.003$) aspects of the lateral DRN. As in other DRN regions, exposure to stress elevated the number of double positive cells in the rostral and mid aspects of the lateral DRN ($P < 0.05$ in all comparisons). Physical activity status did not affect the number of c-Fos/5-HT-positive neurons in the lateral DRN.

No significant correlations within running groups were found between either total distance run or average weekly distance run and the number of double c-Fos/5-HT-labeled cells in any sub-region of the DRN.

3.4. c-Fos expression in the BNST

The BNST is a potential source of CRH or EAAs to the DRN and thus could contribute to hyperactivation of DRN 5-HT neurons during uncontrollable stress. Indeed, activation of the BNST elicits behaviors resembling stress [13] and lesions of the BNST prevent the expression of LH behaviors produced by prior uncontrollable shocks [47]. These data suggest attenuation of stress-induced BNST cellular activity may contribute to the attenuation of DRN 5-HT neural responses and the prevention of LH by wheel running. To examine this hypothesis, uncontrollable stress-induced c-Fos IR in the BNST was compared between sedentary and physically active rats, following either 3 weeks or 6 weeks of wheel running.

Fig. 5 is a photomicrograph showing c-Fos IR in the BNSTld and BNSTlv of a sedentary rat exposed to uncontrollable tail shock. The effects of stress and activity status on mean c-Fos expression in the BNSTld and BNSTlv are shown in Fig. 6. ANOVA revealed a significant effect of group on c-Fos in the BNSTld ($F(4, 23) = 73.14$, $P < 0.0001$). Exposure to stress increased c-Fos IR above

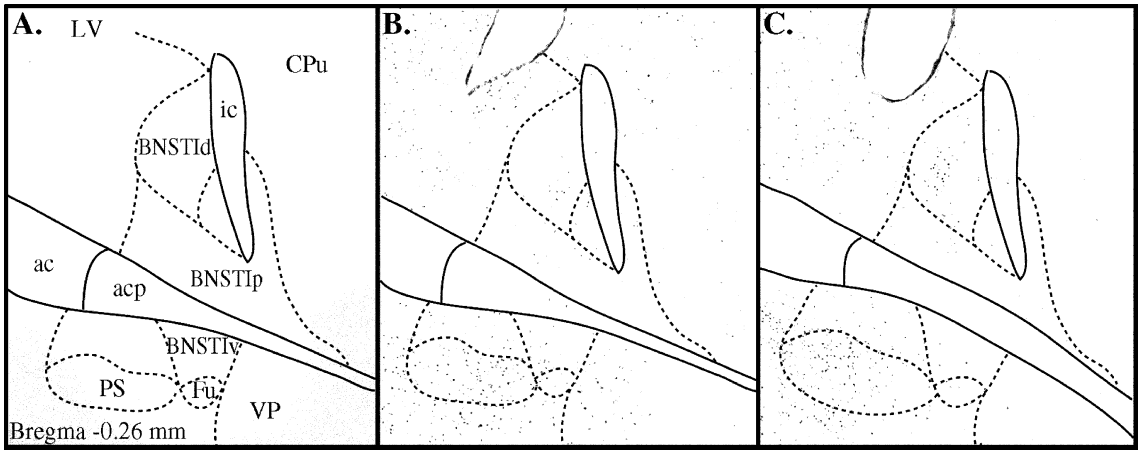

Fig. 5. Photomicrographs (8× magnification) showing c-Fos immunoreactivity in coronal sections through the region of the bed nucleus of the stria terminalis (BNST) in a sedentary control rat (A), a sedentary rat exposed to uncontrollable stress (B), and a 6-week runner exposed to uncontrollable stress (C). *LV*, lateral ventricle; *ic*, internal capsule; *ac*, anterior commissure; *BNSTld*, lateral dorsal region of the BNST; *BNSTlv*, lateral ventral region of the BNST; *Fu*, fusiform region of the BNST; *BNSTlp*, lateral posterior region of the BNST; *VP*, ventral pallidum; *PS*, parastrial nucleus; *CPu*, caudate putamen; *acp*, posterior part of the anterior commissure.

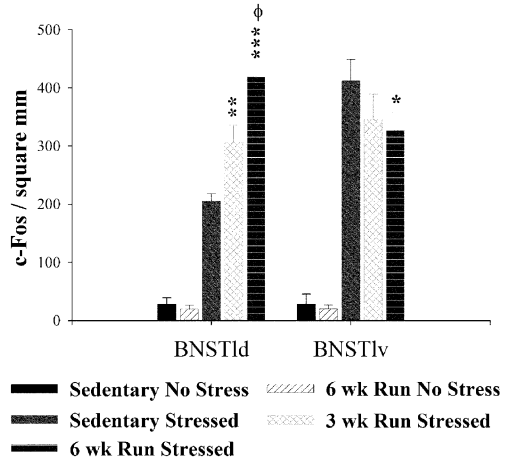

Fig. 6. Uncontrollable stress-induced activation of the lateral dorsal (BNSTld) and lateral ventral (BNSTlv) regions of the bed nucleus of the stria terminalis (BNST). Sedentary rats and rats allowed either three (3-week Run) or six (6-week Run) weeks of voluntary access to running wheels were exposed to uncontrollable shock (Stressed) or remained in their home cages (No Stress). Ninety minutes following the final tail shock, brains were removed and processed for c-Fos immunoreactivity. Values represent mean number of c-Fos-positive cells per square millimeter ± SEM. Fisher's-PLSD: *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ with respect to sedentary stressed group. ϕ$P < 0.01$ with respect to 3-week Run group.

control values in the BNSTld of all stressed groups ($P < 0.0001$ for all comparisons). Interestingly, wheel running dose-dependently potentiated stress-induced c-Fos IR in the BNSTld. Compared to sedentary stressed rats, both 3 weeks ($P = 0.005$) and 6 weeks ($P < 0.0001$) of wheel running significantly augmented the effect of tail shock on c-Fos IR in the BNSTld. The effect of stress was further increased above 3-week runners in the BNSTld of 6-week runners ($P = 0.005$).

ANOVA also revealed significant group differences in c-Fos IR in the BNSTlv ($F(4, 20) = 34.22$, $P < 0.0001$). Post hoc comparisons revealed that stressor exposure increased c-Fos IR above control values in the BNSTlv of all stressed groups ($P < 0.0001$ for all comparisons). Compared to sedentary stressed rats, the effect of stress was attenuated by 6 weeks ($P < 0.05$), but not 3 weeks ($P = 0.15$), of prior wheel access.

No significant correlations within running groups were revealed by simple regression between total or average weekly distance run and c-Fos immunoreactivity in either the BNSTld or BNSTlv.

3.5. c-Fos Expression in the AMG

Like the BNST, the AMG could contribute to the hyperactivation of DRN 5-HT neurons during uncontrollable stress. Therefore, a reduction in cellular activity of the AMG during stress may be involved in the mechanisms by which wheel running prevents LH. To investigate this hypothesis, uncontrollable stress-induced c-Fos IR in the BLA and CeA was compared between sedentary and physically active rats, following either 3 weeks or 6 weeks of wheel running.

Fig. 7 is a photomicrograph showing c-Fos IR in the BLA and CeA of a sedentary rat exposed to uncontrollable tail shock. The effects of stress and activity status on mean c-Fos expression in the BLA and CeA are shown in Fig. 8. ANOVA revealed a significant effect of group on c-Fos in the BLA ($F(4, 34) = 17.8$, $P < 0.0001$). Exposure to stress increased c-Fos IR above control values in the BLA of all stressed groups ($P < 0.0001$ for all comparisons). Wheel running, regardless of duration, had no effect on c-Fos IR in the BLA.

Similar to the BLA, ANOVA revealed significant group differences in c-Fos IR in the CeA ($F(4, 34) = 27.05$, $P < 0.0001$). Post hoc comparisons revealed that stressor exposure increased c-Fos IR above control values in the CeA of all stressed groups ($P < 0.0001$ for all comparisons). Wheel running, regardless of duration, had no effect on c-Fos IR in the CeA.

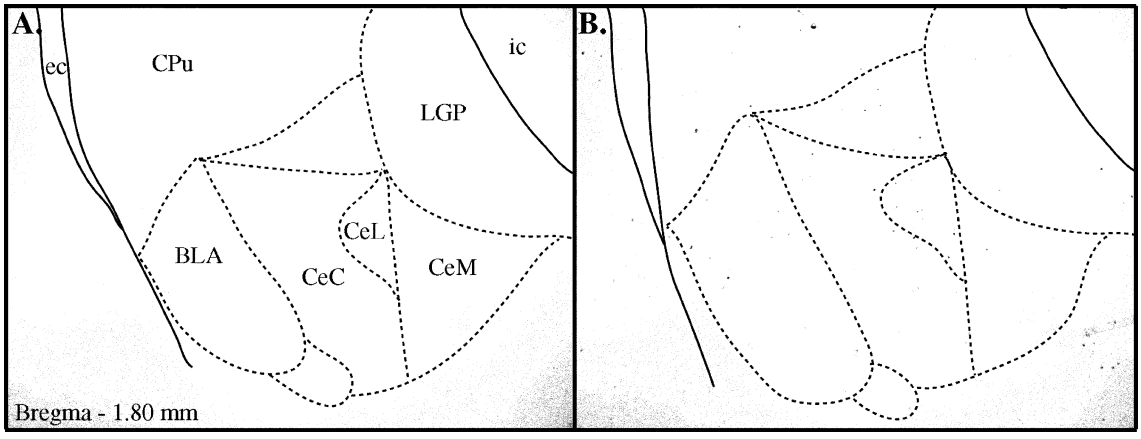

Fig. 7. Photomicrographs (8× magnification) showing c-Fos immunoreactivity in coronal sections through the region of the amygdala in a sedentary control rat (A) and a sedentary rat exposed to uncontrollable stress (B). *BLA*, basolateral amygdala; *CeC*, capsular region of central amygdala; *CeL*, lateral central amygdala, *CeM*, medial central amygdala; *CPu*, caudate putamen; *LGP*, lateral globus pallidus; *ic*, internal capsule; *ec*, external capsule. The CeC, CeL, and CeM make up the central amygdala.

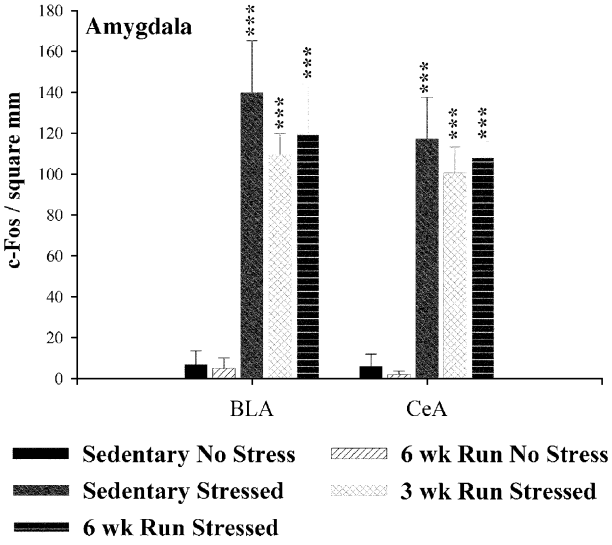

Fig. 8. Uncontrollable stress-induced activation of the basolateral (BLA) and central (CeA) nuclei of the amygdala. Sedentary rats and rats allowed either three (3-week Run) or six (6-week Run) weeks of voluntary access to running wheels were exposed to uncontrollable shock (Stressed) or remained in their home cages (No Stress). Ninety minutes following the final tail shock, brains were removed and processed for c-Fos immunoreactivity. Values represent mean number of c-Fos-positive cells per square millimeter ± SEM. Fisher's-PLSD: ***$P < 0.0001$ with respect to sedentary non-stressed group.

3.6. c-Fos Expression in the LHb

The LHb is another potential source of EAAs to the DRN and thus could contribute to hyperactivation of DRN 5-HT neurons during uncontrollable stress. Lesions of the LHb prevent the typical behavioral consequences of uncontrollable stress [4]. These data suggest that a reduction in cellular activity of the LHb during stress may contribute to the attenuation of DRN 5-HT neural responses and the prevention of LH by wheel running. To examine this hypothesis, uncontrollable stress-induced c-Fos IR in the LHb was compared between sedentary and physically active rats, following either 3 weeks or 6 weeks of wheel running.

Fig. 9 contains photomicrographs showing c-Fos IR in the LHb. The effects of stress and activity status on mean c-Fos expression in the LHb is shown in Fig. 10. ANOVA revealed a significant effect of group on c-Fos in the LHb ($F(4, 30) = 14.0$, $P < 0.0001$). Exposure to stress increased c-Fos IR above control values in the LHb of all stressed groups ($P < 0.003$ for all comparisons). Wheel running, regardless of duration, had no effect on c-Fos IR in the LHb.

4. Discussion

4.1. LH behaviors

Exposure to uncontrollable tail shock produces behaviors in sedentary rats that resemble symptoms of human depression and anxiety [60,63,86,93] and can be prevented by antidepressant and anxiolytic drugs [64,66,69,72]. Consistent with the antidepressant and anxiolytic properties of physical exercise in humans [11,28,70,83] and rodents [12,23,74,87], we have previously reported that 6 weeks of voluntary wheel running prevents LH behaviors typically induced by exposure to uncontrollable shocks in sedentary rats [42]. The current data replicate our previous findings and also indicate that, in contrast to 6 weeks, 3 weeks of wheel running has no affect on LH behaviors.

Interestingly, the behavioral consequences of uncontrollable stress appear to be more sensitive to the duration of wheel access rather than the distance run. There were no significant correlations between the total or average weekly distance run and LH behaviors, in either the 3-week or 6-week run groups. Furthermore, 3-week and 6-week runners ran equal distances the week prior to uncontrollable stress, suggesting that simply reaching the maximum average weekly running distance is not sufficient to prevent LH. Instead, some minimum duration of time, greater than 3 weeks, may be required to provide resistance against the

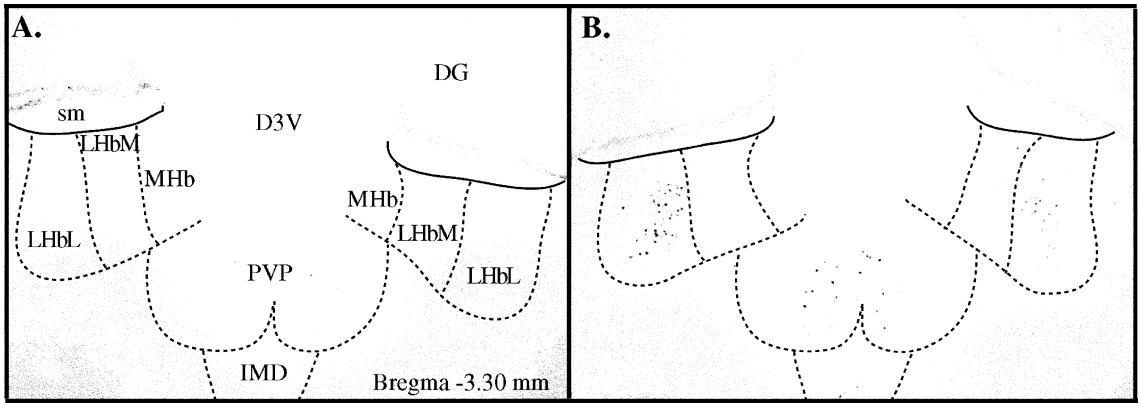

Fig. 9. Photomicrographs (10× magnification) showing c-Fos immunoreactivity in coronal sections through the region of the lateral habenula in a sedentary control rat (A) and a sedentary rat exposed to uncontrollable tail shock stress (B). *MHb*, medial habenula; *LHbM*, lateral habenula medial portion; *LHbL*, lateral habenula lateral portion; *D3V*; dorsal third ventricle; *PVP*, posterior paraventricular thalamic nucleus; *sm*, stria medullaris of thalamus; *DG*, dentate gyrus; *IMD*, intermediodorsal thalamic nucleus.

behavioral consequences of uncontrollable stress. Similar duration-dependent effects of wheel running have recently been reported by Burghardt et al. [12] who observed that 8 weeks, but not 4 weeks, of voluntary wheel running increased defensive responding in the elevated plus maze. These differences were observed despite the fact that 8-week and 4-week runners ran equal distances in the 24-h period prior to behavioral testing. In addition, there were no correlations between running distance and behavioral outcomes [12]. Together, these data suggest that the behavioral adaptations that occur with wheel running are dependent upon the duration of time that the animals are allowed access to the wheels.

4.2. Role of the DRN

Consistent with our prior work [42], 6 weeks of wheel running attenuated uncontrollable stress-induced c-Fos expression in 5-HT IR neurons of the DRN, indicative of decreased DRN 5-HT neuronal firing during uncontrollable stress. Although resulting in a trend for an attenuation of stress-induced c-Fos in 5-HT-positive cells of the dorsal DRN, 3 weeks of wheel running did not significantly affect c-Fos expression in 5-HT neurons of the DRN. The observations that attenuation of uncontrollable stress-induced activity of DRN 5-HT neurons only occurs following a duration of wheel running sufficient to prevent LH (6 weeks), but does not occur following a duration of wheel running insufficient to prevent LH (3 weeks), support our hypothesis that the time-dependent effects of wheel running on attenuation of stress-induced c-Fos expression in 5-HT neurons of the DRN corresponds to the time-dependent effects of wheel running on LH behaviors. These data are also consistent with the hypothesis that constraining DRN 5-HT neural activity during uncontrollable stress contributes to the prevention of LH by wheel running. Previous reports that activation of 5-HT neurons in the DRN is both necessary [66,68] and sufficient [67] to produce LH behaviors also support this idea.

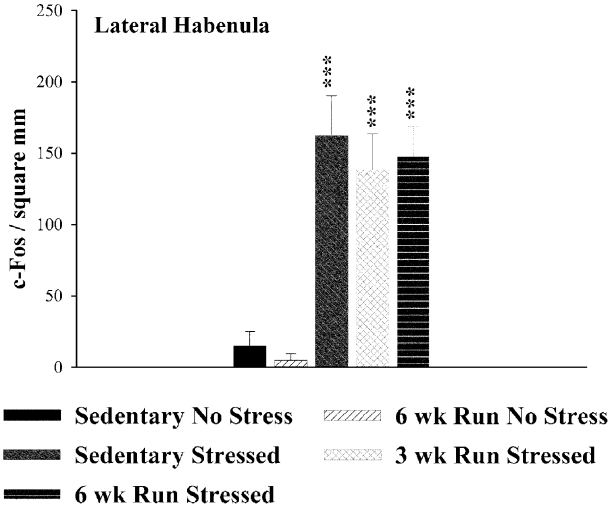

Fig. 10. Uncontrollable stress-induced activation of the lateral habenula (LHb). Sedentary rats and rats allowed either three (3-week Run) or six (6-week Run) weeks of voluntary access to running wheels were exposed to uncontrollable shock (Stressed) or remained in their home cages (No Stress). Ninety minutes following the final tail shock, brains were removed and processed for c-Fos immunoreactivity. Values represent mean number of c-Fos-positive cells per square millimeter ± SEM. Fisher's-PLSD: ***$P < 0.0001$ with respect to sedentary non-stressed group.

Although uncontrollable stress increases c-Fos IR in all DRN sub-regions, 6 weeks of wheel running only affects c-Fos expression in a unique population of DRN 5-HT neurons. The regions affected by wheel running in the present study are similar to those reported earlier [42] and include the dorsal and ventral aspects of the rostral and mid DRN, although the greatest effect of 6 weeks of wheel running on 5-HT neural activity occurs in the dorsal portion. Interestingly, controllable shocks do not induce c-Fos in 5-HT neurons in the mid to caudal DRN to the same extent as do equal amounts of yoked uncontrollable shocks, whereas controllable and uncontrollable tail shocks induce c-Fos in the rostral DRN equally [39]. These anatomical differences suggest that wheel running does not prevent LH by simply changing the DRN response to uncontrollable stress to one resembling controllable stress. Instead, wheel running produces a unique change in the DRN response distinguishable from that produced by controllable stress.

The regional specificity of the effect of wheel running on c-Fos expression in DRN 5-HT neurons is intriguing because the DRN is a heterogeneous structure containing many sub-populations of neurons with unique projections, neurochemical phenotype, and receptor expression [16,21,35,51,58,88]. Consideration of the specific sub-regions of the DRN affected by wheel running may yield insight into other factors that could contribute to the altered 5-HT responses to uncontrollable stress in the DRN of physically active rats. For example, prior work indicates a critical role for CRH in mediating the behavioral consequences of uncontrollable stress [45,46]. Although reduced CRH signaling in the DRN could contribute to the attenuation of 5-HT responses and the prevention of LH by wheel running, a careful analysis of the specific DRN sub-regions affected by wheel running yields a conflicting interpretation. Recall that wheel running only affected c-Fos expression in the rostral–mid DRN. However, CRH has excitatory influences on 5-HT neurons located in the caudal DRN only [59] and CRH-R2 antagonists only prevent the development of LH when injected into the caudal DRN [46]. CRH antagonists have no effect on LH behaviors when injected into the rostral DRN [46]. These data do not rule out a role for CRH in controllability effects; however, they are not consistent with a role for DRN CRH in mediating the effects of wheel running observed in the current study.

4.3. DRN afferents

The BNST, AMG, and LHb have been implicated in LH and contain putative CRH or EAA projections to the DRN

[1,17,24,52,57,73,82]. Contrary to our original hypothesis, uncontrollable shocks activate the AMG and LHb regardless of history of prior wheel running. Therefore, it is unlikely that a reduction in activity of projections from these structures to the DRN contributes to the attenuation of c-Fos induction in DRN 5-HT neurons. Interestingly, however, glutamatergic projections from the BNSTlv to the DRN seem to target precisely the regions of the DRN affected by wheel running, that is, the dorsal and ventral, mid DRN, but not the caudal DRN or lateral wings [57]. Although only speculative, these data are consistent with the possibility that a reduction in BNSTlv-derived EAA release in the rostral–mid DRN during uncontrollable stress may contribute to the attenuation of stress-induced c-Fos in DRN 5-HT neurons and the prevention of LH by wheel running. That 6 weeks of running was required to attenuate c-Fos in the BNSTlv supports this hypothesis.

Compared to sedentary rats, stress-induced activity of the BNSTld was augmented by both 3 weeks and 6 weeks of wheel running; although 6 weeks of wheel running had the greatest potentiating effect. Although it is not yet clear how these changes could affect the DRN and LH behaviors, increased BNSTld activity during stress in physically active rats suggests that the BNSTld could be involved in stress inhibition. In particular, increased cellular activity in the BNSTld could contribute to the attenuation of stress-induced c-Fos expression in the BNSTlv of physically active rats. Consistent with this possibility are high concentrations of γ-aminobutyric acid (GABA)-containing neurons in the BNSTld [20], dense projections from the BNSTld to the BNSTlv [24], and the presence of $GABA_A$ receptors on BNSTlv neurons [48].

Finally, it is important to consider the possibility that some of the behavioral and/or c-Fos differences observed between sedentary and physically active rats may be due to differences in pain sensitivity to the shock procedure. Although wheel running can modify nociceptive responses [54], it is unlikely that the observed group differences in stress-induced c-Fos induction are due to differences in pain sensitivity. If altered pain sensitivity reduced the stress of the shock paradigm, then we would expect that other markers of stress would be different in physically active rats. However, physically active and sedentary rats have equal corticosterone responses during exposure to uncontrollable tail shock [33] and the c-Fos response to uncontrollable stress is not globally attenuated in all stress reactive brain regions, as documented in the current manuscript and in our prior work [43]. Moreover, if physically active rats had altered pain sensitivity to shock, we would expect that, following 2 foot shocks in the shuttle box, physically active rats not exposed to uncontrollable stress 24 h earlier would demonstrate less conditioned fear compared to their sedentary, non-stressed, counterparts. However, wheel running only prevented the exaggeration of conditioned fear produced in sedentary rats by prior uncontrollable stress and did not affect "normal" conditioned fear, as demonstrated by the similar freezing scores of the non-stressed rats in Fig. 2B. Therefore, behavioral and c-Fos changes observed in the current study are not likely simply due to altered pain sensitivity.

4.4. Summary

The data presented here indicate that the behavioral and neurochemical consequences of uncontrollable stress are sensitive to the duration of prior wheel running. Therefore, when studying the effects of wheel running on behavior or stress responses, the duration that the animals are allowed to run may be a critical factor. Results are consistent with the hypothesis that attenuation of 5-HT neural activity in the DRN contributes to the prevention of LH by wheel running. Finally, data suggest a role for the BNST, but not the AMG or the LHb, in the mechanisms by which wheel running prevents LH. The current observations could help further elucidate potential mechanisms underlying the antidepressant and anxiolytic effects of exercise.

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