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Deep Sequencing and High-Resolution Imaging Reveal Compartment-Specific Localization of *Bdnf* mRNA in Hippocampal Neurons

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Abstract

Brain-derived neurotrophic factor (BDNF) is a small protein of the neurotrophin family that regulates various brain functions. Although much is known about how its transcription is regulated, the abundance of endogenous *BDNF* mRNA and its subcellular localization pattern are matters of debate. We used next-generation sequencing and high-resolution in situ hybridization in the rat hippocampus to reexamine this question. We performed 3' end sequencing on rat hippocampal slices and detected two isoforms of *Bdnf* containing either a short or a long 3' untranslated region (3' UTR). Most of the *Bdnf* transcripts contained the short 3' UTR isoform and were present in low amounts relative to other neuronal transcripts. *Bdnf* mRNA was present in the somatic compartment of rat hippocampal slices or the somata of cultured rat hippocampal neurons but was rarely detected in the dendritic processes. Pharmacological stimulation of hippocampal neurons induced *Bdnf* expression but did not change the ratio of *Bdnf* isoform abundance. The findings indicate that endogenous *Bdnf* mRNA, although weakly abundant, is primarily localized to the somatic compartment of hippocampal neurons. Both *Bdnf* mRNA isoforms have shorter half-lives compared with other neuronal mRNAs. Furthermore, the findings show that using complementary high-resolution techniques can provide sensitive measures of endogenous transcript abundance.

Introduction

Brain-derived neurotrophic factor (BDNF) is a small protein of the neurotrophin family (1) that regulates a variety of brain functions, including the development and plasticity of neurons in the central and peripheral nervous system. BDNF is a classic target-derived protein that can promote the growth and survival of sensory neurons (1), as well as the

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Data and materials availability: A text file with the raw 3' end sequencing data from for the *Bdnf* gene is provided as supplementary material.

survival and differentiation of neural stem cells (2). In cultured neurons, BDNF can also promote the directional turning of growth cones (3) and the differentiation and maturation of axons (4). In mature neurons, particularly in the hippocampus, BDNF is a modulator of long-term synaptic plasticity (5). Although many functions have been ascribed to BDNF, the basal (unstimulated) abundance of both BDNF mRNA (6) and protein (7) are low in the brain (8) and throughout development (9). However, the transcription of *BDNF* is regulated by many processes, including diverse promoters (10), DNA methylation (11, 12), and alternative splicing (10), suggesting that there is ample possibility for increased *BDNF* expression.

BDNF mRNA is expressed in many excitatory neurons (13) and is also known to exhibit activity-dependent increased abundance, such as in response to plasticity induced by high-frequency stimulation, potassium-induced depolarization, or epileptogenesis (14–16). Two different *BDNF* mRNA 3′ untranslated region (UTR) isoforms have been identified (17–20), which are reported to influence the localization of *BDNF* mRNA (21). It has been proposed that the short 3′ UTR isoform is restricted to the soma and the long isoform is targeted to dendrites, but most studies rely on the analysis of reporter transcripts typically overexpressed in the proximal aspects of dendrites. Therefore, we examined the abundance and localization of endogenous *Bdnf* transcripts, including the coding sequence (CDS)–containing short and long 3′ UTR isoforms, in the rat hippocampus using various state-of-the-art quantitative techniques.

Results

Deep RNA sequencing and gene counting reveal *Bdnf* mRNA is present in low amounts in the rat hippocampus

To investigate both the abundance of *BDNF* transcripts and the diversity of their 3′ UTRs in the hippocampus, we conducted RNA sequencing of mRNA isolated from the rat hippocampus (22). We obtained 2294 short nucleotide sequences (hereafter called “reads”) that mapped to the rat *Bdnf* transcript sequence (provided by the National Center for Biotechnology Information) predicting two different 3′ UTR isoforms that are 498 and 2887 nucleotides (nt) long (Fig. 1, A and B), which is consistent with previous studies (17–20). The predicted 3′ terminal end of both 3′ UTRs contains a poly(A) (polyadenylate) consensus sequence (Fig. 1, A and B; short = AUUAAA, long = AAUAUA). The relative number of reads for the short and long 3′ UTRs (1500 reads, 0.65 fraction, and 566 reads, 0.25 fraction, respectively) predicted a ratio of 3:1 for the short to long 3′ UTRs in the CA1 region (fig. S1A), which is similar to quantitative reverse transcription polymerase chain reaction (qRT-PCR) data obtained by others (23). We validated these data using qRT-PCR and found a similar ratio (4:1) of short to long 3′ UTRs (Fig. 1, D and E). Because the *Bdnf*-*CDS* transcripts include both the short and long 3′ UTRs at a roughly 4:1 ratio, detection of the *CDS* transcript represents a rough estimate of the short 3′ UTR isoform, and we will therefore refer to the short isoform as *Bdnf*-*CDS*.

To examine the abundance of *Bdnf* relative to other transcripts in the hippocampus, we compared the number of reads we obtained for *Bdnf* mRNA [the short (CDS) and long UTR isoforms] with *Camk2a*-*CDS* mRNA, which codes for a dendritically localized protein that

is abundant in the hippocampus (22, 24, 25). The *Bdnf*-CDS transcript was expressed at about 5% of that observed for *Camk2a* (Fig. 1C), which was confirmed using qRT-PCR (Fig. 1D). We compared the abundance of *Bdnf* to another mRNA of another neurosecretory protein, *Vgf*. We found that *Bdnf*-CDS transcript abundance was about 15% of that observed for the *Vgf* transcript (Fig. 1C), which was confirmed by qRT-PCR (Fig. 1D). Although *Bdnf* transcript amounts were lower compared with those of *Camk2a* and *Vgf*, when compared to the total hippocampal transcriptome, *Bdnf* transcript amounts were higher than that of the median transcript expression (fig. S1B). To validate these results further, we also used NanoString nCounter (26), a technique that permits high-resolution visualization of single mRNA molecules. In 100 ng of total RNA prepared from the CA1 region, 44,000 counts (single mRNA molecules) were detected for *Camk2a*-CDS mRNA compared with only 200 counts for *Bdnf*-CDS mRNA (Fig. 1F), suggesting that the *Bdnf* transcript is present at about 0.5% that of *Camk2a*. Together, these data indicate that *Bdnf* has two 3' UTR isoforms: the short isoform is present in low amounts relative to other neuronal transcripts, and the long isoform is substantially less abundant than the short, indicating that most of the *Bdnf* transcripts contain the short 3' UTR.

Because all of our data were collected from the rat hippocampus, we evaluated whether our findings could extend to other species that express *Bdnf*, namely, mice and humans. We reanalyzed a previously published data set of 3' end sequencing from human, mouse, and rat brain tissues (27). We found that in both human and mouse, the *BDNF* transcript was present at amounts even lower than that observed in the rat hippocampus (fig. S1C). Because *Bdnf* expression is low in all three species and the ratio to another transcript (*Camk2a*) is conserved, these data suggest that the conclusions drawn from our analysis of rat *Bdnf* mRNA abundance and localization may also apply to other species.

The *Bdnf* transcript is localized to neuronal somata

In neurons, some mRNA species are localized to the dendrites where they can be locally translated into protein. We examined the relative distribution of the *Bdnf*-CDS transcript in the somata and neuropil layers of the hippocampus using a suite of techniques (RNA sequencing, qRT-PCR, NanoString) and high-resolution in situ hybridization. Hippocampal slices were microdissected to separate the somatic and neuropil layers (22); the neuropil layer is enriched in dendrites and axons (28). 3' End sequencing was used to determine the abundance of *Bdnf* mRNA. We counted the reads for the *Bdnf* transcript from the somata or neuropil layers and found that most (86%) of the endogenous *Bdnf* transcripts were detected in the somata layer (Fig. 2A). Data obtained from qRT-PCR and NanoString experiments yielded similar results (Fig. 2A and fig. S1A). To compare the layer-specific expression of *Bdnf* to other transcripts, we analyzed the distribution of a well-known, dendritically localized transcript *Camk2a* and a transcript that resides primarily in cellular somata, *Vgf*, by RNA sequencing, qRT-PCR, and NanoString. A comparison of the *Bdnf* transcript expression pattern with that of either *Camk2a* or *Vgf* indicated that it is similar in relative distribution to *Vgf* (fig. S1B), with a greater abundance of transcripts consistently detected in the somata than in the neuropil, regardless of the technique used (Fig. 2A and fig. S2, A to C).

High-resolution visualization of *Bdnf* transcripts is revealed by in situ hybridization

To directly visualize the *Bdnf* transcript in its native environment, we used high-resolution in situ hybridization in either dissociated rat hippocampal neuronal cultures (Fig. 2B) or rat hippocampal slices (Fig. 2C) using probes designed to detect the *Bdnf-CDS* or the *Bdnf-UTR_{long}* transcript. These experiments revealed endogenous *Bdnf-CDS* particles in the neuronal cell somata but little or no particles in the dendrites, which were identified by immunostaining for the microtubule-associated protein 2 (MAP2) (Fig. 2, B and C, and fig. S3A). In cultured hippocampal neurons, a cluster of *Bdnf-CDS* transcripts could be identified in the soma, but no particles were observed in the dendrites (Fig. 2B). In the CA1 area of the mature hippocampus, a similar pattern was observed (Fig. 2C), in which a few particles were occasionally observed within the first 20 μ m of proximal dendrite, a region that is often considered an extension of the cell body. The few particles that were observed in the synaptic neuropil were often associated with nuclei, indicating a probable somatic location in a displaced pyramidal neuron, interneuron, or glial cell. The in situ signal for the *Bdnf-UTR_{long}* transcript was detected at even lower levels than the *Bdnf-CDS* transcript. Although a small number of positive particles were clearly detected in the cell bodies, weak or no signal was visible in the dendrites of either dissociated neurons (Fig. 2B) or neurons in the CA1 region from hippocampal slices (Fig. 2C). These data are consistent with the relative proportion of reads we obtained for the long 3'UTR *Bdnf* transcript in the somata and neuropil (fig. S1A). We also analyzed the distribution of endogenous *Bdnf* transcripts in other hippocampal subfields, including CA3 and the dentate gyrus (DG) (fig. S3B). The expression and distribution pattern of *Bdnf* transcripts in CA3 and DG were comparable to those observed in CA1 (fig. S3, C and D). For comparison, in situ hybridization for endogenous *Camk2a* showed abundant signal in both the somata and the dendrites of dissociated hippocampal neurons in culture and in the CA1 subfield of the hippocampus (Fig. 2, B and C), which is similar to previous reports (22, 29, 30). In summary, four different techniques indicated that the *Bdnf* transcript is present at markedly low amounts in the somata of all hippocampal subfields and the DG but is barely detectable in the neuropil.

Although the endogenous, basal abundance of *Bdnf* mRNA is low, there is clear evidence that *Bdnf* transcript abundance can be increased by a variety of activity-dependent mechanisms (14–16, 20, 31, 32). We examined the sensitivity of our techniques to detect increases in either the *Bdnf-CDS* or the 3'UTR_{long} isoforms. We tested whether enhanced neural activity using the γ -aminobutyric acid type A (GABA_A) receptor antagonist bicuculline alters the abundance or localization of the *Bdnf* transcripts. The in situ hybridization fluorescent signal for *Bdnf-CDS* in bicuculline-treated hippocampal cells was significantly increased compared with that in untreated cells (Fig. 3, A to C). The same analysis using a probe targeting the long 3'UTR_{long} isoform did not reveal a significant difference, suggesting that new transcripts contained primarily the short 3'UTR isoform (Fig. 3C). We also stimulated neurons by treating cells with the neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP) in conditions previously shown to elicit long-lasting bursts of action potential firing (33). The in situ fluorescent signal for *Bdnf-CDS* in PACAP-treated hippocampal cells was significantly increased compared with that in untreated cells (Fig. 3, D and E). The same analysis using the probe targeting the long 3'UTR isoform did not reveal a significant difference, indicating a shift of the CDS/UTR_{long}

ratio to favor the short transcript in PACAP-treated cells (Fig. 3F). Regarding localization in an activated context, *Bdnf* transcripts were detected almost exclusively in somata and proximal dendrites in neurons that exhibited the highest abundance of *Bdnf* signal in situ. We conclude that both bicuculline and PACAP treatments induced increased expression of *Bdnf*, with new transcripts more likely to have the short 3'UTR isoform (fig. S4, A and B). This was confirmed with qRT-PCR, in which a significant increase in the amount of *Bdnf*-*CDS* out of total isolated RNA was observed in both bicuculline- and PACAP-treated hippocampal cells (Fig. 3, G and H). Using qRT-PCR, we also detected a bicuculline- or PACAP-induced increase in the long 3'UTR isoform (Fig. 3, G and H). However, this increase was smaller than the corresponding increase in the CDS transcript (Fig. 3, G and H), again indicating that most of the PACAP-induced *Bdnf* transcripts contained the short 3'UTR isoform.

Differences in 3'UTR isoforms can confer differences in the localization, translational regulation, and half-life of an mRNA. It has been proposed that mRNA isoforms that are transported and localized to dendrites might be endowed with longer half-lives to take into account the fraction of the mRNA life span spent in transport to its destination, often hundreds of micrometers away from the cell body (34, 35). We investigated by qRT-PCR whether there were differences in estimated half-lives for the short and long 3'UTR isoforms of *Bdnf* in dissociated hippocampal neurons. After inhibiting transcription, the long 3'UTR isoform exhibited a half-life about one-half of the value measured for the short *Bdnf*-*CDS* transcript (Fig. 3I and fig. S4C). As expected for an activity-induced gene, the measured half-lives of both *Bdnf* isoforms (6.8 hours for the CDS, 3.2 hours for the 3'UTR_{long}) were short compared to the half-lives of other (not activity-induced) neuronal mRNAs, which range from 16 to 24 hours (36–39). Our data suggest that the long 3'UTR *Bdnf* transcript has a shorter half-life than the short 3'UTR isoform and thus may not be consistent with the hypothesis that it is transported to the dendrites.

Discussion

Most studies of *Bdnf* mRNA localization have relied on transient transfection or viral expression of exogenous constructs in which a *Bdnf* 3'UTR is placed downstream from a reporter molecule. Although these techniques can highlight different localization patterns for different mRNAs, they can also result in extremely high transcript abundance, distorting native patterns of expression. Using next-generation sequencing and the latest high-resolution mRNA counting and in situ hybridization techniques, we reexamined this issue to determine both the abundance and pattern of expression of endogenous *Bdnf* transcripts within the somata and neuropil layer of the mature rat hippocampus. Our 3' end sequencing data indicate that *Bdnf* has markedly low expression in the rat hippocampus. These data were validated with three independent methods: qRT-PCR, direct mRNA detection using nCounter NanoString, and in situ hybridization. The results obtained with these techniques are consistent with one another and represent independent observations because the two hybridization methods (NanoString and in situ hybridization) used probes that hybridize to different regions of the *Bdnf* mRNA. Our data show that endogenous expression of *Bdnf* is quite low and primarily localized to the somatic compartment. The extremely low or absent signal in the dendrites implies that in basal (unstimulated) conditions, there is limited

potential for local translation of *Bdnf* mRNA. We observed that enhanced neural activity (with bicuculline treatment) or stimulation with PACAP increased the amount of *Bdnf* transcript, primarily in the vicinity of neuronal cell bodies. Others have observed activity-dependent increases in *Bdnf* transcripts in response to plasticity induced by high-frequency stimulation, potassium-induced depolarization, or epileptogenesis; these transcripts were also observed primarily in or near cell bodies and proximal dendrites (14–16, 20).

As observed in previous studies (17–21, 23), both a long and a short 3'UTR isoform of the *Bdnf* mRNA are present in the rat hippocampus, but we found that the short 3'UTR isoform was the predominant *Bdnf* transcript—its abundance was about three to four times greater than that of the long isoform. Other studies have suggested that the long 3'UTR isoform harbors signals for dendritic localization (21). Our data, which focused exclusively on the localization of endogenous transcripts rather than exogenously expressed reporter constructs, do not support this claim. Indeed, in unstimulated neurons, the *Bdnf* transcript (both short and long isoforms) is rare or absent from distal dendrites, and both transcript isoforms are very sparsely represented in proximal, but not distal, dendrites. These data contrast with a previous study that reported a 0.8 ratio of long/short isoforms in the hippocampus; in that report, the long 3'UTR was reported to be expressed at amounts sixfold higher in synaptoneurosome (21). Here, the synaptoneurosome were isolated biochemically, and semiquantitative PCR was used to calculate the abundance of the *Bdnf*-CDS transcript and the long 3'UTR; the fraction of total RNA used for the synaptoneurosome reaction was 20 times lower than that used for the cell body reaction, potentially leading to artifacts associated with nonlinear amplification. Here, we used multiple quantitative techniques, all of which indicated that the long 3'UTR is not preferentially localized to or concentrated in the synaptic neuropil. We also determined the half-lives of the 3'UTR long isoform and the *Bdnf*-CDS by inhibiting transcription in cultured hippocampal neurons. We infer from these data that the short 3'UTR isoform was twice as stable as the long isoform. This difference in half-life is not consistent with the hypothesis that the long 3'UTR isoform is targeted to dendrites.

Here, we show that high-resolution techniques used side by side can be used to address the localization of any endogenous transcript with high sensitivity. Our results are consistent with a recently published study showing that the BDNF protein is located in presynaptic dense core vesicles and is not detected at appreciable amounts in postsynaptic compartments (7). In contrast, Orefice *et al.* recently reported that viral overexpression of a BDNF construct can increase the amount of BDNF detected in the dendrites (40). In unstimulated cells, we find little evidence for *Bdnf* mRNA localization outside of neuronal somata. These data suggest that under basal conditions, BDNF protein translation is most likely to take place in the somatic compartment where the mRNA is located. When BDNF is overexpressed, it is possible to detect it in other compartments (40), and it is also possible that some forms of physiological stimulation result in high transcript abundance and, consequently, the presence of the mRNA and/or protein in dendrites.

Materials and Methods

High-resolution in situ hybridization and immunostaining

Dissociated rat hippocampal neurons were prepared and maintained as previously described (41). We performed in situ hybridization with the QuantiGene ViewRNA kit from Panomics as previously described (22, 42). In brief, cultured neurons (DIV 21) were fixed for 30 min at room temperature with a 4% paraformaldehyde solution (4% paraformaldehyde, 5.4% glucose, 0.01 M sodium metaperiodate in lysine-phosphate buffer). After completion of the hybridization protocol, neurons were incubated in blocking buffer (4% goat serum in 1× phosphate-buffered saline) for 1 hour. Thereafter, neurons were immunostained with standard methods (41). Dendrites were stained with an antibody against MAP2 (Millipore, 1:1000), and nuclei were stained for 1 min with DAPI. Subsequently, z-stack images were acquired with a Zeiss LSM 780 confocal microscope with 1024 × 1024 pixel resolution. Images were processed with ImageJ. For in situ hybridization in sections, 500-μm hippocampal slices were processed as previously described (22). Slices were cryosectioned at 7-μm thickness, and in situ hybridization was performed as described above for hippocampal neurons with an additional washing step after adding the probes. Slices were incubated with a primary antibody against MAP2 (Millipore, 1:1000) for 3 hours at room temperature. For imaging of the CA1 region, a z-stack that spanned the entire thickness of the slice was obtained. For visualization purposes, in all presented images, the channels representing mRNA signals were converted to binary images, and puncta were dilated once. The same threshold was used for all mRNA channels. A mask was created using the MAP2 and DAPI signals, and in situ signals were inserted with a different transparency and color range. The probe Bdnf-CDS (NM_012513.3) is located at nucleotides 637 to 1410, and for Bdnf-UTR_{long} (NM_012513.3) at nucleotides 2589 to 3732. The probe Bdnf sense is the sense version of Bdnf-CDS.

Automated signal detection with MATLAB

Random field stack images were taken with a 40× objective. Only neurons in which processes could be well identified were used in analyses. Data were collected with a MATLAB script to automatically identify cells and measure the fluorescence intensity. In the maximum-intensity projection, neurons were detected using the DAPI channel. To create a mask, a threshold was applied to the DAPI signal, then dilated with factor 1.6 (arbitrary), and the mean intensity of the in situ puncta was measured.

Tissue microdissection and RNA isolation

Hippocampal slices (500 μm) from 4-week-old adult male rats were prepared as previously described (41). CA1 cell bodies and the neuropils were microdissected manually from each slice as described previously (22). Total RNA and protein were extracted using TRIzol (Invitrogen) according to the manufacturer's instructions. From 25 slices, we obtained about 5 μg of total RNA from each of the somata and neuropil compartments.

Quantitative reverse transcription polymerase chain reaction

RNA was treated with deoxyribonuclease (DNase) I and cleaned with RNeasy MinElute Clean-up Kit (Qiagen). RNA (500 ng) was reverse-transcribed with the QuantiTect Reverse Transcription Kit (Qiagen). qRT-PCR was performed with SYBR Green (Applied Biosystems), and reaction setup and cycling parameters were recommended by the QuantiTect primer assays (Qiagen): *Bdnf* (QT00375995), *Camk2a* (QT02479988), *Rnr1* (QT00199374), and *Vgf* (QT00493556). Custom-made primers were used for *Bdnf* 3'UTR_{long} (forward, 5'-GCTCCATGTCGGTGGTTTAT-3'; reverse, 5'-AACAGGACGGAAACAGAACG-3'). qRT-PCR was run on a StepOnePlus Real-Time PCR System (Applied Biosystems).

Treatment of cells with bicuculline or PACAP

Hippocampal neurons were plated at a density of 400,000 cells in a 60-mm dish and treated with 10 nM PACAP for 2 hours or with 40 μ M bicuculline for 12 hours. For controls (vehicle), cells were treated with water. Cells were harvested and RNA was isolated using TRIzol according to the manufacturer's instructions.

Treatment of cells with transcription inhibitors

Hippocampal neurons were plated at a density of 400,000 cells in a 60-mm dish and treated with a cocktail of actinomycin D (8 μ M), 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB; 100 μ M), and triptolide (1 μ M) in dimethyl sulfoxide (DMSO) for 0, 2, 4, 6, 8, and 10 hours. The cocktail of inhibitors was used to maximize the inhibition of transcription. Cells were harvested at each time point, and RNA was isolated using TRIzol (Invitrogen) according to the manufacturer's instructions. To determine the mRNA stability, qRT-PCR was performed for every time point obtained. mRNA half-lives were calculated from an exponential decay curve that was fitted to the time points obtained. The half-lives were determined in three independent experiments, and an average half-life was calculated.

Digital analysis of gene expression using nCounter NanoString

Each mRNA was detected by two probes each of 50-nt length: a target-specific capture probe and a reporter probe linked to a fluorescent barcode (26). All probes were designed against the coding region. Total RNA (100 ng) (DNase I-treated and cleaned using RNeasy MinElute Kit, Qiagen) was used for probe hybridization. Data were processed by the nCounter Digital Analyzer as described in (22).

3' End sequencing

A 3' fragment complementary DNA library was prepared by GATC Biotech AG, and deep RNA sequencing was performed with an Illumina HiSeq 2000. Total RNA was fragmented by sonication. Poly(dT) primers were used to extract poly(A)-containing fragments. After gel separation, fragments with the size of 300 to 500 nt were used for ligation of sequencing adapters. Single reads had a length of 101 nt and were aligned to the rat genome (assembly March 2012/rn5) using Bowtie (43). Consecutive A or T mismatches on the genome during alignment were interpreted as a sign for poly(A)-containing reads and further used for identification of cleavage sites. Identification of poly(A) signal hexamers and prediction of

3'UTR isoforms were done with custom-written Perl scripts. For visualization, the University of California, Santa Cruz, Genome Browser was used (44). Data were normalized with the DESeq R package (45). Raw 3' end sequencing data are provided in the supplementary materials online (data file S1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Endogenous *Bdnf* mRNA is weakly expressed in the hippocampus.

(A) Schematic gene structure showing the long (top) and short (bottom) 3'UTR isoforms of *Bdnf*. (B) Genome browser view showing the 3' end sequencing reads mapping to *Bdnf*. The reads and the resulting isoforms are highlighted in gray. Peaks of the 3' end base represent the position and expression of 3' ends in UTR isoforms and determine the prediction of isoforms. For comparison, RefSeq annotation is displayed. The data are representative of eight hippocampi from four rats. (C) Analysis of 3' end sequencing (3' end-Seq) showing the expression of the two *BDNF* isoforms (*Bdnf*-CDS denotes the

short isoform) relative to *Vgf* and *Camk2a* in rat hippocampal total RNA. **(D)** Relative expression values for *Bdnf* isoforms compared to *Vgf* and *Camk2a* determined by qRT-PCR. Data are means $\pm$ SEM from three independent experiments. **(E)** Ratio of the long UTR isoform to the *BDNF-CDS* in the hippocampus determined by 3' end sequencing and qRT-PCR. Data are means $\pm$ SEM from three independent experiments. **(F)** NanoString counts for *Bdnf-CDS* or *Camk2a* in 100 ng of total RNA extracted from hippocampal tissue. Data are means $\pm$ SEM from three independent experiments.

Fig. 2. Endogenous *BDNF* mRNA is predominantly expressed in neuronal somata.

(A) Bar graphs showing the expression of *Bdnf* in the somata and neuropil layer of the rat hippocampus determined by 3' end sequencing, qRT-PCR, and NanoString. The 3' end sequencing data are from a representative of eight hippocampi. Data for qRT-PCR and NanoString are means $\pm$ SEM from three independent experiments. (B) High-resolution in situ hybridization to detect *Bdnf* or *Camk2a* transcripts (magenta) in dissociated hippocampal neurons after 21 days in vitro (DIV 21). Dendrites were immunostained with an antibody for MAP2 (red in the first panel, and gray in the others); nuclei were stained

with DAPI (4',6-diamidino-2-phenylindole) (blue in the first panel, and green in the others). Scale bar, 20 μ m. First panel (black background), an original image is shown; the other images were processed for better visualization of the hybridization signal for *Bdnf-CDS*, *Bdnf-UTR_{long}*, or *Camk2a*. **(C)** High-resolution in situ hybridization in 7- μ m-thick hippocampal slices taken from 4-week-old rats. Images were processed and presented as in **(B)**, except that dendrite staining was removed from the processed images. **(D)** Genome browser view showing the distribution of 3' end sequencing reads between the soma and neuropil, representative of data obtained from the hippocampi from four rats.

Fig. 3. The expression of *Bdnf* changes with activity.

(A and B) High-resolution in situ hybridization for *Bdnf-CDS* mRNA (green) in dissociated hippocampal neurons at DIV 21. MAP2 (gray) marks dendrites; DAPI (blue) marks nuclei. Scale bar, 20 μ m. Neurons were treated with either vehicle (A) or 40 μ M bicuculline (Bic) for 12 hours (B). (C) Scatter plot of the mean fluorescence intensity from in situ hybridization in control cells (A) or cells treated with bicuculline (B). au, arbitrary units; ns., not significant. Data are from 50 cells over three independent experiments. $**P < 0.0088$, Mann-Whitney test. (D and E) Hybridization for *Bdnf-CDS* as in (A) and (B); neurons were

treated either with vehicle (D) or with 10 nM PACAP for 2 hours (E). Scale bar, 20 μ m. (F) Scatter plot of the mean fluorescence intensity from (D) and (E). Data are from 100 cells over three independent experiments. $**P < 0.0063$, Mann-Whitney test. (G and H) Bar graph of the expression of *Bdnf*-CDS or *Bdnf*-UTR_{long} after treatment with bicuculline (G) or PACAP (H), determined by qRT-PCR. Data are means $\pm$ SEM from three independent experiments. $***P < 0.0001$, independent *t* tests. (I) Scatter plot of the stability of the *Bdnf* isoforms (short, CDS; long, UTR) determined by qRT-PCR. Data show one representative experiment out of three independent experiments; data from each independent experiment are shown in fig. S4C.