Neurobiology of Disease

Voluntary Exercise Boosts Striatal Dopamine Release: Evidence for the Necessary and Sufficient Role of BDNF

Guendalina Bastioli,[1] Jennifer C. Arnold,[2] Maria Mancini,[1] Adam C. Mar,[1] Begoña Gamallo-Lana,[1] Khalil Saadipour,[3] Moses V. Chao,[3] and Margaret E. Rice[1,2]

[1]Departments of Neuroscience and Physiology and, [2]Neurosurgery, New York University Grossman School of Medicine, New York, New York 10016, and [3]Skirball Institute of Biomolecular Medicine, New York University Grossman School of Medicine, New York, New York 10016

Physical exercise improves motor performance in individuals with Parkinson's disease and elevates mood in those with depression. Although underlying factors have not been identified, clues arise from previous studies showing a link between cognitive benefits of exercise and increases in brain-derived neurotrophic factor (BDNF). Here, we investigated the influence of voluntary wheel-running exercise on BDNF levels in the striatum of young male wild-type (WT) mice, and on the striatal release of a key motor-system transmitter, dopamine (DA). Mice were allowed unlimited access to a freely rotating wheel (runners) or a locked wheel (controls) for 30 d. Electrically evoked DA release was quantified in *ex vivo* corticostriatal slices from these animals using fast-scan cyclic voltammetry. We found that exercise increased BDNF levels in dorsal striatum (dStr) and increased DA release in dStr and in nucleus accumbens core and shell. Increased DA release was independent of striatal acetylcholine (ACh), and persisted after a week of rest. We tested a role for BDNF in the influence of exercise on DA release using mice that were heterozygous for BDNF deletion ($BDNF^{+/-}$). In contrast to WT mice, evoked DA release did not differ between $BDNF^{+/-}$ runners and controls. Complementary pharmacological studies using a tropomyosin receptor kinase B (TrkB) agonist in WT mouse slices showed that TrkB receptor activation also increased evoked DA release throughout striatum in an ACh-independent manner. Together, these data support a causal role for BDNF in exercise-enhanced striatal DA release and provide mechanistic insight into the beneficial effects of exercise in neuropsychiatric disorders, including Parkinson's, depression, and anxiety.

Key words: **brain slices; fast-scan cyclic voltammetry; nAChRs; nucleus accumbens; Parkinson's disease; running wheel**

Significance Statement

Exercise has been shown to improve movement and cognition in humans and rodents. Here, we report that voluntary exercise for 30 d leads to an increase in evoked DA release throughout the striatum and an increase in BDNF in the dorsal (motor) striatum. The increase in DA release appears to require BDNF, indicated by the absence of DA release enhancement with running in $BDNF^{+/-}$ mice. Activation of BDNF receptors using a pharmacological agonist was also shown to boost DA release. Together, these data support a necessary and sufficient role for BDNF in exercise-enhanced DA release and provide mechanistic insight into the reported benefits of exercise in individuals with dopamine-linked neuropsychiatric disorders, including Parkinson's disease and depression.

Introduction

The basal ganglia are a group of subcortical nuclei in which the neurotransmitter dopamine (DA) plays key roles in movement, motor learning, reward, motivation, and mood (Schultz, 1998; Cagniard et al., 2006; Nestler and Carlezon, 2006; Calabresi et al., 2007; Duman et al., 2008; Palmiter, 2008; Smith and Villalba, 2008; Gerfen and Surmeier, 2011; Surmeier et al., 2011; Tye et al., 2013; Howe and Dombeck, 2016; Sulzer et al., 2016; Schultz et al. 2017; Athalye et al., 2018). The pivotal role of DA in movement is highlighted by the motor deficits that accompany DA neuron loss in Parkinson's disease (PD; Albin et al., 1989; Carlsson, 2002; Carta and Bezard, 2011; Wichmann and Dostrovsky, 2011). Motor striatum receives dense DA input from midbrain DA neurons in the substantia nigra pars compacta (SNc), with the axonal arbor of one SNc DA neuron providing DA to an area up to 6% of striatal volume (Matsuda et al., 2009). Axonal DA release in striatum and

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Correspondence should be addressed to Margaret E. Rice at margaret.rice@nyu.edu.

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somatodendritic DA in SNc are regulated dynamically and locally (Rice et al., 2011; Sulzer et al., 2016). The most striking local regulator of striatal DA release is acetylcholine (ACh) from cholinergic interneurons (ChIs), which can drive DA release via nicotinic ACh receptors (nAChRs) on DA axons (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Cachope et al., 2012; Patel et al., 2012; Threlfell et al., 2012).

Other factors that influence striatal DA release include insulin (Patel et al., 2012; Stouffer et al., 2015) and brain-derived neurotrophic factor (BDNF; Dluzen et al., 2001; Goggi et al., 2002; Bosse et al., 2012; Apawu et al., 2013). Insulin enters the brain from the periphery and enhances DA indirectly by activating ChIs (Stouffer et al., 2015). In contrast, striatal BDNF arises from glutamatergic corticostriatal afferents and projections from midbrain DA neurons (Altar et al., 1997; Conner et al., 1997; Kolbeck et al., 1999). Notably, both corticostriatal and nigrostriatal BDNF transport can be disrupted by the PD-related protein α-synuclein (Miller et al., 2021). In the brain, BDNF acts primarily via tropomyosin receptor kinase B (TrkB) receptors (Lu, 2004; Lu et al., 2013; Park and Poo, 2013). Whether BDNF acts directly on striatal DA axons or indirectly via ChIs has not been addressed. Consistent with the roles of BDNF and other neurotrophins in neuronal survival and synaptic plasticity, increasing evidence implicates these factors in benefits of physical exercise on cognition and memory (van Praag et al., 1999; Cotman et al., 2007; Kobilo et al., 2011; Voss et al., 2013; Sleiman and Chao, 2015; Svensson et al., 2015; Sleiman et al., 2016; Vivar and van Praag, 2017; Choi et al., 2018). Exercise has been shown to slow DA neurodegeneration in toxin models of PD (Gerecke et al., 2010; Zhou et al., 2017), ameliorate motor deficits in PD patients (Fontanesi et al., 2016; Mak et al., 2017; Zhou et al., 2017; Ahlskog, 2018), and improve symptoms of depression and anxiety (Salmon, 2001; Duman et al., 2008). Although underlying mechanisms have not been identified, motor improvements in PD patients are correlated with increases in serum BDNF levels and peripheral BDNF-TrkB signaling (Zoladz et al., 2014; Fontanesi et al., 2016; O'Callaghan et al., 2020).

Based on this body of evidence, we tested the hypothesis that exercise enhances striatal DA release in a BDNF-dependent manner. We found that voluntary wheel-running exercise boosts stimulated DA release in dorsal striatum (dStr) and in nucleus accumbens (NAc) core and shell in *ex vivo* striatal slices from male mice allowed free access to a running wheel, compared with controls housed with a locked wheel. We found that enhanced DA release is independent of ACh and show that BDNF plays a necessary role in this process in dStr and NAc core. Complementary pharmacological experiments showed that TrkB receptor activation enhances DA release throughout the striatum, involving phospholipase C (PLC) in dStr and NAc core and shell and also phosphoinositide 3-kinase (PI3K) in NAc shell. These studies provide insight into the beneficial effects of exercise that could be harnessed for therapeutic approaches for PD and other neuropsychiatric disorders.

Materials and Methods

Animal handling. Studies were conducted using male C57BL6/J mice or heterozygous BDNF knock-out ($BDNF^{+/-}$) mice (Ernfors et al., 1994) on a C57BL6/J background (stock #002266, The Jackson Laboratory); mice were generated by crossing wild-type (WT) females with $BDNF^{+/-}$ males. Animal procedures were in accordance with the National Institutes of Health guidelines and approved by the New York University School of Medicine Animal Care and Use Committee.

Running paradigm. For studies involving voluntary wheel running, WT mice were 4 weeks old on arrival, and 10–12 weeks old by the end of each study; $BDNF^{+/-}$ mice were 8 weeks old on arrival. Cohorts included 12 mice, six runners and six controls, unless noted otherwise. Diurnal running pattern, daily running distance, body weight, and food consumption data were pooled from multiple cohorts. On arrival, mice were housed individually with food and water *ad libitum* with a modified 12 h reverse light/dark cycle with lights off from 10:00 A.M. to 10:00 P.M. local time.

After 14 d of acclimation to the modified light/dark cycle (Fig. 1*A*), mice were randomly assigned at staggered intervals to either runner (freely rotating wheel) or control (locked wheel) groups and given unlimited, voluntary access to the wheel and to food and water for 30 d (van Praag et al., 1999; Sleiman et al., 2016; Fig. 1*A*). Both groups were housed continuously in these cages, except when they were removed briefly for weekly weighing. Running distance (as number of revolutions) across the diurnal cycle was monitored using custom-designed software. Body weight and food consumption for each subject were assessed weekly.

Ex vivo *slice preparation for DA and BDNF tissue contents and for DA release.* For slice preparation, mice were anaesthetized with isoflurane, then the brain removed and place into ice-cold HEPES-buffered artificial cerebrospinal fluid (aCSF) containing the following (in mM): 120 NaCl, 20 $NaHCO_3$, 10 glucose, 6.7 HEPES acid, 5 KCl, 3.3 HEPES sodium salt, 2 $CaCl_2$, and 2 $MgSO_4$, equilibrated with 95% O_2/5% CO_2 (Rice and Cragg, 2004). Coronal corticostriatal slices (300 µm) were cut in this same solution using a Leica VT1200S vibrating blade microtome (Leica Microsystems). For studies of evoked DA release, slices were maintained in HEPES-buffered aCSF at room temperature for 1 h before experimentation.

High-performance liquid chromatography for DA tissue content. On the last day of the wheel-running regimen (day 30), mice were anesthetized with isoflurane, the brains removed, and corticostriatal slices (300 µm) were cut using a Leica VT1200S. Tissue samples were dissected from the dStr and ventral striatum (vStr) of one hemisphere. Individual samples were weighed (1–5 mg), frozen immediately on dry ice, then stored at −80°C until analysis. Tissue content of DA and its metabolite dihydroxyphenylacetic acid (DOPAC) were determined using high-performance liquid chromatography (HPLC) with electrochemical detection, as described previously (Stouffer et al., 2015). On the day of analysis, samples were diluted 1:10 with ice-cold mobile phase that was deoxygenated with argon, sonicated, and spun for 2 min at 13,000 × *g* before direct injection into the HPLC.

Western blotting. For BDNF analysis, dStr and vStr were dissected from the second hemisphere of brains sliced for HPLC analysis of DA tissue content. Samples from each region were pooled for each mouse, frozen on dry ice, and stored at −80°C until processing. Levels of BDNF were determined using methods described previously (Sleiman et al., 2016). A total of 70 µg protein was loaded on 16.5% Tris-tricine gels. Membranes were washed with 1× phosphate-buffered saline, blocked with 3% BSA/TBS-T for 1 h, and incubated with anti-BDNF (Icosagen) and anti-β-Actin (Sigma-Aldrich) overnight at 4°C. Antibodies were diluted 1:500. A peroxidase conjugated goat anti-mouse antibody (1:5000 dilution) was used for detection of both proteins. The presence of proteins on the membranes was detected by chemiluminescence. Antibody specificity was indicated by staining only at the bands corresponding to the molecular weight of the protein examined.

Fast-scan cyclic voltammetry for monitoring evoked DA release. All DA release experiments were conducted in a submersion recording chamber maintained at 32°C and with aCSF flowing at 1.5 mL/min and containing the following (in mM): 124 NaCl, 3.7 KCl, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.3 KH_2PO_4, and 10 glucose, equilibrated with 95% O_2/5% CO_2. Slices were allowed to equilibrate in this environment for 30 min before recording was initiated (Rice and Cragg, 2004; Patel et al., 2012; Stouffer et al., 2015). Evoked DA release was monitored in striatal slices using fast-scan cyclic voltammetry (FSCV) with carbon-fiber microelectrodes that were constructed in house from 7 µm diameter carbon fibers (Goodfellow) in pulled glass capillaries, with the fiber extending 30–75 µm beyond the glass insulation and electrical contact made using Woods metal, as described previously (Patel and Rice, 2013). A

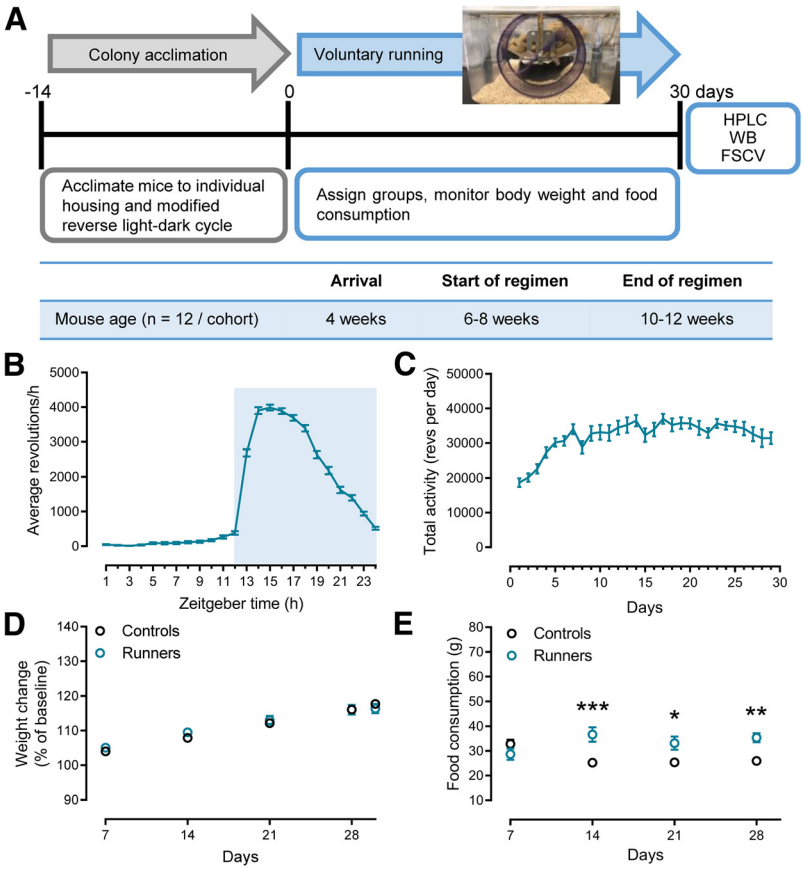

Figure 1. Voluntary wheel running. ***A***, Timeline for voluntary wheel-running protocol; for each study, 12 randomly assigned mice were housed individually with either a freely rotating wheel (runners; $n = 6$) or a locked wheel (controls; $n = 6$). After each 30 d study, brain tissue was collected for HPLC analysis of DA and DOPAC tissue content, Western blotting (WB) for BDNF expression, or FSCV for evaluation of evoked DA release. ***B***, Average time course of wheel running activity for three cohorts of 6 mice each ($n = 18$ runners) showing diurnal variation, with greater activity in the dark phase (shaded light blue) than in the light. ***C***, Average total running per day ($n = 18$ runners). ***D***, Change in body weight monitored weekly, with the first day of housing with a wheel taken as baseline 100%, shows a comparable increase in weight over the experimental period between runners and controls ($p < 0.0001$ initial *vs.* final weight, $n = 18$ mice per group, unpaired *t* test). ***E***, Average weekly food consumption for three cohorts assessed weekly (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ runners vs controls; $n = 18$ per group in study; 2-way ANOVA, Bonferroni *post hoc* test).

Millar voltammeter was used for FSCV; scan rate was 800 V/s and scan range was −700 mV to +1300 mV then back to −700 mV versus a Ag/AgCl reference electrode; no potential was applied to the electrode between scans, minimizing DA adsorption. Scans were initiated immediately after the electrode was positioned in superfusing aCSF in the recording chamber and were repeated continuously at 100 ms intervals (controlled by a Master-8 timing circuit, AMPI) throughout the experiment. No conditioning treatment was used.

Increases in extracellular DA concentration ($[DA]_o$) were evoked using local electrical stimulation (0.1 ms duration pulses). Single-pulse stimulation was used in dStr (dorsolateral portion) and in the NAc core, and a five-pulse stimulus (100 Hz) in NAc shell to increase the reliability of DA release detection in that region (Stouffer et al., 2015). The substance detected in each region was identified as DA by the characteristic oxidation and reduction peaks of recorded voltammograms. In *ex vivo* striatal slices, there is no ambiguity that DA is the electroactive substance detected in all three striatal subregions (Patel and Rice, 2013). To evaluate the influence of wheel-running exercise on DA release, evoked $[DA]_o$ in dStr and NAc core and shell in aCSF was recorded from three to five sites per region in each of typically two slices from runners and controls. We use this multisite sampling protocol because evoked $[DA]_o$ in the striatum shows site-to-site variability that exceeds average differences between slices; sampling multiple sites across slices minimizes sampling bias from this variability within a single slice (Li et al., 2010; Patel et al., 2012; Karayannis et al., 2014; Stouffer et al., 2015; Patel et al., 2019; Longo et al., 2021). Average evoked $[DA]_o$ in control slices was 1.2 ± 0.1 μM for dStr ($n = 79$ sites from 12 mice), 0.9 ± 0.1 μM for NAc core ($n = 77$ sites from 12 mice), and 0.7 ± 0.1 μM for NAc shell ($n = 60$ sites from 12 mice), consistent with previous data from these regions in mouse striatal slices (Patel et al., 2019). Initial sampling of evoked $[DA]_o$ was completed in 20–30 min. Immediately afterward, superfusion with aCSF was either continued or the medium changed to aCSF plus a nAChR antagonist dihydro-β-erythroidine (DHβE; 1 μM) for 20 min, then recording of evoked release was repeated (Stouffer et al., 2015; Patel et al., 2019). For pharmacological studies of the effect of a TrkB agonist, LM22A-4 (1 μM), on evoked DA release, evoked $[DA]_o$ was recorded from three to five sites per region in each of two slices per mouse, three mice per condition. In initial experiments, we compared evoked $[DA]_o$ in dStr and NAc core and shell after superfusion of LM22A-4 for 1 h or 2 h with time-matched sampling in aCSF alone. Subsequently, peak evoked $[DA]_o$ after 2 h in LM22A-4 was compared with time-matched superfusion of LM22A-4 plus DHβE (1 μM), LM22A-4 plus a PI3K inhibitor, LY294002 (1 μM), or LM22A-4 plus a PLC inhibitor, U73122 (1 μM). Evoked increases in $[DA]_o$ were quantified by postexperiment calibration of the electrode in the recording chamber at 32°C. Each electrode was repositioned near the chamber inlet without exposing the tip to air, and the current was recorded during superfusion of DA (1 μM) in aCSF alone then in each drug solution tested on a given day.

Statistical analysis. Data are given as means ± SEM; normality of each dataset was assessed using D'Agostino–Pearson's test ($\alpha = 0.05$ for normality). Significance then was assessed using an unpaired Student's *t* test (parametric), Mann–Whitney *U* test (nonparametric), or two-way ANOVA with Bonferroni *post hoc* tests using Prism 8.0 (GraphPad) software. For FSCV data, *n* is the number of recording sites, given that site-to-site variability within a striatal subregion is greater than interanimal or interslice variability (Rice and Cragg, 2004; Patel et al., 2012; Stouffer et al., 2015). For pharmacological experiments, control groups were time matched with the experimental groups to evaluate drug efficacy.

Results

Effect of wheel-running exercise on DA tissue content and BDNF levels in wild-type mice

We investigated consequences of exercise in DAergic motor and reward centers of the striatum from mice that experienced voluntary wheel running for 30 d (runners) and from nonexercised mice (controls) that were housed with a fixed running wheel (Fig. 1*A*). When allowed free access to a running wheel, mice ran primarily during the dark phase, which began at 12:00 Zeitgeber time (Fig. 1*B*). Runners showed an increase in total revolutions per day over the first few days, then reached a plateau of ~32,000 total revolutions per day after ~6 d (Fig. 1*C*). The averaged diurnal pattern of running (Fig. 1*B*) and the pattern over the 30 d of the experiment (Fig. 1*C*) were consistent across three cohorts (six runners/cohort) tested ($p > 0.05$, two-way ANOVA, Bonferroni *post hoc* test, for diurnal running pattern; $p > 0.05$ for

pattern across days, two-way ANOVA, Bonferroni *post hoc* test), so that running data were pooled (Fig. 1*B*,*C*). Body weight and food consumption data from these three cohorts were also pooled (Fig. 1*D*,*E*). Weekly assessment of body weight showed a comparable increase in weight over the experimental period in runners and controls, with an overall increase from starting weight (~25 g) of 16.0 ± 1.4% for runners and 16.1 ± 0.8% for controls by day 28 ($p < 0.0001$, $n = 18$ mice per group, unpaired *t* test; Fig. 1*D*). We also measured weekly food consumption of controls and runners over the time course of the experiment and found significantly greater food consumption by runners versus controls at day 14 ($p = 0.0005$, $n = 18$ mice per group), day 21 ($p = 0.031$), and day 28 ($p = 0.005$; two-way ANOVA, Bonferroni *post hoc* test; Fig. 1*E*).

The first cohort, six runners and six controls, was used to assess the effect of exercise on striatal DA and BDNF levels at the end of the 30 d running paradigm. We found no difference in DA tissue content between runners and controls in either dStr ($p = 0.75$, $n = 26$ samples per group; unpaired *t* test) or in vStr ($p = 0.23$; $n = 26$ samples per group, unpaired *t* test; Fig. 2*A*,*B*). Similarly, there was no difference in DOPAC tissue content between runners and controls in dStr ($p = 0.64$, $n = 24$–26 samples per group, unpaired *t* test) or vStr ($p = 0.88$, $n = 25$–29 samples per group, unpaired *t* test; Fig. 2*C*,*D*). However, when we examined BDNF expression in the same cohort, we found increased BDNF expression in dStr in runners versus controls ($p = 0.018$, $n = 6$ mice per group; unpaired *t* test), but not in vStr ($p = 0.27$, $n = 6$ mice per group, unpaired *t* test; Fig. 2*E*,*F*).

Wheel-running exercise increases evoked $[DA]_o$ in an ACh-independent manner and persists after 7 d of rest

In a separate cohort, we assessed the influence of exercise dynamic DA release in striatum using multisite sampling of locally evoked $[DA]_o$ in dorsolateral dStr, NAc core, and NAc shell in each corticostriatal slice (Fig. 3*A*). Detection of DA was confirmed by its characteristic voltammetric signature, with oxidation and reduction peaks in each region that matched those of DA in calibration solutions (Fig. 3*A*). Despite the lack of change in DA tissue content with running, evoked $[DA]_o$ increased significantly in all striatal subregions from runners compared with controls, with an enhancement of ~40% in dStr, ~35% in NAc core, and ~30% in NAc shell (dStr, $p = 0.0001$ vs controls, $n = 79$–103 sites from 12 mice per group, unpaired *U* test: NAc core, $p = 0.0023$, $n = 77$–100 sites from 12 mice per group, unpaired *U* test; NAc shell, $p = 0.0016$; $n = 60$ sites from 12 mice per group, unpaired *t* test; Fig. 3*B*–*D*). As noted in the Introduction, striatal DA release evoked by local electrical stimulation can be triggered by ACh that is concurrently released with local electrical stimulation (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004). We therefore tested possible nAChR dependence in dStr and NAc using a nAChR antagonist, DHβE (1 μM; Rice and Cragg, 2004); NAc shell was not examined because of the potentially confounding increase in pulse-train-evoked $[DA]_o$ when nAChRs are blocked (Patel et al., 2012). Notably, the difference between runners and controls remained when nAChRs were antagonized by DHβE, with significantly higher evoked $[DA]_o$ in runners versus controls in both dStr and NAc core (dStr, $p = 0.0157$, $n = 29$–42 sites from six mice per group; NAc core, $p = 0.0062$, $n = 33$–35 sites from six mice per group, unpaired *U* tests; Fig. 3*E*,*F*). These data indicate that exercise-enhanced evoked $[DA]_o$ in runners versus controls is independent of cholinergic involvement and therefore reflects a primarily cell-autonomous effect on axonal DA release.

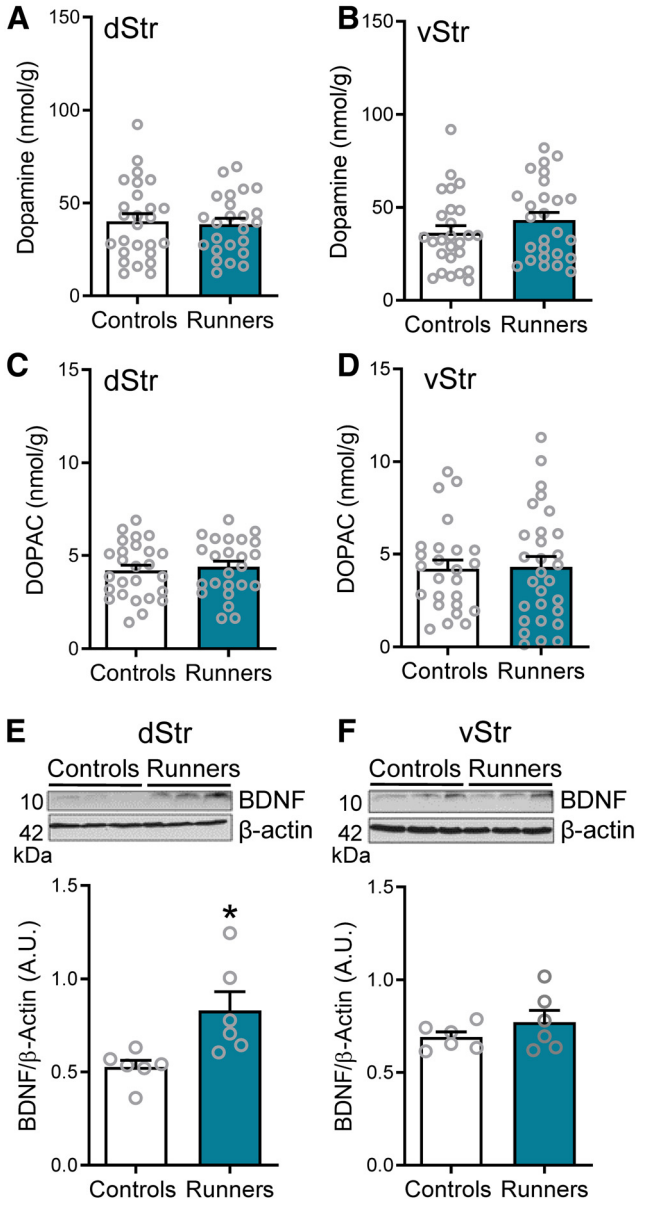

Figure 2. DA and DOPAC tissue contents and BDNF expression in dStr and vStr after 30 d of voluntary wheel running. ***A***, ***B***, Tissue content of DA in dStr and vStr did not differ between runners and controls ($n = 26$ samples from 6 mice per group; unpaired *t* test). ***C***, ***D***, Tissue content of the DA metabolite DOPAC did not differ between runners and controls in either dStr or vStr ($n = 24$–29 samples from 6 mice per group; unpaired *t* test). ***E***, ***F***, BDNF expression in dStr and vStr after 30 d wheel running; quantitative data were normalized to β-actin (*$p < 0.05$ runners vs controls; $n = 6$ mice per group, 1 sample per mouse; unpaired *t* test).

We then assessed whether exercise-enhanced DA release was enduring by examining evoked $[DA]_o$ in striatal slices from runners and controls housed with locked wheels for an additional 7 d beyond the usual 30 d running period (Fig. 4*A*). We found that evoked $[DA]_o$ remained elevated in the dStr and NAc core of runners versus controls after 7 d of rest (dStr, $p = 0.0003$, $n = 70$–80 sites from four mice per group; NAc core, $p = 0.0064$, $n = 70$–80 sites from four mice per group; unpaired *U* tests; Fig. 4*B*,*C*). The difference between the groups was lost in NAc shell, however ($p = 0.3601$, $n = 40$ sites from four mice per group, unpaired *U* test; Fig. 4*D*). Evoked $[DA]_o$ in the dStr and NAc core of runners versus controls persisted in the presence of the nAChR antagonist, DHβE (dStr, $p = 0.0051$, $n = 70$–79 sites

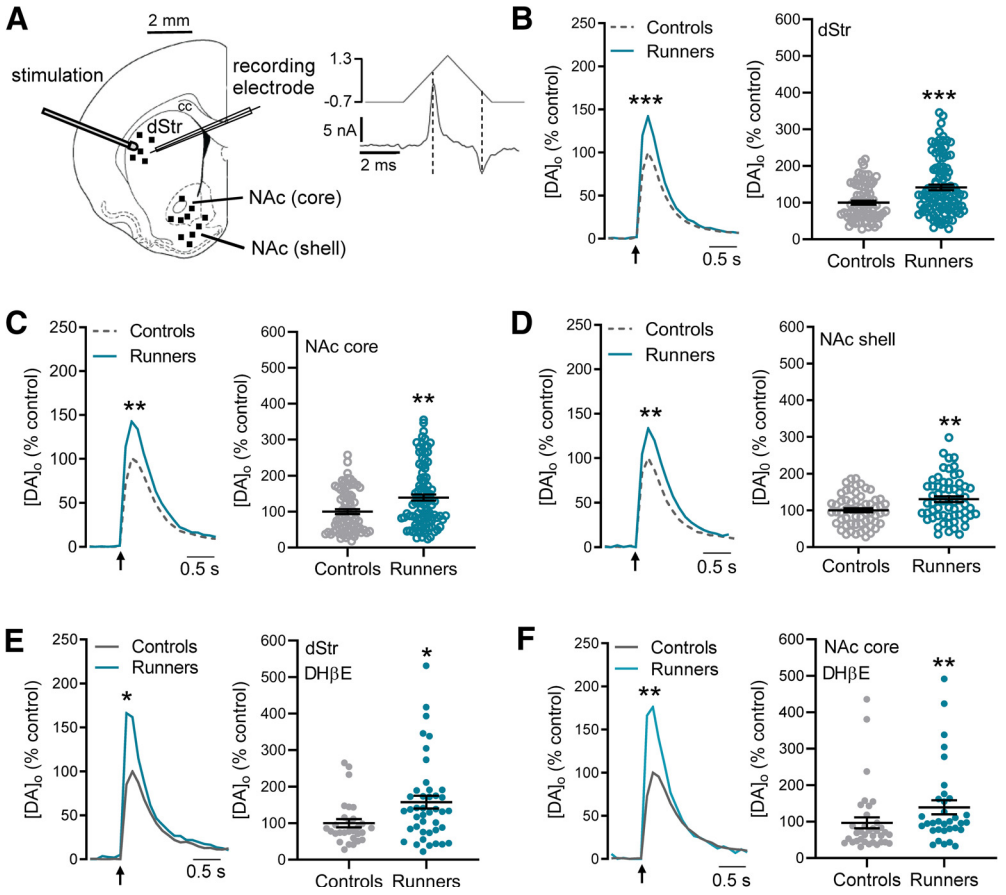

Figure 3. Increased evoked $[DA]_o$ in dStr, NAc core and NAc shell in *ex vivo* striatal slices after 30 d of voluntary wheel running. ***A***, Left, Coronal section of mouse brain showing typical level of forebrain slices used to study axonal DA release (modified from Franklin and Paxinos, 2008). At this level, local electrical stimulation can be used to evoke DA release in dorsolateral dStr and in the NAc core and shell in the same slice. Right, Representative voltammogram recorded in the dStr following local, single-pulse stimulation (1 pulse), showing characteristic DA oxidation (+0.61 vs AgAgCl) and reduction (−0.24 V vs Ag/AgCl) peak potentials; similar voltammograms were obtained in NAc core and shell. ***B–D***, Left, Average evoked increases in $[DA]_o$ in dStr, NAc core (single-pulse stimulation) and NAc shell (5 pulse, 100 Hz) in *ex vivo* slices from runners and controls, normalized to mean peak $[DA]_o$ for each region in controls (error bars omitted); arrow indicates time of stimulation. Right, Data summary for evoked $[DA]_o$ in each region for runners versus controls (n = 60–103 sites per region, 2 slices per mouse, 12 mice per group; unpaired U or t tests). ***E***, ***F***, Left, Average evoked increases in $[DA]_o$ in dStr and NAc core (single-pulse stimulation) in the same slices examined in ***B–D*** after superfusion of DHβE (1 μM), a nAChR antagonist. Data are normalized to mean peak evoked $[DA]_o$ in DHβE for each region in controls. Right, Data summary; evoked $[DA]_o$, dStr, and NAc core in the presence of DHβE (n = 29–42 sites per region, 2 slices per mouse, 6 mice per group; unpaired U tests). ***B–F***, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

from four mice per group; NAc core, $p = 0.0386$, n = 68–80 sites from four mice per group; unpaired one-tailed U test; Fig. 4*F*). These data show that the influence of exercise on DA release can be maintained beyond the exercise period.

Absence of effect of wheel-running exercise on evoked $[DA]_o$ in $BDNF^{+/-}$ mice

To test a possible causal role of BDNF in exercise-induced amplification of DA release, we examined heterozygous BDNF knockout ($BDNF^{+/-}$) mice in our 30 d voluntary wheel-running paradigm (Figs. 5, 6). As seen with WT mice, $BDNF^{+/-}$ mice were active in the dark phase, although with a somewhat different pattern of activity than seen in WT mice (Fig. 5*A*). Compared with WT mice, $BDNF^{+/-}$ mice ran less during the first 7 h of the dark cycle ($p = 0.0001$, n = 6 $BDNF^{+/-}$ mice, n = 18 WT; 2-way ANOVA, Bonferroni *post hoc* test) but ran more later in the dark phase (last 4 h, $p < 0.001$, 2-way ANOVA Bonferroni *post hoc* test; Fig. 5*A*). Overall, however, there was no difference in the number of revolutions per day across the 30 d paradigm between $BDNF^{+/-}$ (six mice) and WT mice (18 mice; $p > 0.99$, two-way ANOVA Bonferroni *post hoc* test; Fig. 5*B*). These findings are consistent with previous behavioral studies showing a lack of difference in spontaneous locomotor activity between young $BDNF^{+/-}$ mice and WT mice (Dluzen et al., 2001; Bosse et al., 2012).

Initial body weight for $BDNF^{+/-}$ mice was ~27 g. This was slightly higher than that for WT, but consistent with the slightly older age of the $BDNF^{+/-}$ mice, as well as previous observations that $BDNF^{+/-}$ mice weigh more than WT as they age (Horger et al., 1999; Boger et al., 2011). Neither body weight (Fig. 5*C*) nor food consumption (Fig. 5*D*) differed between $BDNF^{+/-}$ runner and control groups. In contrast to WT mice (Fig. 1*D*), neither control nor runner $BDNF^{+/-}$ mice showed an increase of body weight over the running period ($p > 0.05$, n = 6 mice per group, unpaired t test). As expected, BDNF expression in $BDNF^{+/-}$ mice was ~50% less than that in WT mice (compare Figs. 2*E*,*F*, 5*E*,*F*). Evaluation of BDNF expression in $BDNF^{+/-}$ mice revealed no difference between runners and controls in dStr ($p = 0.575$, n = 6 mice per group; unpaired t test; Fig. 5*E*) or in vStr ($p = 0.95$, n = 6 mice per group, unpaired t test; Fig. 5*F*).

After this characterization, we used FSCV to evaluate evoked $[DA]_o$ in $BDNF^{+/-}$ runners and controls. Previous studies showed robust depolarization-induced DA release in the dStr

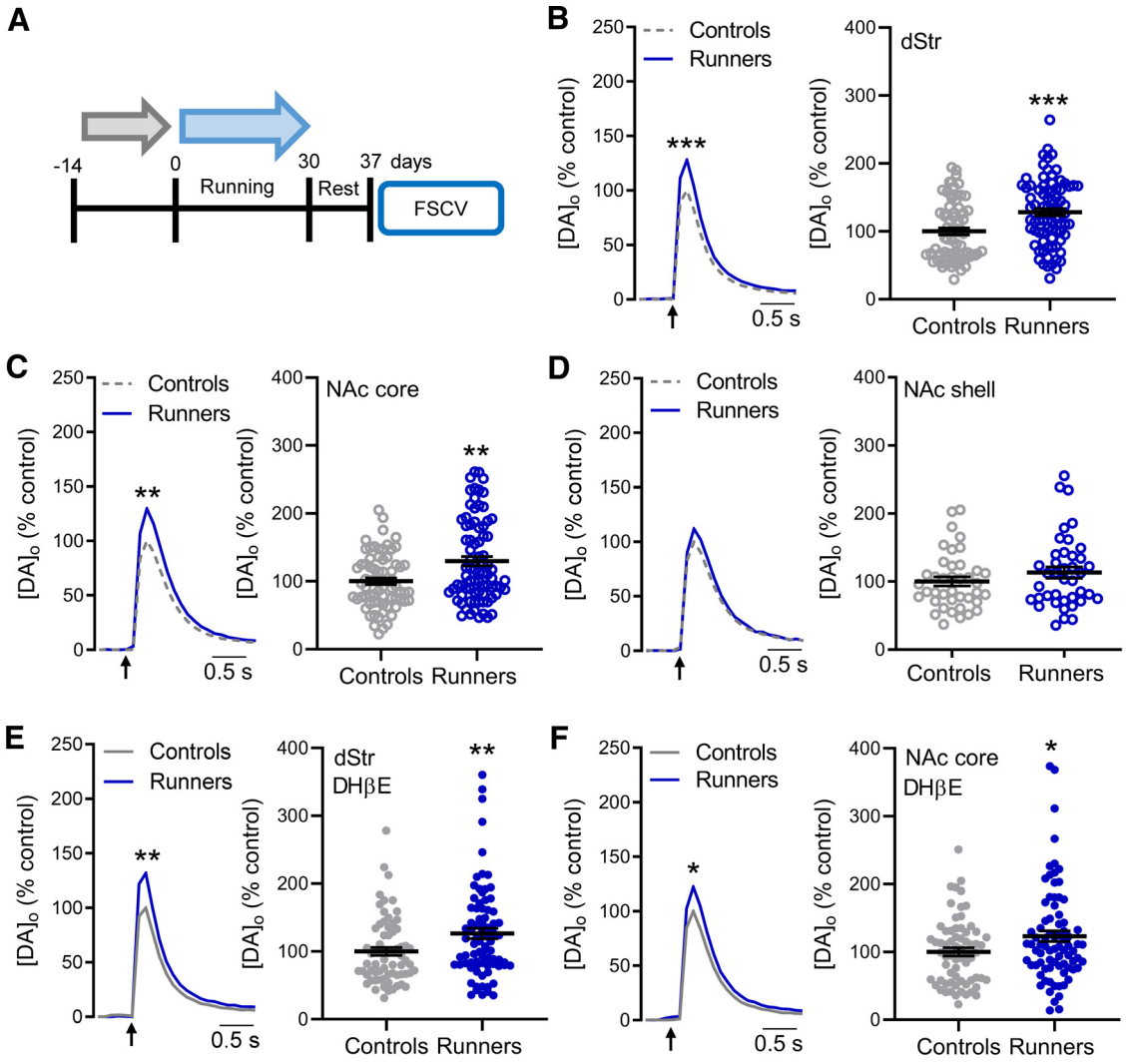

Figure 4. Enduring enhancement of evoked $[DA]_o$ in dStr, NAc core, and NAc shell after 7 d rest. ***A***, Timeline for voluntary wheel running for 30 d followed by 7 d of rest (locked running wheel for runners as well as controls; $n = 4$ mice per group). ***B–D***, Left, Average evoked increases in $[DA]_o$ in dStr, NAc core, and NAc shell in slices from runners and controls, normalized to mean peak $[DA]_o$ for each region in controls (error bars omitted). Right, Data summary; evoked $[DA]_o$ remained higher in runners than controls in dStr and NAc core, but the difference in NAc shell was lost ($n = 40–80$ sites per region, 4 slices per mouse, 4 mice per group; unpaired *U* tests). ***E***, ***F***, Left, Averaged evoked increases in $[DA]_o$ in dStr and NAc in the presence of DHβE (1 μM), normalized to mean peak evoked $[DA]_o$ in DHβE for each region in controls. Right, Data summary; evoked $[DA]_o$ in DHβE in dStr and NAc core from slices for runners versus controls ($n = 68–80$ sites per region, 4 slices per mouse, 4 mice per group; unpaired, one-tailed *U* tests). ***B–F***, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

from control $BDNF^{+/-}$ mice, albeit with differences from WT that vary depending on age, sex, and stimulation method (Dluzen et al., 2004; Bosse et al., 2012; Apawu et al., 2013; Birbeck et al., 2014). For example, DA release in dStr from male $BDNF^{+/-}$ mice at 3–5 months of age was found to be lower than in WT with electrical stimulation in slices, no different from WT with superfusion of high K^+ in slices, and higher than WT with K^+ applied via reverse microdialysis *in vivo* (Dluzen et al., 2004; Bosse et al., 2012; Apawu et al., 2013). Here, we found that average electrically evoked increases in $[DA]_o$ in slices from control male $BDNF^{+/-}$ mice were 1.2 ± 0.1 μM in dStr ($n = 54$ sites from six mice), 1.1 ± 0.1 μM for NAc core ($n = 41$ sites from six mice), and 1.1 ± 0.1 μM for NAc shell ($n = 40$ sites from six mice). Evoked $[DA]_o$ in both dStr and NAc core from $BDNF^{+/-}$ mice was indistinguishable from those in male WT, whereas evoked $[DA]_o$ in NAc shell was higher than in WT (dStr, $p = 0.306$ vs WT; NAc core, $p = 0.104$; NAc shell, $p < 0.001$; unpaired *U* test for each region).

Strikingly, we found that after 30 d of voluntary wheel running, $BDNF^{+/-}$ mice showed no increase in evoked $[DA]_o$ runners versus controls in dStr ($p = 0.596$, $n = 54–57$ sites from six mice per group, unpaired *t* test) or in NAc core ($p = 0.597$, $n = 41–46$ sites from six mice per group, unpaired *t* test; Fig. 6*A*, *B*). In NAc shell, however, an exercise-induced increase in evoked $[DA]_o$ was still seen ($p = 0.0073$, $n = 40–48$ sites from six mice per group, unpaired *t* test; Fig. 6*C*) that was comparable to that seen in WT NAc shell (Fig. 3*D*). The lack of difference between $BDNF^{+/-}$ runners and controls was maintained in the dStr when nAChRs were antagonized by DHβE ($p = 0.922$, $n = 42–49$ sites from six mice per group, unpaired *U test*; Fig. 6*D*), although a small elevation in evoked $[DA]_o$ emerged in runners versus controls in NAc core with nAChR antagonism ($p = 0.0336$, $n = 37–39$ sites from six mice per group; unpaired *t* test.; Fig. 6*E*). It is unlikely that the slight age difference between WT and $BDNF^{+/-}$ cohorts contributed to the difference in the effect of running on evoked $[DA]_o$ for two reasons. First, we found similar enhancement slices in slices from WT mice that started wheel running immediately after the acclimation period and those that started 2 weeks later under our staggered assignment protocol. Second, pilot data from 12-month-old WT male mice indicate a robust increase in evoked

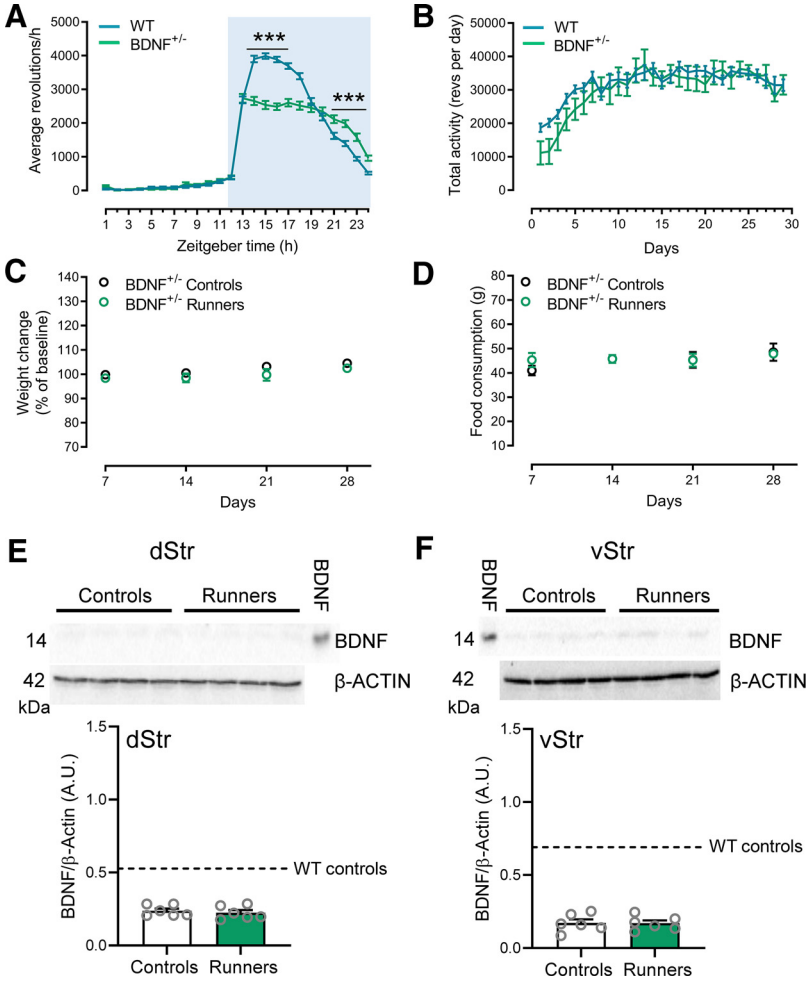

Figure 5. Voluntary wheel running in BDNF$^{+/-}$ mice. ***A***, Average time course of running-wheel activity for WT mice (Figure 1*B*, blue line) and BDNF$^{+/-}$ mice (green line) shows diurnal variation, with greater activity in the dark phase (shaded light blue) than in the light, albeit with a different pattern than seen in WT mice (***$p < 0.001$; $n = 18$ WT mice, $n = 6$ BDNF$^{+/-}$ mice; 2-way ANOVA, Bonferroni *post hoc* test). ***B***, Average total daily running over the 30 d running period did not differ between WT mice (Figure 1*C*) and BDNF$^{+/-}$ mice (2-way ANOVA, Bonferroni *post hoc* test; $n = 18$ WT mice, $n = 6$ BDNF$^{+/-}$ mice). ***C***, Body weight of BDNF$^{+/-}$ mice did not change over the running period for either runner or control BDNF$^{+/-}$ mice and did not differ between runners and controls at any point during the running period; the first day of the running period was taken as baseline (runners vs controls; $n = 6$ mice per group; 2-way ANOVA, Bonferroni *post hoc* test). ***D***, Average weekly food consumption also did not differ between runner and control BDNF$^{+/-}$ mice (runners vs controls; $n = 6$ mice per group; 2-way ANOVA, Bonferroni *post hoc* test). ***E***, ***F***, BDNF expression in dStr and vStr on the last day of the 30 d wheel running did not differ between runner and control BDNF$^{+/-}$ mice; quantitative data normalized to β-actin ($n = 6$ mice per group, runners vs controls; unpaired *t* test); dashed lines indicate average BDNF content for the corresponding striatal region from WT mice.

$[DA]_o$ in runners versus controls (M. Mancini, G. Bastioli, J.A. Arnold, unpublished observations).

Activation of TrkB receptors in *ex vivo* slices enhances evoked striatal dopamine release

Previous studies have shown that TrkB activation by BDNF or other TrkB agonists can potentiate depolarization-induced nigrostriatal DA release in cultured DA neurons, synaptosomes, and striatal slices (Blöchl and Sirrenberg, 1996; Goggi et al., 2002, 2003; Bosse et al., 2012; Apawu et al., 2013). To test the sufficiency of BDNF/TrkB to enhance DA release throughout the striatal complex, we examined the effect of a TrkB receptor agonist, LM22-A4 (1 μM; Massa et al., 2010; Nguyen et al., 2019), on evoked $[DA]_o$ in dStr, NAc core, and NAc shell in slices from WT mice. In initial studies, we found that 1 h superfusion of LM22-A4 caused a significant increase in evoked $[DA]_o$ in the dStr and NAc shell, but not in NAc core, compared with evoked $[DA]_o$ in time-matched control slices superfused with aCSF alone (data not shown). When exposure time was increased to 2 h, however, LM22-A4 caused a significant increase in all three striatal regions versus time-matched controls (dStr, 135 ± 10%, $p = 0.0099$, $n = 30$ sites per condition in slices from three mice; NAc core, 150 ± 18%, $p = 0.024$, $n = 24$ sites per condition; NAc shell, $p = 0.0001$, 180 ± 16%, $n = 23$ sites per condition; unpaired *t* test for each region; Fig. 7*A–C*). Similar to the increase in evoked $[DA]_o$ seen in our running-wheel experiments, LM22-A4-induced increases in dStr and NAc core persisted when nAChRs were antagonized with DHβE (1 μM), compared with time-matched controls in DHβE alone in dStr ($p = 0.046$, $n = 27$ sites per condition from three mice; unpaired *t* test) and in NAc core ($p = 0.0003$, $n = 24$ sites per condition, unpaired *t* test; Figs. 7*D*,*E*).

We then examined downstream pathways that might be involved in the effect of TrkB receptor activation on evoked $[DA]_o$. Two key signaling pathways that can be activated by TrkB receptors are PI3K and PLC (Deinhardt and Chao, 2014). To test the involvement of one or both of these, we superfused an inhibitor of either PI3K or PLC with LM22-A4 for 2 h, then compared evoked $[DA]_o$ in each region with evoked $[DA]_o$ in time-matched control slices superfused with the tested inhibitor alone. In dStr and NAc core, TrkB agonist-induced enhancement of evoked $[DA]_o$ persisted in the presence of a PI3K inhibitor, LY294002 (1 μM; Stouffer et al., 2015), when compared with evoked $[DA]_o$ in the inhibitor alone in dStr ($p = 0.037$; n = 36–37 sites in slices from three mice per condition; unpaired *t* test) and in NAc core ($p = 0.042$; n = 27–30 sites in slices from three mice per condition, unpaired *t* test; 7*F*,*G*). This indicates that DA release enhancement by TrkB receptor activation in dStr and NAc core does not involve PI3K. In the NAc shell, however, LY294002 did prevent the LM22-A4 induced in evoked $[DA]_o$ when applied together with LM22-A4 ($p = 0.192$, n = 27–28 sites in slices from three mice per condition, unpaired *t* test; Fig. 7*H*), implicating a role for PI3K in the shell. In contrast to the regional dependence of PI3K involvement, inhibition of PLC using U73122 prevented the usual TrkB-induced increases in evoked $[DA]_o$ in all three regions (dStr, $p = 0.166$, n = 18–28 sites in slices from three mice per condition; NAc core, $p = 0.828$, n = 23 sites in slices from three mice per condition; NAc shell, p = 0.486, n = 24 sites in slices from three mice per condition, unpaired *t* test; Fig. 7*I–K*).

Discussion

Interest in the influence of exercise on brain health is long standing, with a large literature showing not only improvements in

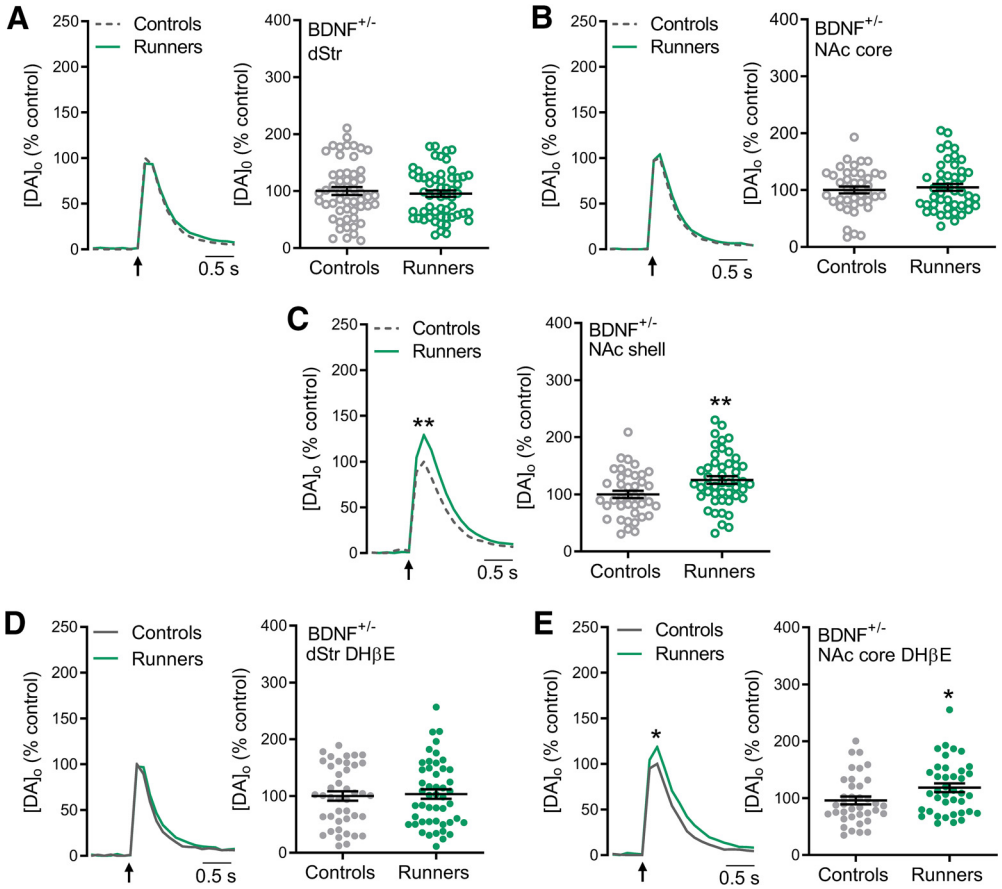

Figure 6. Loss of effect of voluntary wheel running on nigrostriatal DA release in BDNF$^{+/-}$ mice. ***A–C***, Left, Average evoked $[DA]_o$ in dStr, NAc core, and NAc shell, normalized to mean peak evoked $[DA]_o$ for each region in controls. Right, Data summary for BDNF$^{+/-}$ runners vs controls (n = 40–57 sites, 2 slices per mouse, 6 mice per group; unpaired t test). ***D***, ***E***, Left, Average evoked $[DA]_o$ in dStr and NAc core (error bars omitted) in the same slices examined in ***A–C*** after superfusion of DHβE (1 μM). Right, Data summary for evoked increases in $[DA]_o$ in the presence of DHβE in slices from BDNF$^{+/-}$ runners and controls (n = 37–49 sites, 2 slices per mouse, 6 mice per group; unpaired t or U tests). ***C***, ***E***, *$p < 0.05$, **$p < 0.01$.

cognition but also preservation of DA neurons and motor activity in neurotoxin models of PD (Cotman et al., 2007; Petzinger et al., 2007, 2013; Gerecke et al., 2010; Zigmond and Smeyne, 2014; Zhou et al., 2017; Mattson et al., 2018; Voss et al., 2019) and amelioration of depression and anxiety (Salmon, 2001; Duman et al., 2008). Mechanistic studies in hippocampus have implicated BDNF and other growth factors in the benefits of exercise on cognition (van Praag et al., 1999; Cotman et al., 2007; Kobilo et al., 2011; Voss et al., 2013; Sleiman and Chao, 2015; Svensson et al., 2015; Vivar and van Praag, 2017; Choi et al., 2018).

Here, we report that voluntary wheel-running exercise leads to an increase in BDNF levels in dStr, which receives rich DA innervation from SNc DA neurons via the nigrostriatal pathway (Matsuda et al., 2009). Although DA tissue content in dStr and vStr was not altered by this exercise regimen, dynamic DA release was enhanced in dStr, NAc core, and NAc shell in runners versus controls. As noted in the Introduction, local DA release can be triggered by ACh, which is concurrently released from ChIs with local electrical stimulation (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004). Notably, enhanced evoked $[DA]_o$ in runners versus controls was independent of cholinergic involvement; enhancement persisted when nAChRs were blocked, indicating a cell-autonomous effect of exercise on axonal DA release. Moreover, the increase in DA release persisted after 7 d of rest, which shows not only that the influence of exercise on DA release is enduring, but also that the increase seen in runners is not simply a transient effect on DA from their engagement in motor activity at the time of sampling. A limitation of the present findings is that only males were examined. We chose males for this initial experimental series in part because men have a two-fold greater risk for developing PD than females (Cerri et al., 2019). However, we recently conducted a pilot study with female mice using the same wheel-running paradigm. Supporting the generality of our findings, females also showed significant increases in evoked $[DA]_o$ release in dStr and NAc in runners versus controls (G. Bastioli, unpublished observations). Whether this also involves BDNF will require additional investigation, which is beyond the scope of the present report.

Exercise and neuroprotection in PD models

Previous studies of the effect of exercise on brain DA pathways have focused primarily on evidence for neuroprotection and improved motor and/or cognitive function, primarily in neurotoxin PD models in rodents (Tillerson et al., 2003; Petzinger et al., 2007; Tajiri et al., 2010; Wu et al., 2011; Petzinger et al., 2013; Regensburger et al., 2014; Zigmond and Smeyne, 2014; Churchill et al., 2017; Hou et al., 2017; Chen et al., 2018). Not all studies have shown nigrostriatal protection or behavioral restoration, however (Howells et al., 2005; Aguiar et al., 2014; Landers et al., 2014). When motor improvements are seen with treadmill exercise after MPTP in mice (Petzinger et al., 2007) or 6-OHDA in rats (Chen et al., 2018), these are correlated with increased evoked striatal DA

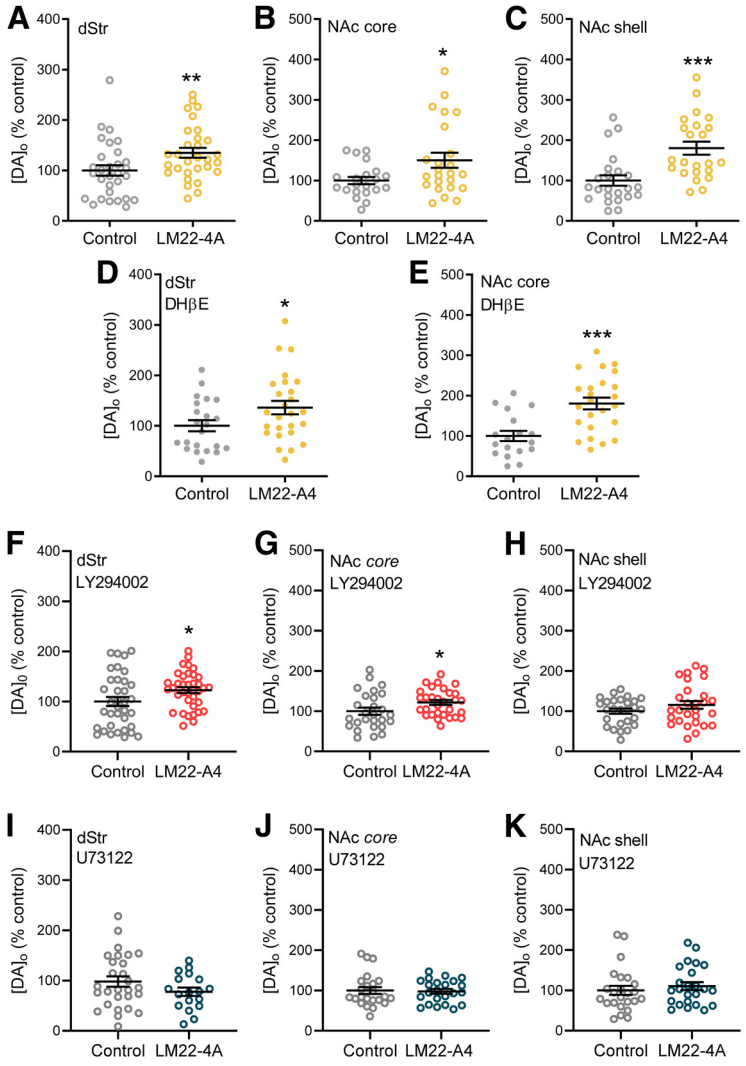

Figure 7. Activation of TrkB receptors in *ex vivo* striatal slices enhances evoked $[DA]_o$. ***A–D***, Summary of peak evoked $[DA]_o$ in dStr, in *ex vivo* slices after 2 h exposure to LM22-A4 (1 μM; a TrkB receptor agonist), to LM22-4A + DHβE (1 μM), to LM22-4A + LY29004 (1 μM), to LM22-4A + U73122 (1 μM), and in time-matched control slices with aCSF alone or the corresponding inhibitor, normalized to mean peak $[DA]_o$ in each region for time-matched controls (n = 24–30 sites per region, 2 slices per mouse, 3 mice per group; unpaired t tests). ***E–H***, Summary of peak evoked $[DA]_o$ in NAc core; in *ex vivo* slices after 2 h exposure to LM22-A4, to LM22-4A + DHβE, to LM22-4A + LY29004, and to LM22-4A + U73122; and in time-matched control slices, every group with corresponding inhibitor, normalized to mean peak $[DA]_o$ in each region for controls (n = 27–37 sites, 2 slices per mouse, 3 mice per group; unpaired t tests). ***I–K***, Summary of peak evoked $[DA]_o$ in NAc shell; in *ex vivo* slices after 2 h exposure to LM22-A4, to LM22-4A + LY29004, and to LM22-4A + U73122; and in time-matched control slices, each group with the corresponding inhibitor, normalized to mean peak $[DA]_o$ in each region for controls (unpaired t test, n = 18–30 sites, 2 slices per mouse, 3 mice per group; unpaired t tests). ***A–G***, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

release in MPTP-lesioned runners versus nonexercised controls. In contrast to the present results, those studies found no difference in evoked $[DA]_o$ between nonlesioned runners and controls. The lack of effect on DA release levels in those previous studies could reflect several differences from the present work, including the use of forced running, the time of day examined (running and testing in the light cycle, when rodents are least active), and the shorter duration of exercise (typically 30–60 min, 5 d per week for 4 weeks; Petzinger et al., 2007; Chen et al., 2018).

Voluntary running was used in the present investigation to minimize stress that can accompany forced running (Moraska et al., 2000; Howells et al., 2005; Landers et al., 2014), given that stress can negatively affect brain chemistry and synaptic plasticity (Stein-Behrens and Sapolsky, 1992; Hill et al., 2012; McEwen and Morrison, 2013). Of particular relevance for our studies is that voluntary running leads to greater increases in brain BDNF than forced or involuntary exercise (Albeck et al., 2006; Ke et al., 2011). A possible contributing factor is that forced exercise can increase release of corticosterone, a stress hormone that decreases levels of BDNF mRNA and protein (Schaaf et al., 1998). Notably, corticosterone is not increased by the voluntary wheel-running paradigm used in the present work (van Praag et al., 1999).

Necessary and sufficient role of BDNF in enhancing nigrostriatal DA release?

The lack of amplification of evoked $[DA]_o$ in the dStr and NAc core from $BDNF^{+/-}$ mice implies that BDNF is necessary for the boosting of DA release seen after voluntary wheel running in these regions (compare Figs. 2*B*,*C*, 6*A*,*B*). The source of BDNF remains to be determined. Likely contributors include midbrain DA neurons and corticostriatal afferents (Altar et al., 1997; Conner et al., 1997; Kolbeck et al., 1999); however, other sources cannot be ruled out given that the mice examined had global BDNF depletion. In contrast to dStr and NAc core, exercise-enhanced evoked $[DA]_o$ release persisted in NAc shell from $BDNF^{+/-}$ mice. The NAc shell receives DA innervation exclusively from mesolimbic DA projections from the ventral tegmental area, whereas the dStr and NAc core receive exclusive (dStr) or partial (NAc core) DA input from the nigrostriatal DA pathway (Dahlström and Fuxe, 1964; Haber et al., 2000). Persistence of release enhancement in the shell, together with the lack of increase in BDNF protein in vStr (combined core and shell) after 30 d of exercise, suggests involvement of factors in addition to BDNF in exercise-enhanced DA release in NAc shell.

The BDNF dependence of exercise-enhanced nigrostriatal DA release is consistent with evidence from cultured DA-producing neurons that BDNF/TrkB signaling promotes neuronal survival and differentiation (Hyman et al., 1991; Spina et al., 1992), even after toxic insult (Spina et al., 1992), as well as increased DA synthesis, depolarization-induced DA release, and DA transporter uptake capacity (Hyman et al., 1994; Blöchl and Sirrenberg, 1996). Here, we tested the sufficiency of TrkB receptor activation on striatal DA release using a TkrB receptor agonist instead of BDNF. We found that TrkB receptor activation by LM22-A4 equals increased evoked $[DA]_o$ in dStr and in NAc core and shell. Mirroring the increases in DA release seen after voluntary wheel running, the increases in dStr and NAc core persisted in the presence of DHβE, an antagonist of nAChRs (compare Figs. 4*E*,*F*, 7*D*,*E*). As noted in Results, five-pulse trains were used in NAc shell to ensure robust DA release, so DHβE was not tested in the shell to avoid the confounding effect of phasic stimulation (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Patel et al., 2012). Examination of PI3K and PLC involvement (Deinhardt and Chao, 2014) revealed differential

regulation in dStr and NAc core versus NAc shell. Although inhibition of PI3K had no effect on TrkB-enhanced evoked $[DA]_o$ in dStr or NAc core, PIK3 inhibition did prevent release enhancement in the shell, indicating a downstream role for PI3K. In contrast to the selective involvement of PI3K in NAc shell, inhibition of PLC prevented TrkB-induced increases in evoked $[DA]_o$ in all three regions. These pharmacological data provide insight into signaling pathways underlying BDNF/TrkB-dependent enhancement of striatal DA release. Determining additional mechanism(s) involved in DA release amplification by BDNF/TrkB awaits future investigation. Previous studies in cultured cortical and hippocampal neurons suggest a role for enhanced Ca^{2+} influx (Li et al., 1998), as well as recruitment of Ca^{2+} from inositol 1,4,5-trisphosphate (IP3)-sensitive intracellular stores, which can occur downstream from either PLC or PI3K activation (Numakawa et al., 2002; He et al., 2005).

Summary and conclusions

We show here that exercise can increase dynamic DA release throughout the striatum. This enhancement is not simply from increased DA tissue content, which was not altered by exercise, and does not involve ACh acting at nAChRs. Instead, our findings support a necessary and sufficient role for BDNF/TrkB signaling in exercise-enhanced DA release in dStr and NAc core that receive nigrostriatal input. Although enhanced DA release was also seen in NAc shell with exercise or TrkB receptor activation, a necessary role for BDNF was not shown, implying involvement of other factors in that region.

Together, these findings suggest a role for enhanced DA release in the motor improvement seen in PD patients with exercise (Fontanesi et al., 2016; Mak et al., 2017; Zhou et al., 2017; Ahlskog, 2018), as well as the alleviation of symptoms in other disorders, including depression and anxiety (Salmon, 2001; Duman et al., 2008). Enduring enhancement of evoked $[DA]_o$ might be reflected in the long-lasting motor improvement seen in PD patients after cessation of an exercise program (Mak et al., 2017). Enhanced DA release could also contribute to other benefits of exercise seen in rodent PD models, including increased dendritic spine density and improved motor learning (Petzinger et al., 2007; Toy et al., 2014) and a slowing of motor-symptom progression (Zhou et al., 2017; Hsueh et al., 2018). Finally, our results provide mechanistic insight into imaging studies from PD patients who were habitual exercisers or enrolled in controlled exercise sessions; both groups show enhanced [^{11}C]raclopride displacement with transcranial magnetic stimulation in caudate nucleus (rodent dStr) and greater neuronal activity in response to reward in vStr compared with nonexercising PD controls (Sacheli et al., 2018, 2019). Overall, the present studies indicate underlying factors in the beneficial effects of exercise in motor and reward pathways and may point to new therapeutic options to boost DA release.

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