Published in final edited form as:

Cell Mol Life Sci. 2017 December ; 74(23): 4369–4385. doi:10.1007/s00018-017-2589-7.

Distinct effects on the dendritic arbor occur by microbead versus bath administration of Brain-Derived Neurotrophic Factor

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Abstract

Proper communication among neurons depends on an appropriately formed dendritic arbor, and thus, aberrant changes to the arbor are implicated in many pathologies, ranging from cognitive disorders to neurodegenerative diseases. Due to the importance of dendritic shape to neuronal network function, the morphology of dendrites is tightly controlled and is influenced by both intrinsic and extrinsic factors. In this work, we examine how brain-derived neurotrophic factor (BDNF), one of the most well-studied extrinsic regulators of dendritic branching, affects the arbor when it is applied locally via microbeads to cultures of hippocampal neurons. We found that local application of BDNF increases both proximal and distal branching in a time-dependent manner and that local BDNF application attenuated pruning of dendrites that occurs with neuronal maturation. Additionally, we examined whether cytosolic PSD-95 interactor (cypin), an intrinsic regulator of dendritic branching, plays a role in these changes and found strong evidence for the involvement of cypin in BDNF-promoted increases in dendrites after 24 but not 48 hours of application. This current study extends our previous work in which we found that bath application of BDNF for 72 hours, but not shorter times, increases proximal dendrite branching and that this increase occurs through transcriptional regulation of cypin. Moreover, this current work illustrates how dendritic branching is regulated differently by the same growth factor depending on its spatial localization, suggesting a novel pathway for modulation of dendritic branching locally.

Keywords

hippocampal neurons; dendrite arborization; BDNF; cytosolic PSD-95 interactor (cypin); Sholl analysis; local stimulation

INTRODUCTION

The precise patterning of dendrites is crucial for proper neuronal communication. The process of dendritic arborization is tightly regulated, and this regulation often occurs locally,

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Conflict of Interest: The authors declare that they have no conflicts of interest.

such as at small regions of the dendrite (reviewed in [1]). Brain-derived neurotrophic factor (BDNF) is one of most well-studied extrinsic regulators of dendrite development [2–14]. A member of the neurotrophin family, BDNF is essential for neuronal development and dendrite outgrowth. As a protein, it exists in two states: pro-BDNF, an unprocessed form that binds to the p75 neurotrophin receptor (p75^{NTR}) with high affinity, and mature BDNF, the proteolytically processed form that preferentially binds to the tropomyosin-related kinase B (TrkB) receptor. The two receptors activate distinct pathways. Activation of p75^{NTR} leads to apoptosis while activation of the TrkB receptor promotes neuronal development and dendritic outgrowth [15–20].

Recent studies have revealed much about how BDNF regulates the dendritic arbor. Several studies have shown that BDNF is a mediator of activity-dependent dendrite branching [3,21,22,5]. Additionally, our laboratory and others have investigated how bath application of BDNF to neuronal cultures influences dendritic branching. Bath application of BDNF increases primary dendrite branching in cortical neurons via the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways in as little as 5 hours [7]. Moreover, our laboratory has shown that global stimulation of hippocampal neurons with BDNF increases proximal branching via CRE-binding protein (CREB)-dependent transcriptional regulation of cytosolic PSD-95 interactor (cypin) when cultures are treated for at least 72 hours [23]. Additionally, treatment with BDNF causes dendrites to be more active; that is, dendrites are both gained and lost more quickly than when no treatment is present [8]. Importantly, release of BDNF from single cells elicits local dendritic growth in nearby neurons, but this occurs only if the source of BDNF is within 4.5 μm of the recipient neuron [24], suggesting that local dendritic stimulation with BDNF may result in different effects on the arbor than bath BDNF application. For example, local puff application of BDNF to dendrites of hippocampal neurons in culture results in phosphorylation of S6, which is a mediator of mTOR-induced local protein synthesis [25].

Outgrowth of proximal and distal dendrites from neuronal cell bodies is regulated by different mechanisms. For example, in cortical neurons, overexpression of the full length TrkB receptor increases proximal dendrite branching while overexpression of the truncated TrkB receptor T1 (TrkB.T1) results in elongation of distal dendrites [26]. In addition, developmental expression patterns of these receptor isoforms differ [27], suggesting the possibility of differential control of dendrite development mediated by distinct receptors or TrkB receptor subtypes. Local stimulation of dendrites can occur when BDNF is released synaptically, and global stimulation of neurons in culture simulates BDNF release by astrocytes, which would more broadly activate signals in the dendritic arbor. Therefore, we used our hippocampal neuron culture system to ask how local stimulation with BDNF affects both proximal and distal dendrites. Importantly, we consider these results in the context of our previous study [23]. In this work, we found that application of a local source of BDNF to hippocampal neuron cultures increased both proximal and distal dendrite branching and that local exposure of dendrites to BDNF on beads prevents pruning of higher order dendrites. Notably, these results differ from our previous study [23], which demonstrated that bath application of BDNF to hippocampal neuron cultures resulted in changes to proximal, but not distal, dendrite branching. Additionally, we investigated whether these changes to the arbor are mediated by cypin, and we found that knockdown of

cypin blocks increases in branching resulting from 24, but not 48, hours of exposure to BDNF coupled to beads. Thus, our results strongly suggest that BDNF affects the dendritic arbor differently, depending on how it is applied to neurons in culture.

MATERIALS AND METHODS

Primary culture of hippocampal neurons

Neuronal cultures were prepared from the hippocampi of rat embryos at 18 days of gestation (E18) as described previously [28,23]. The hippocampi were dissociated using manual trituration, and cells were plated onto poly-D-lysine (PDL; Sigma)-coated 12 mm glass coverslips (Fisher) in 24 well plates (Corning) at a density of 1800 cells/mm². Cultures were maintained in Neurobasal medium (Life Technologies) supplemented with B27, GlutaMAX, and penicillin/streptomycin (all Life Technologies) and kept in a humidified 37 °C incubator with 5% CO₂. Cells were grown for 7 days *in vitro* (DIV) prior to treatment and used for specific experiments as indicated below. All studies were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) standards.

Transfection of cultured cells

To visualize dendritic arbors, cultured hippocampal neurons were transfected at DIV 6 with cDNA encoding GFP or at DIV 5 with shRNAs using Lipofectamine LTX and PLUS reagent following the manufacturer's protocol (Invitrogen). The pSuper GFP vector (Oligoengine) contained shRNAs against the cypin transcript or an unrelated sequence as a negative control (GST) as previously described [29,23].

Preparation, imaging, and analysis of BSA- and BDNF-coated microbeads

Fluorescent carboxylated microparticles (641 nm for excitation wavelength; Polysciences, Inc., Warrington, PA) were used for coupling BDNF (Promega) to beads by activating carboxyl groups. BDNF was coupled using the PolyLink Protein Coupling Kit (Polysciences, Inc., Warrington, PA) following the manufacturer's protocol. Briefly, microparticles were resuspended in PolyLink Coupling Buffer (50 mM MES, pH 5.2, 0.05% Proclin-300) and activated by adding 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) solution. BDNF (250 µg) was incubated with activated microparticles for 1 hour at room temperature with gentle end-to-end mixing. After washing with PolyLink wash buffer (10 mM Tris, pH 8.0, 0.05% bovine serum albumin (BSA; Fisher), 0.05% Proclin-300), BDNF-coupled particles were resuspended in wash buffer and stored at 4°C. Using this method, nearly all BDNF protein bound to the beads (Figure 1A). BSA was used as the control protein bound to beads since it should not alter dendrite branching.

To determine whether BDNF is released from the beads over time, neuronal cultures were grown in 6 well plates and treated with 50 µl beads, and after various treatment lengths (5, 24, 48, and 72 h), beads were isolated from the medium. We used this volume of beads for these experiments because no visible pellet was observed after isolating 10 µl of beads from 24 well plates as we used to treat neurons in branching experiments. Prior to Western blot analysis, the beads were boiled in protein loading buffer (10% β-Mercaptoethanol, 0.05% bromophenol blue, 10% glycerol, 4% sodium dodecyl sulfate, Tris (50 mM, pH 6.8)) for 10

min at 95 °C and pelleted in a microcentrifuge at $4500 \times g$ for 5 min. Extracted protein was resolved by SDS-polyacrylamide gel electrophoresis. We observed that minimal amounts of BDNF and BSA protein are released from the beads in culture over time (Figure 1B).

To determine coating efficiency, BSA- and BDNF-coated beads were immunostained with antibodies recognizing the coated proteins. Beads were prepared as described above, washed in phosphate-buffered saline (PBS), and incubated in blocking buffer (PBS with 0.1% Triton X-100, 2% normal goat serum, and 0.02% sodium azide). Beads were incubated in primary antibody (mouse anti-BDNF, 1:50 dilution, Abcam; or rabbit anti-BSA, 1:50 dilution, Fisher) at room temperature for 1 hr. Beads were washed in PBS and incubated in secondary antibody (AlexaFluor 488 anti-mouse or AlexaFluor 555 anti-rabbit, both at 1:25 dilution) at room temperature for 45 min. Beads were washed in PBS and mounted onto microscope slides in Fluoromount G. Immunofluorescence images were obtained using an Olympus FV1000 oil-immersion 60 $\times$ objective (1.3 N.A.) with sequential-acquisition setting. Approximately ten images were taken in 1 μ m sections from the top to the bottom of the beads [30,31]. Additionally, STORM/PALM was set up on an Olympus IX73 with an LED excitation light source as previously described [31]. Briefly, an Olympus 60 $\times$ 1.3 N.A. UPlanSApo objective was used to acquire 512×512 -pixel images, and a spinning disk was included to block excess light. Software was used to correct for lateral drift using the StackReg plugin from ImageJ. Five thousand images were acquired for each field of view. Blinking events were identified by the change in fluorescence at individual pixels [31]. Centroid determination and image reconstruction were completed using QuickPALM [32]. Immunostaining and imaging show that all microbeads were evenly coated with protein (Figure 1C).

Treatment of neuronal cultures with BSA- and BDNF-coated beads

To mimic local exposure to BDNF, neurons were treated with 1.75 μ m fluorescent beads. Each well contained 0.7 ml of medium, and 10 μ l of a 0.0625% aqueous suspension of beads (either coupled to BDNF or BSA) was added to each well of a 24 well plate (10^7 beads/well). Cultures were treated with BDNF- or BSA-coated beads for the indicated duration at DIV 7 prior to imaging. To obtain an estimate for the number of beads that contacted each dendrite, the number of beads within 4.5 μ m of a dendrite was counted. The estimates were obtained by choosing 10 neurons randomly per condition and evaluating 3–5 dendrites per neuron. The number of beads per dendrite ranged from 1.36 to 2.21 for the first set of experiments and 1.58 to 2.47 for the second set of experiments (data not shown), and the average number of beads per dendrite was not significantly different between any of the conditions (determined by one-way ANOVA followed by Bonferroni's Multiple Comparisons test). Moreover, we determined the distribution of beads along the dendritic arbor. Ten neurons per condition were chosen, and bead distance from the cell body was measured if beads were within 4.5 μ m of a dendrite. The distributions were plotted in 20 μ m increments and compared using correlation analysis (Supplementary Figure 1). These distribution curves mirror the Sholl curves because the highest number of beads contacting dendrites occurs in the same location as the highest density of dendrites (i.e. close to the cell body). Pearson's r for BSA-versus BDNF-coated beads ranged from 0.9371–0.9787 for the first set of experiments and 0.8708–0.9769 for the second set of experiments.

Live imaging of neurons

Live images were taken using an Olympus Optical (Tokyo, Japan) IX50 microscope with a Cooke Sencam charge-coupled device cooled camera fluorescence imaging system and Image Pro software (Media Cybernetics, Silver Spring, MD). The plate containing the cultures remained on a heated stage for the duration of imaging. Transfected neurons were identified under the GFP channel, and beads were identified under the far red channel. Fluorescent images of transfected neurons were taken live with no fixation since we observed bead movement when we attempted to fix the cultures.

Dendrite assessment and statistics

Dendrites were assessed as previously described [33,34,23] using the most common Sholl analysis, also known as the Inside-Out labeling scheme [35]. We used the “Bonfire” program developed by our laboratory to perform semi-automated Sholl analysis with a 6 μm ring interval starting at 9.3 μm from the soma. The experimenter was blinded to conditions during all data analyses. Dendrites less than 3 μm in length were not counted [36,37]. Prism (Graphpad) was used for statistical analyses. Two-way ANOVA followed by Bonferroni's Multiple Comparisons test was used to evaluate Sholl curves, and Student *t*-tests were used for analysis of dendrite numbers and lengths.

RESULTS

Treatment with BDNF-coated beads increases overall dendrite branching in a location and time-dependent manner

To mimic a source of local BDNF, we seeded microbeads coated with covalently-bound BDNF (or BSA, as the control) in neuronal cultures. Microbeads were distributed across the entire neuron, from proximal to distal areas of the dendritic arbor, and made multiple contacts with dendrites (Figure 1D,E).

To investigate how exposure to local sources of BDNF affects dendrite branching over time, hippocampal cultures were treated at DIV 7 with control (BSA-coated) or BDNF-coated beads for 5, 24, 48, and 72 hours. Representative images are shown in Figure 2A. We then performed Sholl analysis and found that treatment for 24 hours or less does not have a significant effect on the overall dendritic arbor (Figure 2B,F and Figure 2C,G). However, treatment with BDNF-coated beads significantly increases dendrites when neurons are treated for 48 hours and more. Interestingly, the location of increased branching differs when neurons are treated with BDNF-coated beads for 48 hours versus 72 hours. After 48 hours (Figure 2D,H), significant increases in branching occur distally (93.3 to 159.3 μm from the soma), whereas at 72 hours (Figure 2E,I), significant increases in branching occur both proximally (21.3 to 29.3 μm from the soma) and distally (123.3 to 153.3 μm from the soma). This change to the dendritic arbor over time indicates that local sources of BDNF affect the arbor as it develops. A subset of proximal and distal dendrites represented on Sholl curves decrease between 48 and 72 hours in the control condition whereas the decrease in these dendrites is attenuated when the neurons are treated with BDNF-coated beads (compare Figure 2D,H and Figure 2E,I). Moreover, the increases observed after exposure to local

sources of BDNF differ when compared to those we previously observed after neurons were treated with bath application of BDNF [23].

We also performed order-specific Sholl analysis (Figure 3; [34,35]), which sheds light on the mechanism behind the observed increases in certain regions of the overall dendritic arbor by categorizing dendrites according to proximity to the cell body, as denoted by order (primary, secondary, or tertiary and above). The labeling scheme used in this study is the one that is most commonly used for Sholl analysis, termed Inside-Out, in which dendrite order increases with distance from the cell body.

Treatment with BDNF-coated beads for 5 hours, 24 hours, and 72 hours significantly increases primary dendrites (Figure 3A, D, and J, respectively). The most prominent increase was observed at 24 hours (Figure 3D), with significantly increased branching occurring at 9.3 to 57.3 μm from the soma. Secondary dendrites significantly increase at all time points of BDNF-coated bead treatment (Figure 3B, E, H, and K, respectively). After 5 hours of incubation (Figure 3B), neurons exposed to BDNF-coated beads show decreased secondary dendrites proximal to the soma (15.3 to 33.3 μm from the soma) and increased secondary dendrites distally (69.3 to 75.3 μm from the soma). After longer incubation periods with beads, exposure to BDNF results in increases in secondary dendrites at similar distances from the soma (between 21.3 and 45.3 μm from the soma). Furthermore, increases in tertiary and higher order dendrites are observed distally and only after 48 hours and 72 hours of exposure to BDNF-coated beads (Figure 3I and L, respectively).

To better understand how treatment with BDNF-coated beads affects dendritic arborization, we grouped the order-specific and total Sholl curves according to condition and re-graphed our data, as shown in Figure 4. Interestingly, treatment with BDNF-coated beads changes development of the dendritic arbor for all orders of dendrites. For primary dendrites, decreases in Sholl distribution curves are observed between each successive time point in the control (Figure 4A) but not in cultures treated with BDNF-coated beads (Figure 4D). In addition, Sholl distributions for secondary dendrites significantly decrease in control cultures over time (Figure 4B). The natural pruning process that occurs between 5 and 24 hours is attenuated with exposure to BDNF-coated beads (Figure 4E). Pruning of tertiary and higher order dendrites occurs between 5 and 24 hours in control cultures (Figure 4C) and is blocked by treatment with BDNF-coated beads (Figure 4F).

Treatment with BDNF-coated beads affects dendrite number and length

In addition to performing multiple Sholl analyses, we quantified how exposure to local sources of BDNF changes the number and length of dendrites, both overall and in an order-specific manner, versus control treatment (BSA-coated beads). The data are summarized in Table 1. Treatment with BDNF-coated beads significantly increases the total number dendrites after at least 48 hours of treatment (Supplementary Figure 2). Primary dendrite number is significantly increased after 24 hours and 72 hours of treatment (Supplementary Figure 3), consistent with primary dendrite-specific Sholl curves at these time points. Secondary dendrites significantly decrease after 5 hours of treatment with BDNF-coated beads, do not change after 24 or 48 hours of treatment, and then significantly increase after 72 hours of treatment (Supplementary Figure 3). Finally, tertiary and higher order dendrites

significantly increase after 48 hours of treatment (Supplementary Figure 3). In particular, the data from tertiary and higher order dendrites indicate that BDNF does not promote addition of new dendrites to the neuron but instead blocks pruning of higher order dendrites that occurs after DIV 7.

The average length of dendrites does not change when all dendrites are grouped together (Supplementary Figure 4); however, order-specific analysis demonstrates that the length of tertiary and higher order dendrites increases only after 72 hours of treatment with BDNF-coated beads (Supplementary Figure 5). Treatment with BDNF-coated beads does not alter primary dendrite length or secondary dendrite length after any length of treatment (Supplementary Figure 5).

Knockdown of cytosolic PSD-95 interactor (cypin) blocks a subset of effects resulting from treatment with BDNF-coated beads

Previously, our laboratory reported that cytosolic PSD-95 interactor (cypin) is an intrinsic regulator of dendrite branching [38,35,37,39,29]. Specifically, overexpression of cypin results in an increase in dendrite branching while knockdown of cypin results in a decrease in dendrite branching. Recently, we demonstrated that bath application of BDNF to hippocampal cultures increases dendrite branching and that this increase occurs through transcriptional regulation of cypin mRNA [23]. We wanted to extend these previous studies by investigating whether local exposure to BDNF via microbeads signals through cypin to alter the dendritic arbor. Therefore, we transfected neurons with shRNA against cypin, which decreases cypin by approximately 40% [29], or with control shRNA against GST, and then we treated each set of cultures with either BSA- or BDNF-coated beads at DIV 7 for a period of 5, 24, and 48 hours. We did not investigate the effects of a 72 hour treatment because neurons must be imaged live and GFP expressed from the pSUPER plasmid does not express at the high levels observed when GFP is expressed from the pEGFP-C1 plasmid (as was done for experiments in Figures 2–4). In this current set of experiments, control neurons are transfected with GST shRNA and treated with BSA-coated beads.

We performed Sholl analysis for all conditions and found that knockdown of cypin blocks some of the effects resulting from treatment with BDNF-coated beads (Figure 5). Representative images from each condition are shown in Figure 5A. Importantly, we observed significant decreases at all time points for neurons transfected with cypin shRNA and treated with BSA-coated beads compared with the control (neurons transfected with GST shRNA and treated with BSA-coated beads). As expected, the most prominent decreases occurred at the 48 hour time point (Figure 5D,G; green stars) [29]. Additionally, in neurons expressing the control GST shRNA, we also observed that treatment with BDNF-coated beads increased dendrite branching proximally at 24 hours and 48 hours, although only at two distances from the cell body (Figure 5C,F and Figure 5D,G, respectively; blue stars). Similarly, at the 5 hour time point (Figure 5B,E), cultures transfected with cypin shRNA and treated with BDNF-coated beads resulted in restoration of dendritic arborization to control levels (red vs. black lines). After 24 hours of treatment with beads, not only does cypin knockdown decrease dendrites but also blocks increases in dendrites promoted by BDNF-coated beads (Figure 5C,F; purple stars). The difference in the effect of knockdown

of cypin on changes to dendrites by exposure to BDNF-coated beads between 48 and 72 hours of knockdown (5 hours and 24 hours of bead exposure, respectively) is most likely due to the fact that maximal knockdown of cypin protein occurs after 48 hours [29,23]. After 48 hours of treatment with beads (96 hours of cypin knockdown), cypin knockdown does not attenuate the increases in dendrite branching promoted by BDNF-coated beads (Figure 5D,G). Since BDNF may increase endogenous cypin levels between 24 and 72 hours of exposure [23], this may account for the inability of cypin knockdown to block the effects of BDNF-coated beads after 48 hours of treatment.

We also utilized order-specific Sholl grouped by condition in this set of data to illuminate pruning patterns. Very little pruning of primary dendrites is observed in control cultures (Figure 6A). Interestingly, treatment with BDNF-coated beads increases primary dendrites proximal to the soma over time (Figure 6D). As expected, cypin knockdown results in decreased primary dendrites proximal to the soma over time (Figure 6G), and this decrease is seen even with treatment with BDNF-coated beads (Figure 6J), though at an earlier time. In contrast, secondary dendrites are pruned over time in control cultures (Figure 6B), and treatment with BDNF-coated beads not only blocks this pruning but actually increases secondary dendrites after 48 hours (Figure 6E). Knockdown of cypin increases pruning after 48 hours of control bead treatment (Figure 6H), and treatment with BDNF-coated beads partially blocks the increased pruning seen with cypin knockdown (Figure 6K). Furthermore, tertiary and higher order dendrites appear to be subject to the greatest pruning over time in control cultures (Figure 6C). Exposure to BDNF-coated beads significantly attenuates this pruning and even increases higher order dendrites after 24 hours, but treatment exerts no effect on the pruning of these dendrites after 48 hours in culture (Figure 6F), suggesting a transient effect of local sources of BDNF on arbor pruning. Cypin knockdown increases higher order dendrite pruning at 48 hours (Figure 6I), and treatment with BDNF-coated beads attenuates pruning, resulting in a pattern similar to that of control cultures (compare Figure 6C and Figure 6L). These results suggest that local sources of BDNF may act, in part, through cypin to regulate pruning of the dendritic arbor.

DISCUSSION

BDNF alters the dendritic arbor when applied locally using beads

The major finding of the current study is that local stimulation of cultured hippocampal neurons with BDNF results in changes to dendritic arbor and that these changes are not evident until after 48 hours of stimulation. Furthermore, these changes are observed distal from the cell body after 48 hours and both proximally and distally after 72 hours, suggesting that localized stimulation with BDNF actively shapes neuronal morphology. Moreover, BDNF attenuates pruning of dendrites, specifically higher order dendrites, resulting in an increase in the overall Sholl curve representing the arbor. Our results strongly suggest that BDNF signals in part through a cypin-dependent mechanism. A model representing the effects of BDNF on the dendritic arbor is shown in Figure 7.

How do the effects of local stimulation of the dendritic arbor, as we have done in this study, differ from those observed when neurons are exposed to bath application of BDNF? To answer this question, we compare our current results with those from our previous work

[23], where all experimental conditions were the same except that cultures were treated with BDNF as a bath application. Similarities between the two studies include the following: 1) greater than 24 hours of treatment with either bath or bead application of BDNF is needed to result in overall changes to the dendritic arbor as observed by Sholl analysis; 2) increases in primary and secondary dendrite number are observed after 72 hours of treatment for both studies; and 3) both types of BDNF treatment depend on cypin to promote effects on the arbor. Differences include the following: 1) five hours of treatment with bead, but not bath, application of BDNF results in a transient decrease in secondary dendrites; 2) bath application of BDNF increases dendrites close to the soma while bead application increases dendrites both proximal and distal to the soma; and 3) cypin knockdown affects increases in dendrites promoted after 24 hours of bead application of BDNF but only after 72 hours of bath application. Taken together, the two studies suggest that there is partial, but not complete, overlap of signaling mechanisms that mediate changes to the dendritic arbor by the two types of stimulation with BDNF.

Our previous data demonstrate a role for MAPK and CREB-mediated cypin transcription in how global BDNF stimulates the promotion of dendritogenesis [23]. Although we do not rule out a role for this signaling pathway in mediating the effects of local BDNF stimulation on the dendritic arbor, our data suggest additional pathways for local BDNF signaling since knockdown of cypin has no effect on BDNF-mediated increases at 48 hours for local application of BDNF via beads. One possibility is that, in this scenario, BDNF promotes the local translation of cypin, as it has been reported that local BDNF application in cultured hippocampal neurons phosphorylates the S6 protein, which is involved in mTOR-dependent local protein synthesis [25]. Furthermore, global BDNF treatment increases proximal dendrite branching, resulting from increased cypin transcription and protein levels [23]. However, localized treatment using BDNF-coated microbeads increases branching in both proximal and distal areas of the dendrite in a time-dependent manner, suggesting that there exists distinct regulation of proximal and distal dendrites. Moreover, in the current study, BDNF is covalently bound to microbeads and is minimally released, and thus, it is unlikely to be internalized by the cell [40,41], which could explain distinct downstream pathway activation from that stimulated by bath application of BDNF. Thus, BDNF presented on beads versus bath application may act differently through cypin to alter the dendritic arbor.

The complex actions of BDNF on the dendritic arbor may be mediated, in part, by multiple receptors, resulting from alternative splicing of the full length TrkB receptor (TrkB.FL). Like other neurotrophins, BDNF exists in two states, proteolytically processed, which is the active form that can bind Trk receptors, or unprocessed, which allows BDNF to bind with high affinity to p75^{NTR}. Three alternative splice variants of TrkB.FL have been identified. The full-length TrkB.FL contains a cytoplasmic tyrosine kinase domain, which is responsible for autophosphorylation and clustering of the receptor when activated by BDNF [15,2]. Two truncated isoforms, termed TrkB.T1 and TrkB.T2, have also been identified, and both lack the cytoplasmic tyrosine-kinase domains [17]. TrkB.FL and TrkB.T2 are expressed exclusively in neurons, and TrkB.T1 is expressed in both neurons and nonneuronal cells [42–46]. Importantly, stimulation of TrkB.FL increases proximal branching, and activation of TrkB.T1 increases distal dendrites [26]. Bath treatment of neurons would result in synchronized activation of both TrkB receptors [26,47] while local stimulation with BDNF-

coated beads could result in differential spatial stimulation of these two receptors, minimizing receptor cross-talk and regulating proximal and distal dendritogenesis separately [48]. In addition, TrkB.T1 has been shown to regulate dendritic growth via p75^{NTR} [47], again suggesting that global versus local stimulation of neurons by BDNF acts via different signaling cascades. Since there are variations in expression and localization of the different BDNF receptors between brain regions [49–51], different distributions of these receptors on the neuron most likely play a role in localized BDNF function.

Finally, covalent binding of BDNF to microbeads likely not only blocks BDNF internalization, but BDNF may also be oriented differently on individual microbeads [40,41], affecting how the TrkB receptor is stimulated. Since linkage of BDNF to microbeads likely prevents BDNF internalization, distinct downstream signaling pathways are likely to be activated. While it has been reported that inhibition of BDNF-TrkB internalization does not affect TrkB kinase activity [52], there are other reports that exogenously applied BDNF is recycled for activity-dependent secretion, maintenance of LTP, and other functions [53–55]. While stimulation of neurons with BDNF-coated microbeads may activate similar pathways to those stimulated when BDNF is internalized, specific cellular functions require internalization [56] and would not be activated by application of BDNF-coated microbeads. Moreover, it is technically challenging to ensure that all beads are applied to neuronal cultures as single beads. As can be seen in the lower panel of Figure 1B, occasionally, beads will cluster, and it is possible that the clusters affect dendrite branching differently than do the single beads. Future work will determine the detailed pathways BDNF activates when bound to microbeads.

Local BDNF treatment blocks pruning in addition to adding new dendrites

As neurons develop, the dendritic arbor undergoes age-dependent changes [57–59]. Periods of active branching are followed by periods of pruning as neurons undergo development [37,60,61]. Most studies have focused on the role of BDNF in axonal pruning (reviewed in [62]); the fact that our current study focuses on dendritic pruning provides a novel role for BDNF in shaping neuronal morphology. Importantly, acute versus chronic exposure of neurons to BDNF influences activation of different transcription factors [63], which may explain changes to dendrite pruning by BDNF over time. Our data suggest that local BDNF treatment reduces the pruning of all types of branches, with the most prominent attenuation of pruning occurring to tertiary and higher order branches. Stimulation of p75^{NTR} by BDNF is important for developmental axonal pruning [64], supporting the idea that binding of BDNF to this receptor, and potentially TrkB.T1, distally plays an important role in shaping the dendritic arbor by localized but not global BDNF exposure. In contrast, our previous studies suggest that global application of BDNF increases terminal dendrites with no effect on intermediate dendrites [34]. Thus, the mechanism by which the different modes of BDNF stimulation increase dendritic arbor complexity differ.

Moreover, the time period used in the current study (DIV 7–10) represents a highly dynamic, unstable stage of neuronal development. Previous studies indicate that dendrites undergo frequent extensions and retractions from DIV 8–10 [65,66]. Moreover, it is important to recognize that the arbor is in a natural state of flux, and the results from this

study shed light not on how BDNF stimulation affects a mature, stable dendritic arbor but rather how it shapes an immature, developing dendritic arbor. In addition, it is possible that some of the observed differences in Sholl curves are due to transient changes to the arbor that occur as a result of development and that the mature arbor sculpted by BDNF will differ from these morphologies. Future studies will include time-lapse imaging to better understand whether BDNF and/or cypin contribute to the natural dynamics of the arbor that occur during this time.

Potential endogenous sources of BDNF: global versus local stimulation of neurons

What is the significance of global stimulation versus local dendritic stimulation of neurons with BDNF? BDNF is secreted from both neurons and astrocytes [45,67,68]. Astrocytes can release BDNF in an exocytotic manner [69,70], resulting in exposure of neurons to BDNF similar to that seen with bath application. In addition, BDNF can act in a peri-synaptic, or more localized, manner. Recycling of synaptic BDNF by astrocytes stabilizes long-term potentiation and mediates memory retention [54]. Furthermore, neurons release BDNF synaptically, where it can act in a localized manner at postsynaptic sites (reviewed in [62] and [71]). Moreover, BDNF secretion modulates not only neuronal activity at the cellular level but also cognitive function. For example, the Val66Met single nucleotide polymorphism (SNP) rs6265 in humans affects BDNF packaging and secretion [72]. This mutation does not alter constitutive BDNF secretion but instead reduces activity-dependent secretion, which affects brain structure and cognitive processes and is associated with susceptibility to psychiatric disorders [73–76,72]. Thus, local stimulation with BDNF-coated beads can help us uncover mechanisms underlying changes to dendrites by synaptic or peri-synaptic BDNF.

The neuron itself may serve as a determinant of localized production and release of BDNF. Different transcript variants of BDNF exist, and these variants localize BDNF mRNA – and, thus, BDNF protein – to different parts of the neuron. There are BDNF transcripts that contain distinct 3' untranslated regions (UTRs). BDNF transcripts containing the short 3' UTR are restricted to cell bodies, and transcripts containing the long 3' UTR are localized to both cell bodies and dendrites. Expression of BDNF from these transcripts results in different actions on dendritic spines but, importantly, encodes the same protein [77,78]. Furthermore, the RNA binding protein HuD binds to a region in the long 3' UTR, regulating expression from this mRNA but not the mRNA containing the short 3' UTR [79]. Moreover, the rodent BDNF gene consists of eleven alternative 5' UTRs, resulting in different developmental profiles of expression of these BDNF transcripts [80,48]. The different 5' UTRs selectively alter outgrowth of proximal or distal dendrites, depending on where BDNF mRNA is localized by the 5' UTRs, and these changes in dendritic arborization may be related to selective activation of TrkB in these dendritic regions [48]. Additionally, trafficking of the BDNF 5' UTR splice variant resulting from exon 6 is impaired in mice expressing the BDNF human variant Val66Met [81], having implications for BDNF action on the dendritic arbor. Finally, as the Val66Met mutation results in decreased transport of BDNF mRNA in the dendrites (as reviewed in [82]), and thus a disruption of the BDNF mRNA spatial code [83], it is possible that local stimulation of the dendritic arbor by BDNF-coated beads may alleviate some of the issues caused by this dysregulation in BDNF mRNA

transport. Thus, expression of specific BDNF mRNAs is crucial for the proper regulation of dendritogenesis and other cellular processes. Ongoing work in our laboratory addresses how BDNF resulting from these transcripts acts to regulate the dendritic arbor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is funded in part by National Science Foundation grants IOS-0919747 and IOS-1353724 (to BLF). KMO is supported by the National Institutes of Health under the Ruth L. Kirschstein National Research Service Award T32 GM8339 from the NIGMS, a Predoctoral Fellowship from the New Jersey Commission on Brain Injury Research #CBIR13FEL002, and a Predoctoral GAANN Fellowship #P200A150131 from the DOE. KED was awarded Aresty Research Funding from Rutgers University. The authors would also like to thank Dr. Kelvin Kwan (Rutgers University) for his generous help with imaging of the microbeads.

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Figure 1. Local stimulation of dendrites using microbeads

A. Western blot analysis of coating efficiency of beads with BDNF. Load and flow through (FT) indicate that no detectable BDNF remained in solution after BDNF was conjugated to the beads. BDNF is detected on BDNF-coated beads but not on BSA-coated beads. **B.** Western blot analysis of BDNF- and BSA-coated beads after incubation with neuronal culture over time. BDNF and BSA remain bound to beads over the various treatment times and are not released into the medium. **C.** STORM images of BSA-coated beads (top) or BDNF-coated beads (bottom). Scale bars indicate 10 μ m. **D.** Representative images of neurons treated with BSA-coated beads (top) or BDNF-coated beads (bottom). Arrows indicate bead-dendrite contact close to a branching point, and arrowheads indicate bead-dendrite contact where no branching point was observed. Four sites of dendrite bead-contact were indicated for each condition. **E.** Boxed regions from **A** at higher magnification showing BSA-coated (top) and BDNF-coated (bottom) beads in contact with dendrites. The number of beads per dendrite ranged from 1.36 to 2.21 for the first set of experiments and 1.58 to 2.47 for the second set of experiments (data not shown), and the average number of beads per dendrite was not significantly different between any of the conditions. Scale bars indicate 20 μ m.

Figure 2. Sholl analysis of hippocampal neurons shows that treatment with BDNF-coated beads increases branching

A. Representative images from each condition (BSA- or BDNF-coated bead treatment) at each of the time points (5, 24, 48, and 72 hours). Scale bar indicates 100 μ m. **B.** No significant changes in the Sholl curves are observed after 5 hours of treatment with BDNF-coated beads compared with the control treatment (BSA-coated beads). **C.** No significant changes in the Sholl curves are observed after 24 hours of treatment with BDNF-coated beads compared with the control treatment. **D.** Treatment with BDNF-coated beads for 48 hours significantly increases distal branching (93.3–159.3 μ m; * p <0.05). **E.** Treatment with BDNF-coated beads for 72 hours significantly increases both proximal (21.3–39.3 μ m) and distal (123.3–153.3 μ m) branching (both * p <0.05). **F, G, H, and I.** Plots showing the first 160 μ m of the Sholl curve from **B, C, D, and E** (respectively). For 5 hour treatment: $n(\text{BSA})=40$ and $n(\text{BDNF})=50$. For 24 hour treatment: $n(\text{BSA})=48$ and $n(\text{BDNF})=35$. For 48 hour treatment: $n(\text{BSA})=64$ and $n(\text{BDNF})=93$. For 72 hour treatment: $n(\text{BSA})=91$ and $n(\text{BDNF})=104$. Statistics calculated by Two-Way ANOVA followed by Bonferroni's Multiple Comparisons test. Error bars indicate SEM.

Figure 3. Order-specific Sholl analysis reveals that BDNF exerts effects on different orders of dendrites

Stars indicate exact levels of significance, and bars indicate significance of at least $*p<0.05$.

A–C. Order-specific Sholl analysis of neurons treated for 5 hours with BSA- or BDNF-coated beads. **A.** Sholl analysis of primary dendrites shows that treatment with BDNF-coated beads significantly increases dendrites at 15.3 to 33.3 μm from the soma ($*p<0.05$). **B.** Sholl analysis of secondary dendrites shows that treatment with BDNF-coated beads significantly increases dendrites at 15.3 to 33.3 μm ($**p<0.01$) and at 69.3 to 75.3 μm ($*p<0.05$) from the soma. **C.** Sholl analysis of tertiary and higher order dendrites shows that treatment with BDNF-coated beads does not significantly alter branching of these dendrites. **D–F.** Order-specific Sholl analysis of neurons treated for 24 hours with BSA- or BDNF-coated beads. **D.** Treatment with BDNF-coated beads significantly increases primary dendrites at 9.3 to 57.3 μm from the soma ($*p<0.05$). **E.** Treatment with BDNF-coated beads significantly increases secondary dendrites at 33.3 μm ($**p<0.01$) and at 39.3 μm ($*p<0.05$) from the soma. **F.** Treatment with BDNF-coated beads does not significantly alter the Sholl

curves of tertiary and higher order dendrites. **G–I.** Order-specific Sholl analysis of neurons treated for 48 hours with BSA- or BDNF-coated beads. **G.** Treatment with BDNF-coated beads does not significantly alter the Sholl curves of primary dendrites. **H.** Treatment with BDNF-coated beads significantly increases secondary dendrites at 45.3 μm from the soma (** $p < 0.01$). **I.** Treatment with BDNF-coated beads significantly increases tertiary and higher order dendrites at 105.3 to 111.3 μm and at 129.3 to 159.3 μm from the soma (both * $p < 0.05$). **J–L.** Order-specific Sholl analysis of neurons treated for 72 hours with BSA- or BDNF-coated beads. **J.** Treatment with BDNF-coated beads significantly increases primary dendrites at 9.3 to 21.3 μm from the soma (* $p < 0.05$). **K.** Treatment with BDNF-coated beads significantly increases secondary dendrites at 21.3 to 45.3 μm from the soma (** $p < 0.01$). **L.** Treatment with BDNF-coated beads significantly increases tertiary and higher order branching at 105.3 μm and at 123.3 to 153.3 μm from the soma (both * $p < 0.05$). Statistics calculated by Two-Way ANOVA followed by Bonferroni's Multiple Comparisons test. Error bars indicate SEM.

Figure 4. Grouping order-specific Sholl analysis by condition reveals how BDNF modulates pruning of higher order dendrites

To indicate sites of significant differences in dendritic arborization, the following notation is used: 1) green stars/bars to compare the 5 hour and 24 hour time points (black vs. green lines); 2) red stars/bars to compare the 24 hour and 48 hour time points (green vs. red lines); and 3) blue stars/bars to compare the 48 hour and 72 hour time points (red vs. blue lines). Stars indicate exact levels of significance, and bars indicate significance of at least $*p < 0.05$.

A–C. Order-specific Sholl analysis of neurons treated with BSA-coated beads. **A.** Primary dendrites at 9.3 μm from the soma are significantly decreased between 5 and 24 hours (green stars, $****p < 0.0001$). Primary dendrites significantly increase between 24 and 48 hours (red bar/star, 9.3–27.3 and 39.3 μm ; $***p < 0.001$). Primary dendrites significantly increase between 48 and 72 hours (blue bar, 15.3–39.3 μm ; $*p < 0.05$). **B.** Secondary dendrites at 15.3 to 21.3 μm from the soma are significantly decreased between 5 and 24 hours (green bar; $**p < 0.01$). Secondary dendrites at 15.3 μm from the soma significantly decrease between 48 and 72 hours (blue stars, $****p < 0.0001$). **C.** Tertiary and higher order dendrites are significantly decreased at 33.3 μm and at 75.3–165.3 μm from the soma between 5 and 24 hours (green star/bar; both $*p < 0.05$). Tertiary and higher order dendrites at 141.3 to 183.3 μm from the soma are also significantly decreased between 48 and 72 hours (blue bar; $*p < 0.05$).

D–F. Order-specific Sholl analysis of neurons treated with BDNF-coated beads. **D.** BDNF attenuates the changes that occurs between 5 and 24 hours and between 24 and 48 hours of treatment, and it significantly increases primary dendrites after 72 hours of treatment (blue bar, 9.3–27.3 μm ; $**p < 0.01$). **E.** Treatment with BDNF-coated beads not only attenuates the pruning seen between 5 and 24 hours of treatment but also significantly increases secondary branching at 33.3 μm from the soma (green stars; $**p < 0.01$). Secondary dendrites at 15.3 μm from the soma remain significantly decreased between 48 and 72 hours of treatment (blue stars; $***p < 0.001$). **F.** Treatment with BDNF-coated beads attenuates pruning seen between 5 and 24 hours, but the pruning that occurs between 48 and 72 hours

is still observed: tertiary and higher order dendrites are significantly decreased between 48 and 72 hours at 129.3 to 189.3 μm from the soma (blue bar, $*p<0.05$). Statistics calculated by Two-Way ANOVA followed by Bonferroni's Multiple Comparisons test. Error bars indicate SEM.

Figure 5. Sholl analysis reveals a role for cypin in alterations to the dendritic arbor resulting from treatment with BDNF-coated beads

To indicate sites of significant differences in dendritic arborization, the following notation is used: 1) blue bars/stars compare control neurons (black line) to those transfected with GST shRNA and treated with BDNF-coated beads (blue line); 2) green stars/bars compare control neurons (black line) to those transfected with cypin shRNA and treated with BSA-coated beads (green line); 3) purple stars/bars compare neurons transfected with cypin shRNA and treated with BDNF-coated beads (red line) to neurons transfected with GST shRNA and treated with BDNF-coated beads (blue line); and 4) black stars/bars compare neurons transfected with cypin shRNA and treated with BDNF-coated beads (red line) to neurons transfected with cypin shRNA and treated with BSA-coated beads (green line). Stars indicate exact levels of significance, and bars indicate significance of at least $*p < 0.05$. **A.** Representative images from each condition. Scale bar indicates 100 μm . **B.** Sholl analysis for 5 hour timepoint. **C.** Sholl analysis for 24 hour timepoint. **D.** Sholl analysis for 48 hour timepoint. **E.** The first 100 μm of the Sholl curve from panel B. BDNF treatment significantly increases branching at 93.3 μm from the soma (blue vs. black lines, blue stars; $*p < 0.05$). Cypin knockdown significantly decreases branching at 21.3 to 27.3 μm from the soma (green vs. black lines, green bar; $**p < 0.01$). The Sholl curve for cypin knockdown + BDNF-coated beads (red line) is significantly increased over cypin knockdown + BSA-coated beads (green line) at 15.3 to 27.3 μm and at 81.3 to 87.3 μm from the soma (black bars; both $*p < 0.05$). Compared with the Sholl curve for the control neurons treated with BDNF-coated beads (blue line), the Sholl curve for cypin knockdown + BDNF-coated beads (red line) is significantly increased at 15.3 to 21.3 μm and significantly decreased at 33.3 to 39.3 μm from the soma (purple bars; both $*p < 0.05$). **F.** The first 100 μm of the Sholl curve from panel C. Treatment with BDNF-coated beads significantly increases branching at 27.3 to 33.3 μm from the soma (blue vs. black lines, blue bar; $**p < 0.01$). Cypin knockdown significantly decreases branching at 33.3 μm from the soma (green vs. black lines; green stars; $**p < 0.01$). The Sholl curve for cypin knockdown + BDNF-coated beads (red line) is significantly decreased versus cypin knockdown + BSA-coated beads (green line) at 39.3 to 45.3 μm from the soma (black bar; $**p < 0.01$) and at 57.3 to 63.3 μm from the soma (black bar; $*p < 0.05$). Compared with the Sholl curve for the control neurons treated with BDNF-coated beads (blue line), the Sholl curve for cypin knockdown + BDNF-coated beads (red line) is significantly decreased at 21.3 to 57.3 μm from the soma (purple bar;

*** $p < 0.0001$). **G.** The first 100 μm of the Sholl curve from panel D. Treatment with BDNF-coated beads significantly increases branching at 15.3 to 21.3 μm from the soma (blue vs. black lines, blue bar; $*p < 0.05$). Cypin knockdown significantly decreases branching at 27.3 to 45.3 μm from the soma (green vs. black lines, bar stars; $**p < 0.01$). The Sholl curve for cypin knockdown + BDNF-coated beads (red line) is significantly decreased versus cypin knockdown + BSA-coated beads (green line) at 21.3 to 39.3 μm and at 51.3 μm from the soma (black bar/star; both $*p < 0.05$). GST KD indicates neurons that were transfected with GST shRNA, and Cypin KD indicates neurons that were transfected with Cypin shRNA. For 5 hour treatment: $n(\text{GST}+\text{BSA})=46$, $n(\text{GST}+\text{BDNF})=50$, $n(\text{Cypin}+\text{BSA})=40$, $n(\text{Cypin}+\text{BDNF})=49$. For 24 hour treatment: $n(\text{GST}+\text{BSA})=67$, $n(\text{GST}+\text{BDNF})=43$, $n(\text{Cypin}+\text{BSA})=48$, $n(\text{Cypin}+\text{BDNF})=54$. For 48 hour treatment: $n(\text{GST}+\text{BSA})=42$, $n(\text{GST}+\text{BDNF})=53$, $n(\text{Cypin}+\text{BSA})=17$, $n(\text{Cypin}+\text{BDNF})=29$. Statistics calculated by Two-Way ANOVA followed by Bonferroni's Multiple Comparisons test. Error bars indicate SEM.

Figure 6. Order-specific Sholl analysis illustrates that treatment with BDNF-coated beads may act, in part, through cypin to regulate pruning of the dendritic arbor

To indicate significance, green stars/bars show significance between the 5 hour and 24 hour time points (black vs. green lines), and black stars/bars show significance between the 24 hour and 48 hour time points (green vs. red lines). Stars indicate exact levels of significance, and bars indicate significance of at least $*p < 0.05$. **A–C.** Order-specific Sholl analysis of neurons transfected with cDNA encoding GST shRNA and treated with BSA-coated beads. **A.** Primary dendrites significantly decrease between 5 and 24 hours at 9.3 μm from the soma (green star; $*p < 0.05$). **B.** Between 5 and 24 hours, secondary dendrites significantly decrease at 21.3 μm from the soma (green stars; $****p < 0.0001$) and significantly increase at 33.3 to 51.3 μm from the soma (green bar; $**p < 0.01$). Secondary dendrites significantly decrease between 24 and 48 hours at 15.3 to 27.3 μm from the soma (black bar; $*p < 0.05$). **C.** Tertiary and higher order dendrites significantly decrease between 5 and 24 hours at 69.3 to 135.3 μm from the soma (green bar; $*p < 0.05$). Between 24 and 48 hours, tertiary and higher order dendrites significantly decrease at 21.3 to 45.3 μm from the soma (black bar; $*p < 0.05$), and

they significantly increase at 105.3 to 111.3 μm and at 123.3 to 147.3 μm from the soma (black bars; both $*p<0.05$). **D–F.** Order-specific Sholl analysis of neurons transfected with cDNA encoding GST shRNA and treated with BDNF-coated beads. **D.** Primary dendrites significantly decrease between 5 and 24 hours at 15.3 to 21.3 μm from the soma (green bar; $*p<0.05$), but they significantly increase between 24 and 48 hours at 9.3 to 33.3 μm from the soma (black bar; $**p<0.01$). **E.** Between 5 and 24 hours, secondary dendrites significantly increase at 15.3 μm from the soma (green stars; $****p<0.0001$) and significantly decrease at 39.3 μm from the soma (green stars; $*p<0.05$). Between 24 and 48 hours, secondary dendrites significantly increase at 21.3 to 27.3 μm (black bar; $****p<0.0001$) and at 63.3 μm from the soma (black star; $*p<0.05$). **F.** Tertiary and higher order dendrites significantly increase between 5 and 24 hours at 21.3 to 45.3 μm and at 57.3 μm from the soma (green bar/star; both $*p<0.05$). Between 24 and 48 hours, tertiary and higher order dendrites significantly decrease at 21.3 to 69.3 (black bar; $*p<0.05$). **G–I.** Order-specific Sholl analysis of neurons transfected with cDNA encoding cypin shRNA and treated with BSA-coated beads. **G.** Primary dendrites significantly decrease between 24 and 48 hours at 9.3 to 21.3 μm from the soma (black bar; $****p<0.0001$). **H.** Secondary dendrites significantly increase between 5 and 24 hours at 21.3 to 27.3 μm from the soma (green bar; $**p<0.01$). The Sholl curve shifts at 48 hours, resulting in a significant increase at 15.3 μm and significant decreases at 21.3 to 39.3 μm from the soma (black bar; $**p<0.01$). **I.** Tertiary and higher order dendrites significantly decrease between 24 and 48 hours at 33.3 to 75.3 μm from the soma (black bar; $*p<0.05$). **J–L.** Order-specific Sholl analysis of neurons transfected with cDNA encoding cypin shRNA and treated with BDNF-coated beads. **J.** Primary dendrites significantly decrease between 5 and 24 hours at 9.3 to 15.3 μm from the soma (green bar; $****p<0.0001$). **K.** Secondary dendrites significantly decrease between 5 and 24 hours at 15.3 to 27.3 μm from the soma (green bar; $***p<0.001$). Between 24 and 48 hours, secondary dendrites significantly increase at 15.3 μm (black stars; $****p<0.0001$) and at 51.3 μm (black stars; $**p<0.01$) from the soma. **L.** Tertiary and higher order dendrites significantly decrease at 33.3 to 45.3 μm from the soma (green bar; $**p<0.01$). GST KD indicates neurons that were transfected with GST shRNA, and Cypin KD indicates neurons that were transfected with Cypin shRNA. Statistics calculated by Two-Way ANOVA followed by Bonferroni's Multiple Comparisons test. Error bars indicate SEM.

Figure 7. Schematic illustrating the changes that occur over time as a result of treatment with BDNF-coated beads

Primary dendrites are shown in blue, secondary dendrites in green, and tertiary and higher order dendrites in red. **Part 1.** Treatment with BDNF-coated beads causes an increase in primary dendrites at 5 hours and then results in a transient decrease in secondary dendrites at 24 hours. At 48 hours, the increase in primary dendrites is no longer present, but tertiary and higher order dendrites increase. After 72 hours of treatment, the overall number of primary and secondary dendrites increases, and distal tertiary and higher order dendrite numbers and length increase. Pruning over time for both conditions is also shown. **Part 2.** Treatment of control neurons (transfected with cDNA encoding GST shRNA) with BDNF results in increased tertiary dendrites after 24 hours and increased secondary dendrites after 48 hours. Neurons with cypin knockdown and BSA-coated bead treatment have fewer secondary dendrites after 5 hours, fewer tertiary and higher order dendrites after 24 hours, and even fewer primary, tertiary, and higher order dendrites after 48 hours. Neurons with cypin knockdown and BDNF-coated bead treatment did not show these decreases. There was no change in dendrites of any order at 5 hours, a loss of primary, tertiary, and higher order dendrites after 24 hours, and no further loss of dendrites at 48 hours. Pruning over time for all conditions is also shown. GST KD indicates neurons that were transfected with GST shRNA, and Cypin KD indicates neurons that were transfected with Cypin shRNA.

Table 1

Summary showing how treatment with BDNF-coated beads affects dendrite number and length.

	5h	24h	48 h	72h
Total Dendrite #	n.s.	n.s.	+	+
Primary Dendrite #	n.s.	++	n.s.	+
Secondary Dendrite #	-	n.s.	n.s.	+
Tertiary+ Dendrite #	n.s.	n.s.	+	n.s.
Avg. Dendrite Length	n.s.	n.s.	n.s.	n.s.
Primary Dendrite Length	n.s.	n.s.	n.s.	n.s.
Secondary Dendrite Length	n.s.	n.s.	n.s.	n.s.
Tertiary+ Dendrite Length	n.s.	n.s.	n.s.	+

The following symbols are used to indicate changes: 'n.s.' indicates no significant change versus treatment with BSA-coated beads, '+' indicates that BDNF-coated beads significantly increase the parameter, and '-' indicates that BDNF-coated beads significantly decrease the parameter. After 5 h treatment with BDNF-coated beads, secondary dendrite number significantly decreases (- p<0.05). After 24 h treatment with BDNF-coated beads, primary dendrite number significantly increases (++ p<0.01). After 48 h treatment with BDNF-coated beads, total dendrite number and tertiary and higher order dendrite number significantly increase (+ p<0.05). After 72 h treatment with BDNF-coated beads, total dendrite number, primary dendrite number, and secondary dendrite number significantly increase (+ p<0.05). Only tertiary and higher order dendrite length significantly increases after 72 h treatment with BDNF-coated beads (+ p<0.05). Statistics calculated by Student's t-test.