Neurotrophin-mediated dendrite-to-nucleus signaling revealed by microfluidic compartmentalization of dendrites

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Signaling from dendritic synapses to the nucleus regulates important aspects of neuronal function, including synaptic plasticity. The neurotrophin brain-derived neurotrophic factor (BDNF) can induce long-lasting strengthening of synapses in vivo and this effect is dependent on transcription. However, the mechanism of signaling to the nucleus is not well understood. Here we describe a microfluidic culture device to investigate dendrite-to-nucleus signaling. Using these microfluidic devices, we demonstrate that BDNF can act directly on dendrites to elicit an anterograde signal that induces transcription of the immediate early genes, *Arc* and *c-Fos*. Induction of Arc is dependent on dendrite- and cell body-derived calcium, whereas induction of c-Fos is calcium-independent. In contrast to retrograde neurotrophin-mediated axon-to-nucleus signaling, which is MEK5-dependent, BDNF-mediated anterograde dendrite-to-nucleus signaling is dependent on MEK1/2. Intriguingly, the activity of TrkB, the BDNF receptor, is required in the cell body for the induction of Arc and c-Fos mediated by dendritically applied BDNF. These results are consistent with the involvement of a signaling endosome-like pathway that conveys BDNF signals from the dendrite to the nucleus.

gene expression | mRNA translation

The neurotrophin brain-derived neurotrophic factor (BDNF) has emerged as a key regulator of synaptic plasticity (1). Deletion of *bdnf*, or its receptor kinase *trkB*, or treatment with function-blocking antibodies impairs long-term potentiation (LTP) induced by high-frequency stimulation (2–7). Exogenously applied BDNF can induce LTP at medial perforant path-granule cell synapses in vivo (8), and this effect is blocked by the transcription inhibitor actinomycin D (9). Additionally, BDNF induces genes known to regulate synapse function, such as activity-regulated cytoskeletal (*Arc*) protein (10–12). Because the effects of BDNF on transcription are frequently explored by bath application, it is not clear if BDNF-mediated transcription is because of activation of TrkB in dendrites, axons, or the cell body (13). If dendritically localized TrkB receptors can regulate gene expression, mechanisms to convey the signal from dendrites to the nucleus, a distance that can be several hundred micrometers, would be required.

How might BDNF transduce a signal from dendrites to the nucleus? Intracellular calcium and the MAPK pathway are among the downstream effectors of BDNF-TrkB (10–12). Several mechanisms for conveying the BDNF signal from dendrites to the nucleus can be envisioned, among them: (*i*) propagation of a calcium wave to the soma, and subsequent activation of calcium-dependent transcription, as has been previously suggested (14) or (*ii*) diffusion of signaling molecules from dendrites to the nucleus, as has been suggested for phosphorylated MAPK (6). To determine the mechanisms by which dendritic stimulation leads to transcriptional effects, selective stimulation or inhibition of dendritic signaling is required.

Here we describe the design of a microfluidic device to investigate BDNF-mediated dendrite-to-nucleus signaling. CNS neurons can be cultured in this device and their dendrites can be fluidically isolated from their cell bodies. Using this device, we investigated intradendritic as well as dendrite-to-nucleus signaling. We find that BDNF can act on dendrites to activate gene transcription. This anterograde signaling requires selective roles for calcium, as well as MAPK and TrkB activity, in each compartment. Finally, we find that anterograde neurotrophin signaling from dendrites to the nucleus exhibits marked differences compared with retrograde signaling from axons to the nucleus. Taken together, these data identify a unique mechanism of dendritic signaling that may mediate the effects of dendritic BDNF.

Results

Dendritic Compartmentalization Using a Microfluidic Device. To investigate dendrite-to-nucleus signaling, we developed an approach to selectively stimulate dendrites. Recently, polydimethylsiloxane (PDMS)-based microfluidic culturing devices were developed to fluidically isolate axons from cell bodies, allowing selective stimulation of distal axons (15). These culturing devices comprise two compartments, the cell body compartment and axon compartment, separated by embedded microgrooves (10 μm × 450 μm). Because axons can grow long distances in essentially straight lines, these devices are suitable for compartmentalizing axons. However, because dendrites are branched and substantially shorter, it is not clear whether dendrites would be compatible with a microfluidic culturing system.

We designed a microfluidic device containing two separate compartments (1.5-mm wide, 7-mm long) separated by 7 μm × 75 μm microgrooves (Fig. 1 *A* and *B*). To determine if dendrites would grow through the microgrooves, embryonic day 18 (E18) rat cortical neurons were plated on poly-D-lysine (PDL)/laminin in the cell body compartment. E18 cortical neuronal dendrites crossed into the neurite compartment as early as day in vitro 7 (DIV7) and exhibited characteristic highly branched processes by DIV14 (Fig. 1*C*). Most crossed dendrites arose from cell bodies that were within ~100 μm of the microgrooves in the cell body compartment (Fig. 1*C*). As expected, axons also crossed and were readily detected in the neurite compartment by DIV14 (Fig. 1*D*). Similar results were obtained for E18 rat hippocampal

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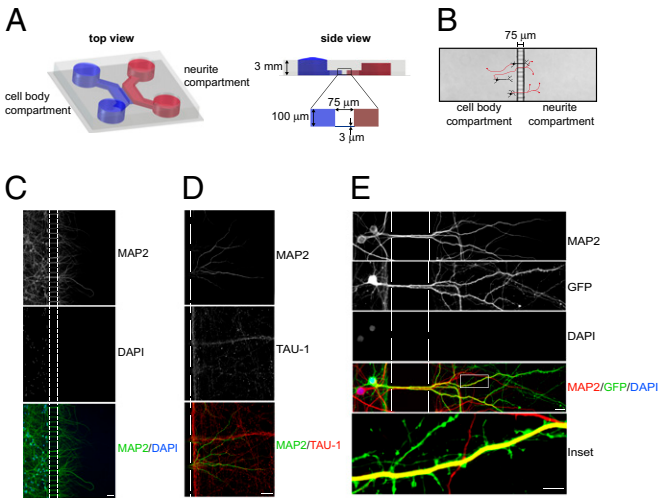

Fig. 1. A microfluidic device to fluidically isolate dendrites from the cell body. (*A*) Schematic representation of the neuronal microfluidic device. The device is fabricated from a PDMS mold containing mirror image compartments (1.5-mm wide, 7-mm long, 100-μm high) connected by microgrooves (10-μm wide, 3-μm high). (*B*) Phase-contrast image of the microfluidic device with 75-μm long microgrooves illustrating schematic representations of CNS neurons projecting dendrites and axons into the neurite compartment. (*C–E*) Immunofluorescence analysis of E18 rat cortical neurons (DIV14) cultured in the microfluidic device. (*C*) Extensive crossing of dendrites (MAP2, green) into the neurite compartment is observed for cell bodies within ~100 μm of the microgrooves. Cell bodies (DAPI, blue) are restricted to the cell body compartment. (Scale bar, 50 μm.) (*D*) Axons (TAU-1, red) project extensively into the neurite compartment and considerably farther from the microgrooves than the dendrites (MAP2, green), which remain close to the microgrooves. (Scale bar, 20 μm.) (*E*) A cortical neuron expressing GFP (green) reveals the presence of dendritic spines on dendrites (MAP2, red) in the neurite compartment. (Scale bar, 20 μm.) (*Inset*) Higher magnification image of a segment of dendrite enclosed by the white box in the merged image. (Scale bar, 2 μm.)

neurons, albeit less crossing into the neurite compartment was observed (Fig. S1).

Because both axons and dendrites grow into the neurite compartment, we examined dendrites for evidence of synaptic connections. At DIV14, spines were observed in crossed dendrites of GFP-expressing neurons (Fig. 1*E*). The average spine density (0.461 ± 0.052 spines per micrometer) of crossed dendrites is similar to previously reported spine densities (16). Additionally, we detected punctate staining for α-actinin, a protein enriched at excitatory synapses (17) (Fig. S1). Together, these results demonstrate that dendrites can grow through the 75-μm long microgrooves, exhibit characteristic branched morphology, and form synaptic connections in the neurite compartment.

Fluidic Isolation of Neurites and Cell Bodies. Next, we examined whether the neurite and cell body compartments connected by significantly shortened microgrooves were fluidically isolated. We incubated the neurite compartment with Alexa Fluor-647 hydrazide (20 μg/mL), a low molecular weight fluorescent dye. After 24 h, only a trace amount (~3%) of the dye was detected in the cell body compartment (Fig. S2). This amount of dye leakage was only slightly greater than that determined for the device with 450-μm long microgrooves (~1%), indicating that despite the six-times shorter length of the 75-μm long microgrooves, near-complete fluidic isolation can be achieved.

To further assess fluidic isolation, we examined whether cycloheximide (CHX) applied to the neurite compartment can affect protein synthesis in the cell body compartment. Protein synthesis was measured by metabolic labeling with the methionine bioisostere, azidohomoalanine (AHA), which can be labeled with Alexa Fluor-488 alkyne using click chemistry (18). Consistent with a dose-response study, application of CHX (0.5 μM) to the cell body compartment resulted in near-complete inhibition of protein synthesis (Fig. S2). In contrast, treatment of the neurite compartment with CHX did not affect protein synthesis in the cell body compartment (Fig. S2). Taken together, these results demonstrate that discrete chemical microenvironments can be maintained with 75-μm long microgrooves.

Intradendritic mRNA Translation Is Required for Dendritic Growth. To examine intradendritic signaling pathways, we first focused on potential functions of dendritic mRNA translation. Dendrites contain mRNAs that encode cytoskeletal proteins (19), suggesting an involvement of local translation in processes such as dendritic growth. However, because it has not been possible to maintain selective application of protein-synthesis inhibitors to dendrites over several days, a role for intradendritic protein synthesis in dendritic growth has not been addressable. To test this idea, we monitored the effect of CHX on basal dendrite growth over 7 d. The cumulative distributions of dendritic length of crossed dendrites after 7 d of CHX treatment demonstrated that neuritic CHX treatment reduced the total dendritic length by 35% compared with vehicle control (median, 847.2 vs. 1,308 μm, respectively) (Fig. S3). To rule out the possibility that the effects of CHX on dendritic growth could reflect inhibition of intra-axonal protein synthesis, we stained neurites for ribosomal protein S6. Immunolabeling of DIV9 neurons reveals discrete puncta in dendrites, but not axons (Fig. S3), indicating the absence of ribosomes in mature axons. This finding is consistent with previous studies demonstrating that mature axons of the central nervous system lack the capacity for protein synthesis (20, 21). These results indicate that intradendritic protein synthesis is required for basal dendritic growth in cortical neurons.

BDNF Induces a Dendrite-to-Nucleus Signal to Regulate Gene Expression. We next asked whether BDNF can elicit a signal that is conveyed from the dendrite to the nucleus. Consistent with previous studies (10, 12), bath application of BDNF induced the expression of the immediate early genes (IEGs) *Arc* and *c-Fos* (Fig. S4). To determine if the transcriptional effects of BDNF can be elicited from dendrites, we selectively applied BDNF to the neurite compartment and measured the expression of Arc and c-Fos in the cell body compartment. Treatment of the neurite compartment with BDNF resulted in a significant induction of Arc (twofold) and c-Fos (fourfold) protein (Fig. 2 *A* and *B*, white arrowheads) and mRNA (Fig. S4) levels in a subset of neurons, which exhibited dendrites that had crossed to the neurite compartment. The increase in Arc and c-Fos protein mediated by neurite application of BDNF was abrogated by the transcription inhibitor actinomycin D (Fig. 2 *C* and *D*). To rule out the possibility that the induction of c-Fos or Arc might reflect activation of TrkB on axonal processes, we selectively applied BDNF to axons of cortical neurons grown in microfluidic devices with 450-μm long microgrooves. Application of BDNF to axons did not induce IEG expression (Fig. S5), indicating that the effects of BDNF do not reflect retrograde signaling from axons to the nucleus. Importantly, previous studies using Campenot chambers, where the distance between axons and cell bodies is 1 mm, have shown that neurotrophins can convey signals from axons to the nucleus in peripheral system neurons within the time course of our experiments (22, 23).

We next sought to compare the magnitude of gene induction mediated by dendritically applied versus cell body-applied BDNF. Unlike application of BDNF to the neurite compartment, direct application of BDNF to the cell body compartment induced the expression of Arc and c-Fos in nearly all cells (Fig. 2 *E* and *F*). However, the magnitude of c-Fos induction was nearly

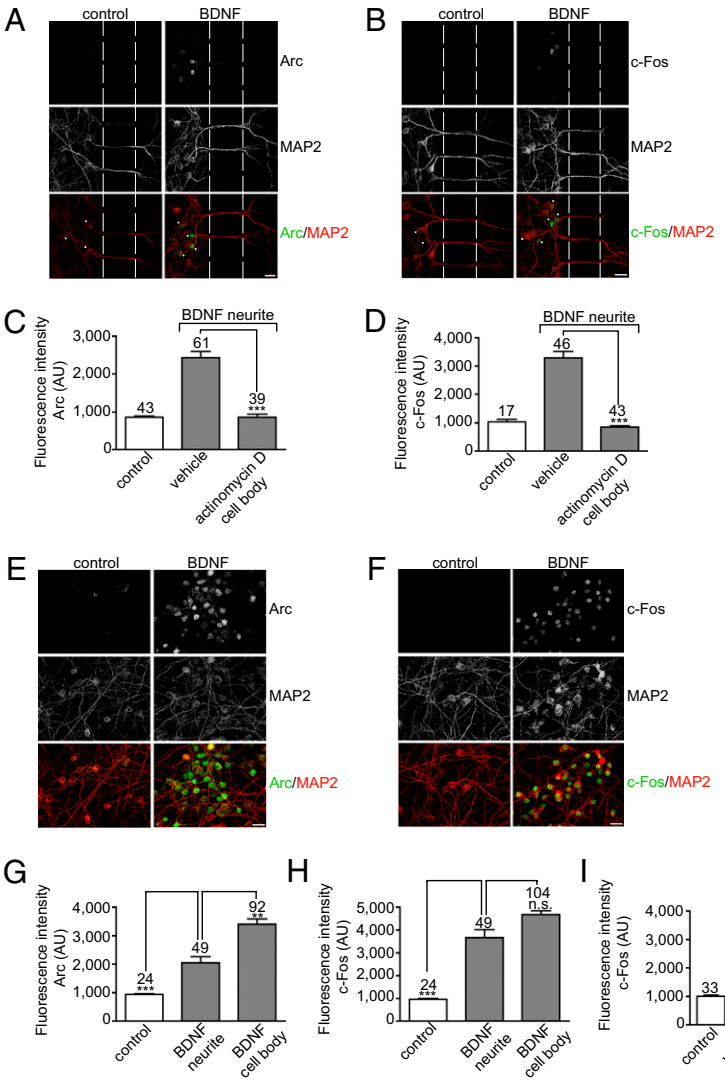

Fig. 2. BDNF acts at dendrites to induce the expression Arc and c-Fos. On DIV10 BDNF (100 ng/mL) or vehicle control was applied to the neurite (*A* and *B*) or the cell body (*E* and *F*) compartment. After 2 h, cells were fixed and immunolabeled with antibodies against Arc (green, *A* and *E*), c-Fos (green, *B* and *F*), and MAP2 (red). (Scale bars, 20 μm.) (*A* and *B*) The cell bodies in the cell body compartment that have dendrites projecting into the neurite compartment are indicated by white arrowheads. (*C* and *D*) Induction of Arc and c-Fos expression upon dendritic BDNF stimulation is because of new transcription. On DIV10, actinomycin D (2 μg/mL) or vehicle control was added to the cell body compartment for 1 h followed by the addition of BDNF (100 ng/mL) to the neurite compartment for 2 h. Cells were fixed and immunolabeled with antibodies against c-Fos and MAP2. (*G* and *H*) Quantification of results from *A*, *B*, *E*, and *F*. (*I*) Local protein synthesis is not required for c-Fos induction mediated by dendritic BDNF stimulation. On DIV10, CHX (10 μM) or vehicle control was added to the indicated compartment for 1 h followed by the addition of BDNF (100 ng/mL) to the neurite compartment for 2 h. Cells were fixed and immunolabeled with antibodies against c-Fos and MAP2. The error bars represent SEM, $***P < 0.0001$, $**P = 0.006$ (unpaired, two-tailed *t* test); *n* values listed above the bars represent the number of cell bodies analyzed.

identical in both treatment paradigms (Fig. 2*H*) and the magnitude of Arc induction mediated by neurite application of BDNF was slightly less than that for cell body application (Fig. 2*G*). Taken together, these results demonstrate that BDNF acts on dendrites to generate a signal that is conveyed to the nucleus to induce changes in gene expression.

Because BDNF enhances mRNA translation in dendrites (24), we wondered if intradendritic mRNA translation is required for BDNF-mediated dendrite-to-nucleus signaling. Selective application of CHX to the neurite compartment did not affect dendritic BDNF-mediated c-Fos induction. In contrast, direct application of CHX to the cell body compartment attenuated BDNF-induced increases in c-Fos protein levels (Fig. 2*I*). Hence, intradendritic mRNA translation is not required for gene induction mediated by BDNF signaling at dendrites.

Dendritic BDNF-Induced Arc and c-Fos Expression Does Not Require Glutamate Signaling. BDNF can rapidly potentiate excitatory synaptic transmission in cultured cortical and hippocampal neurons by stimulating glutamate release from presynaptic terminals (25, 26). To determine if glutamatergic transmission is required for dendrite-to-nucleus signaling mediated by dendritic BDNF, cortical neurons were treated with kynurenic acid and $MgCl_2$ for 48 h before the addition of BDNF to the neurite compartment. These conditions completely blocked c-Fos induction mediated by the $GABA_A$ receptor antagonist bicuculline, indicating complete inhibition of glutamatergic signaling (Fig. S6). Neither Arc nor c-Fos induction by dendritic BDNF was significantly changed in the presence of kynurenic acid and $MgCl_2$ (Fig. S6). Thus, glutamatergic transmission is not required for BDNF-mediated dendrite-to-nucleus signaling. Because the basal levels of Arc and

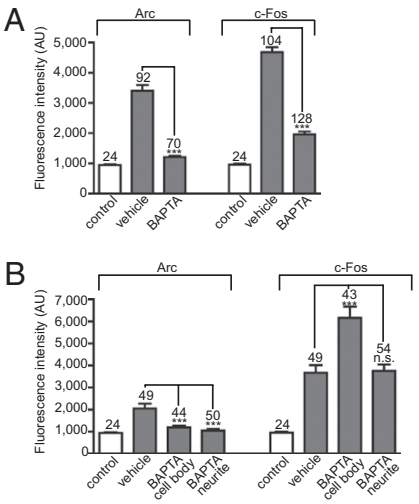

Fig. 3. Induction of Arc by dendritically-applied BDNF is dependent on dendrite- and cell body-derived calcium, whereas induction of c-Fos is calcium-independent. (*A*) On DIV10, BAPTA-AM (32 μM) or vehicle control was added to the cell body compartment followed by the addition of BDNF (100 ng/mL) to the cell body (*A*) or neurite (*B*) compartment for 2 h. Cells were fixed and immunolabeled with antibodies against Arc, c-Fos, and MAP2. The error bars represent SEM, ***$P < 0.0001$ (unpaired, two-tailed t test); n values listed above the bars represent the number of cell bodies analyzed.

c-Fos were reduced in the presence of kynurenic acid and $MgCl_2$ all subsequent experiments were performed with media containing these compounds.

Intracellular Calcium Is Differentially Required for Dendritic BDNF-Induced Arc and c-Fos Expression. We next asked if BDNF-mediated dendrite-to-nucleus signaling requires calcium in either the cell body or dendrites. Induction of Arc and c-Fos by cell body application of BDNF was blocked by cell body application of the intracellular calcium chelator BAPTA-AM (Fig. 3*A*), consistent with previous studies (12). When BDNF was applied to neurites, Arc induction was blocked by chelating calcium in either the neurite or cell body compartment, indicating a requirement for calcium in both compartments in mediating dendritic BDNF signals. However, induction of c-Fos by dendritic BDNF was independent of calcium in dendrites and was potentiated by chelation of cell body-derived intracellular calcium (Fig. 3*B*). The 1.7-fold increase in dendritic BDNF-mediated c-Fos induction in the presence of cell body BAPTA-AM could reflect an inhibitory role for intracellular calcium in regulating c-Fos expression. Taken together, these results demonstrate a differential requirement for intracellular calcium in dendritic BDNF-induced Arc and c-Fos expression.

Dendritically Applied BDNF Induces Arc and c-Fos Through Trk Activity in the Cell Body. We next examined the mechanism by which BDNF signals are conveyed from dendrites to the nucleus. We first asked whether the induction of c-Fos and Arc mediated by dendritic application of BDNF reflects an increase in the release of BDNF from the cell body. To test this idea, we used TrkB-Fc, a membrane-impermeable scavenger of BDNF, to block the effects of extracellular BDNF (6). Application of TrkB-Fc to the neurite compartment before dendritic BDNF stimulation blocked dendritic BDNF-mediated c-Fos and Arc induction (Fig. S7). In contrast, application of TrkB-Fc to the cell body compartment did not affect gene expression induced by dendritically applied BDNF (Fig. S7). These data argue against a model where dendritic application of BDNF leads to the release of BDNF from the cell body.

Selective application of neurotrophins to PNS axons leads to Trk receptor endocytosis and subsequent retrograde trafficking of neurotrophin-bound Trk to the cell body, where it activates signaling pathways leading to transcription (23, 27–30). To determine if a similar mechanism occurs in dendrites, we first asked if endocytosis is required for BDNF-mediated dendrite-to-nucleus signaling. Previous findings demonstrated that the GTPase dynamin is required for TrkB receptor internalization (31). To assess the requirement of dynamin-mediated endocytosis for dendritic BDNF-induced IEG expression, we used the selective dynamin inhibitor dynasore (32). Selective treatment of the neurite compartment with dynasore (100 μM) before dendritic BDNF stimulation significantly blocked Arc and c-Fos induction (Fig. S8).

We next determined if TrkB activity in the cell body is required for BDNF-mediated dendrite-to-nucleus signaling. To test this idea, we sought to pharmacologically inhibit TrkB activity in the cell body after dendritic application of BDNF. Although K252a is commonly used as a Trk inhibitor to investigate the roles of Trks (TrkA, TrkB, and TrkC) in retrograde axonal signaling, it is not selective for Trks (33, 34). We therefore sought to confirm our results using a panel of structurally distinct Trk inhibitors. The bis-indole Gö6976 is a well-described potent Trk inhibitor (35). We also considered GW2580, a pyrimidine derivative that was initially described as a highly selective inhibitor of the colony stimulating factor-1 receptor (CSF-1R) (36), a macrophage-enriched kinase that is not expressed at detectable levels in the cortex or hippocampus (37). A selectivity profile of GW2580 against a panel of over 300 kinases using in vitro competition binding assays revealed marked selectivity for CSF-1R and TrkB, and to a lesser extent, TrkA and TrkC (38). To determine if GW2580 can inhibit Trk activity in cells, we treated TrkB-expressing human embryonic kidney (TrkB-HEK) cells (39) with increasing concentrations of GW2580 before BDNF stimulation. GW2580 inhibited BDNF-induced Tyr490 phosphorylation of Trk in a dose-dependent manner (EC_{50} ~70 nM) with complete inhibition achieved by 3 μM (Fig. S9).

We next tested the effects of these inhibitors on dendritic BDNF-induced IEG expression in the microfluidic chambers. Selective application of either K252a (1 μM), Gö6976 (50 nM) or GW2580 (3 μM) to the cell body compartment completely blocked Arc and c-Fos induction mediated by dendritic BDNF (Fig. 4 *A* and *B* and Fig. S9), indicating that Trk activity in the cell body is necessary for BDNF-mediated dendrite-to-nucleus signaling. In contrast, selective treatment of the neurite compartment with the Trk inhibitors before dendritic BDNF stimulation only partially blocked Arc induction and did not significantly inhibit c-Fos induction (Fig. 4 *A* and *B* and Fig. S9). Taken together, these data support the idea that dendritic application of BDNF induces TrkB endocytosis and subsequent translocation of TrkB to the cell body, where its activity is required for c-Fos and Arc induction.

BDNF Uses the MEK1/2 Pathway for Dendrite-to-Nucleus Signaling. We next examined the signaling pathways downstream of BDNF-TrkB dendrite-to-nucleus signaling. In PNS neurons, neurotrophins use the MAPK kinase 5 (MEK 5) to elicit retrograde survival signaling (40). BDNF can activate both MEK1/2 and MEK5 in cortical neurons (41, 12); however, it is unclear which of these kinases is required for BDNF-mediated IEG transcription. To determine if the MEK1/2 pathway regulates dendritic BDNF-induced IEG expression, we treated the cell body compartment with the selective MEK1/2 inhibitor PD 0325901 before dendritic BDNF stimulation (42). PD 0325901 significantly inhibited Arc and c-Fos induction mediated by neuritic application of BDNF (Fig. 4 *C* and *D*). We next sought to determine if p90 ribosomal protein s6 kinases (RSKs), which are major downstream effectors of MEK1/2 in CNS neurons (43), could regulate BNDF-mediated dendrite-to-nucleus signaling. Treat-

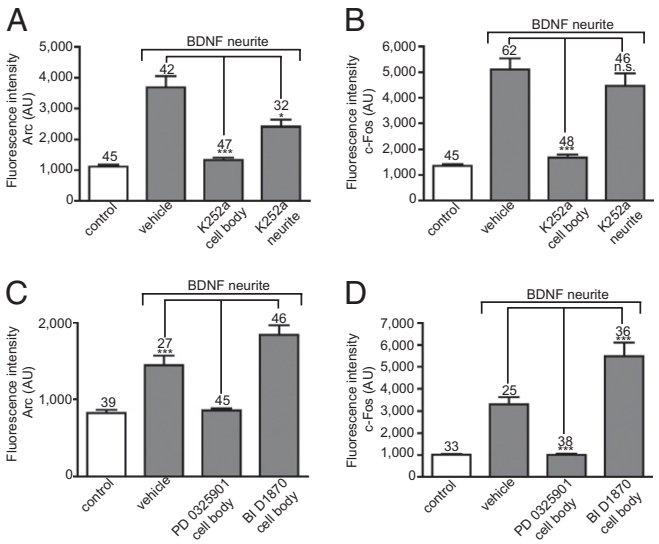

Fig. 4. Trk and Erk1/2 activity in the cell body are required for the induction of Arc and c-Fos mediated by dendritically applied BDNF. (*A* and *B*) On DIV10, K252a (1 μM) or vehicle control was added to the indicated compartment followed by the addition of BDNF (100 ng/mL) to the neurite compartment for 2 h. Cells were fixed and immunolabeled with antibodies against Arc (*A*), c-Fos (*B*), and MAP2. (*C* and *D*) On DIV10, PD 0325901 (150 nM) or BI D1870 (10 μM) or vehicle control was added to the cell body compartment followed by the addition of BDNF (100 ng/mL) to the neurite compartment for 2 h. Cells were fixed and immunolabeled with antibodies against Arc (*A*), c-Fos (*B*), and MAP2. The error bars represent SEM, $***P < 0.0001$, $*P < 0.05$ (unpaired, two-tailed *t* test); *n* values listed above the bars represent the number of cell bodies analyzed.

ment of the cell body compartment with the selective RSK inhibitor BI D1870 (44) did not block Arc or c-Fos induction (Fig. 4 *C* and *D*), but instead resulted in an increase in c-Fos induction compared with BDNF alone. The potentiation of dendritic BDNF-mediated c-Fos induction by BI D1870 suggests that RSK is involved in a negative feedback loop that suppresses the MEK1/2 pathway. Indeed, previous studies have shown that BI D1870 can augment the kinase activity of ERK1/2, which are direct upstream activators of RSK (44). Taken together, these results demonstrate anterograde dendrite-to-nucleus signaling requires MEK1/2 activity and that RSKs are not downstream effectors in this pathway.

Discussion

The transcriptional effects of BDNF have largely been investigated by bath application of BDNF to cultured neurons. As a result, it is not clear if these effects are a result of the activation of the BDNF receptor TrkB in the cell body or if dendritic TrkB can generate a signal that is conveyed to the nucleus to regulate gene transcription. By selectively applying BDNF to dendrites using microfluidic culturing devices, we find that dendritic TrkB can elicit a signal that is conveyed to the nucleus to induce transcription. Dendritically applied BDNF elicited both a calcium-dependent and a calcium-independent signaling cascade, regulating Arc and c-Fos, respectively. BDNF signaling from dendrites to the nucleus requires dynamin-dependent endocytosis in dendrites as well as MEK1/2 and TrkB activity in the cell body. These data suggest BDNF acting at dendrites regulates transcription by inducing TrkB endocytosis and translocation to the cell body, where it activates a MEK1/2 signaling pathway to induce c-Fos and Arc. Taken together, these data demonstrate the existence of a mechanism for conveying signals from dendrites to the nucleus, which may mediate the long-lasting effects of BDNF on synaptic plasticity in vivo.

The finding that dendritic BDNF-induced c-Fos expression was calcium-independent was unanticipated, because we found that intracellular calcium was required for the expression of c-Fos following bath application of BDNF. Additionally, previous studies have suggested that BDNF induces c-Fos expression in a calcium-dependent manner (14). One explanation for this discrepancy is that the signaling pathway induced by dendritically localized TrkB is calcium independent, and was masked by the global activation of TrkB. Indeed, numerous signaling proteins are expressed in specific compartments in the cell (45), and may therefore not be accessible to TrkB depending on its localization in cells. Our results point to the potential differences between TrkB pathways elicited in the cell body and dendrites, and highlight the importance of selective stimulation of dendritic TrkB using approaches such as the microfluidic devices described here.

There are at least three differences between anterograde neurotrophin-mediated dendrite-to-nucleus and retrograde axon-to-nucleus signaling that are worth noting. First, whereas retrograde axonal signaling requires Trk activity in the axon (30), dendrite-to-nucleus signaling does not necessarily require Trk activity in dendrites. Second, whereas neurotrophin-mediated axon-to-nucleus signaling uses MEK5 to mediate nuclear responses (40), BDNF-mediated dendrite-to-nucleus signaling uses MEK1/2 to mediate Arc and c-Fos induction. The specific MAPK pathway that is activated most likely determines which downstream signaling pathways are activated to mediate transcription of specific genes. Finally, local mRNA translation promotes retrograde signaling in axons (46), and intradendritic mRNA translation is dispensable for the induction of c-Fos by dendritically applied BDNF.

What does the requirement for Trk activity in the cell body for dendritically mediated BDNF induction of IEGs suggest about the mechanism of neurotrophin-mediated dendrite-to-nucleus signaling? A major mechanism for axon-to-nucleus signaling in PNS neurons is the signaling endosome model (47). In this model, the neurotrophin-bound Trk receptor is endocytosed in distal axons and is then translocated as an endosome to the cell body, where it activates its downstream effectors, leading to gene transcription. Our data showing the requirement for Trk activity in the cell body and dynamin-mediated endocytosis in dendrites for BDNF-mediated dendrite-to-nucleus signaling is consistent with a novel dendrite-derived signaling endosome. It will be important to image BDNF-TrkB endosome trafficking from dendrites to the cell body, and to determine if these signaling endosomes contribute to BDNF-mediated changes in synaptic plasticity in a physiological context. Previous studies have shown that theta-burst stimulation induces the release of endogenous BDNF from mossy fibers, as well as Schaffer collaterals onto the dendrites of CA3 and CA1 pyramidal neurons, respectively, and this mediates long-lasting changes in potentiation or depression (3, 48, 6, 49). Moreover, a recent study demonstrated that release of BDNF from cortical axons onto dendrites of medium spiny neurons in the dorsal striatum induced postsynaptic LTP (50). It is conceivable that dendritically derived BDNF-TrkB endosomes have a role in these pathways.

We envision the investigation of other potential dendrite-to-nucleus signaling pathways using the microfluidic device described here. Because the microfluidic platform is compatible with live cell imaging, intracellular trafficking of signaling molecules from dendrites to the cell body can be imaged following selective dendritic synapse activation using these devices. Recently, Schuman and colleagues described a different microfluidic device to investigate synaptic signaling (51). In this device, neurons are cultured in two compartments, and project axons and dendrites toward a narrow central perfusion chamber. Both of these devices provide an opportunity to investigate the molecular

pathways that couple dendritic stimulation with changes in gene expression.

Materials and Methods

The polydimethylsiloxane microfluidic devices with 75-μm microgrooves were fabricated as previously described (15). The preparation of the microfluidic devices for compartmentalization of dendrites are described in *SI Materials and Methods*.

The materials used and detailed methods are described in *SI Materials and Methods*.

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