International Journal of Neuropsychopharmacology, Page 1 of 11. © CINP 2012
doi:10.1017/S1461145712000168

ARTICLE

CINP

Induction of the plasticity-associated immediate early gene *Arc* by stress and hallucinogens: role of brain-derived neurotrophic factor

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Abstract

Exposure to stress and hallucinogens in adulthood evokes persistent alterations in neurocircuitry and emotional behaviour. The structural and functional changes induced by stress and hallucinogen exposure are thought to involve transcriptional alterations in specific effector immediate early genes. The immediate early gene, activity regulated cytoskeletal-associated protein (*Arc*), is important for both activity and experience dependent plasticity. We sought to examine whether trophic factor signalling through brain-derived neurotrophic factor (BDNF) contributes to the neocortical regulation of *Arc* mRNA in response to distinct stimuli such as immobilization stress and the hallucinogen 2,5-dimethoxy-4-iodoamphetamine (DOI). Acute exposure to either immobilization stress or DOI induced *Arc* mRNA levels within the neocortex. BDNF infusion into the neocortex led to a robust up-regulation of local *Arc* transcript expression. Further, baseline *Arc* mRNA expression in the neocortex was significantly decreased in inducible BDNF knockout mice with an adult-onset, forebrain specific BDNF loss. The induction of *Arc* mRNA levels in response to both acute immobilization stress or a single administration of DOI was significantly attenuated in the inducible BDNF knockout mice. Taken together, our results implicate trophic factor signalling through BDNF in the regulation of cortical *Arc* mRNA expression, both under baseline conditions and following stress and hallucinogen exposure. These findings suggest the possibility that the regulation of *Arc* expression via BDNF provides a molecular substrate for the structural and synaptic plasticity observed following stimuli such as stress and hallucinogens.

Received 12 October 2011; Reviewed 1 December 2011; Revised 31 January 2012; Accepted 6 February 2012

Key words: Activity regulated cytoskeletal-associated protein, BDNF knockout mouse, DOI, neocortex, immobilization stress.

Introduction

Stress and hallucinogen exposure evokes changes in neuronal structure and function often observed to persist long after the cessation of the original stimulus (Abraham, 1983; Arnsten, 2009; Lupien *et al.* 2009). Rapid transcriptional changes that arise following initial exposure to stressful and hallucinogenic stimuli are implicated in establishing molecular changes that eventually result in long-lasting alterations of both structural and synaptic plasticity (Leslie & Nedivi, 2011). The effector immediate early gene (IEG), activity regulated cytoskeletal-associated protein (*Arc*) is rapidly up-regulated in response to stress and hallucinogen treatment and is implicated in coupling activity and experience related stimuli to neuronal plasticity (Lyford *et al.* 1995; Nichols & Sanders-Bush, 2002; Ons *et al.* 2004; Pei *et al.* 2004). *Arc* through its crosstalk with the cytoskeleton influences actin dynamics and through its interactions with endophilin and dynamin modulates neurotransmitter receptor trafficking, thus regulating structural and synaptic plasticity (Chowdhury *et al.* 2006; Messaoudi *et al.* 2007). In particular, among the effector IEGs, *Arc* is ideally poised to rapidly translate experience-induced changes in neuronal activity into sustained structural and functional alterations at the synapse

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(Alberi *et al.* 2011; Fosnaugh *et al.* 1995; Peebles *et al.* 2010; Steward & Worley, 2002).

The regulation of *Arc* expression has been best studied in the hippocampus, where it plays a key role in modulating synaptic plasticity associated with learning and memory (Guzowski *et al.* 2000; Peebles *et al.* 2010). The neurotrophin brain-derived neurotrophic factor (BDNF) has been implicated in contributing to the hippocampal regulation of *Arc* expression, in particular in the context of stimuli that evoke long-term potentiation, a cellular correlate of learning and memory (Ying *et al.* 2002). Infusion of BDNF into the dentate gyrus subfield of the hippocampus up-regulates Arc levels (Ying *et al.* 2002). Further, BDNF has been shown to increase the translation of Arc in hippocampal synaptoneurosomal fractions (Yin *et al.* 2002). However, it is poorly understood whether BDNF signalling regulates *Arc* expression in a similar manner across other brain regions.

We hypothesized that BDNF may be important to the regulation of neocortical *Arc* mRNA expression in response to stimuli such as stress and hallucinogen exposure, which evoke persistent changes in structural and synaptic plasticity. Using BDNF infusions and the forebrain and adult specific, inducible BDNF knockout (KO) mice, we addressed the role of BDNF in the regulation of *Arc* mRNA under baseline conditions and in response to acute immobilization stress or a single dose of the hallucinogen, 2,5-dimethoxy-4-iodoamphetamine (DOI). Our results demonstrate that neocortical BDNF infusion leads to an increase in *Arc* mRNA levels, whereas adult-onset loss of BDNF in the forebrain decreases levels of *Arc* mRNA in the neocortex, implicating BDNF in the maintenance of baseline *Arc* mRNA expression. Strikingly, inducible BDNF KO mice exhibit a significant attenuation of the stress and DOI induced *Arc* transcript expression in the neocortex. These results highlight an important role for BDNF in the signalling pathways downstream of stress and hallucinogen exposure, culminating in the transcription of the synaptic plasticity associated IEG *Arc*.

Method

Animal treatment paradigms: Sprague–Dawley rats

Male Sprague–Dawley rats bred in the Tata Institute of Fundamental Research (TIFR) colony were used in all rat experiments. Animals were group housed and maintained on a 12 h light–dark cycle (lights on 07:00 hours) with access to food and water *ad libitum*. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the TIFR Institutional Animal Ethics Committee.

Animal treatment paradigms: inducible BDNF KO mice

In order to address the role of BDNF in the regulation of *Arc* mRNA by stress and hallucinogens, we used the inducible BDNF KO mice (Monteggia *et al.* 2004). Trigenic (NSE-tTA/TetOp-Cre/floxed BDNF) mice were bred on doxycycline (1 mg/ml; Sigma, Germany) administered in their drinking water to suppress recombination until they were aged 3 months. The mice were then divided into two cohorts, one maintained on doxycycline throughout [wild-type (WT)] and the other with doxycycline withdrawal to induce recombination in adult mice to avoid confounds of developmental compensation (BDNF KO mice). BDNF KO mice were taken off doxycycline and maintained on drinking water for 3 months prior to killing for experiments to ensure complete recombination. Both male and female mice were used for these experiments.

Immobilization stress

We used the immobilization stress paradigm to address the stress-mediated regulation of *Arc* mRNA and the role of BDNF in such a regulation. Adult (aged 2–3 months) Sprague–Dawley rats or the inducible BDNF KO mice were subjected to the acute immobilization stress paradigm as previously described (Nair *et al*. 2007).

Immobilization stress: Sprague–Dawley rats

Sprague–Dawley rats were subjected to immobilization stress by placing them in plastic restrainer cones (Stoelting Co., USA) for 2 h and they were killed at the end of this period. Controls were handled and left in the home cage until the time of killing. At the time of killing, rats were decapitated, their brains rapidly dissected and frozen prior to *in situ* hybridization. This experiment involved two groups: control (Con) and acute immobilization stress (AIS), $n = 7–9$/group.

Immobilization stress: inducible BDNF KO mice

The BDNF KO mice and their WT controls were subjected to immobilization stress by placing them in 50 ml perforated plastic tubes for 2 h, while the non-stressed groups were handled and left undisturbed in

the home cage until the time of killing. Mice were killed by anaesthetizing using thiopentone and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA) and brains were stored prior to sectioning. The experimental design to examine the role of BDNF in acute stress-induced *Arc* mRNA levels involved four groups: WT, BDNF KO mice (BDNF KO), WT mice subjected to acute immobilization stress (AIS) and BDNF KO mice subjected to acute immobilization stress (BDNF KO + AIS), $n = 3$–5/group.

Pharmacological treatment: Sprague–Dawley rats

The regulation of *Arc* mRNA by the hallucinogen DOI was studied by administering a single i.p. injection of DOI (8 mg/kg, Sigma) or vehicle (0.9% saline) and killing 2 h later (Vaidya *et al.* 1997). At the end of 2 h, the rats were rapidly anaesthetized, brains dissected out and immediately frozen prior to processing for *in situ* hybridization. The experiment involved two groups: vehicle-treated control group (Con) and the DOI treated group (DOI), $n = 3$–4/group.

Pharmacological treatment: inducible BDNF KO mice

The BDNF KO and WT control mice were anaesthetized with thiopentone and transcardially perfused with standard saline followed by 4% PFA. Brains were dissected out, fixed in 4% PFA and cryoprotected using 30% sucrose in 4% PFA. The experiment consisted of four cohorts: WT mice injected with saline; WT mice injected with DOI (DOI); BDNF KO mice injected with saline (BDNF KO); BDNF KO mice injected with DOI (BDNF KO + DOI), $n = 4$–6/group.

Neocortical infusion of BDNF in Sprague–Dawley rats

Sprague–Dawley rats (aged 3–4 wk) were used for this experiment. Rats were anaesthetized with a ketamine (50 mg/kg; Sigma)–xylazine (20 mg/kg; Sigma) cocktail and placed in a stereotactic rig (Stoelting). Recombinant human BDNF (5 μg; Promega, USA) was infused into the neocortex (1.5 mm posterior to bregma, 2.0 mm lateral to the midline and 1.0 mm below the dura) using a Hamilton syringe over a period of 5 min. Sham-treated animals received 5 μl saline. All animals were killed 3 h following infusion of BDNF. This experiment consisted of three cohorts: a cohort that was subjected to the entire surgical procedure (sham); a cohort that was infused with vehicle (vehicle); a cohort that received BDNF infusion (BDNF), $n = 3$–4/group. The *Arc* mRNA expression on the side ipsilateral to the surgery was normalized to that on the side contralateral to the infusion site.

In situ *hybridization*

In situ hybridization on fresh frozen rat brains was carried out as previously described (Nair *et al*. 2007). At the end of the stress exposure, animals were killed by rapid decapitation, brains dissected out and stored at $-70\,^{\circ}$C. Cryostat (Leica, Germany) cut 14 μm thick sections were thaw mounted onto ribonuclease free probe-on plus slides (Electron Microscopy Services, USA). The slides were treated with 4% PFA, acetylated, dehydrated and stored at $-70\,^{\circ}$C. [^{35}S]UTP labelled (PerkinElmer, USA) riboprobes against *Arc* mRNA were generated from a transcription competent plasmid kindly provided by Dr Oswald Steward (Johns Hopkins University, USA). Sections were then incubated with the [^{35}S]UTP labelled riboprobe (1×10^6 cpm/150 μl) in hybridization buffer (50% formamide, $1 \times$ SSC, $25 \times$ Denhardt's solution, 40 mM dithiothreitol, 150 μg/ml yeast tRNA, 10% dextran sulfate, 400 μg/ml salmon sperm DNA) for 20 h at 60 °C. Following hybridization, all sections were washed in ribonuclease A (20 μg/ml; USB corporation, USA), followed by stringent washes in decreasing concentrations of SSC, air dried and exposed to hyper film β-max (GE Healthcare, USA) for 7 d. To determine cellular localization, sections were also dipped in emulsion (NTB2, Kodak, USA) and developed 6 wk later. Slides were counterstained with Toluidine Blue to show the silver grains.

Brains from experiments involving the inducible BDNF KO mice were sectioned (30 μm) using a freezing microtome (Leica, Germany), fixed (4% PFA), blocked (0.1 M glycine) and acetylated. The sections were then incubated in pre-hybridization buffer (50% formamide, $1 \times$ SSC, 0.025% BSA, 0.025% polyvinyl pyrolidone, 0.025% Ficoll, 10% dextran sulphate, 40 mM dithiothreitol, 150 μg/ml yeast tRNA, 400 μg/ml salmon sperm DNA and 0.03% Triton X-100) at 60 °C for 1 h. [^{35}S]UTP labelled riboprobes against *Arc* mRNA were generated from a transcription competent plasmid. Sections were then incubated with the [^{35}S]UTP labelled riboprobe (1×10^6 cpm/150 μl) added to the pre-hybridization buffer (without Triton X-100) for 20 h at 60 °C. Following hybridization, sections were washed in ribonuclease A (20 μg/ml), followed by stringent washes in decreasing concentrations of SSC, air dried and exposed to hyper film β-max for 7–10 d. Levels of *Arc* mRNA were quantified using Scion Image (Scion, USA) and calibrated using ^{14}C standards to correct for nonlinearity.

Equivalent areas of the neocortex were outlined and optical density measurements were determined (3–4 sections/animal).

Immunohistochemistry

Cryostat cut sections were used to show Arc protein in the neocortex following acute immobilization stress. Sections were first treated with 3% H_2O_2 for 10 min followed by a 1 h incubation in blocking solution (0.1% Triton X-100, 10% horse serum in 0.1 M PB). Sections were then washed with 0.1 M PB and incubated with polyclonal goat anti-Arc antibody (1:1000, Santa Cruz Biotechnology, USA) in 0.1 M PB containing 0.1% Triton X-100 overnight at 4 °C. Following washes with 0.1 M PB, sections were incubated with biotinylated secondary antibody (donkey anti-goat, 1:500; Santa Cruz Biotechnology) for 2 h at room temperature. A Vectastain ABC Elite kit (Vector Laboratory, USA) and diaminobenzidine (Sigma) was used to visualize Arc immunoreactivity. The immunohistochemical signal was imaged on a brightfield microscope (Zeiss Axioskop, Zeiss, Germany).

Statistical analysis

Statistical analysis was performed using the software Prism (Graphpad, USA). Results were subjected to the Student's *t* test for experiments with two groups. Experiments with three groups were subjected to one-way analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test. Two-way ANOVA analysis followed by Bonferroni's *post-hoc* test was used for experiments with four groups. Differences were considered statistically significant at p values <0.05.

Results

Acute exposure to immobilization stress or the hallucinogen DOI increases levels of Arc *mRNA in the neocortex of adult rats*

We sought to address the regulation of *Arc* mRNA levels in response to emotionally salient cues such as acute immobilization stress and the phenethylamine hallucinogen, DOI. Acute immobilization stress is a strong emotional stressor and has been shown to induce neuronal activation across specific neocortical regions (Cullinan *et al.* 1995; Ons *et al.* 2004). At the end of the 2 h acute immobilization stress, *Arc* mRNA levels were significantly increased in the rat neocortex with differential induction observed across several cortical layers (Fig. 1*a*; $p=0.008$, Student's *t* test; $n=7$–9/group). Emulsion autoradiography revealed induction of *Arc* mRNA levels across several neocortical layers, with the strongest induction seen within layer IV (Fig. 1*b*). Immunohistochemistry for Arc protein also revealed an increase in somatodendritic immunoreactivity for Arc in the neocortex of rats subjected to acute immobilization stress (Fig. 1*c*). In striking contrast, at the time-point examined, immobilization stress did not alter *Arc* mRNA expression in any of the hippocampal subfields (Fig. 1*a*). This observation differs from the regulation of *Arc* mRNA in response to novelty exposure, a milder stressor that does induce *Arc* mRNA expression within hippocampal subfields (Guzowski *et al.* 1999). Thus, it appears that induction of *Arc* mRNA in neocortical regions may be a common theme of stress exposure with specific elements relating to stressor type determining the participation of the hippocampus.

We next addressed the regulation of *Arc* mRNA levels in Sprague–Dawley rats upon exposure to the hallucinogen DOI. DOI is a phenethylamine hallucinogen and a partial agonist at the 5-HT_2 receptor. Treatment with DOI led to a robust induction of *Arc* mRNA levels in the rat neocortex (Fig. 2; $p=0.009$, Student's *t* test, $n=3$–4/group) and not in the hippocampus, consistent with previous observations (Pei *et al.* 2000).

Neocortical infusion of BDNF increases Arc *mRNA levels in the rat neocortex*

We next asked whether the neurotrophin BDNF, which has been implicated in the regulation of Arc in the hippocampus, influences neocortical *Arc* mRNA expression. This was addressed by infusing recombinant BDNF into the neocortex of Sprague–Dawley rats and measuring local expression of *Arc* mRNA. Infusion of BDNF led to a robust and significant induction of *Arc* mRNA levels in the neocortex, proximal to the site of infusion in comparison to sham surgery or vehicle infusion (Fig. 3*a*; one-way ANOVA: $F_{1,9}=48.12$, $p<0.0001$; $n=3$–4/group). Interestingly, previous studies indicate that infusion of BDNF into the hippocampus also increases local *Arc* mRNA levels (Ying *et al.* 2002).

Adult forebrain specific inducible BDNF KO mice exhibit a decline in baseline Arc *mRNA levels in the neocortex*

Given that BDNF infusions increased levels of *Arc* mRNA in the rat neocortex, we used the inducible BDNF KO mice to investigate whether loss of BDNF affects neocortical *Arc* mRNA levels. A role for BDNF in cortical *Arc* mRNA expression in adulthood has

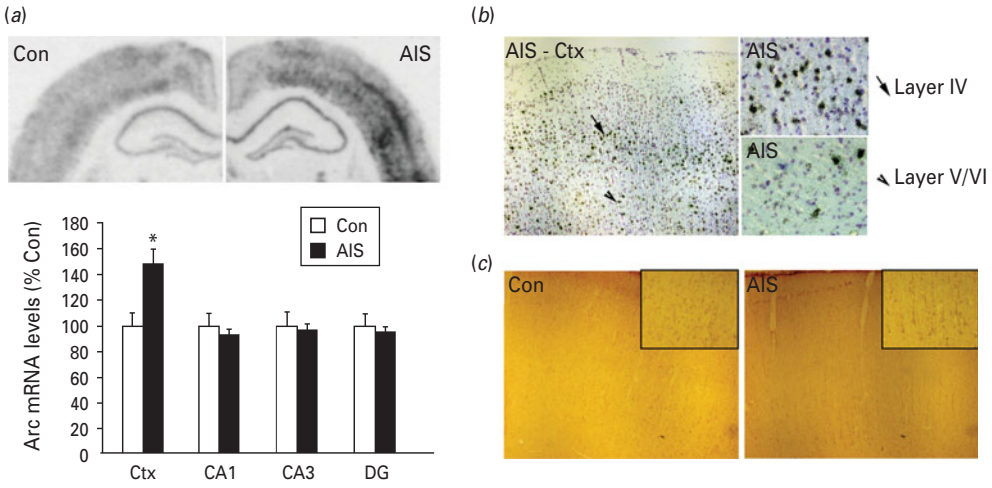

Fig. 1. Influence of acute immobilization stress on activity regulated cytoskeletal-associated protein (*Arc*) mRNA levels in the rat neocortex (Ctx) and hippocampal subfields. (*a*) Levels of *Arc* mRNA in the Ctx and hippocampus of rats belonging to the control (Con) and acute immobilization stress (AIS) groups. Exposure to AIS led to a robust induction of *Arc* mRNA in the Ctx and not the CA1, CA3 and dentate gyrus (DG) subfields of the hippocampus. The results are represented as percentage of Con and are the mean ± S.E.M. ($n = 7$–9/group). * $p < 0.05$ compared to Con; Student's t test. (*b*) Cellular localization of *Arc* mRNA induction in the Ctx following AIS. Shown is a representative brain section from an AIS animal with a strong expression of black silver grains indicating hybridization to *Arc* mRNA within layer IV (arrow) together with scattered cells in layer V/VI (arrowhead). (*c*) The distribution of Arc protein in the Ctx in response to AIS. Immunohistochemical analysis using an antibody against Arc shows somatodendritic labelling (inset – 20 × magnification) and a clear induction in the regional expression of Arc protein in the Ctx.

been difficult to investigate, owing to the lack of KO mice with a specific depletion of BDNF protein in the forebrain only in adulthood. The BDNF heterozygote mice (Lyons *et al.* 1999) and the BDNF conditional KO mice (Rios *et al.* 2001) exhibit a loss of BDNF during embryonic and early post-natal life, thus making it difficult to interpret experiments done in adulthood. To circumvent any possible compensatory effects because of loss of BDNF during developmental critical periods, we used inducible BDNF KO mice, which allow for inducible temporal and spatial control of BDNF expression in adulthood (Monteggia *et al.* 2004). Inducible BDNF KO mice have been previously shown to exhibit a significant decline in BDNF mRNA and protein within the neocortex and the hippocampus (~70%), with residual levels of BDNF still observed due to the nature of recombination, which does not occur in every neuron (Monteggia *et al.* 2004). The inducible BDNF KO mice were divided into two cohorts: one cohort was maintained on doxycycline to preserve normal levels of BDNF (WT) while the other cohort was maintained on drinking water to induce a loss of BDNF protein, specifically within the forebrain for a period of 3 months to ensure complete recombination prior to commencing experiments. Analysis of *Arc* mRNA levels using *in situ* hybridization revealed a decrease in baseline *Arc* mRNA levels in the neocortex of the BDNF KO mice (Fig. 3*b*; $p = 0.004$, Student's t test; $n = 5$/group). It is noteworthy that the decline in *Arc* mRNA levels in BDNF KO mice was restricted to the neocortex with no change in baseline expression observed in the hippocampal subfields examined. These results suggest that BDNF-mediated signalling contributes to maintaining baseline expression of *Arc* mRNA levels in the neocortex. Taken together with our results, this suggests that, while infusion of BDNF protein leads to the induction of *Arc* mRNA levels both in the neocortex and the hippocampus, loss of BDNF expression affects the baseline levels of *Arc* mRNA only in the neocortex. This suggests the possibility that, while neocortical *Arc* expression is sensitive to a depletion of BDNF levels, baseline *Arc* expression within the hippocampus may still be maintained despite a significant depletion of BDNF.

Neocortical induction of Arc *mRNA levels by acute immobilization stress is significantly attenuated in inducible BDNF KO mice*

We next addressed whether acute immobilization stress-evoked increases in neocortical *Arc* mRNA expression are altered in BDNF KO mice. Acute immobilization stress robustly induced levels of *Arc*

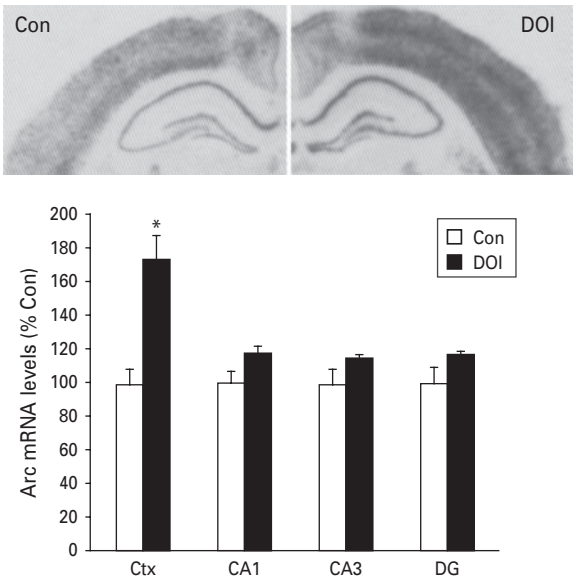

Fig. 2. Regulation of activity regulated cytoskeletal-associated protein (*Arc*) mRNA in the rat neocortex (Ctx) and hippocampus following acute 2,5-dimethoxy-4-iodoamphetamine (DOI) treatment. Shown are the levels of *Arc* mRNA in the rat Ctx, CA1, CA3 and dentate gyrus (DG) hippocampal subfields quantified using densitometric analysis. Acute DOI robustly increased levels of *Arc* mRNA in the Ctx and not the hippocampal subfields examined. Levels of *Arc* mRNA from the control (Con) and acute DOI groups are represented as percentage of Con and are the mean ± S.E.M. ($n = 3$–4/group). * $p < 0.05$ compared to Con; Student's *t* test).

mRNA in the neocortex but not the hippocampus of WT control mice. In striking contrast, the induction of *Arc* mRNA in response to acute immobilization stress was significantly attenuated within the neocortex of BDNF KO mice (Fig. 4). Two-way ANOVA analysis revealed a significant BDNF KO × acute immobilization stress interaction ($F_{1,12} = 10.45$, $p = 0.0072$, $n = 3$–5/group). These results demonstrate a role for BDNF signalling in the induction of *Arc* mRNA transcripts within the neocortex in response to acute immobilization stress.

Induction of Arc *mRNA levels by DOI in the neocortex is significantly attenuated in inducible BDNF KO mice*

The hallucinogen DOI leads to a robust induction of *Arc* mRNA levels in the neocortex and not the hippocampus (Fig. 2). To address the role of BDNF in the DOI-induced regulation of *Arc* mRNA levels, WT and BDNF KO mice were treated with DOI. While WT control mice showed a significant increase in neocortical *Arc* mRNA levels, this induction of *Arc* transcript was not observed in BDNF KO mice (Fig. 5). Two-way ANOVA analysis indicated a significant BDNF KO × DOI interaction ($F_{1,17} = 15.78$, $p = 0.001$, $n = 4$–6/group). These findings demonstrate a requirement of BDNF for the effects of DOI on *Arc* mRNA within the neocortex.

Discussion

Arc has been hypothesized to serve as a key molecular substrate involved in translating experience dependent transcriptional regulation into synaptic and structural plasticity (Bramham *et al.* 2008). *Arc* mRNA is regulated in response to neuronal activity and is robustly enhanced by diverse environmental stimuli, including stress and hallucinogen exposure (Ons *et al.* 2004; Pei *et al.* 2004). Our results indicate that the regulation of *Arc* mRNA within the neocortex in response to acute immobilization stress or treatment with the hallucinogen, DOI, requires BDNF signalling. Using adult-onset, forebrain specific inducible BDNF KO mice, we show that BDNF signalling is necessary to maintain baseline expression of *Arc* mRNA levels in the neocortex. We also demonstrate that infusion of BDNF in the neocortex evokes a robust induction in *Arc* transcript expression. Strikingly, the ability of immobilization stress and DOI treatment to regulate cortical *Arc* expression is lost in BDNF KO mice. Prior reports have implicated BDNF in *Arc* regulation within the hippocampus in the context of learning and memory. We find that such a role for BDNF in mediating the regulation of the effector IEG *Arc* may be conserved for quite distinct stimuli and across cortical brain regions.

Exposure to acute immobilization stress and the hallucinogen DOI increases *Arc* mRNA levels in the neocortex (Ons *et al.* 2004; Pei *et al.* 2004). These data suggest that the IEG *Arc* is potentially a convergent target for pathways involved in structural remodelling events in response to acute stress and hallucinogens. Indeed, it is tempting to speculate that components of pathways that contribute to the regulation of *Arc* mRNA may be shared for such distinct stimuli as acute immobilization stress and the hallucinogen DOI. Electrophysiological and activity dependent marker expression studies indicate that stress and hallucinogen exposure evoke neuronal activity in overlapping circuits within the neocortex. Both acute immobilization stress and acute DOI induce the expression of several IEGs in the neocortex (Cullinan *et al.* 1995; Tilakaratne & Friedman, 1996). Further, both stress and DOI increase excitatory post-synaptic

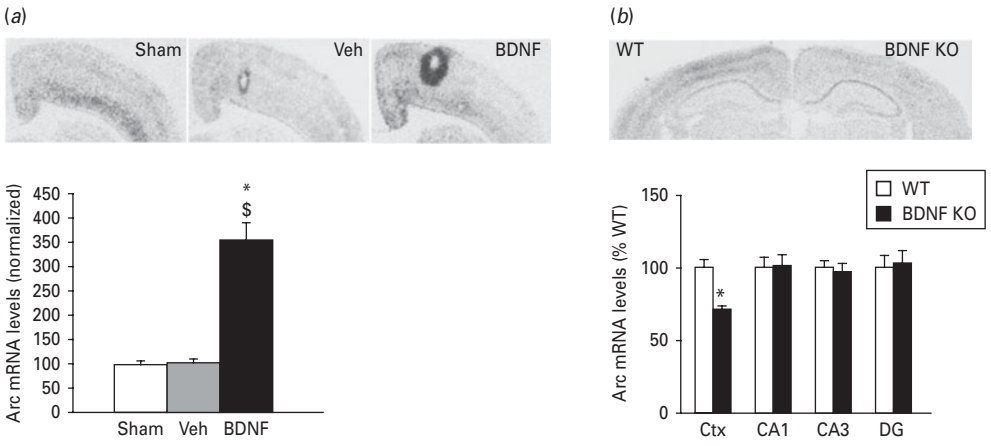

Fig. 3. Brain-derived neurotrophic factor (BDNF) regulates basal levels of activity regulated cytoskeletal-associated protein (*Arc*) mRNA in the neocortex (Ctx) of Sprague–Dawley rats (*a*) and inducible BDNF knockout (KO) mice (*b*). (*a*). Shown are levels of *Arc* mRNA in response to infusion of recombinant BDNF protein into the rat Ctx. Infusion of BDNF into the Ctx led to a robust induction of *Arc* mRNA levels proximal to the site of infusion. The data for the sham, vehicle (Veh) and BDNF infusion groups are normalized to the side contralateral to the infusion site and are the mean ± S.E.M. ($n = 3$–4/group). (* $p < 0.001$ compared to Sham; $^{\$}$ $p < 0.001$ compared to Veh; analysis of variance and Bonferroni's *post-hoc* test). (*b*). Shown are levels of *Arc* mRNA in wild-type (WT) and inducible BDNF knockout (KO) mice. Baseline *Arc* mRNA levels in the Ctx of BDNF KO mice were significantly lower as compared to WT, but were not altered within the dentate gyrus (DG) and CA subfields of the hippocampus as compared to WT controls. The results are presented as percentage of WT and are the mean ± S.E.M. ($n = 5$/group) (* $p < 0.05$ compared to WT; Student's *t* test).

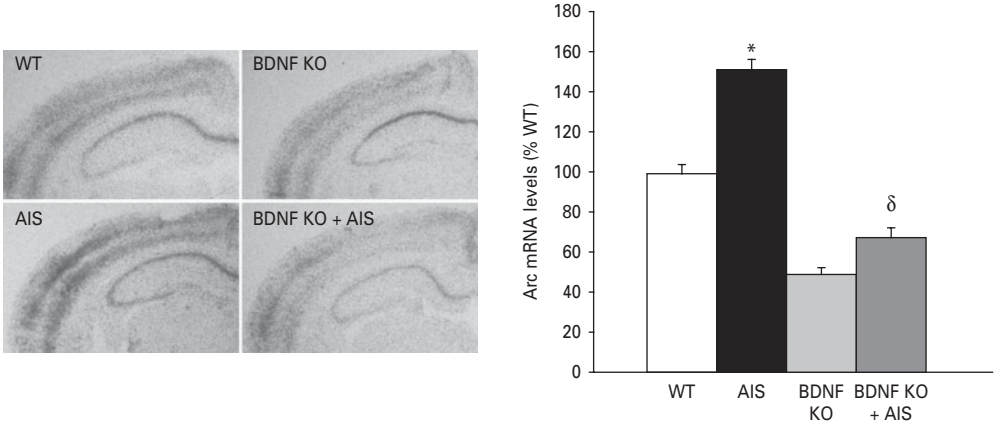

Fig. 4. The neocortical induction of activity regulated cytoskeletal-associated protein (*Arc*) mRNA levels by acute immobilization stress is significantly attenuated in inducible brain-derived neurotrophic factor (BDNF) knockout (KO) mice. Shown are *Arc* mRNA levels in the neocortex of both wild-type (WT) and inducible BDNF KO mice subjected to acute immobilization stress (AIS). AIS evoked a robust induction of *Arc* mRNA levels in the neocortex of WT mice. Inducible BDNF KO mice showed a significant attenuation of the AIS evoked increase in neocortical *Arc* mRNA levels. The results are represented as percentage of WT and are the mean ± S.E.M. ($n = 3$–5/group). * $p < 0.001$ significantly different in comparison to WT; $^{\delta}$ $p < 0.001$ significantly different from AIS; analysis of variance and Bonferroni's *post-hoc* test.

currents (EPSCs) and glutamate release in the neocortex (Gilad *et al.* 1990; Scruggs *et al.* 2003) and stimulation of AMPA or NMDA glutamatergic receptors increases *Arc* mRNA levels (El-Sayed *et al.* 2011). Excitation of neocortical neurons may in turn evoke trophic factor signalling pathways that feed into a downstream regulation of *Arc* transcription. Indeed, in the hippocampus, the induction of *Arc* mRNA by BDNF *in vitro* has been known to require ongoing neuronal activity (Rao *et al.* 2006). In this regard, it is

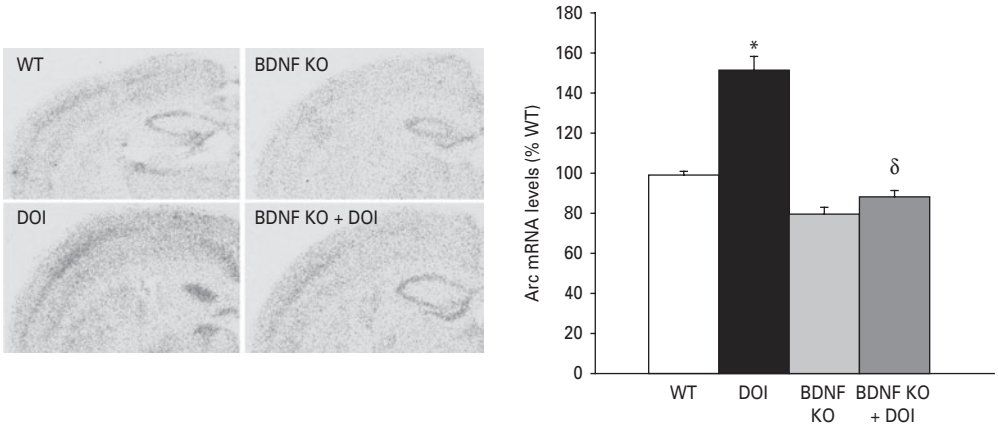

Fig. 5. The neocortical induction of activity regulated cytoskeletal-associated protein (*Arc*) mRNA levels by acute 2,5-dimethoxy-4-iodoamphetamine (DOI) treatment is significantly attenuated in inducible brain-derived neurotrophic factor (BDNF) knockout (KO) mice. Shown are *Arc* mRNA levels in response to acute DOI treatment in wild-type (WT) and inducible BDNF KO mice. While DOI led to a robust induction of *Arc* mRNA levels in the neocortex of WT mice, this induction was not observed in inducible BDNF KO mice. The results are represented as percentage of WT and are the mean ± S.E.M. ($n = 4–6$/group). * $p < 0.001$ significantly different from WT; $^{\delta}$ $p < 0.001$ significantly different from DOI; analysis of variance and Bonferroni's *post-hoc* test.

noteworthy that BDNF itself evokes neuronal firing and may serve to further enhance local neuronal activity within the neocortex (Desai *et al.* 1999). Thus, enhanced activity coupled to increased glutamate release and increased BDNF release may constitute a common pathway that regulates the *Arc* IEG response to both stress and hallucinogen exposure.

Another neurotransmitter pathway that may be commonly recruited by stress and hallucinogen exposure is the serotonergic pathway. Serotonin regulates *Arc* mRNA expression in the neocortex in a 5-HT_2 receptor dependent manner (Pei *et al.* 2000). The serotonergic system and, in particular, the 5-HT_2 receptor has been shown to be a target of stress and hallucinogenic effects in the neocortex (Vaidya *et al.* 1997). The effects of DOI and stressors such as novelty exposure on neocortical *Arc* mRNA regulation are mediated through the 5-HT_2 receptor (Pei *et al.* 2004; Santini *et al.* 2011). This raises the intriguing possibility that the effects of stress and DOI on neocortical, but not hippocampal, *Arc* mRNA expression arise as a consequence of distinct effects of 5-HT_2 receptors on neuronal activation within these circuits. While the 5-HT_{2A} receptor is predominantly expressed on pyramidal neurons in the rat cortex (Willins *et al.* 1997), its expression in the hippocampus is largely on GABAergic interneurons (Pompeiano *et al.* 1994; Wright *et al.* 1995). 5-HT_2 receptor stimulation increases EPSPs in the cortex (Aghajanian & Marek, 1999), whereas in the hippocampus 5-HT_2 receptor activation leads to an increase in spontaneous γ-aminobutyric acid (GABA) release and inhibitory post-synaptic current (IPSC) frequency (Piguet & Galvan, 1994). This may be one possible reason why the activity-responsive IEG Arc is up-regulated by DOI and immobilization stress in neocortical regions, but does not appear to be regulated within hippocampal subfields. In this context, it is also noteworthy that both increased total 5-HT levels and enhanced 5-HT_2 stimulation have previously been shown to induce neocortical BDNF as well as *Arc* expression (Pei *et al.* 2000, 2004; Vaidya *et al.* 1997). A reciprocal relationship between the BDNF and serotonergic systems is revealed by results that show that post-natal BDNF KO mice exhibit a range of defects in the serotonergic system, including a reduction in cortical 5-HT_{2A} receptor function (Klein *et al.* 2010; Rios *et al.* 2006). It is therefore possible that the role of BDNF in stress and DOI-induced regulation of *Arc* may involve a common role for 5-HT and 5-HT_2 receptor signalling. Indeed, the fact that relatively diverse stimuli such as acute immobilization stress and DOI both appear to mediate their effects on *Arc* via BDNF suggests the possibility that recruitment of common pathways involving serotonin receptors, neuronal activity or both may act as the common triggers to BDNF-dependent *Arc* transcriptional regulation.

Previous studies have examined the role of BDNF in contributing to DOI-evoked cortical IEG regulation using conditional BDNF KO mice that show an early post-natal onset of BDNF loss across the brain (Klein *et al.* 2010). In conditional BDNF KO mice that exhibit disrupted 5-HT_{2A} receptor responses, DOI-induced head twitch behaviour and enhanced c-fos positive

cell number in the neocortex did not differ from WT controls. Further, basal c-fos positive cell number was also not changed in conditional BDNF KO mice. This finding is quite different from what we observe *vis-à-vis* the baseline and DOI-evoked regulation of cortical *Arc* expression in adult-onset, inducible BDNF KO mice. This difference raises the possibility that intact BDNF signalling may be necessary for *Arc* transcription but may not be key to the regulation of c-fos levels in response to hallucinogenic stimuli.

Enhanced BDNF via autocrine or paracrine mechanisms could signal through its receptor, TrkB, to eventually cause an induction of *Arc* mRNA. In the hippocampus, it has been shown that ERK1/2-induced cyclic AMP response element-binding protein (CREB) phosphorylation contributes to the effects of BDNF on *Arc* transcript expression (Chotiner *et al.* 2010; Ying *et al.* 2002). On the other hand, ERK can also phosphorylate the transcription factor Elk-1 and thus enhance its ability to form a complex with serum response factor (SRF; Gille *et al.* 1995). The *Arc* promoter has serum response elements at position −936 and −1581 (Waltereit *et al.* 2001) and has been known to be regulated by SRF (Pintchovski *et al.* 2009). Recent results suggest that the effects of BDNF on *Arc* transcription in hippocampal neurons are also strongly dependent on whether enhanced BDNF signalling occurs in an acute or gradual manner (Ji *et al.* 2010). While several studies suggest that within hippocampal neurons the effects of BDNF on *Arc* transcription involve ERK signalling and CREB or SRF mediated transcription, the signalling events that mediate the BDNF-induced up-regulation of *Arc* in the neocortex are currently unclear. Future studies are required to address the signalling pathways that mediate the BDNF-induced regulation of *Arc* transcription across different cortical brain regions.

Arc transcription is thought to be one of the initial events that serve to bridge environmental experience and neuronal plasticity, eventually contributing to synaptic remodelling and changes in synaptic strength (Beique *et al.* 2011; Bramham *et al.* 2008; Guzowski *et al.* 2005). The major finding of our study is that BDNF signalling may serve as a key intermediate step in the regulation of neocortical *Arc* expression in response to distinct cues, such as stress and hallucinogen exposure. This observation suggests strong similarities with learning and memory paradigms, in which trophic signalling through BDNF has been strongly implicated in the regulation of hippocampal *Arc* expression (Ying *et al.* 2002). Our results indicate that, within the neocortex, stress and hallucinogen exposure may also utilize similar trophic mechanisms to induce rapid changes in *Arc* expression. These data then raise the possibility that diverse stimuli that evoke long-lasting changes in neuronal plasticity involving a role for dynamic changes in Arc may utilize trophic factor mediated mechanisms to execute the initial events of rapid *Arc* transcriptional regulation. Future studies are required to understand in-depth the pathways through which BDNF influences *Arc* mRNA regulation and to understand how BDNF and Arc may act in concert to shape changes within local neocortical circuitry.

Acknowledgements

This research was supported by a TIFR intramural grant (V.V.) and a National Institute of Health grant MH 070727 (L.M.M.).

Statement of Interest

None.

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