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SYNAPTIC MECHANISMS

Brain-derived neurotrophic factor rapidly increases AMPA receptor surface expression in rat nucleus accumbens

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Abstract

In the rodent nucleus accumbens (NAc), cocaine elevates levels of brain-derived neurotrophic factor (BDNF). Conversely, BDNF can augment cocaine-related behavioral responses. The latter could reflect enhancement of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPAR) transmission, because AMPARs in the NAc mediate some cocaine-induced behaviors. Furthermore, *in vitro* studies in other cell types show that BDNF can promote AMPAR synaptic delivery. In this study, we investigated whether BDNF similarly promotes AMPAR trafficking in the adult rat NAc. After unilateral intracranial injection of BDNF into NAc core or shell, rats were killed at post-injection times ranging from 30 min to 3 days. NAc core or shell tissue from both injected and non-injected hemispheres was analysed by Western blotting. A protein cross-linking assay was used to measure AMPAR surface expression. Assessment of tropomyosin receptor kinase B signaling demonstrated that injected BDNF was biologically active. BDNF injection into NAc core, but not NAc shell, led to a protein synthesis- and extracellular signal-regulated kinase-dependent increase in cell surface GluA1 and a trend towards increased total GluA1. This was detected 30 min post-injection but not at longer time-points. GluA2 and GluA3 were unaffected, suggesting an effect of BDNF on homomeric GluA1 Ca^{2+}-permeable AMPARs. These results demonstrate that exogenous BDNF rapidly increases AMPAR surface expression in the rat NAc core, raising the possibility of a relationship between increases in endogenous BDNF levels and alterations in AMPAR transmission observed in the NAc of cocaine-experienced rats.

Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is widely accepted as an important regulator of synaptic function and plasticity, including plasticity induced by drugs of abuse (Russo *et al.*, 2009; Ghitza *et al.*, 2010; McGinty *et al.*, 2010). BDNF exerts its major biological functions through binding to tropomyosin receptor kinase B (TrkB), which is linked to a number of signal transduction cascades, including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase and phospholipase C-γ pathways (Reichardt, 2006). BDNF can influence plasticity through multiple mechanisms. For example, it exerts both presynaptic and postsynaptic effects, and influences both early-phase and late-phase long-term potentiation (Bramham & Messaoudi, 2005; Waterhouse & Xu, 2009).

Regulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking plays an important role in synaptic plasticity and learning (Shepherd & Huganir, 2007; Kessels & Malinow, 2009; Keifer & Zheng, 2010). Recent work has highlighted the importance of AMPARs lacking the GluA2 subunit. These Ca^{2+}-permeable AMPARs (CP-AMPARs) have high conductance and exhibit inward rectification due to voltage-dependent polyamine block (Cull-Candy *et al.*, 2006; Isaac *et al.*, 2007; Liu & Zukin, 2007). *In vitro* studies have shown that activation of BDNF-TrkB signaling increases synaptic delivery of CP-AMPARs (Caldeira *et al.*, 2007; Li & Keifer, 2008, 2009). Whether BDNF similarly promotes CP-AMPAR trafficking *in vivo* is unknown.

Cocaine exposure can increase BDNF expression in addiction-related brain regions, including the nucleus accumbens (NAc; Zhang *et al.*, 2002; Grimm *et al.*, 2003; Le Foll *et al.*, 2005; Filip *et al.*, 2006; Liu *et al.*, 2006; Pu *et al.*, 2006; Fumagalli *et al.*, 2007; Graham *et al.*, 2007; Im *et al.*, 2010; Lu *et al.*, 2010; Sadri-Vakili *et al.*, 2010), and can also modulate TrkB expression and phosphorylation in the NAc (Freeman *et al.*, 2003; Filip *et al.*, 2006; Graham *et al.*, 2009; Crooks *et al.*, 2010). Elevation of BDNF levels in rodent NAc is associated with enhancement of cocaine-related behavioral responses, including locomotor sensitization, responding for conditioned reward, conditioned place preference and cocaine-seeking after withdrawal (Horger *et al.*, 1999; Grimm *et al.*, 2003; Graham *et al.*, 2007; Bahi *et al.*, 2008), although this may depend on whether BDNF elevation occurs in core or shell (see Discussion). While the underlying mechanism is unclear, it is intriguing to speculate that it may involve the effects of BDNF on AMPAR trafficking, based on the importance of AMPAR plasticity in the NAc for some behavioral consequences of repeated cocaine exposure (Wolf & Ferrario, 2010).

As a first step towards testing this hypothesis, we measured AMPAR surface expression after acute injection of BDNF into the

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NAc core or shell of adult rats. Cell surface levels of GluA1, but not other AMPAR subunits, were increased in the core 30 min after BDNF injection. This effect was dependent on protein synthesis and extracellular signal-regulated kinase (ERK) activity, dissipated at longer times after BDNF injection, and was not detected in the shell. Our results suggest that BDNF produces a rapid and transient increase in cell surface levels of CP-AMPARs in the NAc core.

Materials and methods

Subjects

Male Sprague–Dawley rats (270–320 g) were housed in groups of three with food and water available *ad libitum*. A 12 h/12 h light/dark cycle was used, with lights on at 07:00 h. All intracranial injections were performed between 08:00 and 16:00 h. All procedures were approved by the Institutional Animal Care and Use Committee of Rosalind Franklin University of Medicine and Science. Rats were allowed to acclimate 7–10 days prior to injection.

BDNF intracranial injections

Rats were anesthetized with a ketamine–xylazine cocktail (80 and 10 mg/kg, respectively) and mounted onto a stereotaxic frame. A Hamilton syringe was slowly lowered, and either vehicle [sterile phosphate-buffered saline (PBS)–saline, 1 : 1, v/v] or BDNF (0.75 μg/0.5 μL or 0.25 μg/0.5 μL, dissolved in vehicle solution, recombinant human BDNF, 248-BD; R&D Systems, Minneapolis, MN, USA) was unilaterally microinjected into NAc core or shell (0.1 μL/min). Injectors were left in place for 2 min after the injection. The doses of BDNF were chosen based on previous studies (Lu *et al.*, 2004). The coordinates for NAc core were: anteroposterior (AP) + 1.2 mm; lateral (L) + 2.6 mm (6° angle); and dorsoventral (DV) −7.0 mm (Paxinos & Watson, 1998). The coordinates for NAc shell were: AP + 1.4 mm; L + 1.5 mm (6° angle); and DV −7.0 mm. BDNF or vehicle was infused into a randomly assigned hemisphere. The other hemisphere served as a non-injected control. Rats were decapitated at different post-injection times (10 min, 30 min, 3 h, 24 h and 3 days). To determine if the effect of BDNF on GluA1 at the 30-min time-point required protein synthesis or ERK activation, anisomycin (150 mg/kg, i.p., A9789; Sigma, St Louis, MO, USA) or SL327 (30 mg/kg, i.p., Asc-082; Ascent Scientific, Princeton, NJ, USA) was administered ∼30 min before microinjection of BDNF into the core. Systemic injection of anisomycin has been used by several groups to produce a long-lasting reduction in protein synthesis (e.g. Lattal & Abel, 2001; Tronel *et al.*, 2005; Bernardi *et al.*, 2007). Approximately 90% inhibition is produced with the dose and timing used in our study (see Flood *et al.*, 1973). The dose of the ERK inhibitor, SL327, and the timing of its injection were chosen based on previous studies (e.g. Selcher *et al.*, 1999; Wang *et al.*, 2003; Ferguson & Robinson, 2004; Valjent *et al.*, 2006a,b).

Protein cross-linking and immunoblotting

Rats were decapitated at different times after intracranial injection of BDNF as described above. NAc core and shell subregions were rapidly dissected from a 2-mm coronal section obtained using a brain matrix as described previously (McCutcheon *et al.*, 2011). During the dissection, we verified that infusion sites were located within the boundaries of our standard core and shell dissections. For all time-points except 10 min, NAc core or shell tissue was collected and processed for BS^3 [bis(sulfosuccinimidyl) suberate] cross-linking and Western blotting as described previously to distinguish surface-expressed and intracellular AMPAR subunits (Boudreau & Wolf, 2005; Ferrario *et al.*, 2010). Tissue from the 10-min time-point was homogenized without cross-linking. The following primary antibodies were used: GluA1 (PA1-37776, 1 : 1000; Thermoscientific, Rockford, IL, USA), GluA2 (L21/32, 1 : 200; UC Davis/NIH NeuroMab Facility, Davis, CA, USA), GluA2/3 (AB1506, 1 : 1000; Millipore, Billerica, MA, USA), BDNF (sc-546, 1 : 500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), pTrkB (Tyr 706/707; 4621, 1 : 1000; Cell Signaling, Danvers, MA, USA), TrkB (07-225, 1 : 2000; Millipore), phosphorylated (p)ERK (4377, 1 : 1000; Cell Signaling) and ERK (4695, 1 : 1000; Cell Signaling). GAPDH (glyceraldehyde-3-phosphate dehydrogenase, CB1001, 1 : 10 000; Calbiochem, San Diego, CA, USA) was used as a loading control. Results from injected hemispheres (I) are normalized to the non-injected hemisphere (N). Non-injected hemispheres for BDNF and vehicle rats did not differ significantly for any measure (data not shown).

Statistical analysis

Quantity One analysis software (BioRad, Hercules, CA, USA) was used to quantify bands of interest. A background value was obtained, and diffuse densities for surface and intracellular bands in each lane were determined. Total protein levels were determined by summing surface and intracellular values. Surface, intracellular and total protein values were then normalized to a loading control (GADPH), except for pTrkB and pERK, which were normalized to total TrkB and ERK levels determined with a phosphorylation-independent antibody. Unpaired *t*-tests (two-tailed) were conducted to compare protein levels between non-injected and injected hemispheres within each experimental group. Significance was set at $P < 0.05$.

Results

Effects of BDNF in NAc core

To verify that injected BDNF was biologically active, we first measured the activation of TrkB receptors and ERK 10 min after injection of the mature form of BDNF (14 kDa; 0.75 μg/0.5 μL) into the NAc core. As expected, a robust increase in mature BDNF protein was detected in the injected hemisphere (Fig. 1, BDNF). At the same time, phosphorylation of the TrkB receptor and both isoforms of ERK

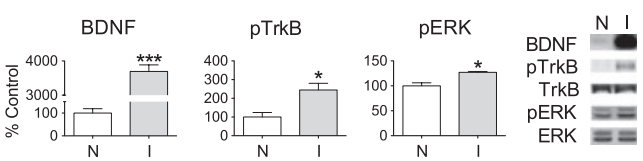

FIG. 1. Brain-derived neurotrophic factor (BDNF) is biologically active after intracranial injection into the nucleus accumbens (NAc) core. Rats ($n = 3$) received a unilateral injection of BDNF (0.75 μg/0.5 μL) and were killed 10 min later. Compared with the non-injected hemisphere (N), the injected hemisphere (I) showed elevated levels of BDNF protein ($t_4 = 18.95$, $^{***}P < 0.0005$), phosphorylated tropomyosin receptor kinase B (pTrkB; $t_4 = 3.34$, $^{*}P < 0.05$) and phosphorylated extracellular signal-regulated kinase (pERK; $t_4 = 4.25$, $^{*}P < 0.05$). pERK levels are expressed as the sum of pERK1 and pERK2 bands; phosphorylation of both ERK1 and ERK2 was increased by BDNF. Representative Western blots are shown (molecular weights: BDNF, 14 kDa; TrkB, 140 kDa; ERK1 and ERK2, 44 and 42 kDa, respectively). All data (mean ± SEM) are expressed as percent of mean values in the non-injected (N) hemisphere.

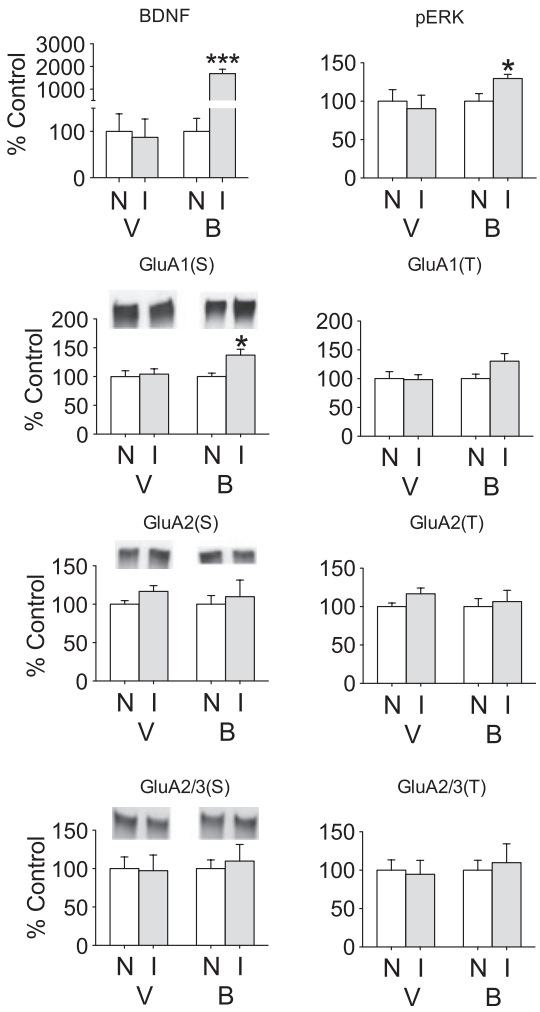

FIG. 2. An increase in GluA1 surface expression was observed 30 min after brain-derived neurotrophic factor (BDNF) injection into the nucleus accumbens (NAc) core. Each rat received a unilateral injection of vehicle (V; sterile PBS–saline, 1 : 1, v/v) or BDNF (B; 0.75 μg/0.5 μL), and the injected hemisphere (I) was compared with the non-injected hemisphere (N). BDNF injection increased levels of BDNF protein ($t_{10} = 8.01$, $^{***}P < 0.0005$) and phosphorylated extracellular signal-regulated kinase (pERK; expressed as the sum of pERK1 and pERK2 bands; $t_{10} = 2.60$, $^{*}P < 0.05$). BDNF also increased surface (S) GluA1 ($t_{10} = 3.19$, $^{*}P < 0.05$) and produced a trend towards increased total (T) GluA1 ($t_{10} = 2.00$, $P = 0.08$), whereas GluA2 and GluA2/3 immunoreactivity were unaffected. Representative surface bands are shown above bars. Total AMPAR subunit levels were determined by summing surface and intracellular bands. $N = 6$ rats/group. All data (mean ± SEM) are expressed as percent of mean values in the non-injected (N) hemisphere.

were significantly elevated in the BDNF-injected hemisphere (I) compared with the non-injected hemisphere (N; Fig. 1, pTrkB and pERK). The TrkB phospho-specific antibody used in our studies detects phosphorylation of Tyr706/707, which is required for TrkB activation (see Discussion). These results show that injected BDNF activates TrkB-related signaling cascades in the NAc core.

Next, we evaluated AMPAR surface expression 30 min after BDNF injection into the NAc core (0.75 μg/0.5 μL; Fig. 2). For rats that received a unilateral BDNF injection (B), BDNF levels were significantly increased in the injected hemisphere (I) compared with the non-injected hemisphere (N; Fig. 2, BDNF), while no differences was detected in rats that received vehicle injections (V). At the 30-min time-point, we also detected elevated ERK phosphorylation in the BDNF-injected side. Most importantly, we found a significant increase in surface (S) expression of GluA1 [Fig. 2, GluA1(S)] in the BDNF-injected hemisphere compared with the non-injected hemisphere, whereas no changes were detected when different aliquots of the same tissue were probed with GluA2 or GluA2/3 antibodies [Fig. 2, GluA2(S) and GluA2/3(S)]. Total GluA1 levels, derived by summing surface and intracellular bands, showed a trend towards an increase after BDNF injection [Fig. 2, GluA1(T); $P = 0.08$], whereas no changes or trends were observed for total GluA2 or GluA2/3 [Fig. 2, GluA2(T) and GluA2/3(T)]. Vehicle injection did not alter surface or total levels of any AMPAR subunit (Fig. 2).

In other systems, BDNF has been shown to increase AMPAR synaptic targeting through ERK- and protein synthesis-dependent mechanisms (see Discussion). In fact, the trend towards an increase in total GluA1 30 min after BDNF injection (Fig. 2) suggested a possible effect of BDNF on GluA1 protein synthesis. To test involvement of protein synthesis and ERK, rats received an i.p. injection of anisomycin (an inhibitor of protein synthesis) or SL327 (an inhibitor of ERK activation) ~30 min prior to intracranial injection of BDNF (0.75 μg/0.5 μL). Results from these rats are shown in Fig. 3 by the middle and right pairs of bars in each graph, labeled 'A + B' to indicate anisomycin + BDNF treatment and 'S + B' to indicate SL327 + BDNF treatment, respectively. Controls received i.p. saline followed by vehicle microinjection into the NAc core (V + V; left pair of bars). BDNF injection produced an elevation of BDNF levels in anisomycin- and SL327-pretreated rats (Fig. 3A), similar to that observed in rats that were not pretreated (Fig. 2). However, anisomycin and SL327 completely blocked the effect of BDNF on surface and total GluA1 [Fig. 3C, GluA1(S) and GluA1(T)]. To verify that SL327 was blocking ERK activation, we measured pERK in all treatment groups. As expected, SL327 pretreatment blocked BDNF-induced ERK activation (Fig. 3B, left) and reduced basal pERK levels by ~70% (Fig. 3B, right), whereas ERK activation was not affected by anisomycin (Fig. 3B, left and right). Together, these results suggest that BDNF produces a rapid ERK-dependent increase in GluA1 protein synthesis that leads to increased GluA1 surface expression. To determine if anisomycin or SL327 given alone had effects on AMPAR surface expression, we compared the non-injected hemispheres of the anisomycin- or SL327-pretreated rats with the non-injected hemisphere of the saline-pretreated group. No differences were found, indicating that 30 min of protein synthesis inhibition or ERK inhibition did not significantly alter AMPAR subunit surface expression in the NAc (data not shown).

To determine if the effect of BDNF on GluA1 persisted longer than 30 min, rats were injected unilaterally into the NAc core with either BDNF or vehicle, and killed 3 h, 24 h or 3 days later for comparison of AMPAR levels in non-injected and injected hemispheres (Fig. 4). No changes in GluA1 or GluA2/3 surface levels [Fig. 4, GluA1(S), GluA2/3(S)] or total levels (data not shown) were detected at these time-points. These experiments used the same BDNF dose as the 30-min time-point (0.75 μg/0.5 μL), except for the 3-day experiment, which used 0.25 μg/0.5 μL BDNF. Both doses of BDNF had a similar effect in a prior study demonstrating BDNF-mediated enhancement of the incubation of cocaine craving (Lu *et al.*, 2004), a phenomenon relevant to the goals of the present study because incubation is associated with increased levels of CP-AMPARs in the NAc (Conrad *et al.*, 2008). Furthermore, because our goal is to understand the consequences of cocaine-induced elevation of NAc BDNF, both BDNF concentrations used here are relevant as both produce a greater elevation of NAc BDNF levels than those observed after cocaine treatment (e.g. Grimm *et al.*, 2003; Huang *et al.*, 2011). We also examined the BDNF-induced activation of signaling

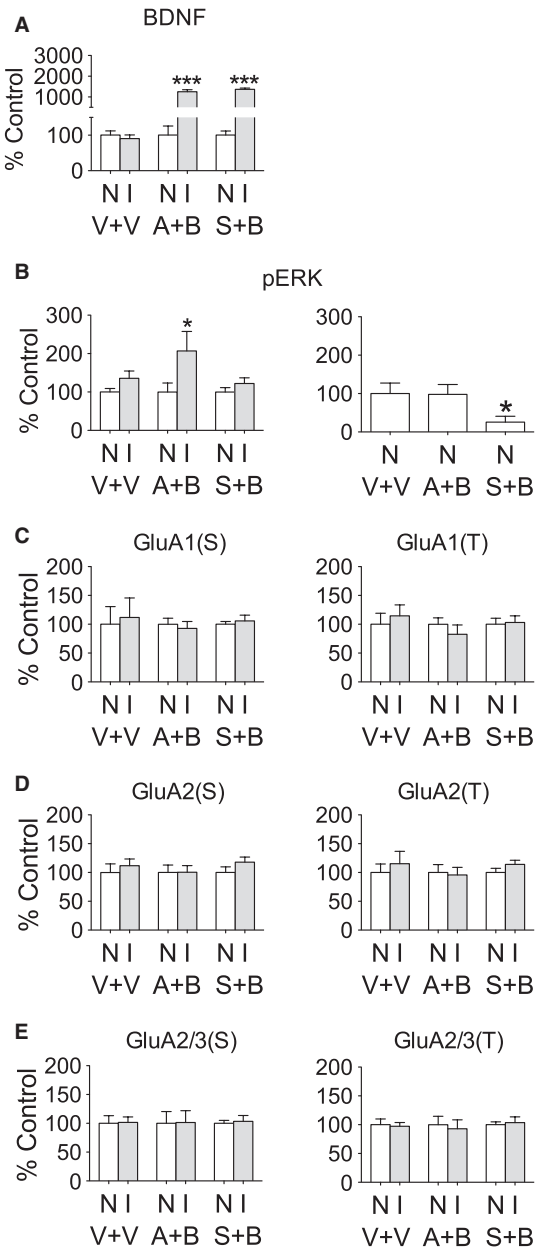

FIG. 3. The increased GluA1 surface expression observed 30 min after brain-derived neurotrophic factor (BDNF) injection into the nucleus accumbens (NAc) core was dependent on both protein synthesis and extracellular signal-regulated kinase (ERK) activation. Three groups of rats were pretreated with vehicle (V; saline, i.p.), anisomycin (A; 150 mg/kg, i.p.) or SL327 (S; 30 mg/kg, i.p.). Thirty minutes later, vehicle rats (V + V, $n = 6$) received unilateral vehicle injection (V; sterile PBS–saline, 1 : 1, v/v) into the NAc core, while the other two groups (A + B and S + B, $n = 6$ and $n = 10$, respectively) received unilateral BDNF injection (0.75 μg/0.5 μL). (A) BDNF levels were significantly elevated on the injected side (A + B, $t_{10} = 11.06$; S + B, $t_{18} = 20.13$; both $^{***}P < 0.05$ compared with non-injected hemisphere). The elevated BDNF levels observed in anisomycin- and SL327-pretreated rats did not differ from those observed in rats that were not pretreated (compare to Fig. 2). (B, left) The BDNF-induced elevation in pERK ($t_{10} = 2.36$, $^{*}P < 0.05$) was intact in anisomycin-pretreated rats (compare to Fig. 2), while SL327 completely blocked the effect of BDNF on pERK. Data in this panel are normalized to the non-injected side of each group. (B, right) Comparison of pERK in the non-injected sides of vehicle-, anisomycin- and SL327-pretreated rats revealed that SL327 reduced basal pERK levels by ~70% (ANOVA, $F_{2,19} = 4.41$; $^{*}P < 0.05$, Dunnett's test). Data in this panel are normalized to the vehicle + vehicle group. (C) Both anisomycin and SL327 pretreatment abolished the effect of BDNF on GluA1 surface (S) and total (T) protein levels. (D and E) GluA2 and GluA2/3 surface (S) and total (T) levels were unaffected by BDNF injection regardless of pretreatment. All data (mean ± SEM) are expressed as percent of mean values in the non-injected (N) hemisphere, except for the right-hand graph in (B) (see above for explanation of this panel).

pathways at the later time-points. While pERK remained elevated 3 h after BDNF injection (Fig. 4A), no significant differences were detected between the BDNF-injected and non-injected hemispheres after 24 h and 3 days (data not shown). Taken together, these results indicated a transient, protein synthesis- and ERK-dependent effect of BDNF on GluA1 surface expression in the NAc core.

Effects of BDNF in NAc shell

We checked the biological activity of injected BDNF by measuring levels of phosphorylated TrkB and ERK. As shown in Fig. 5A, levels of BDNF and pTrkB were significantly elevated 10 min after BDNF injection (0.75 μg/0.5 μL) into the NAc shell, and there was a trend towards increased pERK. The magnitude of BDNF elevation in shell was somewhat less than occurred when the same amount of BDNF was injected into core (compare with Fig. 1), possibly due to more diffusion into the ventricle following shell injections. However, the level of TrkB activation was at least as great in the shell, and the magnitude of the trend towards increased pERK was similar to the significant increase observed in the core (see Fig. 1).

BDNF levels remained elevated 30 min after BDNF injection (0.75 μg/0.5 μL) into the NAc shell (Fig. 5B). However, in contrast to results obtained in the core, pERK levels were no longer elevated, and no changes in cell surface (S) or total (T) AMPAR subunit levels were observed at this time-point (Fig. 5B). There were also no changes in AMPAR surface expression at the 3-h and 3-day time-points (Fig. 5C and D, respectively; these experiments used the lower BDNF dose of 0.25 μg/0.5 μL). Total AMPAR subunit levels were also unaltered after 3 h or 3 days (data not shown).

Discussion

We undertook these studies to bridge three prior areas of investigation: (i) studies demonstrating that cocaine can increase BDNF levels in the NAc and that this can affect cocaine-related behaviors; (ii) studies implicating NAc AMPAR transmission in cocaine-seeking; and (iii) studies showing that BDNF modulates AMPAR expression and trafficking *in vitro* (see Introduction). Our results demonstrate that the acute injection of exogenous BDNF into the NAc core of adult rats rapidly (30 min) upregulated GluA1 surface expression in the core via a protein synthesis- and ERK-dependent mechanism. This effect of BDNF dissipated quickly and had no delayed consequences for AMPAR expression, as no changes in surface GluA1 were found 3 h, 24 h or 3 days after BDNF injection. When injected into the shell, BDNF had no effect on shell GluA1 levels. BDNF did not alter GluA2 or GluA2/3 levels in either subregion. To our knowledge, this is the first demonstration that BDNF alters AMPAR trafficking *in vivo*.

It is important to acknowledge that our studies were restricted to drug-naïve and anesthetized rats, that intracranial injection of BDNF does not reproduce temporal features of cocaine-induced increases in endogenous BDNF levels, and that the BDNF concentrations achieved after intracranial injection are much higher than those reported after cocaine exposure (e.g. Grimm *et al.*, 2003; Huang *et al.*, 2011). Nevertheless, our results raise the possibility that cocaine-induced

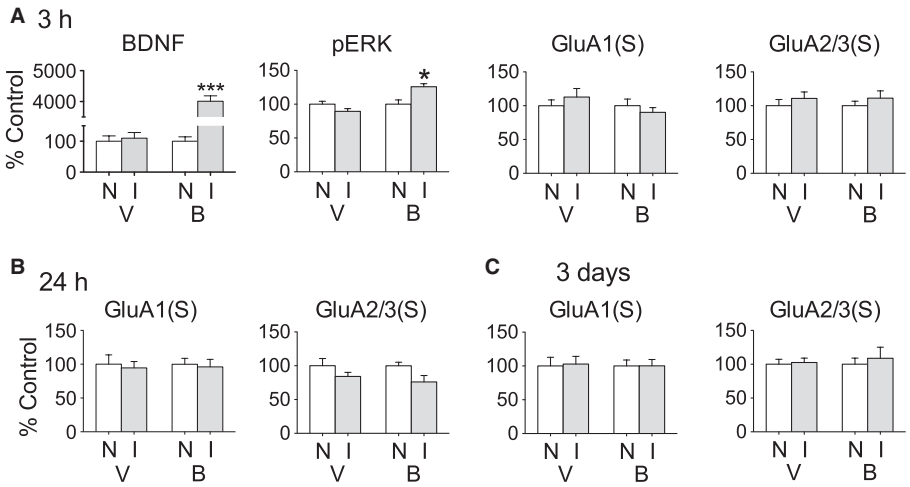

FIG. 4. No effect on AMPAR expression was observed at longer times following unilateral brain-derived neurotrophic factor (BDNF) injection into the nucleus accumbens (NAc) core. (A) Three hours after NAc core injection of vehicle (V) or BDNF (B; 0.75 μg/0.5 μL), no differences in surface (S) GluA1 or GluA2/3 were observed between N and I hemispheres, whereas elevated levels of BDNF (t_{12} = 22.14, ***P < 0.0005) and pERK (t_{12} = 3.30, *P < 0.05) were still detected in the BDNF-injected hemisphere. N = 7 rats/group. (B) No changes in surface (S) GluA1 or GluA2/3 were found 24 h after BDNF injection into the NAc core (0.75 μg/0.5 μL). N = 10 rats/group. (C) No changes in surface (S) GluA1 or GluA2/3 were found 3 days after BDNF injection into the core (0.25 μg/0.5 μL). N = 6 rats/group. Total AMPAR subunit levels were also unchanged (data not shown). BDNF levels in the injected hemisphere remained significantly elevated at the 24-h and 3-day time-points (data not shown). All data (mean ± SEM) are expressed as percent of mean values in the non-injected (N) hemisphere.

elevations in endogenous BDNF levels lead to enhanced AMPAR transmission in the NAc and therefore have potentially important implications for understanding the mechanisms by which BDNF influences drug-seeking and other behaviors in cocaine-experienced rats.

Potential explanations for different BDNF effects in the core and shell of drug-naïve rats

Although core and shell differ in a number of properties, it is unclear which of these may explain our observation that BDNF increased AMPAR surface expression in core but not shell. Different roles for core and shell in particular behaviors have been attributed both to differences in connectivity (Groenewegen *et al.*, 1999; Voorn *et al.*, 2004) and to different intrinsic properties of core and shell neurons, including differences in neuron size, dendritic organization, spine density, and subcellular localization of dopamine and glutamate receptors (Meredith & Totterdell, 1999; Meredith *et al.*, 2008). Differences in intrinsic properties seem more likely to explain our results, as we are applying BDNF directly into the NAc and measuring AMPAR distribution at the same location. For example, perhaps TrkB receptors in the core are located closer to spines where AMPAR trafficking or local protein synthesis may be occurring. Although no studies have assessed such possibilities, a difference in BDNF signaling in the two subregions may be indicated by our observation that pERK in the core was significantly elevated 10 min, 30 min and 3 h after BDNF injection, whereas pERK in the shell was completely normalized by 30 min. In fact, even at an earlier time-point (10 min after BDNF injection), pERK showed only a trend towards elevation in the shell, despite very robust increases in BDNF levels and TrkB phosphorylation. More studies are needed to determine the molecular basis for core–shell differences in the effects of BDNF on ERK and AMPAR trafficking.

Core and shell play distinct functional roles in cocaine-experienced rats (e.g. Ito *et al.*, 2004), and some adaptations produced by repeated cocaine exposure differ between the subregions, including adaptations potentially related to local protein synthesis (e.g. Wang *et al.*, 2010). Therefore, it is possible that the core–shell selectivity we have observed in drug-naïve rats is lost or altered due to neuroadaptations produced by repeated cocaine exposure. For example, a relationship between elevated BDNF and AMPAR trafficking may emerge in the shell of cocaine-experienced rats, or be altered in the core. Exploring these possibilities is an important goal for future studies because it may help explain some results discussed in the next section pertaining to core–shell differences in the role of BDNF in cocaine-seeking behavior.

Possible core–shell differences in the role of BDNF after cocaine exposure

There is growing evidence for distinct BDNF–cocaine interactions in core vs. shell. For example, BDNF mRNA levels were increased in the shell but not the core after acute or repeated cocaine injections, while TrkB mRNA levels declined more robustly in core than shell (Filip *et al.*, 2006). Furthermore, a transient increase in BDNF protein levels was found in the shell but not in the core immediately after cocaine self-administration, but not 1 day later; this increase was linked to maintaining higher cocaine intake and facilitating relapse to cocaine-seeking after abstinence (Graham *et al.*, 2007). TrkB levels were also elevated in shell but not core immediately after cocaine self-administration, and knocking down TrkB in the NAc reduced cocaine reward in cocaine place conditioning experiments and produced a downward shift in the cocaine self-administration dose–response curve (Graham *et al.*, 2009). These data provide evidence for facilitation of cocaine reward by BDNF–TrkB activation in the NAc shell.

In contrast to these results, there is evidence that elevation of BDNF in the prefrontal cortex (PFC) is a homeostatic response that opposes drug-seeking (Berglind *et al.*, 2007, 2009; Sadri-Vakili *et al.*, 2010; Whitfield *et al.*, 2011; but see Lu *et al.*, 2010). Interestingly, a single injection of BDNF into the dorsal PFC, which is sufficient to suppress subsequent cocaine-seeking, increased BDNF levels not only in the PFC but also in the NAc (presumably via anterograde transport in PFC–NAc projections; Berglind *et al.*, 2007). Intra-PFC injection of

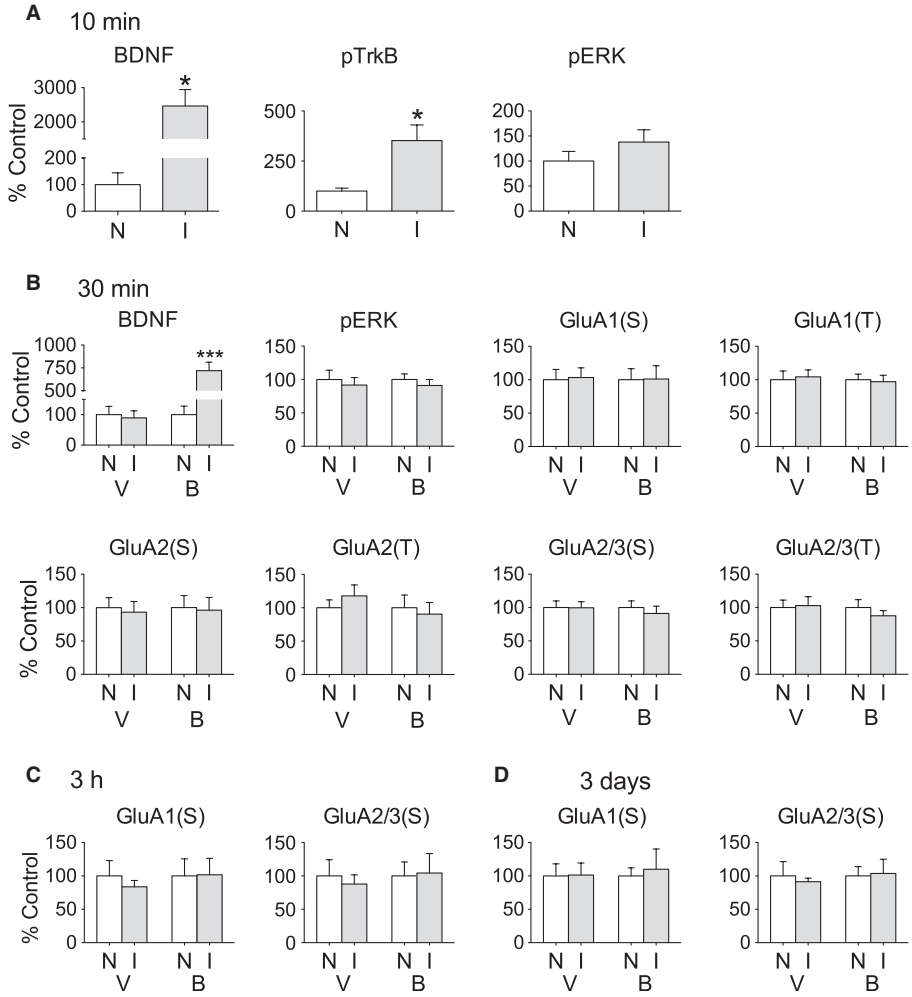

FIG. 5. No effect on AMPAR expression was observed after unilateral brain-derived neurotrophic factor (BDNF) injection into the nucleus accumbens (NAc) shell. (A) Ten minutes after BDNF injection into NAc shell (0.75 μg/0.5 μL), BDNF protein levels ($t_6 = 4.91$, $^*P < 0.05$) and phosphorylated TrkB (pTrkB; $t_6 = 3.17$, $^*P < 0.05$) were significantly elevated in the injected hemisphere (I) compared with the non-injected hemisphere (N). $N = 4$ rats/group. (B) Tissue was analysed 30 min after injection of BDNF (B; 0.75 μg/0.5 μL) or vehicle (V) into the NAc shell. While BDNF was still significantly elevated 30 min after BDNF injection ($t_{14} = 6.38$, $^{***}P < 0.0005$), no change in pERK levels or AMPAR surface (S) expression was found in the injected hemisphere compared with the non-injected hemisphere. Total AMPAR subunit levels (T) were also unchanged. $N = 8$ rats/group. (C and D) No changes in AMPAR subunit surface expression were found 3 h or 3 days after BDNF injection into the NAc shell (0.25 μg/0.5 μL). Total AMPAR subunit levels were also unchanged (data not shown). BDNF levels in the injected hemisphere remained significantly elevated 3 h but not 3 days after BDNF injection (data not shown; $N = 8$ rats/group for 3-h time-point and 5 rats/group for 3-day time-point). All data (mean ± SEM) are expressed as percent of mean values in the non-injected hemisphere.

BDNF also normalized alterations in NAc glutamate transmission linked to reinstatement of cocaine-seeking (Berglind *et al.*, 2009). Microdialysis probes in the latter study were primarily located in the core, and the region of PFC injected with BDNF in both studies projects primarily to the NAc core (see Pierce *et al.*, 1998). Thus, one way to reconcile these results with those of Graham *et al.* (2007, 2009) is to propose that elevation of BDNF–TrkB signaling in the core suppresses cocaine-seeking, whereas the opposite relationship holds in the shell. However, it is also possible that presynaptic and postsynaptic effects of BDNF in the NAc promote different behaviors, and that PFC infusion mimics a predominantly presynaptic effect of BDNF. Furthermore, the temporal pattern of BDNF release likely differed between studies, and it has been shown that the temporal aspects of BDNF application can significantly alter the duration of TrkB activation and its downstream consequences (Ji *et al.*, 2010).

In addition to core–shell differences, evidence for cell-type specificity of BDNF action in the NAc of cocaine-experienced rodents is provided by a recent study showing that TrkB deletion had opposite effects on cocaine reward in D1 receptor-positive vs. D2 receptor-positive NAc neurons (Lobo *et al.*, 2010). Studies that did not distinguish between NAc subregions or cell types generally suggest that elevating NAc BDNF signaling promotes cocaine-related behaviors (Horger *et al.*, 1999; Grimm *et al.*, 2003; Bahi *et al.*, 2008).

BDNF and CP-AMPARs

Our observation that BDNF increases surface expression of GluA1 but not GluA2 or GluA2/3 implicates homomeric GluA1 receptors, which are CP-AMPARs. Some previous results also suggest a selective effect of BDNF on CP-AMPARs: (i) In hippocampal cultures, incubation with BDNF for 30 min produced a protein synthesis-dependent increase in cell surface GluA1 but not GluA2 that normalized by 3 h, suggesting a transient increase in CP-AMPARs very similar to what we have observed *in vivo* (Caldeira *et al.*, 2007). (ii) In pond turtle brainstem, bath application of BDNF for 80 min increased synaptic

delivery of GluA1 and GluA4, reproducing cellular changes associated with classical conditioning in this *in vitro* model (Li & Keifer, 2008, 2009). (iii) Mutant mice with lower BDNF levels showed reduced hippocampal expression of GluA1 but not GluA2 or GluA3 (Giralt *et al.*, 2009). However, other results suggest that BDNF can regulate protein levels of GluA2 and GluA3 as well as GluA1 (Narisawa-Saito *et al.*, 1999; Caldeira *et al.*, 2007), and induce a rapid translocation of GluA2-containing AMPARs to the cell surface (Narisawa-Saito *et al.*, 2002). Furthermore, our preliminary studies in cultured NAc neurons have found that 30 min of incubation with BDNF increased cell surface GluA1 and GluA2 levels, as well as their co-localization (Reimers *et al.*, 2010). Together, these results indicate that BDNF can selectively modulate CP-AMPARs, but also influences GluA2-containing AMPARs under some conditions.

TrkB signaling after BDNF infusion

Our results at the 30-min time-point in the core showed that the transient effect of BDNF on GluA1 surface expression is ERK-dependent, which is consistent with results obtained in the turtle brainstem (Li & Keifer, 2009). ERK activation is also implicated in upregulation of GluA1/A2-containing AMPARs in the NAc of cocaine-sensitized rats (Boudreau *et al.*, 2007; Schumann & Yaka, 2009). However, some of the present results indicate dissociation between ERK activation and GluA1 surface expression. Thus, pERK remained elevated at the 3-h time-point, whereas surface GluA1 had normalized. Furthermore, while anisomysin prevented the BDNF-induced increase in surface GluA1 at the 30-min time-point, ERK activation was not altered. Similarly, a reduction in BDNF levels can be associated with decreased GluA1 expression in the absence of altered ERK phosphorylation (Giralt *et al.*, 2009). It should be noted that our study measured pERK in tissue homogenates, leaving open the possibility of compartmentalized changes in ERK activation that parallel the observed changes in GluA1 surface expression.

We also monitored TrkB phosphorylation at Tyr706/707 to verify biological activity of BDNF after *in vivo* injection. It is well established that positionally equivalent residues in TrkA, B and C correspond to autophosphorylation sites in the catalytic domain; phosphorylation of these sites is necessary for TrK activation and subsequent activation of downstream pathways including the MAPK pathway (Segal *et al.*, 1996; Cunningham *et al.*, 1997; Huang & Reichardt, 2003). In our studies, we found a significant elevation in pTrkB 10 min after BDNF injection into either the core or shell, indicating that infused BDNF triggered TrkB activation. Unfortunately, we were unable to measure pTrkB at later time-points, as tissue collected at these time-points was cross-linked with BS^3 to enable measurement of AMPAR surface expression, and the pTrkB antibody was not able to detect cross-linked TrkB receptors.

Due to the rapid protein synthesis-dependent nature of the BDNF-induced increase in GluA1 in the NAc core, we speculate that the increased GluA1 protein was translated from local mRNA. Indeed, some results in other systems are consistent with an ability of BDNF to modulate local translation of GluA1 (e.g. Narisawa-Saito *et al.*, 1999; Slipczuk *et al.*, 2009). However, we failed to detect changes in levels or phosphorylation of some candidate signaling molecules that could contribute to local protein synthesis regulation after BDNF, including AKT (protein kinase B) and mTOR (mammalian target of rapamycin; data not shown). Our results could be explained by the involvement of diverse mechanisms in BDNF-induced local protein synthesis (Santos *et al.*, 2010).

In conclusion, our *in vivo* results extend prior *in vitro* findings suggesting that BDNF can selectively modulate CP-AMPARs (Caldeira *et al.*, 2007; Li & Keifer, 2008, 2009). Thus, future studies are warranted to determine if there is a relationship between chronic cocaine-induced changes in BDNF levels (Ghitza *et al.*, 2010) and AMPAR transmission (Wolf & Ferrario, 2010), particularly in animal models of cocaine addiction in which CP-AMPARs are implicated (Conrad *et al.*, 2008; Mameli *et al.*, 2009; Ferrario *et al.*, 2011; McCutcheon *et al.*, 2011).

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Marina E. Wolf has no biomedical financial interests, but has a patent on A Possible Therapy For Cue-Induced Cocaine Craving Leading to Relapse in Abstinent Cocaine Abusers Based on Blockade of GluR2-lacking AMPA Receptors in the Nucleus Accumbens. Xuan Li reports no biomedical financial interests or potential conflicts of interest.

Abbreviations

AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF, brain-derived neurotrophic factor; BS^3, bis(sulfosuccinimidyl) suberate; CP-AMPAR, Ca^{2+}permeable AMPA receptor; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; NAc, nucleus accumbens; PBS, phosphate-buffered saline; pERK, phosphorylated extracellular signal-regulated kinase; PFC, prefrontal cortex; pTrkB, phosphorylated tropomyosin receptor kinase B; TrkB, tropomyosin receptor kinase B.

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