Behavioural Brain Research 272 (2014) 252–263

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Wheel running alters patterns of uncontrollable stress-induced cfos mRNA expression in rat dorsal striatum direct and indirect pathways: A possible role for plasticity in adenosine receptors

Peter J. Clark[a,*], Parsa R. Ghasem[a], Agnieszka Mika[a], Heidi E. Day[a,b], Jonathan J. Herrera[a], Benjamin N. Greenwood[a], Monika Fleshner[a]

[a] Integrative Physiology, University of Colorado Boulder, 354 UCB, Boulder, CO 80309, United States
[b] Department of Psychology & Neuroscience, University of Colorado Boulder, Muenzinger D244, 345 UCB, Boulder, CO 80309, United States

HIGHLIGHTS

- Long-term wheel running and acute uncontrollable stress decrease adenosine 1 and 2A receptor mRNA levels in the dorsal and ventral striatum.
- Wheel running modestly increases dopamine 2 receptor mRNA levels in the dorsal and ventral striatum.
- Long-term running potentiates stress-induced cfos in direct pathway and attenuates stress-induced cfos in indirect pathway neurons of the dorsal striatum.

ARTICLE INFO

Article history:
Received 24 February 2014
Received in revised form 22 May 2014
Accepted 3 July 2014
Available online 11 July 2014

Keywords:
Exercise
Striatum
Adenosine
Dopamine
Stress
Running

ABSTRACT

Emerging evidence indicates that adenosine is a major regulator of striatum activity, in part, through the antagonistic modulation of dopaminergic function. Exercise can influence adenosine and dopamine activity, which may subsequently promote plasticity in striatum adenosine and dopamine systems. Such changes could alter activity of medium spiny neurons and impact striatum function. The purpose of this study was twofold. The first was to characterize the effect of long-term wheel running on adenosine 1 (A_1R), adenosine 2A ($A_{2A}R$), dopamine 1 (D_1R), and dopamine 2 (D_2R) receptor mRNA expression in adult rat dorsal and ventral striatum structures using *in situ* hybridization. The second was to determine if changes to adenosine and dopamine receptor mRNA from running are associated with altered cfos mRNA induction in dynorphin- (direct pathway) and enkephalin- (indirect pathway) expressing neurons of the dorsal striatum following stress exposure. We report that chronic running, as well as acute uncontrollable stress, reduced A_1R and $A_{2A}R$ mRNA levels in the dorsal and ventral striatum. Running also modestly elevated D_2R mRNA levels in striatum regions. Finally, stress-induced cfos was potentiated in dynorphin and attenuated in enkephalin expressing neurons of running rats. These data suggest striatum adenosine and dopamine systems are targets for neuroplasticity from exercise, which may contribute to changes in direct and indirect pathway activity. These findings may have implications for striatum mediated motor and cognitive processes, as well as exercise facilitated stress-resistance.

1. Introduction

The striatum is a basal ganglia structure critically involved in a wide range of motor, learning, and motivational processes. Abnormalities in the striatum have been suggested to contribute to a diverse array of motor and mental health pathologies including Huntington's disease, Parkinson's disease, schizophrenia, drug abuse, and depression (for review see [1–5]). A growing literature suggests that exercise promotes neuroplasticity in the striatum, which may have therapeutic benefits for mental and physical health [6–11]. Moreover, recent findings indicate rodents that engage in running are protected against uncontrollable stress-induced enhancement of drug preference and cognitive deficits that involve the striatum [12,13]. Therefore, exercise may promote neuroplasticity in the striatum that protects against the deleterious effects of

* Corresponding author at: National Institute on Aging, 251 Bayview Boulevard, Baltimore, MD 21224, United States. Tel.: +1 410 558 8393; fax: +1 410 558 8323.
E-mail address: peter.clark@nih.gov (P.J. Clark).

http://dx.doi.org/10.1016/j.bbr.2014.07.006
0166-4328/

stress. However, few studies have characterized exercise-induced plasticity in the striatum that may contribute to stress-resistance [7,11,14].

Emerging evidence indicates that the endogenous purine nucleoside adenosine is a major regulator of striatum activity under periods of increased metabolic demand, in part through its potent modulation of dopamine signaling (for review see [15,16]). Presynaptic adenosine A_1 and A_{2A} receptors located on terminals of glutamate, dopamine, and cholinergic neurons in the striatum can fine tune neurotransmission at synapses of medium spiny neurons (for further review see [16]). Additionally, post-synaptic adenosine A_1R and $A_{2A}R$ in the striatum are located in abundance on distinct networks of medium spiny neurons [17,18], where they antagonistically modulate the binding and function of dopamine D_1 and D_2 receptors respectively [17–21]. Dopamine transmission in the striatum is critically involved in many cognitive and motor processes including instrumental learning, working memory, reward, motivation, and movement (as reviewed in [22,23]). Therefore, neuroplasticity in adenosine receptor systems in the striatum may have profound effects on cognitive and motor processes that require normal dopamine signaling, and may be a target for the stress protective properties of exercise.

Prolonged or acute exposure to A_1R and $A_{2A}R$ agonists is associated with increases or decreases of receptor mRNA in both cell culture and *in vivo* models [24–29]. Therefore, treatments that increase adenosine, such as exercise, may result in changes to A_1R and $A_{2A}R$ receptor mRNA in the striatum. Acute bouts of running increase brain-wide adenosine concentrations [30] and metabolic activity in the striatum [31,32]. Repeated increases of adenosine that may follow several bouts of wheel running could thus be sufficient to change adenosine receptor mRNA levels in the striatum. Additionally, evidence suggests that a particularly augmented adenosine activity in the striatum may contribute to the development of depression-like behaviors in rats following uncontrollable stress [33–37]. Indeed, brain adenosine receptor activation or blockade can, respectively, mimic or prevent depression-like deficits in shuttle box escape following exposure to uncontrollable tail shock [36,37] that are thought to involve the striatum [13] and are prevented by exercise [38,39]. Therefore, wheel running may alter adenosine receptor mRNA in a manner consistent with protection against the development of depression-like behavior. Changes to A_1R and $A_{2A}R$ mRNA levels are commonly associated with similar changes in radioligand binding affinity or protein concentration in the brain [40–56]. Thus, altered A_1R and $A_{2A}R$ mRNA levels may have importance for adenosine receptor expression, which may ultimately influence dopamine-mediated medium spiny neuron activity and striatum function. However, to the best of our knowledge, the impact of long-term wheel running on A_1R and $A_{2A}R$ mRNA levels in the striatum remains unknown.

The first aim of this study was to characterize the effects of long-term wheel running on A_1R, $A_{2A}R$, D_1R, and D_2R mRNA expression in the rat dorsal medial (DMS) and lateral striatum (DLS), as well as structures in the ventral striatum including the core (AcbC), lateral shell (AcbShL), and medial shell (AcbShM) of the nucleus accumbens. We examined dorsal and ventral structures, because the striatum has been traditionally separated into functionally distinct components. The dorsal striatum has been generally implicated in control of movement and motor learning processes, whereas the ventral striatum has been thought to be involved in motivation and reinforcement, although considerable functional overlap has been reported between striatal divisions (for review see [57]). Moreover, structures within the dorsal and ventral striatum have been further subdivided by function. The DMS has been associated with goal directed motor behavior [58,59], whereas the DLS has been implicated in habit learning [60]. Furthermore, evidence suggests ventral striatum subdivisions including AcbC, AcbShL, and AcbShM may serve distinct roles in processing reward [61–65]. Therefore, running-induced regional changes to A_1R, $A_{2A}R$, D_1R, and D_2R gene expression in the striatum may have significance for different striatum-mediated processes.

The second aim of this study was to determine if potential changes to A_1R, $A_{2A}R$, D_1R, or D_2R gene expression following wheel running are associated with differences in cfos (neural activity marker) induction from uncontrollable tail shock in two distinct classes of dorsal striatum medium spiny neurons. The first class, striatonigral "direct pathway" neurons, almost exclusively expresses the opioid dynorphin and post-synaptic D_1R [66]. The second class, striatopallidal "indirect pathway" neurons, nearly exclusively expresses the opioid enkephalin, as well as post-synaptic $A_{2A}R$ and D_2R [67]. Through antagonistic A_1R–D_1R and $A_{2A}R$–D_2R interactions, adenosine can reduce post-synaptic D_1R-mediated activation of direct pathway and D_2R-mediated inactivation of indirect pathway neurons in the dorsal striatum [17–21]. Resulting changes to dorsal striatum A_1R, $A_{2A}R$, D_1R, or D_2R mRNA following running may thereby be related to differential stimulation of dynorphin-expressing direct and enkephalin-expressing indirect pathway neurons during exposure to a stressor that has been suggested to increase adenosine and dopamine activity [35,68]. Direct and indirect pathways have been suggested to serve opposing roles for processing reward [69], with increased direct pathway activity promoting rewarding and indirect pathway activity signaling aversive experiences [70]. Therefore, potential changes to net activity in direct or indirect pathway neurons during tail shock exposure in running rats could play a role in signaling the degree of reward or aversion experienced during a stressful event, and thus have important implications for stress buffering properties of exercise [69,71].

We report that 6 weeks of wheel running reduced A_1R and $A_{2A}R$ mRNAs in the rat dorsal and ventral striatum. Wheel running had no impact on D_1R mRNA levels, but modestly increased D_2R mRNA striatum sub-regions. Finally, cfos mRNA induction was potentiated in direct pathway and attenuated in indirect pathway neurons of running rats following tail shock. These data are consistent with a reduced efficacy of adenosine-mediated inhibition of dopamine signaling which could be related to observed reductions of A_1R and $A_{2A}R$ mRNA in medium spiny neurons of exercising animals. Taken together, these data suggest the striatum adenosine system may be a target for neuroplasticity from exercise. Therefore, these findings may have implications for striatum-mediated motor and cognitive processes, as well as exercise facilitated stress-resistance.

2. Materials and methods

2.1. Subjects and husbandry

Upon arrival, 32 male Fischer344 rats (obtained from Harlan SPF, Indianapolis, IN, USA) weighing approximately 148 g were individually housed in standard laboratory cages (45 cm × 25.2 cm × 14.7 cm) ($n = 16$) or cages with locked running wheels (45 cm × 25.2 cm × 14.7 cm) ($n = 16$). Although stress induced by single housing could potentially impact our dependent measures, rats were single housed in order to accurately monitor individual running distance. Moreover, evidence suggests corticosterone is equivalently low in single-housed sedentary and running rats (see Section 3.2 and [72]), and single housing during adulthood does not exacerbate the development of the depression- and anxiety-like behavioral consequences of uncontrollable tail shock [73]. After 1 week, running wheels were unlocked and rats received free access to wheels for the 6 week remainder of the study. Daily wheel revolutions were recorded digitally using Vital View software (Mini Mitter, Bend, OR, USA). Rooms were controlled for

temperature (21 ± 1 °C) and photo-period (12:12 L:D) for the entire study. Food (Harlan Teklad 7012) and water were provided *ad libitum*. All procedures were approved by the University of Colorado Institutional Animal Care and Use Committee and adhered to NIH guidelines. Special care was taken to minimize animal discomfort during all procedures.

2.2. Uncontrollable tail shock

After 6 weeks of running or sedentary conditions, rats were randomly assigned to either receive uncontrollable tail shocks ($n = 8$ Sedentary Stress, $n = 8$ Running Stress) or no stress ($n = 8$ Sedentary Control; $n = 8$ Running Control). Approximately 2–5 h into the light cycle, rats that received tail shocks were restrained in a Plexiglas tube with the tail protruding from the back where electrodes were placed to deliver 100, 5-s tail shocks on a variable 1 min inter-shock interval. Rats received 1.0 mA tail shocks for 50 min. Shock intensity was then increased to 1.5 mA for the remainder of the session. Uncontrollable tail shock is a well characterized stressor that produces depression- and anxiety-like behaviors after a single episode [74]. Moreover, evidence indicates depression-like behaviors following uncontrollable tail shock involves a *particularly* augmented adenosine signaling in the brain [36,37,75], and can be prevented by 6 weeks of running [38,39]. Control rats that did not receive stress were left undisturbed in home cages.

2.3. Tissue preparation

All rats were euthanized immediately after the termination of the final tail shock (between 4 and 7 h into the light cycle) in pairs (one rat from the Stress and Control groups, alternating exercise condition) by rapid decapitation. Trunk blood was collected in EDTA tubes (Greiner Vacuette, Monroe, NC) and spun for 15 min at 3000 × *g* in a refrigerated centrifuge (4 °C). Plasma supernatant was removed and stored at −80 °C until processing for corticosterone ELISA. Brains were rapidly extracted, frozen in chilled isopentene (−20 °C) for 4 min, and stored at −80 °C until sectioning. Brains were sliced through the rostral to caudal extent of the striatum at 10 μm coronal sections using a cryostat. Slices were thaw-mounted directly on to Superfrost Plus slides (Fisherbrand, Pittsburg, PA) and stored at −80 °C until processing for radioactive or dual label fluorescent *in situ* hybridization.

2.4. Corticosterone ELISA

Plasma corticosterone was measured using a commercially available ELISA (Enzo Life Sciences, Farmingdale, NY) in accordance with the provided instructions.

2.5. Radioactive in situ hybridization

Radioactive *in situ* hybridization was used to detect relative group differences in striatal A_1R, $A_{2A}R$, D_1R, or D_2R mRNA. Radioactive *in situ* hybridization followed our previously published protocols [11]. Briefly, on day 1, a 1-in-20 series (separated by 200 μm) of sections containing the striatum were washed in 4% paraformaldehyde for 1 h, 0.1 M triethanolamine with 0.25% acetic anhydride for 10 min, and then dehydrated in graded ethanol. Complementary (c)RNA riboprobes to A_1R (743 bp, 127–869 coding region), $A_{2A}R$ (949 bp, 1316–2264 coding region), D_1R (460 bp, 383–843 coding region), and D_2R (495 bp, 1174–1665 coding region) were prepared from cDNA subclones in transcription vectors and labeled with [S-35]UTP (Perkin-Elmer, Waltham, MA, USA), using standard transcription methods. Riboprobes were diluted in 50% hybridization buffer containing 50% formamide, 10% dextran sulfate, 2× sodium citrate (SSC), 50 mM phosphate buffer (PBS) at pH 7.4, 1× Denhardt's solution, and 0.1 mg/ml yeast tRNA. Slides containing sections of the striatum were hybridized with the respective probe for approximately 18 h at 55 °C. On day 2, sections were washed in 2× SSC, treated in RNaseA (200 μg/ml) for 1 h at 37 °C. Sections were treated with graded SCC washes (2×, 1×, 0.5×, 0.1×) and placed in 0.1× SSC at 65 °C for 1 h. Sections were then dehydrated in graded ethanol, air dried for approximately 40 min. Finally, slides were placed into X-ray film cassettes and covered with X-ray film (Biomax-MR; Eastman Kodak, Rochester, NY, USA) for 3 days (for $A_{2A}R$, D1, D2) or 7 days (for A_1R). For individual probes (A_1R, $A_{2A}R$, D1, or D2), slides from all rats were processed together to allow for direct comparisons across experimental groups. Labeling with "sense" probes indicated that the signal observed with the "antisense" probes was specific.

2.6. Double fluorescent in situ hybridization (non-radioactive)

Double radioactive *in situ* hybridization was used to detect the proportion of dynorphin (direct pathway) and enkephalin (indirect pathway) containing neurons that co-express cfos (neural activation) in the dorsal striatum following exposure to uncontrollable tail shock. The protocol was similar to *radioactive in situ hybridization* except (c)RNA riboprobes for dynorphin (744 bp, 164–907 coding region) or enkephalin (558 bp, 493–1052 coding region) were labeled with fluorescein UTP (purchased from Roche, Indianapolis, IN) and cfos (574 bp, 596–1171 coding region) was labeled with digoxigenin UTP (purchased from Roche, Indianapolis, IN). Moreover, sections were not exposed to the final graded ethanol dehydration on day 2. Instead, immediately following the 0.1× SSC wash at 65 °C, sections were brought back to room temperature in distilled water and washed with 0.05 M PBS. Sections were then quenched in hydrogen peroxide diluted to 2% in 0.05 M PBS for 30 min, washed in 1× TRIS–buffered saline containing Tween pH 7.5 (TBS-T), and incubated in 0.5% blocking buffer (Perkin-Elmer, Waltman, MA, USA) in 1× TBS for 1 h. Sections were immediately incubated in anti-digoxigenin-horseradish peroxidase (Perkin-Elmer, Waltham, MA, USA) at 1:750 in TBS-T for 30 min. Sections were next washed in TBS-T and then incubated in cyanine 3 (CY3) amplification reagent solution at a 1:100 dilution in 1× amplification diluent (Perkin-Elmer, Waltham, MA, USA). Sections were washed and stored overnight in 0.05 M PBS at 4 °C. On day 3, sections were again quenched in 2% hydrogen peroxide in 0.05 M PBS for 30 min. Sections were next washed in TBS-T and incubated for 90 min in anti-fluorescein-horseradish peroxidase (Perkin-Elmer, Waltham, MA, USA) at 1:100 dilution in TBS-T. Next, sections were again washed in TBS-T and incubated in fluorescein amplification reagent at a 1:100 diultion in 1× amplification diluent (Perkin-Elmer, Waltham, MA, USA) for 1 h. Slides were then washed in PBS and air dried for approximately 30 min. Coverslips were set on slides using ProLong Gold antifade reagent with DAPI (Life Technologies, Grand Island, NY).

2.7. Image analysis for radioactive in situ hybridization

Levels of A_1R, $A_{2A}R$, D_1R, and D_2R were semi-quantitatively analyzed following our previously published protocols [11]. Briefly, images of brain sections on X-ray film were digitally captured using camera (CCD camera, model XC-77; Sony, Tokyo, Japan) interfaced to a computer (see Fig. 1). The relative optical density of the probe on each captured image was determined using Scion Image version 4.0 (Scion, Fredick, MD, USA). For each section, a background sample was taken over an area of white matter (for $A_{2A}R$, D_1R, and D_2R) or off the section (for A_1R, as it is expressed in oligodendrocytes). Next, a sample was taken over the respective brain areas that include DMS, DLS, AcbC, AcbShM, or AcbShL (boundaries defined in Paxinos and Watson rat brain atlas). The signal threshold was

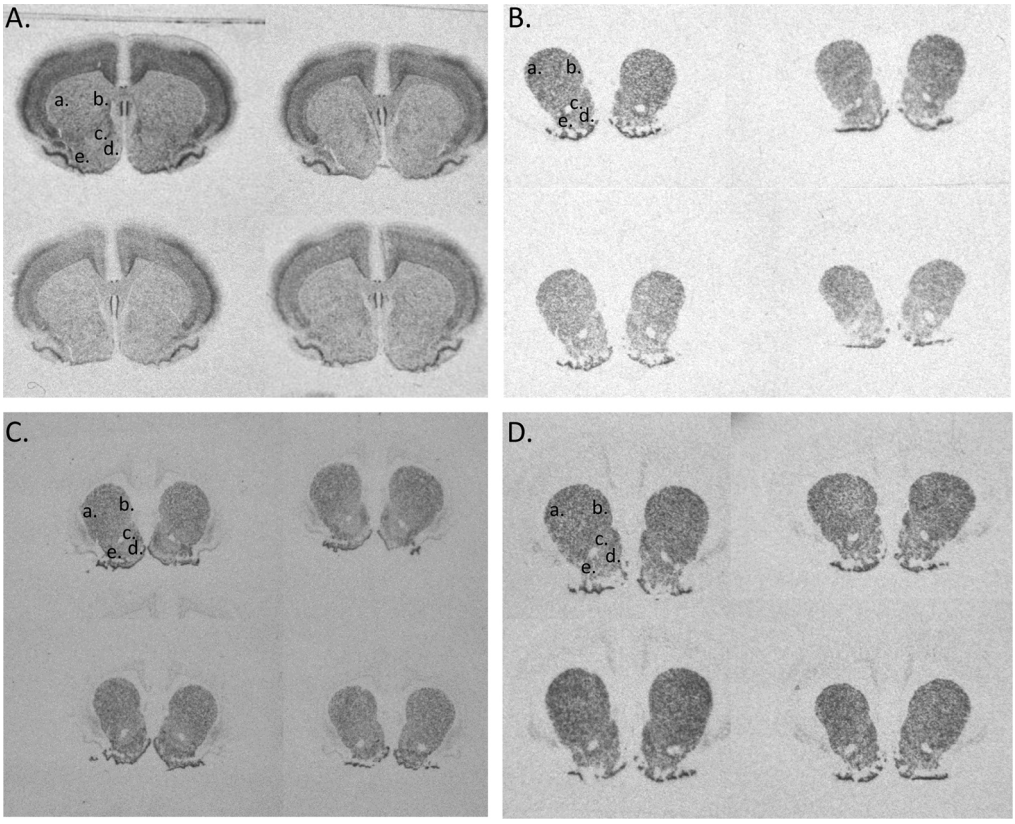

Fig. 1. Radioactive *in situ* hybridization in the striatum. Representative autoradiographs showing *in situ* hybridization for (A) A_1R, (B) $A_{2A}R$, (C) D_1R, and (D) D_2R of Sedentary Control (top left), Sedentary Stress (top right), Running Control (bottom left), and Running Stress (bottom right) in the (a) dorsal medial striatum, (b) dorsal lateral striatum, (c) nucleus accumbens core, (d) medial shell of the nucleus accumbens, and (e) lateral shell of the nucleus accumbens.

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 analyses. The results reflect the mean signal produced by the cRNA probe above the background multiplied by the number of pixels above the assigned background. Each rat's relative optical density for a particular cRNA probe was measured across rostral to caudal axis of the striatum containing 5 sections in the DLS and DMS (coordinates 2.2 to 1.7 mm, 1.6 to 1 mm, 0.2 to −0.3 mm, −0.4 to −0.8 mm, and −0.9 to −1.5 mm from bregma), as well as 4 sections in the AcbC, AcbShM, and AcbShL (coordinates 2.2 to 2.0 mm, 1.9 to 1.6 mm, 1.5 to 1.2 mm, and 1.1 to 0.8 mm from bregma).

2.8. Image analysis for double fluorescent in situ hybridization (non-radioactive)

Images were acquired from a Zeiss AX10 with axioscan Z1 fluorescent microscope interfaced to a computer running axiovision software (Zeiss, Oberkochen, Germany) at 200x total magnification. Fluorescein (dynorphin or enkephalin), Cy3 (cfos), and DAPI (nuclei) emission channels were merged to create a single image. For each rat, two non-overlapping adjacent images were acquired in the DMS and DLS over 3 sections across the rostral to caudal axis of the striatum (coordinates between 2.0 to 1.8 mm, 1.2 to 1.0 mm, and 0 to −0.2 mm from bregma). Images were taken unilaterally alternating right and left striatum for each rat. Each image captured the entire *z*-plane of a striatum. The number of cfos, enkephalin or dynorphin, and co-labeled (enkephalin/cfos or dynorphin/cfos) neurons were counted in each image by an individual blind to rat group assignments. A co-label was confirmed when a dynorphin- or enkephalin-expressing neuron near completely covered and had a similar morphology to a cfos-reactive cell.

2.9. Data analysis

Overall distance run was compared between Running Control and Running Stress groups using Student's *t*-test. Serum corticosterone concentrations were compared by a 2-way ANOVA with exercise (Sedentary *vs.* Running) and stress conditions (Control *vs.* Stress) as factors. Trends in A_1R, $A_{2A}R$, D_1R, and D_2R mRNA expression were generally consistent across the rostral to caudal extent of each striatal area. Therefore, relative optical density for A_1R, $A_{2A}R$, D_1R, and D_2R mRNA levels was reported as an average across analyzed rostral to caudal sections for each brain area (DMS, DLS, AcbC, AcbShM, AcbShL) and compared by a 2-way ANOVA with exercise and stress condition as factors. Relative optical density for each rostral to caudal sub region analyzed (as previously described in Section 2) in dorsal and ventral striatum structures were also compared using 2-way ANOVAs with exercise and stress conditions as factors. These data are included in Supplementary Tables 1–5. A small number of sections contained damage over the region of interest and were subsequently removed from analysis. Moreover, 1–2 of the rats in each group were missing sections from the most rostral level of the nucleus accumbens (2.2–2.0 mm from bregma). However, each group maintained at least a sample size of 6 for comparison at every level of the striatum. Discrepancies in sample sizes are noted by the degrees of freedom in Supplementary tables.

The densities of cfos positive cells, as well as the proportions of enkephalin or dynorphin neurons that co-expressed cfos in the dorsal striatum were compared by a 2-way ANOVA with exercise and stress condition as factors. For ANOVAs, *post hoc* analyses using

Fisher projected least significant difference (PLSD) corrections were completed when appropriate. For all analyses $P < 0.05$ was considered statistically significant.

3. Results

3.1. Wheel running

Daily wheel running distance increased steadily for the first 3 weeks and thereafter maintained a plateau averaging 4.49 km/day (±0.09 SE) the final 3 weeks. Average distance run during the entire experiment was 3.66 km/day (±0.20 SE). Average distance traveled on wheels did not differ between Running Control and Running Stress groups.

3.2. Corticosterone concentrations

Exposure to uncontrollable tail shock potently increased corticosterone concentrations in both sedentary and running conditions [$F(1,28) = 351.8$, $P < 0.0001$]. No effect of exercise condition or significant interaction between exercise and stress condition was observed. Average corticosterone concentrations were 1.9 μg/dl (±0.4 SE) for Sedentary Control, 1.4 μg/dl (±0.4 SE) for Running Control, 61.4 μg/dl (±1.8 SE) for Sedentary Stress, and 72.3 μg/dl (±6.5 SE) for Running Stress groups.

3.3. Dorsal striatum

3.3.1. A_1R gene expression

In the DMS, exercise decreased A_1R mRNA levels [$F(1,28) = 25.5$, $P < 0.0001$] (Fig. 2A). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 5.9$, $P = 0.02$]. *Post hoc* analysis revealed that exposure to stress decreased A_1R mRNA in sedentary rats ($P = 0.007$). Moreover, Sedentary Control maintained greater A_1R mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups. Additionally, Sedentary Stress had a greater A_1R mRNA expression than the Running Control group ($P = 0.03$).

In the DLS, exercise decreased A_1R mRNA levels [$F(1,28) = 23.0$, $P < 0.0001$] (Fig. 2B). A significant interaction was found between exercise and stress conditions [$F(1,28) = 5.8$, $P = 0.02$]. *Post hoc* analysis revealed that exposure to stress decreased A_1R mRNA in sedentary rats ($P = 0.003$). Sedentary Control maintained greater A_1R mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups.

3.3.2. $A_{2A}R$ gene expression

In the DMS, exercise decreased $A_{2A}R$ mRNA levels [$F(1,28) = 94.5$, $P < 0.0001$] (Fig. 2A). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 28.4$, $P < 0.0001$]. *Post hoc* analysis revealed that exposure to stress decreased $A_{2A}R$ mRNA in sedentary rats ($P < 0.0001$). Moreover, Sedentary Control displayed greater $A_{2A}R$ mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups. In addition, Sedentary Stress had a greater $A_{2A}R$ mRNA expression than Running Control ($P = 0.04$) and Running Stress ($P = 0.004$) groups.

In the DLS, exercise decreased $A_{2A}R$ mRNA expression [$F(1,28) = 111.3$, $P < 0.0001$] (Fig. 2B). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 29.5$, $P < 0.0001$]. *Post hoc* analysis revealed that exposure to stress decreased $A_{2A}R$ mRNA in sedentary rats ($P < 0.0001$). Sedentary Control had greater $A_{2A}R$ mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups. Moreover, Sedentary Stress rats had more $A_{2A}R$ mRNA than Running Control ($P = 0.009$) and Running Stress ($P = 0.001$) groups.

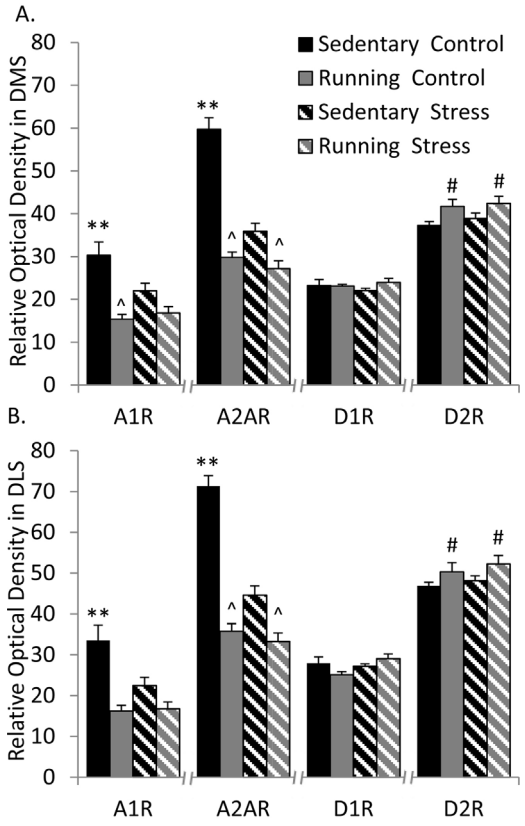

Fig. 2. A_1R, $A_{2A}R$, D_1R, and D_2R gene expression in the dorsal striatum following stress and exercise. Relative levels of A_1R, $A_{2A}R$, D_1R, and D_2R messenger ribonucleic acid (mRNA) averaged across five sections (coordinates 2.2 to 1.7 mm, 1.6 to 1 mm, 0.2 to −0.3 mm, −0.4 to −0.8 mm, and −0.9 to −1.5 mm from bregma) of the (A) dorsal medial striatum and (B) dorsal lateral striatum. Please note, the presented data are not normalized across receptors, as *in situ* hybridization for each receptor-type was completed on different days (see Section 2 for details). $^{**}P < 0.001$ from Running Control, Sedentary Stress, and Running Control within respective receptor, $^{\wedge}P < 0.05$ from Sedentary Stress within respective receptor, $^{\#}P < 0.05$ main effect of exercise within respective receptor.

3.3.3. D_1R gene expression

No significant effects of exercise, stress, or interaction between exercise and stress were observed for D_1R mRNA in the DMS or DLS (Fig. 2A and B).

3.3.4. D_2R gene expression

Exercise increased D_2R mRNA in the DMS [$F(1,28) = 7.8$, $P = 0.009$] (Fig. 2A), as well as in the DLS [$F(1,28) = 4.7$, $P = 0.03$] (Fig. 2B). No significant effect of stress or interaction between exercise and stress was observed.

Values and statistical analysis for A_1R, $A_{2A}R$, D_1R, and D_2R mRNA expression at each level of the DMS and DLS are located in Supplementary Tables 1 and 2.

3.4. Ventral striatum

3.4.1. A_1R gene expression

In the AcbC, exercise decreased A_1R mRNA levels [$F(1,28) = 24.4$, $P < 0.0001$] (Fig. 3A). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 5.5$, $P = 0.03$]. *Post hoc* analysis revealed that exposure to stress decreased A_1R mRNA in sedentary rats ($P = 0.007$). Sedentary Control had greater A_1R mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups. Moreover, Sedentary Stress rats had greater A_1R mRNA expression than Running Control rats ($P = 0.03$).

In the AcbShM, exercise decreased A_1R mRNA levels [$F(1,28) = 36.1$, $P < 0.0001$] (Fig. 3B). A significant interaction was found between exercise and stress conditions [$F(1,28) = 6.6$, $P = 0.02$]. *Post hoc* analysis revealed that exposure to stress decreased A_1R mRNA in sedentary rats ($P = 0.002$). Sedentary Control had greater A_1R mRNA expression than Running Control ($P < 0.0001$), and Running Stress ($P < 0.0001$) groups. Moreover, Sedentary Stress rats had greater A_1R mRNA expression than Running Control ($P = 0.01$) and Running Stress ($P = 0.02$) groups.

In the AcbShL, exercise decreased A_1R mRNA levels [$F(1,28) = 36.8$, $P < 0.0001$] (Fig. 3C). A significant interaction was found between exercise and stress conditions [$F(1,28) = 7.3$, $P = 0.01$]. *Post hoc* analysis revealed that exposure to stress decreased A_1R mRNA in sedentary rats ($P < 0.0001$). Sedentary Control had greater A_1R mRNA expression than Running Control ($P < 0.0001$), and Running IS ($P < 0.0001$) groups. Moreover, Sedentary Stress had greater A_1R mRNA expression than Running Control ($P = 0.02$) and Running Stress ($P = 0.02$) groups.

3.4.2. $A_{2A}R$ gene expression

In the AcbC, exercise decreased $A_{2A}R$ mRNA levels [$F(1,28) = 111.8$, $P < 0.0001$] (Fig. 3A). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 19.2$, $P = 0.0001$]. *Post hoc* analysis revealed that exposure to stress decreased $A_{2A}R$ mRNA in sedentary rats ($P < 0.0001$). Sedentary Control maintained greater $A_{2A}R$ mRNA expression than Running Control ($P < 0.0001$) and Running IS ($P < 0.0001$) groups. Sedentary Stress had more $A_{2A}R$ mRNA than Running Stress rats ($P = 0.002$). Additionally, Running Control had significantly greater expression $A_{2A}R$ mRNA than Running Stress rats ($P = 0.02$).

In the AcbShM, exercise decreased $A_{2A}R$ mRNA levels [$F(1,28) = 68.5$, $P < 0.0001$] (Fig. 3B). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 27.0$, $P < 0.0001$]. *Post hoc* analysis revealed that exposure to stress decreased $A_{2A}R$ mRNA in sedentary rats ($P < 0.0001$). Sedentary Control maintained greater $A_{2A}R$ mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups. Sedentary Stress rats had more $A_{2A}R$ mRNA than Running Stress rats ($P = 0.04$). Moreover, Running Control had greater mRNA expression than Running Stress rats ($P = 0.04$).

In the AcbShL, exercise decreased $A_{2A}R$ mRNA levels [$F(1,28) = 78.6$, $P < 0.0001$] (Fig. 3C). A significant interaction was observed between exercise and stress conditions [$F(1,28) = 24.7$, $P < 0.0001$]. *Post hoc* analysis revealed that exposure to stress decreased $A_{2A}R$ mRNA in sedentary rats ($P < 0.0001$). Sedentary Control maintained greater $A_{2A}R$ mRNA expression than Running Control ($P < 0.0001$) and Running Stress ($P < 0.0001$) groups. Moreover, Sedentary Stress rats had greater $A_{2A}R$ mRNA expression than Running Control ($P = 0.04$) and Running Stress ($P = 0.01$) groups.

3.4.3. D_1R gene expression

No significant effect of exercise, stress, or interaction between exercise and stress was observed for D_1R mRNA in the AcbC, AcbShM, and AcbShL (Fig. 3A–C).

3.4.4. D_2R gene expression

Exercise modestly increased D_2R mRNA [$F(1,28) = 9.4$, $P = 0.005$] in the AcbShM (Fig. 3B), as well as in the AcbShL [$F(1,28) = 11.8$, $P = 0.002$] (Fig. 3C). No significant effect of stress or interaction between exercise and stress was found in AcbC, AcbShM, or AcbShL.

Values and statistical analysis for A_1R, $A_{2A}R$, D_1R, and D_2R mRNA expression at each level of the AcbC, AcbShM, and AcbShL are located in Supplementary Tables 3–5.

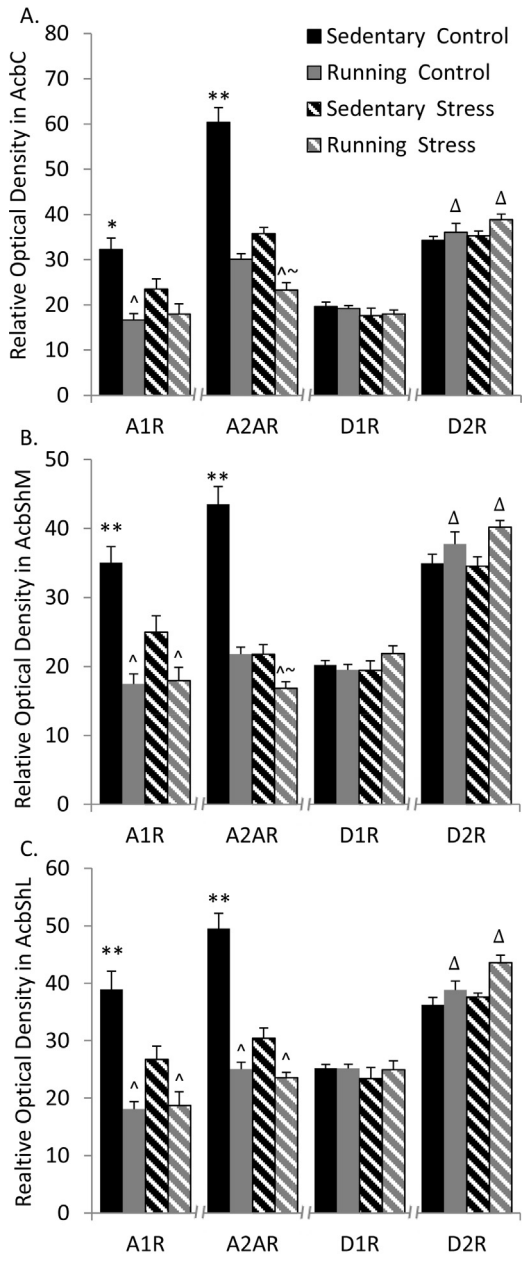

Fig. 3. A_1R, $A_{2A}R$, D_1R, and D_2R gene expression in the ventral striatum following stress and exercise. Relative levels of A_1R, $A_{2A}R$, D_1R, and D_2R messenger ribonucleic acid (mRNA) averaged across four sections (coordinates 2.2 to 2.0 mm, 1.9 to 1.6 mm, 1.5 to 1.2 mm, and 1.1 to 0.8 mm from bregma) of the (A) nucleus accumbens core, (B) medial shell of the nucleus accumbens, and (C) lateral shell of the nucleus accumbens. Please note, the presented data are not normalized across receptors, as *in situ* hybridization for each receptor-type was completed on different days (see Section 2 for details). $^{*}P < 0.01$ from Running Control, Sedentary Stress, and Running Control within respective receptor $^{**}P < 0.001$ from Running Control, Sedentary Stress, and Running Control within respective receptor; $^{\wedge}P < 0.05$ from Sedentary Stress within respective receptor, $^{\sim}P < 0.05$ from Running control within respective receptor; $^{\Delta}P \leq 0.05$ main effect of exercise within respective receptor.

3.5. Fluorescent in situ hybridization

3.5.1. cfos mRNA induction in the dorsal striatum following acute stress

No statistically significant differences were observed for density of cfos mRNA between DLS and DMS in any group. Moreover, cfos induction did not differ through the rostral to caudal striatum. Therefore, cfos density was averaged across the DLS and DMS at all levels (see Fig. 4A). Exposure to stress caused a marked increase

in cfos induction in the dorsal striatum [$F(1,28) = 268.0$, $P < 0.0001$] (Fig. 4B). No effect of exercise or interaction between exercise and stress condition was observed.

3.5.2. Effect of acute stress on cfos induction in dynorphin neurons in the dorsal striatum (see Fig. 4C)

The proportion of dynorphin neurons that co-expressed cfos did not differ between the DMS and DLS. Moreover, proportion of dynophin neurons expressing cfos was similar throughout sections containing rostral to caudal striatum within each group. Therefore, proportion of dynorphin neurons that co-expressed cfos were averaged across the DLS and DMS at all levels. Stress increased the proportion of dynorphin neurons that expressed cfos [$F(1,28) = 193.2$, $P < 0.0001$] (Fig. 4D). Moreover, a statistically significant interaction as observed between stress and exercise condition [$F(1,28) = 7.4$, $P = 0.01$] (Fig. 4D). *Post hoc* analysis revealed stress-induced cfos expression in dynorphin neurons was augmented in running rats ($P = 0.0003$).

3.5.3. Activation of enkephalin neurons in the dorsal striatum from acute stress (see Fig. 4E)

The proportion of enkephalin neurons that expressed cfos did not differ between the DMS and DLS. Moreover, proportion of enkephalin and cfos double labeled neurons were similar throughout sections containing rostral to caudal striatum within each group. Therefore, the proportion of enkephalin cells that co-expressed cfos were averaged across the DLS and DMS at all levels for each group. Stress increased the proportion of enkephalin neurons that expressed cfos [$F(1,28) = 162.9$, $P < 0.0001$] (Fig. 4F). A statistically significant interaction was observed between stress and exercise conditions [$F(1,28) = 5.7$, $P = 0.02$] (Fig. 4F). *Post hoc* analysis revealed stress-induced cfos expression in enkephalin neurons was reduced in running rats ($P = 0.01$).

4. Discussion

The results of the current study show long-term wheel running and acute uncontrollable tail shock decreased striatal A_1R and $A_{2A}R$ mRNA levels across the DMS, DLS, AcbC, AcbShM, and AcbShL (see Figs. 2 and 3). Acute uncontrollable tail shock exposure further reduced $A_{2A}R$ mRNA levels in the AcbC and AcbShM in running rats (see Fig. 3A and B). D_2R mRNA was modestly increased in striatum sub-regions of running rats (see Figs. 2 and 3). Neither running nor stress affected striatal D_1R mRNA expression (see Figs. 2 and 3). Changes in A_1R, $A_{2A}R$, and D_2R gene expression in the dorsal striatum from running were associated with greater stress-induced cfos induction in dynorphin expressing "direct pathway" neurons and attenuated stress-induced cfos induction in enkephalin expressing "indirect pathway" neurons (see Fig. 4D and F). Taken together, these data provide novel evidence indicating running alters striatum A_1R and $A_{2A}R$ mRNA levels, which may affect adenosine-mediated antagonism of dopamine signaling in the striatum. These data suggest the striatum adenosine system may be a target for neuroplasticity following exercise, which may have significance for several behavioral and cognitive processes that involve the striatum.

Changes to A_1R and $A_{2A}R$ mRNA levels are commonly associated with similar changes to protein or radioligand binding [40–56]. If reported decreases of A_1R and $A_{2A}R$ gene expression from running translate to functional reductions in protein, these data may have implications for both normal striatum function and potentially the prevention or treatment of disorders that involve abnormal dopamine signaling. Indeed, A_1R and $A_{2A}R$ are now recognized as major modulators of striatum function. Their relatively high degree of expression in the striatum, predominate post-synaptic location on medium spiny neurons, and antagonistic interaction with dopamine receptors are consistent with a role in motor control, instrumental learning, habit learning, working memory, reward, and motivation [76–85]. Thus, potential changes to A_1R and $A_{2A}R$ expression, related to reduced mRNA from running, may influence a number of striatum-involved cognitive modalities. In addition to normal physiological processes, the A_1R and $A_{2A}R$ may serve as novel targets to treat pathologies associated with striatum dysfunction (as reviewed in [86]). Striatal dopamine receptors have been widely studied as targets for therapeutic treatment of drug abuse, depression, schizophrenia, cognitive dysfunction, and motor control disorders (*e.g.* Parkinson's and Huntington's Disease) with mixed efficacy. Due to their ability to reduce dopamine signaling, adenosine receptors have provided a new direction in the search for novel drugs to treat these disorders [87–95]. In particular, $A_{2A}R$ antagonists have entered clinical trials for treatment of motor degenerative Parkinson's disease, due to its ability to stimulate motor enhancement with a reduced propensity to cause dyskinesia compared to dopamine agents [96]. Given the potential for $A_{2A}R$ antagonist treatment of Parkinson's disease, the reported decrease of $A_{2A}R$ mRNA in the striatum (see Figs. 2 and 3) could contribute to delayed onset of Parkinson's disease symptoms in individuals that exercise (as reviewed in [97]).

In addition to motor control, data from pre-clinical work suggest striatal A_1R and $A_{2A}R$ may represent novel targets to treat mental health disorders like drug abuse or depression. Therefore, decreased striatum adenosine receptor mRNA levels from exercise could be related to reductions in the abusive properties of certain drugs and protect against incidences of depression. Indeed, $A_{2A}R$ antagonism or knockdown has been reported to suppress motivation and drug seeking for morphine in rodents [98–100]. Therefore, reduced $A_{2A}R$ mRNA levels in wheel running rats (see Figs. 2 and 3) may be related to protection from uncontrollable tail shock-induced enhancement of morphine preference in rats [12]. Moreover, the exercise-induced decrease in $A_{2A}R$ mRNA could be relevant for reduced abusive properties of other drugs, as $A_{2A}R$ knockdown or blockade has also been reported to attenuate the reinforcing properties of nicotine and cannabinoid receptor stimulation [101,102]. Finally, striatum adenosine receptors may be involved in the expression of behavioral despair [34,92,93,103], as well as the development of depression-like behavior that follows uncontrollable stress [33,35–37]. Indeed, $A_{2A}R$ knockdown or antagonist treatment has been reported to increase rodent mobility on forced swim and tail suspension tests [92,93], as well as prevent depression-like behavior that develops following reserpine treatment [34]. Furthermore, blockade of brain adenosine receptors has been reported to prevent the development of depression-like shuttle box escape deficits following uncontrollable tail shock [33,35–37] that are known to involve the striatum [13]. Therefore, reduced adenosine receptor mRNA levels in the striatum following wheel running (see Figs. 2 and 3) may contribute to the stress-buffering properties of exercise [38]. Future analyses are required to determine if reported decreases of A_1R and $A_{2A}R$ mRNA from wheel running are translated to functional reduction in receptors, as these changes could have significance for striatum function and dysfunction.

The mechanism by which wheel running reduces A_1R and $A_{2A}R$ mRNA is unknown; however, repeated or excessive ligand binding during exercise could contribute to the observed reduction in A_1R and $A_{2A}R$ mRNA levels. Indeed, prior work indicates that A_1R or $A_{2A}R$ activation can lead to changes in receptor mRNA both *in vitro* and *in vivo*, although inconsistencies in the literature exist in the direction and magnitude of such changes [24–29,104,105]. Moreover, neither of these receptor mRNAs have been well characterized in the brain following *in vivo* exposure to agonists, as only one report exists, to the best of our knowledge, suggesting chronic A_1R activation with the agonist

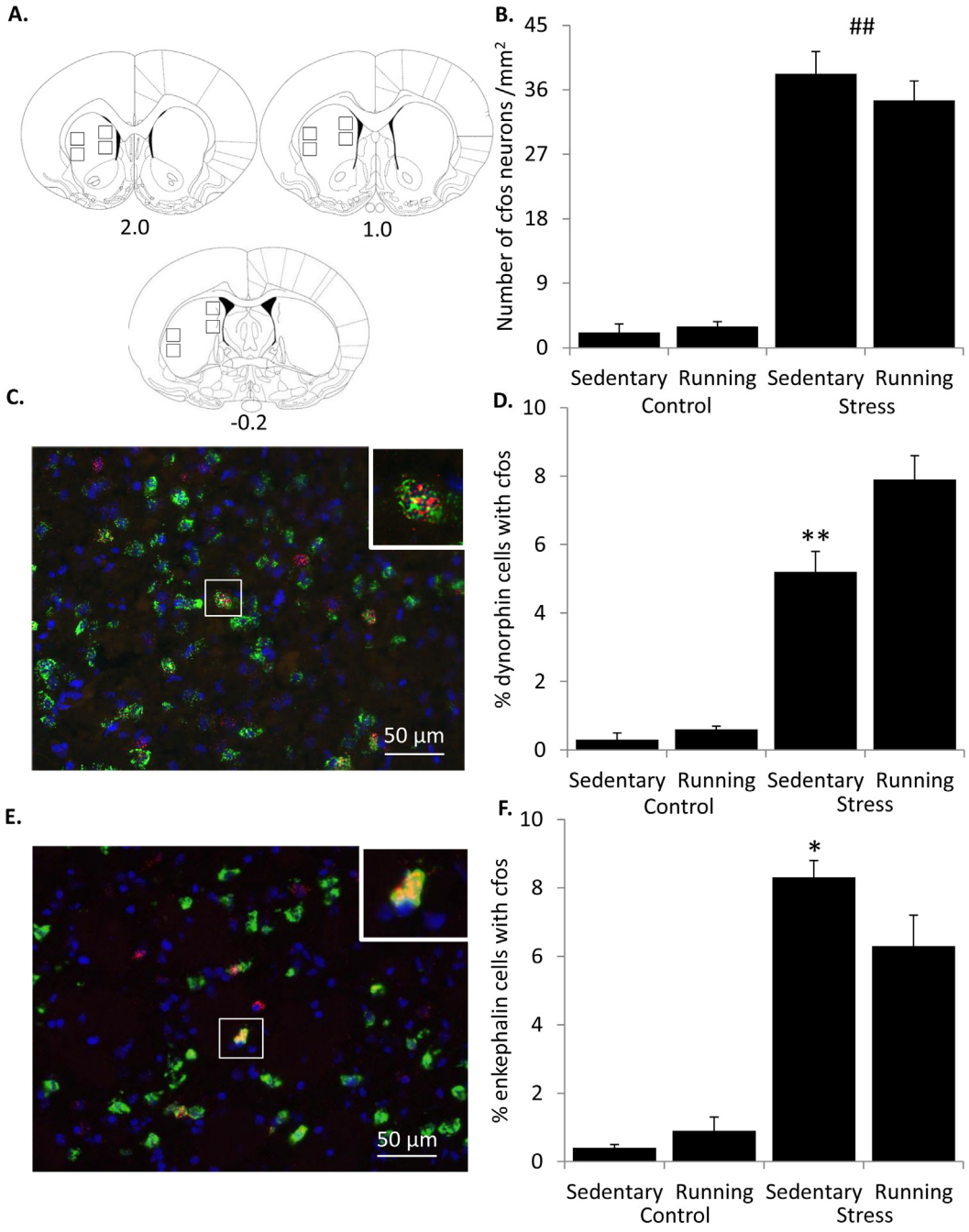

Fig. 4. cfos induction in direct and indirect pathway neurons of the dorsal striatum. (A) Approximate location of images capturing the dorsal lateral and dorsal medial striatum used for analysis. Pictures modified from the Paxinos and Watson rat brain atlas. (B) Number of cfos expressing neurons in the dorsal striatum per square mm from uncontrollable tail shock stress in sedentary and running rats. (C) Representative coronal section of the dorsal striatum stained for dynorphin mRNA (green), cfos mRNA (red), and DAPI (blue). The white box contains a zoomed in image of a dynorphin-positive cell within the section co-expressing cfos. (D) The proportion of dynorphin-positive cells co-expressing cfos in the dorsal striatum following uncontrollable tail shock stress in sedentary and running rats. (E) Representative coronal section of the dorsal striatum stained for enkephalin mRNA (green), cfos mRNA (red), and DAPI (blue). The white box contains a zoomed in image of an enkephalin-positive cell within the section co-expressing cfos. (F) The proportion of enkephalin-positive cells co-expressing cfos in the dorsal striatum following uncontrollable tail shock stress in sedentary and running rats. $^{\#\#}P < 0.0001$ main effect of stress; $^{*}P < 0.05$ or $^{**}P < 0.001$ from Running Stress. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

R-PIA reduces mRNA and surface expression of A_1R in the brain *in vivo* [26]. However, prolonged exposure to agonists of other brain G-protein coupled receptors, like A_1R and $A_{2A}R$, commonly result in decreased receptor mRNA and protein (as reviewed in [106]). Multiple lines of evidence suggest wheel running can increase adenosine activity in the striatum, which may have contributed to the observed reduction of A_1R and $A_{2A}R$ mRNA levels. Adenosine is released into the extracellular space when the rate of adenosine triphosphate (ATP) hydrolysis exceeds synthesis. Thus, adenosine concentrations increase as a function of cellular workload (as reviewed in [16]). Exercise promotes activity-dependent increases of ATP utilization and adenosine concentrations in both peripheral and central tissues [30,107–109]. However, the source of brain adenosine from exercise that may contribute to altered A_1R

and $A_{2A}R$ mRNA expression is not well understood. One possibility is that adenosine becomes released into extracellular space during brain area-specific increases in energy demand. Previous work has shown that bouts of wheel and treadmill running potently increase neural activity and markers of metabolic demand in the striatum [8,31,32,110–112], which may be sufficient to increase local adenosine levels. Consistent with this idea, extracellular adenosine concentrations measured by microdialysis are slightly elevated in the rat striatum across the dark-cycle, a period of increased physical activity, suggesting even moderate increases of locomotor activity can augment striatum adenosine signaling [113]. In addition, adenosine can cross the blood brain barrier [114,115]. Therefore, peripheral increases of adenosine during running could also contribute to the altered receptor mRNA levels by elevating brain-wide adenosine concentrations [30]. Taken together, these data suggest that repeated increases of local or brain-wide adenosine levels during running may be a contributing factor to reduced A_1R and $A_{2A}R$ mRNA levels, and could be a topic for future investigations.

The potential factors contributing to and significance of the rapidly reduced striatum adenosine receptor mRNA immediately following uncontrollable tail shock is more difficult to hypothesize (see Figs. 2 and 3). It is possible that rapid and excessive increases of adenosine in the striatum related to increased neural activity during stress could be a factor contributing to decreased receptor mRNAs (see Fig. 4B) [35], as levels of mRNA coding for the adenosine receptors can become altered within hours following agonist binding in cell culture models [25,27]. However, this effect has not been well characterized *in vivo*. Other factors related to uncontrollable tail shock such as increases of corticosterone (see Section 3.2) [40] and proinflammatory cytokines [116] likely do not contribute to observed reductions because evidence suggests these factors increase, not decrease adenosine receptor mRNA [54,117,118]. Therefore, factors contributing to rapid reductions of both adenosine receptor mRNAs following uncontrollable stress require further investigation. Additionally, stress has been reported to up-regulate $A_{2A}R$ in the striatum, which is related to the development of depression-like behaviors in rats [103]. Therefore, rapid changes to striatum adenosine receptor mRNA observed immediately following tail shock may not be representative of later time points. Other G-protein coupled receptors that display altered mRNA levels immediately following uncontrollable tail shock return to baseline levels within hours [119], or can continue to change expression days later [73]. It is possible that A_1R or $A_{2A}R$ mRNAs may also display differing patterns of expression overtime. The stability of reported adenosine receptor mRNA reductions immediately following uncontrollable stress also requires further exploration in order to better understand how these changes may influence striatum function.

Factors contributing to the activation of medium spiny neurons comprising the direct and indirect pathways are complex, and involve coordinated interactions between several neurotransmitter systems that act both pre- and post-synaptically. However, several lines of evidence suggest potentiated stress-induced cfos in dynorphin-expressing direct pathway and attenuated stress-induced cfos in enkephalin-expressing indirect pathway neurons of physically active rats (see Fig. 4D and F) are consistent with observed changes to A_1R, $A_{2A}R$, and D_2R gene expression (see Fig. 2). Evidence suggests uncontrollable tail shock can increase adenosine and dopamine signaling in the striatum [33,35–37,68]; therefore, potential exercise-induced changes to adenosine or dopamine receptor expression, related altered mRNA, could lead to the observed differences in stress-induced activation of direct and indirect pathway neurons between running and sedentary rats. Indeed, $A_{2A}R$ and D_2R mRNA are nearly exclusively found in enkephalin-expressing striatum indirect pathway neurons, where these receptors are predominately located on dendrites and cell bodies [18,67]. $A_{2A}R$ activation decreases the binding affinity and function of D_2R in indirect pathway neurons [120,121]. Therefore, potential reductions of $A_{2A}R$ and greater D_2R expression [122–124], associated with observed changes to receptor mRNA, in the striatum of physically active rats could lead to a greater inhibitory influence on indirect pathway neurons during tail shock. Furthermore, adenosine can reduce dopamine-mediated activation of direct pathway neurons through post-synaptic A_1R–D_1R heterodimer interactions [19,125,126]. Therefore, decreased A_1R mediated inhibition of D_1R could lead to a greater excitatory influence on direct pathway neurons. Together, observations from the literature along with reported changes in adenosine and dopamine receptor mRNA levels are consistent with a reduced efficacy of adenosine-mediated inhibition of dopamine signaling in medium spiny neurons of exercising animals, thus resulting in potentiated direct pathway and attenuated indirect pathway activation during stress in physically active rats.

This interpretation, albeit consistent with our data, requires caution. A_1R mRNA expression has been reported in direct and indirect pathway neurons, and presumably both A_1R and $A_{2A}R$ mRNA are located in striatum cholinergic interneurons [127]. Therefore, the sources contributing to observed decreases in adenosine receptor mRNAs are not clear. However, given the small proportion of striatum neurons that are estimated to be cholinergic (~0.4%) [128], this source would likely only contribute modestly to the large decreases in adenosine receptor mRNA observed in this study (see Figs. 2 and 3). Furthermore, exposure to uncontrollable tail shock also rapidly reduced A_1R and $A_{2A}R$ mRNA (see Fig. 2), suggesting that functional differences in protein level could be minimal between running and sedentary rats following tail shock. However, loss of G-protein coupled receptors, like A_1R and $A_{2A}R$, that are associated with reductions of mRNA normally occurs in response to a stimulus treatment for several hours or longer (as reviewed in [106,129–131]). Therefore, it is unlikely that the reductions in mRNA levels observed immediately after stress would also be reflected in a change in protein at this time point, whereas potential protein changes in exercising rats would have been in place prior to stress. Finally, activation of the direct pathway and indirect pathways has been shown to promote and inhibit movement respectively [69]. If running rats struggled to a greater degree in restraint tubes during tail shock than sedentary rats, this could be an alternative source for the observed changes in patterns of cfos induction in direct and indirect pathways. However, we have observed no difference in movement between sedentary and running rats during uncontrollable tail shock using accurate biotelemetric recording (*unpublished data*). Therefore, this alternative explanation seems unlikely. While it is difficult to make strong conclusions from the current data that altered striatum A_1R, $A_{2A}R$, or D_2R gene expression from running contributes to increased direct and decreased indirect pathway activation during tail shock, the data are consistent with this hypothesis and should be explored in greater detail by future studies.

Recent evidence has emerged suggesting excitation of direct and indirect pathway neurons in the striatum serve opposing roles for reinforcement learning, which may have significance for stress-buffering properties of exercise. Data from studies using gene modification combined with rodent behavior suggest activation of striatum direct and indirect pathway neurons can contribute to reward and aversion, respectively [70,132]. Differences in the amount of direct and indirect pathway activation; therefore, may be important for varying expression of hedonic states during exposure to rewarding or noxious stimuli [69]. In light of recent findings, the current observation of greater activation of the direct pathway and attenuated activation of the indirect pathway during stress in physically active, compared to sedentary, rats suggests that uncontrollable stress could be a more aversive experience

for sedentary compared to running rats. Consistent with this idea, selective induction of the chronic activity marker ΔFosB in direct and indirect pathway neurons are, respectively, related to stress-resistance and -susceptibility in mice that were previously exposed to repeated social defeat [71]. Together, these data raise the interesting possibility that exercise-induced neuroplasticity in the striatum may facilitate a shift from aversion-related to reward-related neural activation during exposure to stressful events. Such changes in neural activity could play a role in exercise-induced stress resistance and resilience, and should be topics for further investigation.

5. Conclusion

The current data provide novel evidence that the adenosine system in the striatum may be sensitive to physical activity status and acute uncontrollable stress. The observed reduction in A_1R and $A_{2A}R$ mRNA levels following exercise could potentially contribute to reduced efficacy of adenosine-mediated inhibition of dopamine signaling in the striatum. Indeed, the observed changes to direct and indirect pathway neuron activation during uncontrollable stress in running rats are compatible with this hypothesis. These findings could have important implications for striatum function, as well as the prevention and treatment of mental health and motor pathologies involving the striatum.

Acknowledgements

Special thanks to University of Colorado Boulder Animal Care facility. This work was supported by National Institutes of Mental Health Grant 068283.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2014.07.006.

References

[1] Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature 2008;455:894–902.
[2] Feil J, Sheppard D, Fitzgerald PB, Yucel M, Lubman DI, Bradshaw JL. Addiction, compulsive drug seeking, and the role of frontostriatal mechanisms in regulating inhibitory control. Neurosci Biobehav Rev 2010;35:248–75.
[3] Ehrlich ME. Huntington's disease and the striatal medium spiny neuron: cell-autonomous and non-cell-autonomous mechanisms of disease. Neurotherapeutics 2012;9:270–84.
[4] Nikkhah G. Restorative strategies for the dopaminergic nigrostriatal projection pathway. Acta Neurochir Suppl 2013;117:79–85.
[5] Brunelin J, Fecteau S, Suaud-Chagny MF. Abnormal striatal dopamine transmission in schizophrenia. Curr Med Chem 2013;20:397–404.
[6] Lambert TJ, Fernandez SM, Frick KM. Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. Neurobiol Learn Mem 2005;83:206–16.
[7] Marais L, Stein DJ, Daniels WM. Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. Metab Brain Dis 2009;24:587–97.
[8] Werme M, Messer C, Olson L, Gilden L, Thoren P, Nestler EJ, et al. Delta FosB regulates wheel running. J Neurosci 2002;22:8133–8.
[9] Werme M, Thoren P, Olson L, Brene S. Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. Eur J Neurosci 2000;12:2967–74.
[10] Foley TE, Fleshner M. Neuroplasticity of dopamine circuits after exercise: implications for central fatigue. Neuromol Med 2008;10:67–80.
[11] Greenwood BN, Foley TE, Le TV, Strong PV, Loughridge AB, Day HE, et al. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. Behav Brain Res 2011;217:354–62.
[12] Rozeske RR, Greenwood BN, Fleshner M, Watkins LR, Maier SF. Voluntary wheel running produces resistance to inescapable stress-induced potentiation of morphine conditioned place preference. Behav Brain Res 2011;219:378–81.
[13] Strong PV, Christianson JP, Loughridge AB, Amat J, Maier SF, Fleshner M, et al. 5-Hydroxytryptamine 2C receptors in the dorsal striatum mediate stress-induced interference with negatively reinforced instrumental escape behavior. Neuroscience 2011;197:132–44.
[14] Greenwood BN, Strong PV, Loughridge AB, Day HE, Clark PJ, Mika A, et al. 5-HT2C receptors in the basolateral amygdala and dorsal striatum are a novel target for the anxiolytic and antidepressant effects of exercise. PLoS ONE 2012;7:e46118.
[15] Fuxe K, Ferre S, Genedani S, Franco R, Agnati LF. Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav 2007;92:210–7.
[16] Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferre S. Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 2007;83:277–92.
[17] Ferre S, Popoli P, Gimenez-Llort L, Finnman UB, Martinez E, Scotti de Carolis A, et al. Postsynaptic antagonistic interaction between adenosine A1 and dopamine D1 receptors. Neuroreport 1994;6:73–6.
[18] Svenningsson P, Le Moine C, Fisone G, Fredholm BB. Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol 1999;59:355–96.
[19] Ferre S, Popoli P, Tinner-Staines B, Fuxe K. Adenosine A1 receptor-dopamine D1 receptor interaction in the rat limbic system: modulation of dopamine D1 receptor antagonist binding sites. Neurosci Lett 1996;208:109–12.
[20] Dayne Mayfield R, Larson G, Orona RA, Zahniser NR. Opposing actions of adenosine A2a and dopamine D2 receptor activation on GABA release in the basal ganglia: evidence for an A2a/D2 receptor interaction in globus pallidus. Synapse 1996;22:132–8.
[21] Fuxe K, Ferre S, Zoli M, Agnati LF. Integrated events in central dopamine transmission as analyzed at multiple levels. Evidence for intramembrane adenosine A2A/dopamine D2 and adenosine A1/dopamine D1 receptor interactions in the basal ganglia. Brain Res Brain Res Rev 1998;26:258–73.
[22] Do J, Kim JI, Bakes J, Lee K, Kaang BK. Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. Learn Mem 2012;20:21–8.
[23] Cools R, D'Esposito M. Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol Psychiatry 2011;69:e113–25.
[24] Ruiz MA, Leon DA, Albasanz JL, Martin M. Desensitization of adenosine A(1) receptors in rat immature cortical neurons. Eur J Pharmacol 2011;670:365–71.
[25] Saitoh O, Saitoh Y, Nakata H. Regulation of a(2a) adenosine receptor messenger-RNA expression by agonists and Forskolin in Pc12 cells. Neuroreport 1994;5:1317–20.
[26] Leon DA, Castillo CA, Albasanz JL, Martin M. Reduced expression and desensitization of adenosine A1 receptor/adenylyl cyclase pathway after chronic (−)N6-phenylisopropyladenosine intake during pregnancy. Neuroscience 2009;163:524–32.
[27] Vendite D, Sanz JM, Lopez-Alanon DM, Vacas J, Andres A, Ros M. Desensitization of adenosine A1 receptor-mediated inhibition of adenylyl cyclase in cerebellar granule cells. Neurochem Res 1998;23:211–8.
[28] Jajoo S, Mukherjea D, Kumar S, Sheth S, Kaur T, Rybak LP, et al. Role of beta-arrestin1/ERK MAP kinase pathway in regulating adenosine A1 receptor desensitization and recovery. Am J Physiol Cell Physiol 2010;298:C56–65.
[29] Kobayashi S, Millhorn DE. Stimulation of expression for the adenosine A2A receptor gene by hypoxia in PC12 cells. A potential role in cell protection. J Biol Chem 1999;274:20358–65.
[30] Dworak M, Diel P, Voss S, Hollmann W, Struder HK. Intense exercise increases adenosine concentrations in rat brain: implications for a homeostatic sleep drive. Neuroscience 2007;150:789–95.
[31] McCloskey DP, Adamo DS, Anderson BJ. Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus. Brain Res 2001;891:168–75.
[32] Vissing J, Andersen M, Diemer NH. Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 1996;16:729–36.
[33] Minor TR, Chang WC, Winslow JL. Stress and adenosine: I. Effect of methylxanthine and amphetamine stimulants on learned helplessness in rats. Behav Neurosci 1994;108:254–64.
[34] Minor TR, Huang Q, Witt AE. Cytokine-purine interactions in traumatic stress, behavioral depression, and sickness. CNS Neurol Disord Drug Targets 2006;5:547–60.
[35] Minor TR, Hunter AM. Stressor controllability and learned helplessness research in the United States: sensitization and fatigue processes. Integr Physiol Behav Sci 2002;37:44–58.
[36] Minor TR, Rowe MK, Soames Job RF, Ferguson EC. Escape deficits induced by inescapable shock and metabolic stress are reversed by adenosine receptor antagonists. Behav Brain Res 2001;120:203–12.
[37] Minor TR, Winslow JL, Chang WC. Stress and adenosine: II. Adenosine analogs mimic the effect of inescapable shock on shuttle-escape performance in rats. Behav Neurosci 1994;108:265–76.
[38] Greenwood BN, Foley TE, Day HE, Campisi J, Hammack SH, Campeau S, et al. Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. J Neurosci 2003;23:2889–98.
[39] Greenwood BN, Loughridge AB, Sadaoui N, Christianson JP, Fleshner M. The protective effects of voluntary exercise against the behavioral consequences of uncontrollable stress persist despite an increase in anxiety following forced cessation of exercise. Behav Brain Res 2012;233:314–21.
[40] Svenningsson P, Fredholm BB. Glucocorticoids regulate the expression of adenosine A1 but not A(2A) receptors in rat brain. J Pharmacol Exp Ther 1997;280:1094–101.

[41] Leon D, Albasanz JL, Fernandez M, Ruiz MA, Martin M. Down-regulation of rat brain adenosine A1 receptors at the end of pregnancy. J Neurochem 2004;88:993–1002.
[42] Khoa ND, Montesinos MC, Reiss AB, Delano D, Awadallah N, Cronstein BN. Inflammatory cytokines regulate function and expression of adenosine A(2A) receptors in human monocytic THP-1 cells. J Immunol 2001;167:4026–32.
[43] Nguyen DK, Montesinos MC, Williams AJ, Kelly M, Cronstein BN. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. J Immunol 2003;171:3991–8.
[44] Biber K, Fiebich BL, Gebicke-Harter P, van Calker D. Carbamazepine-induced upregulation of adenosine A1-receptors in astrocyte cultures affects coupling to the phosphoinositol signaling pathway. Neuropsychopharmacology 1999;20:271–8.
[45] Malek RL, Nie Z, Ramkumar V, Lee NH. Adenosine A(2A) receptor mRNA regulation by nerve growth factor is TrkA-, Src-, and Ras-dependent via extracellular regulated kinase and stress-activated protein kinase/c-Jun NH(2)-terminal kinase. J Biol Chem 1999;274:35499–504.
[46] Grden M, Podgorska M, Kocbuch K, Szutowicz A, Pawelczyk T. Expression of adenosine receptors in cardiac fibroblasts as a function of insulin and glucose level. Arch Biochem Biophys 2006;455:10–7.
[47] Arslan G, Kull B, Fredholm BB. Anoxia redistributes adenosine A(2A) receptors in PC12 cells and increases receptor-mediated formation of cAMP. Naunyn-Schmiedeberg Arch Pharmacol 2002;365:150–7.
[48] Calon F, Dridi M, Hornykiewicz O, Bedard PJ, Rajput AH, Di Paolo T. Increased adenosine A2A receptors in the brain of Parkinson's disease patients with dyskinesias. Brain 2004;127:1075–84.
[49] Murphree LJ, Sullivan GW, Marshall MA, Linden J. Lipopolysaccharide rapidly modifies adenosine receptor transcripts in murine and human macrophages: role of NF-kappaB in A(2A) adenosine receptor induction. Biochem J 2005;391:575–80.
[50] Othman T, Sinclair CJ, Haughey N, Geiger JD, Parkinson FE. Ethanol alters glutamate but not adenosine uptake in rat astrocytes: evidence for protein kinase C involvement. Neurochem Res 2002;27:289–96.
[51] Aden U, O'Connor WT, Berman RF. Changes in purine levels and adenosine receptors in kindled seizures in the rat. Neuroreport 2004;15:1585–9.
[52] Xie X, Jhaveri KA, Ding M, Hughes LF, Toth LA, Ramkumar V. Expression of striatal adenosine and dopamine receptors in mice deficient in the p50 subunit of NF-kappaB. Life Sci 2007;81:1031–41.
[53] Albasanz JL, Rodriguez A, Ferrer I, Martin M. Adenosine A2A receptors are up-regulated in Pick's disease frontal cortex. Brain Pathol 2006;16:249–55.
[54] Trincavelli ML, Costa B, Tuscano D, Lucacchini A, Martini C. Up-regulation of A(2A) adenosine receptors by proinflammatory cytokines in rat PC12 cells. Biochem Pharmacol 2002;64:625–31.
[55] Varani K, Vincenzi F, Tosi A, Gessi S, Casetta I, Granieri G, et al. A2A adenosine receptor overexpression and functionality, as well as TNF-alpha levels, correlate with motor symptoms in Parkinson's disease. FASEB J 2010;24:587–98.
[56] Cheng JT, Liu IM, Juang SW, Jou SB. Decrease of adenosine A-1 receptor gene expression in cerebral cortex of aged rats. Neurosci Lett 2000;283:227–9.
[57] Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM. Putting a spin on the dorsal–ventral divide of the striatum. Trends Neurosci 2004;27:468–74.
[58] Lex B, Hauber W. The role of dopamine in the prelimbic cortex and the dorsomedial striatum in instrumental conditioning. Cereb Cortex 2010;20:873–83.
[59] Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 2005;22:513–23.
[60] Yin HH, Knowlton BJ, Balleine BW. Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. Behav Brain Res 2006;166:189–96.
[61] Baliki MN, Mansour A, Baria AT, Huang L, Berger SE, Fields HL, et al. Parceling human accumbens into putative core and shell dissociates encoding of values for reward and pain. J Neurosci 2013;33:16383–93.
[62] Chang SE, Holland PC. Effects of nucleus accumbens core and shell lesions on autoshaped lever-pressing. Behav Brain Res 2013;256:36–42.
[63] Fischer KD, Houston AC, Rebec GV. Role of the major glutamate transporter GLT1 in nucleus accumbens core versus shell in cue-induced cocaine-seeking behavior. J Neurosci 2013;33:9319–27.
[64] Saddoris MP, Sugam JA, Cacciapaglia F, Carelli RM. Rapid dopamine dynamics in the accumbens core and shell: learning and action. Front Biosci 2013;5:273–88.
[65] van der Plasse G, Schrama R, van Seters SP, Vanderschuren LJ, Westenberg HG. Deep brain stimulation reveals a dissociation of consummatory and motivated behaviour in the medial and lateral nucleus accumbens shell of the rat. PLoS ONE 2012;7:e33455.
[66] Gerfen CR, Young 3rd WS. Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 1988;460:161–7.
[67] Schiffmann SN, Jacobs O, Vanderhaeghen JJ. Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. J Neurochem 1991;57:1062–7.
[68] Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem 1989;52:1655–8.
[69] Kravitz AV, Kreitzer AC. Striatal mechanisms underlying movement, reinforcement, and punishment. Physiology 2012;27:167–77.
[70] Kravitz AV, Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 2012;15:816–8.
[71] Lobo MK, Zaman S, Damez-Werno DM, Koo JW, Bagot RC, DiNieri JA, et al. DeltaFosB induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli. J Neurosci 2013;33:18381–95.
[72] Speaker KJ, Cox SS, Paton MM, Serebrakian A, Maslanik T, Greenwood BN, et al. Six weeks of voluntary wheel running modulates inflammatory protein (MCP-1, IL-6, and IL-10) and DAMP (Hsp72) responses to acute stress in white adipose tissue of lean rats. Brain Behav Immun 2013;39:87–98.
[73] Greenwood BN, Fleshner M. Exercise, stress resistance, and central serotonergic systems. Exerc Sport Sci Rev 2011;39:140–9.
[74] Maier SF, Watkins LR. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev 2005;29:829–41.
[75] Woodson JC, Minor TR, Job RF. Inhibition of adenosine deaminase by erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) mimics the effect of inescapable shock on escape learning in rats. Behav Neurosci 1998;112:399–409.
[76] Worden LT, Shahriari M, Farrar AM, Sink KS, Hockemeyer J, Muller CE, et al. The adenosine A2A antagonist MSX-3 reverses the effort-related effects of dopamine blockade: differential interaction with D1 and D2 family antagonists. Psychopharmacology 2009;203:489–99.
[77] Mott AM, Nunes EJ, Collins LE, Port RG, Sink KS, Hockemeyer J, et al. The adenosine A2A antagonist MSX-3 reverses the effects of the dopamine antagonist haloperidol on effort-related decision making in a T-maze cost/benefit procedure. Psychopharmacology 2009;204:103–12.
[78] Salamone JD, Farrar AM, Font L, Patel V, Schlar DE, Nunes EJ, et al. Differential actions of adenosine A1 and A2A antagonists on the effort-related effects of dopamine D2 antagonism. Behav Brain Res 2009;201:216–22.
[79] Randall PA, Nunes EJ, Janniere SL, Stopper CM, Farrar AM, Sager TN, et al. Stimulant effects of adenosine antagonists on operant behavior: differential actions of selective A2A and A1 antagonists. Psychopharmacology 2011;216:173–86.
[80] Jones-Cage C, Stratford TR, Wirtshafter D. Differential effects of the adenosine A(2)A agonist CGS-21680 and haloperidol on food-reinforced fixed ratio responding in the rat. Psychopharmacology 2012;220:205–13.
[81] Nam HW, Hinton DJ, Kang NY, Kim T, Lee MR, Oliveros A, et al. Adenosine transporter ENT1 regulates the acquisition of goal-directed behavior and ethanol drinking through A2A receptor in the dorsomedial striatum. J Neurosci 2013;33:4329–38.
[82] Shen HY, Coelho JE, Ohtsuka N, Canas PM, Day YJ, Huang QY, et al. A critical role of the adenosine A2A receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A2A receptor knock-outs. J Neurosci 2008;28:2970–5.
[83] Bastia E, Xu YH, Scibelli AC, Day YJ, Linden J, Chen JF, et al. A crucial role for forebrain adenosine A(2A) receptors in amphetamine sensitization. Neuropsychopharmacology 2005;30:891–900.
[84] Yu C, Gupta J, Chen JF, Yin HH. Genetic deletion of A2A adenosine receptors in the striatum selectively impairs habit formation. J Neurosci 2009;29:15100–3.
[85] Wei CJ, Singer P, Coelho J, Boison D, Feldon J, Yee BK, et al. Selective inactivation of adenosine A(2A) receptors in striatal neurons enhances working memory and reversal learning. Learn Mem 2011;18:459–74.
[86] Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. Int Rev Neurobiol 2005;63:191–270.
[87] Fuxe K, Agnati LF, Jacobsen K, Hillion J, Canals M, Torvinen M, et al. Receptor heteromerization in adenosine A2A receptor signaling: relevance for striatal function and Parkinson's disease. Neurology 2003;61:S19–23.
[88] Orru M, Zanoveli JM, Quiroz C, Nguyen HP, Guitart X, Ferre S. Functional changes in postsynaptic adenosine A(2A) receptors during early stages of a rat model of Huntington disease. Exp Neurol 2011;232:76–80.
[89] Cunha RA, Ferre S, Vaugeois JM, Chen JF. Potential therapeutic interest of adenosine A2A receptors in psychiatric disorders. Curr Pharm Des 2008;14:1512–24.
[90] Hauber W, Neuscheler P, Nagel J, Muller CE. Catalepsy induced by a blockade of dopamine D1 or D2 receptors was reversed by a concomitant blockade of adenosine A(2A) receptors in the caudate-putamen of rats. Eur J Neurosci 2001;14:1287–93.
[91] Wang JH, Short J, Ledent C, Lawrence AJ, van den Buuse M. Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. Behav Brain Res 2003;143:201–7.
[92] El Yacoubi M, Ledent C, Parmentier M, Bertorelli R, Ongini E, Costentin J, et al. Adenosine A2A receptor antagonists are potential antidepressants: evidence based on pharmacology and A2A receptor knockout mice. Br J Pharmacol 2001;134:68–77.
[93] Hodgson RA, Bertorelli R, Varty GB, Lachowicz JE, Forlani A, Fredduzzi S, et al. Characterization of the potent and highly selective A2A receptor antagonists preladenant and SCH 412348 [7-[2-[4-2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine] in rodent models of movement disorders and depression. J Pharmacol Exp Ther 2009;330:294–303.
[94] Hobson BD, O'Neill CE, Levis SC, Monteggia LM, Neve RL, Self DW, et al. Adenosine A1 and dopamine d1 receptor regulation of AMPA receptor phosphorylation and cocaine-seeking behavior. Neuropsychopharmacology 2013;38:1974–83.

[95] O'Neill CE, LeTendre ML, Bachtell RK. Adenosine A2A receptors in the nucleus accumbens bi-directionally alter cocaine seeking in rats. Neuropsychopharmacology 2012;37:1245–56.
[96] Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, et al. Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 1998;43:507–13.
[97] Goodwin VA, Richards SH, Taylor RS, Taylor AH, Campbell JL. The effectiveness of exercise interventions for people with Parkinson's disease: a systematic review and meta-analysis. Mov Disord 2008;23:631–40.
[98] Brown RM, Short JL, Cowen MS, Ledent C, Lawrence AJ. A differential role for the adenosine A2A receptor in opiate reinforcement vs opiate-seeking behavior. Neuropsychopharmacology 2009;34:844–56.
[99] Castane A, Wells L, Soria G, Hourani S, Ledent C, Kitchen I, et al. Behavioural and biochemical responses to morphine associated with its motivational properties are altered in adenosine A(2A) receptor knockout mice. Br J Pharmacol 2008;155:757–66.
[100] Yao L, Fan P, Jiang Z, Mailliard WS, Gordon AS, Diamond I. Addicting drugs utilize a synergistic molecular mechanism in common requiring adenosine and Gi-beta gamma dimers. Proc Natl Acad Sci U S A 2003;100:14379–84.
[101] Castane A, Soria G, Ledent C, Maldonado R, Valverde O. Attenuation of nicotine-induced rewarding effects in A2A knockout mice. Neuropharmacology 2006;51:631–40.
[102] Soria G, Castane A, Berrendero F, Ledent C, Parmentier M, Maldonado R, et al. Adenosine A2A receptors are involved in physical dependence and place conditioning induced by THC. Eur J Neurosci 2004;20:2203–13.
[103] Crema LM, Pettenuzzo LF, Schlabitz M, Diehl L, Hoppe J, Mestriner R, et al. The effect of unpredictable chronic mild stress on depressive-like behavior and on hippocampal A1 and striatal A2A adenosine receptors. Physiol Behav 2013;109:1–7.
[104] Brito R, Pereira MR, Paes-de-Carvalho R, Calaza Kda C. Expression of A1 adenosine receptors in the developing avian retina: in vivo modulation by A(2A) receptors and endogenous adenosine. J Neurochem 2012;123:239–49.
[105] Hettinger BD, Leid M, Murray TF. Cyclopentyladenosine-induced homologous down-regulation of A1 adenosine receptors (A1AR) in intact neurons is accompanied by receptor sequestration but not a reduction in A1AR mRNA expression or G protein alpha-subunit content. J Neurochem 1998;71:221–30.
[106] Collins S, Caron MG, Lefkowitz RJ. From ligand binding to gene expression: new insights into the regulation of G-protein-coupled receptors. Trends Biochem Sci 1992;17:37–9.
[107] Costa F, Diedrich A, Johnson B, Sulur P, Farley G, Biaggioni I. Adenosine, a metabolic trigger of the exercise pressor reflex in humans. Hypertension 2001;37:917–22.
[108] Simpson RE, Phillis JW. Adenosine in exercise adaptation. Br J Sports Med 1992;26:54–8.
[109] Wojciechowska F, Karon H, Blawack M. The effect of short-lasting intensive physical exercise on ATP content in the rat muscles and liver. Acta Physiol Pol 1975;26:313–6.
[110] Shi LH, Luo F, Woodward DJ, Chang JY. Neural responses in multiple basal ganglia regions during spontaneous and treadmill locomotion tasks in rats. Exp Brain Res 2004;157:303–14.
[111] Anderson BJ, Greenwood SJ, McCloskey D. Exercise as an intervention for the age-related decline in neural metabolic support. Front Aging Neurosci 2010;2.
[112] Liste I, Guerra MJ, Caruncho HJ, Labandeira-Garcia JL. Treadmill running induces striatal Fos expression via NMDA glutamate and dopamine receptors. Exp Brain Res 1997;115:458–68.
[113] Huston JP, Haas HL, Boix F, Pfister M, Decking U, Schrader J, et al. Extracellular adenosine levels in neostriatum and hippocampus during rest and activity periods of rats. Neuroscience 1996;73:99–107.
[114] Pardridge WM, Yoshikawa T, Kang YS, Miller LP. Blood–brain barrier transport and brain metabolism of adenosine and adenosine analogs. J Pharmacol Exp Ther 1994;268:14–8.
[115] Cornford EM, Oldendorf WH. Independent blood–brain barrier transport systems for nucleic acid precursors. Biochim Biophys Acta 1975;394:211–9.
[116] Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, et al. Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. Neuroscience 2005;135:1295–307.
[117] Biber K, Lubrich B, Fiebich BL, Boddeke HW, van Calker D. Interleukin-6 enhances expression of adenosine A(1) receptor mRNA and signaling in cultured rat cortical astrocytes and brain slices. Neuropsychopharmacology 2001;24:86–96.
[118] Biber K, Pinto-Duarte A, Wittendorp MC, Dolga AM, Fernandes CC, Von Frijtag Drabbe Kunzel J, et al. Interleukin-6 upregulates neuronal adenosine A1 receptors: implications for neuromodulation and neuroprotection. Neuropsychopharmacology 2008;33:2237–50.
[119] Campeau S, Nyhuis TJ, Kryskow EM, Masini CV, Babb JA, Sasse SK, et al. Stress rapidly increases alpha 1d adrenergic receptor mRNA in the rat dentate gyrus. Brain Res 2010;1323:109–18.
[120] Fenu S, Pinna A, Ongini E, Morelli M. Adenosine A2A receptor antagonism potentiates L-DOPA-induced turning behaviour and c-fos expression in 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 1997;321:143–7.
[121] Ferre S, von Euler G, Johansson B, Fredholm BB, Fuxe K. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci U S A 1991;88:7238–41.
[122] Gilliam PE, Spirduso WW, Martin TP, Walters TJ, Wilcox RE, Farrar RP. The effects of exercise training on [3H]-spiperone binding in rat striatum. Pharmacol Biochem Behav 1984;20:863–7.
[123] MacRae PG, Spirduso WW, Walters TJ, Farrar RP, Wilcox RE. Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolites in presenescent older rats. Psychopharmacology 1987;92:236–40.
[124] MacRae PG, Spirduso WW, Cartee GD, Farrar RP, Wilcox RE. Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolite levels. Neurosci Lett 1987;79:138–44.
[125] Ferre S, Torvinen M, Antoniou K, Irenius E, Civelli O, Arenas E, et al. Adenosine A1 receptor-mediated modulation of dopamine D1 receptors in stably cotransfected fibroblast cells. J Biol Chem 1998;273:4718–24.
[126] Gines S, Hillion J, Torvinen M, Le Crom S, Casado V, Canela EI, et al. Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. Proc Natl Acad Sci U S A 2000;97:8606–11.
[127] Dixon AK, Gubitz AK, Sirinathsinghji DJ, Richardson PJ, Freeman TC. Tissue distribution of adenosine receptor mRNAs in the rat. Br J Pharmacol 1996;118:1461–8.
[128] Oorschot DE. The percentage of interneurons in the dorsal striatum of the rat, cat, monkey and human. A critique of the evidence. Basal Ganglia 2013;3:19–24.
[129] Kelly E, Bailey CP, Henderson G. Agonist-selective mechanisms of GPCR desensitization. Brit J Pharmaco 2008;153(Suppl. 1):S379–88.
[130] Hanyaloglu AC, von Zastrow M. Regulation of GPCRs by endocytic membrane trafficking and its potential implications. Annu Rev Pharmacol Toxicol 2008;48:537–68.
[131] Marchese A, Paing MM, Temple BR, Trejo J. G protein-coupled receptor sorting to endosomes and lysosomes. Annu Rev Pharmacol Toxicol 2008;48:601–29.
[132] Hikida T, Kimura K, Wada N, Funabiki K, Nakanishi S. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. Neuron 2010;66:896–907.