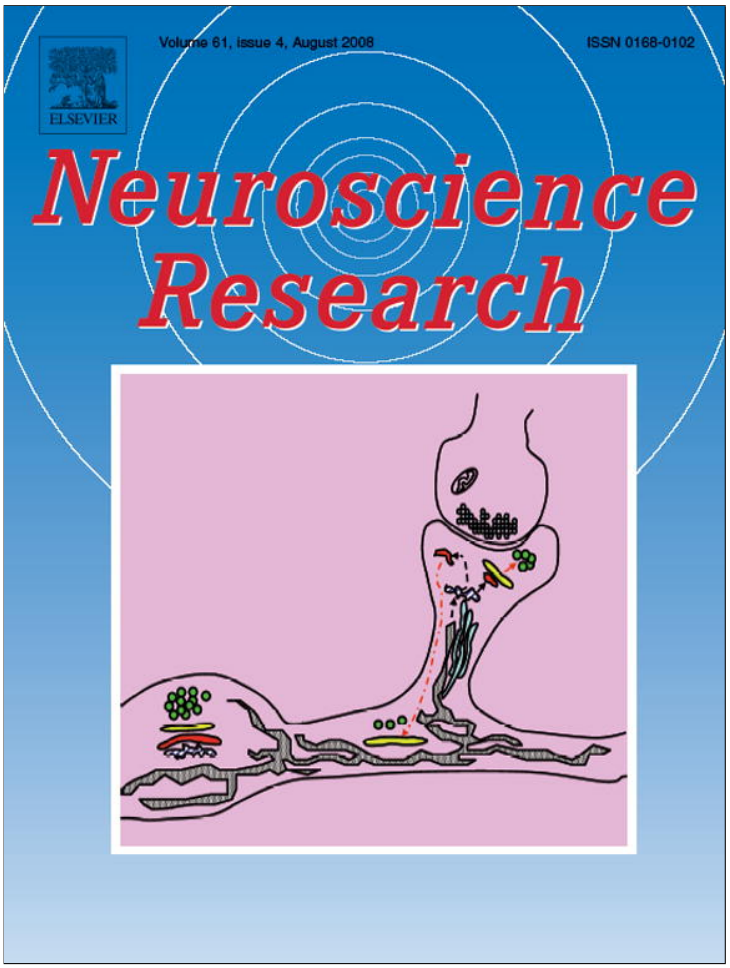

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Review article

Activity-dependent expression of brain-derived neurotrophic factor in dendrites: Facts and open questions

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Abstract

Long-lasting synaptic changes in transmission and morphology at the basis of memory storage, require delivery of newly synthesized proteins to affected synapses. Although many of these proteins are generated in the cell body, several key molecules for plasticity can be delivered in the form of silent mRNAs at synapses in extra somatic compartments where they are locally translated. One of such mRNAs encodes brain-derived neurotrophic factor (BDNF), a key molecule in neuronal development, learning and memory. A single BDNF protein is produced from several splice variants having a different 5′ untranslated region. These mRNA variants have a different subcellular localization (soma, proximal or distal dendritic compartment) and may represent a spatial code for a local control of BDNF availability. This review will highlight current knowledge on the mechanisms of spatial and temporal regulation of activity-dependent BDNF mRNA localization in dendrites in relation with synaptic plasticity.

Keywords: Neurotrophic factors; Dendritic mRNA trafficking; Local protein translation; Dendritic spines; Dendritic arborization; Long-term potentiation

Contents

1. Introduction

Brain-derived neurotrophic factor (BDNF), is the most abundant and widely expressed neurotrophin in the mammalian nervous system. In analogy to the other members of the neurotrophin family, including nerve growth factor, neurotrophin-3, and neurotrophin-4, BDNF has been studied extensively in the context of neuronal development and synaptic plasticity. From these studies, it emerged that neuronal activity-dependent expression of BDNF is a key mechanism that governs long-lasting changes in synaptic efficacy and morphology (reviewed in McAllister et al., 1999; Thoenen, 2000; Poo, 2001; Bramham and Messaoudi, 2005; Lu et al., 2008; Bramham, 2007). Evidence of the role of BDNF in synaptic plasticity is related to the demonstration of reciprocal regulation of BDNF expression and synaptic activity. Accordingly, early *in vitro* studies showed that BDNF transcription is regulated by glutamatergic, GABAergic, and cholinergic

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neurotransmitter systems (Zafra et al., 1990, 1991, 1992). On the other hand, BDNF enhances activity mediated neurotransmitter release from presynaptic terminals (Lohof et al., 1993; Gottschalk et al., 1998), and post-synaptically, enhances transmission via *N*-methyl-D-aspartate (NMDA) receptors (Levine et al., 1995; Suen et al., 1997). *In vivo* studies showed that upregulation of BDNF mRNA levels can be elicited by physiological visual stimuli (Castren et al., 1992) as well as by stimuli leading to hippocampal long-term potentiation (LTP), a widely studied cellular model of synaptic plasticity (Patterson et al., 1992; Castren et al., 1993; Dragunow et al., 1993). Using transgenic BDNF knock out mice, it was demonstrated that appropriate levels of BDNF are critical for the establishment of LTP (Korte et al., 1995, 1996; Pozzo-Miller et al., 1999), spatial learning, and memory (Mu et al., 1999). BDNF was also shown to be able to induce a glutamate receptors-independent form of LTP (BDNF–LTP) in acute hippocampal slices and living animals (Kang and Schuman, 1995; Messaoudi et al., 1998). In addition, several studies demonstrated the capability of BDNF to shape axonal and dendritic arborization and to determine the number and possibly, size of dendritic spines (McAllister et al., 1995; Horch, 2004; Danzer et al., 2002; Matsutani and Yamamoto, 2004; Tyler and Pozzo-Miller, 2003). More recent studies have established a critical role for BDNF in cap-dependent translation initiation in LTP (reviewed in Sutton and Schuman, 2005; Bramham, 2007).

The paradigm establishing a central role of BDNF in activity-dependent synaptic plasticity bears several important consequences, the first of which is that its expression and release, as well as expression or responsiveness of its tropomyosin-related kinase receptor B (TrkB), should be specifically localized to the most active synapses. The second consequence is that availability of these molecules at activated synapses should occur within a time scale consistent with the variation in synaptic plasticity observed. Consistently with these predictions, it was shown that BDNF (protein) is stored in the somatodendritic compartment as well as in axons and that electrical activity, elicited through proepileptic drugs, increases BDNF immunoreactivity in the most active dendrites and axons (Wetmore et al., 1994; Dugich-Djordjevic et al., 1995; Yan et al., 1997; Conner et al., 1997; Fawcett et al., 1998; Tongiorgi et al., 2004). In further agreement with the above predictions, secretion of BDNF from both dendrites and axons occurs mainly through an activity-dependent, regulated pathway mediated by the excitatory neurotransmitters glutamate and acetylcholine or the neurotrophins BDNF, NT3, and NT4/5 (Goodman et al., 1996; Canossa et al., 1997, 2001; Altar and DiStefano, 1998; Griesbeck et al., 1999). In addition, classical theta-burst stimulation (TBS) patterns evoking LTP (four pulses at 100 Hz, interburst interval of 200 ms and an intertrain interval of 20 s), which mimics the typical firing pattern of hippocampal neurons during learning (O'Keefe and Recce, 1993), can instantaneously elicit a large increase in BDNF secretion persisting 5–12 min beyond the stimulation period, in agreement with the expected time course for a role of BDNF already in the early phase of LTP (Balkowiec and Katz, 2002; Aicardi et al., 2004). In contrast, stimulation procedures that induce only the initial phase of LTP lead to a very short-lasting (1 min) increase in BDNF secretion of much smaller magnitude (Balkowiec and Katz, 2002; Aicardi et al., 2004). Taken together, these observations suggest that BDNF is required for LTP occurrence, and that prolonged duration of BDNF secretion at high levels is needed for LTP maintenance.

Two recent studies showed that hippocampal LTP (in CA1) that is blocked by a protein synthesis inhibitor can be fully rescued by adding exogenous mature BDNF (100 ng/ml) from 5 min before to 15 min after TBS. The same BDNF treatment was ineffective in the absence of TBS and results were comparable in both juvenile (Pang et al., 2004) and adult rats (Santi et al., 2006). These studies suggest that during prolonged periods of secretion observed in long-lasting forms of LTP, the endogenous activity-dependent releasable pool of BDNF is rapidly depleted. Under these conditions, new synthesis from pre-existing BDNF mRNA and recycling of secreted BDNF seems to be crucial for providing threshold levels of BDNF to maintain long-lasting synaptic changes underlying memory (Pang et al., 2004; Santi et al., 2006). The lines of evidence summarized above underscore the importance of activity-dependent translation of BDNF as a fundamental mechanism governing long-lasting synaptic changes. Within this paradigmatic view, the finding about 10 years ago, that BDNF mRNA can be transported into neuronal dendrites in an activity-dependent manner assumes a particular relevance (Tongiorgi et al., 1997). In this review article, I describe the present status of knowledge related to BDNF mRNA dendritic targeting and translation and attempt to discuss it within the framework of synaptic plasticity.

2. Discovery of activity-dependent BDNF mRNA trafficking in dendrites

That proteins synthesis may occur outside of the cell body was first suggested when Bodian et al. discovered, by electron microscopy, the presence of ribosomes in dendrites (Bodian, 1965). Subsequently, incorporation of radiolabeled aminoacids in distal dendrites was demonstrated (Kiss, 1977). In the early 1980s Steward and Levy demonstrated the presence of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. These ribosomes were collected in characteristic rosettes, which is the distinctive ultrastructural evidence that they are bound to a messenger RNA and actively engaged in protein synthesis (Steward and Levy, 1982). A number of later studies demonstrated that the components necessary for translation, such as endoplasmic reticulum, tRNA, elongation (eEFs), initiation factors (eIFs), and Golgi apparatus, are constitutively present in dendrites (Spacek, 1985; Tiedge and Brosius, 1996; Gardiol et al., 1999; Spacek and Harris, 1997; Pierce et al., 2000, 2001; Tang et al., 2002; Horton and Ehlers, 2003). Importantly, it was recently shown that, following LTP, the percentage of dendritic spines in the rat hippocampus that contain polyribosomes raises from 12 to 39% and, morphological changes leading to synapse enlargement and increased connectivity occur only in spines bearing polyribosomes formations (Ostroff et al., 2002; Harris et al.,

2003). The dendritic localization of the translation machinery therefore suggests the capability of an autonomous translational control in dendrites, possibly at individual synapses with important consequences for synaptic plasticity (Sutton and Schuman, 2005; Schuman et al., 2006; Bramham and Wells, 2007).

In situ hybridization, PCR and gene-array studies led to the identification of 150–400 different mRNAs in dendrites of hippocampal and cortical neurons (Steward and Schuman, 2001; Eberwine et al., 2002; Zhong et al., 2006; Poon et al., 2006). Considering that mammalian neurons are estimated to express as much as 10–30.000 different transcripts, the fraction of dendritic mRNAs appears to account for only about 1–4% of the entire neuronal transcriptome. Besides the low percentage of transcripts targeted to dendrites, it is now clear that they code for most of the components of postsynaptic density and, more in general, for proteins involved in synaptic plasticity although there is as yet, no final consensus on the number and identity of dendritic mRNAs with the exception of a restricted number of them (Steward and Schuman, 2001; Eberwine et al., 2002; Zhong et al., 2006; Poon et al., 2006). Among such dendritic transcripts, one encodes BDNF.

Although an initial study on animal models of epilepsy showed radioactive *in situ* hybridization data suggesting a proximal dendritic localization of BDNF mRNA in hippocampal neurons (Dugich-Djordjevic et al., 1992), in a number of similar studies BDNF mRNA localization in dendrites was either not detected or not specifically investigated (Wetmore et al., 1990; Ernfors et al., 1990, 1991; Isackson et al., 1991; Kokaia et al., 1993; Miranda et al., 1993; Ringstedt et al., 1993; Timmusk et al., 1993; Castren et al., 1995; Lauterborn et al., 1996; Conner et al., 1997). Notably, all the above mentioned studies showed only low magnification pictures of *in situ* hybridization for BDNF mRNA on hippocampal sections from which it is not possible to determine if dendrites are labelled or not. Another study showed high magnification pictures of radioactive *in situ* hybridization suggesting, but not conclusively demonstrating, localization of BDNF mRNA in the proximal dendritic domain of hippocampal neurons from adult rats treated with pilocarpine (Schmidt-Kastner et al., 1996). This issue was reconsidered, when BDNF mRNA was amplified by PCR from growth cones of immature dendrites (Crino and Eberwine, 1996; Steward, 2002) However, the localization of BDNF mRNA in the dendrites of fully differentiated neurons remained highly controversial, also because it was reported that several mRNAs are temporarily localized in axonal and dendritic growth cones during the early growth phase but eventually disappear from mature dendrites and axons (Steward and Worley, 2001). It was therefore, only in 1997, owing to a nonradioactive *in situ* hybridization study on cultured rat hippocampal neurons, that the first unequivocal demonstration of an activity-dependent dendritic targeting of BDNF mRNA was provided (Tongiorgi et al., 1997). In that study, BDNF mRNA was detected in the initial segment of the dendrites under basal conditions. By increasing KCl level from the physiological concentration of 3.5–10 mM, a tetanic firing activity was induced for 3 h leading to accumulation of BDNF mRNA in distal dendrites. Importantly, this phenomenon does not appear to be an *in vitro* artefact as it can be reproduced *in vivo* with both physiological as well as pathological stimuli. Accordingly, newborn or adult rats were reared in complete darkness for 3 weeks, resulting in a decrease in BDNF expression levels in the visual cortex and disappearance of BDNF mRNA localization in dendrites. However, upon exposure to normal light conditions for as short as 2 h (a physiological stimulus), the mRNA for BDNF reappeared into the apical dendrites of cortical neurons (Capsoni et al., 1999a,b). In a second set of experiments, pharmacologically induced status epilepticus prompted a dramatic accumulation of BDNF mRNA in the hippocampal laminae containing the apical dendrites of pyramidal neurons and granule cells (Tongiorgi et al., 2004) with a time course that perfectly matched that observed in *in vitro* studies. Another remarkable finding is that even the average distance from the cell soma reached by BDNF transcripts along the dendrites is comparable between *in vivo* and *in vitro* studies (Tongiorgi et al., 1997, 2004). The time course and final destination of dendritic BDNF mRNA transport will be further discussed in the next two sections.

3. What is the time course of BDNF mRNA dendritic targeting?

Although BDNF mRNA is detectable in the distal dendritic district only several hours after the stimulation, activity-dependent dendritic targeting of BDNF mRNA is rapidly induced. Fig. 1 shows a collection of *in situ* hybridization results from different studies demonstrating that already after 1 min of stimulation, the mRNA for BDNF translocates into the dendrites and that this accumulation progresses at least, for 3 h (Fig. 1A and B). Calculation of the average distance reached in the apical dendrites of pyramidal neurons within 3 h indicates a global transport velocity of about 8 μm/h for BDNF mRNA targeting induced by KCl (Tongiorgi et al., 1997), in agreement with previous calculations (the global transport speed of recently synthesized total RNA in neuronal dendrites is on average 11 μm/h; Davis et al., 1990; however, single RNA granules may move with instantaneous velocities even greater than 600 μm/h; Dynes and Steward, 2007). In contrast, incubating neurons with 50 ng/ml BDNF appears to induce BDNF mRNA targeting in dendrites with a net translocation speed of only about 2.5 μm/h (Righi et al., 2000). Although these differences in trafficking of BDNF mRNA might also be due, at least in part, to differential effects of BDNF and KCl on *bdnf* gene transcription, these results suggest that dendritic targeting of BDNF mRNA may be regulated by several inducible signals and that signalling through the BDNF receptor TrkB might lead to the recruitment of only a subset of these signals. Recent studies in our laboratory demonstrated that this indeed is the case (Vicario, Baj, Ratti and Tongiorgi, personal communication). Notably, by 3 h KCl stimulation, most apical dendrites of cultured hippocampal neurons were filled with BDNF mRNA for about 2/3 of their length (see Fig. 1C–F). This finding leads to the next question.

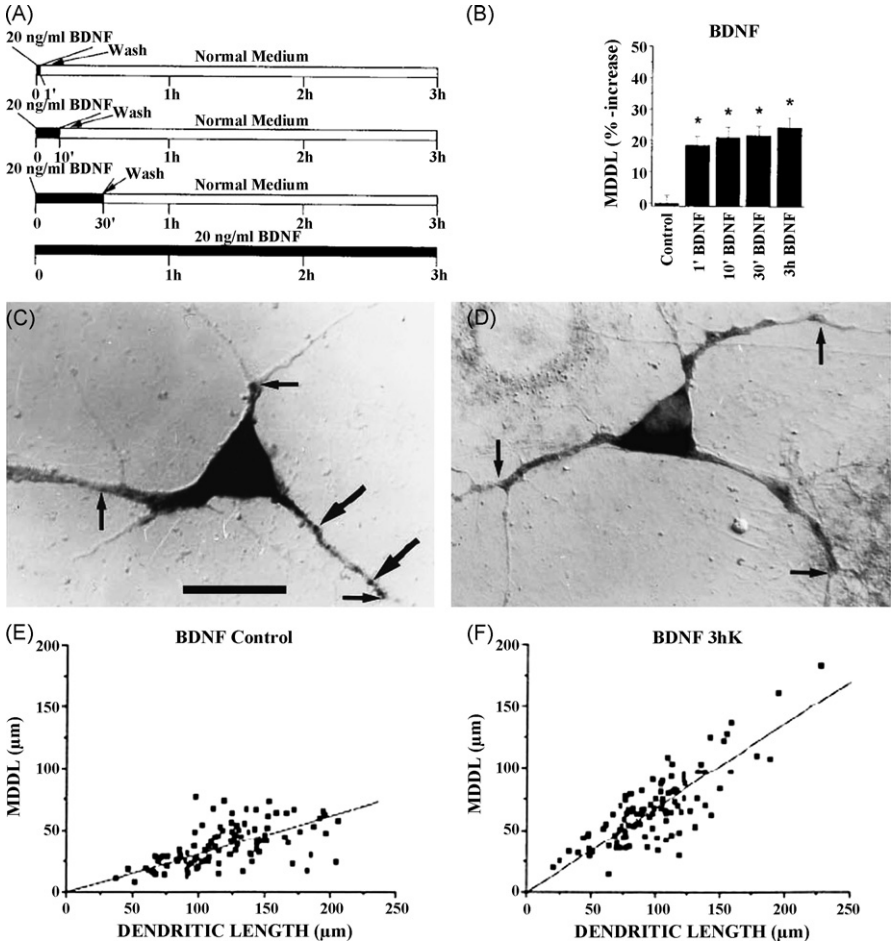

Fig. 1. Quantification of mean distance reached by BDNF mRNA in dendrites of 8-day-old cultured hippocampal neurons. (A) Experimental protocol used to stimulate hippocampal neurons with 20 mg/ml BDNF for different times, (B) effects of incubation with BDNF showing a progressive increase in the mean distance of dendritic labelling (MDDL), from 1 min to 3 h stimulation. Bars represent the average of at least 150 dendrites ± S.E. (*$p < 0.01$ ANOVA with respect to controls). (C) *In situ* hybridization for BDNF mRNA showing proximal localization in unstimulated cultures, (D) depolarization with 10 mM KCl for 3 h increases the distance reached by BDNF mRNA in dendrites. Small arrows indicate the maximal distance of dendritic labelling used to measure MDDL; large arrows indicate spots of intense, discontinuous labelling. Calibration bar in $C = 30$ μm. (E) and (F) Correlation plots of the MDDL vs. dendritic length (μm). Each point represents one MDDL determination, together with the length of the corresponding dendrite, under the experimental conditions indicated. The scatter plots were fitted by linear regression lines through the origin. (E) Slope = 0.30, correlation coefficient $r = 0.57$; *F*, slope = 0.68; $r = 0.80$. (A) and (B) reprinted from Righi et al., 2000, courtesy of Journal of Neuroscience. (C)–(F) reprinted from Tongiorgi et al., 1997, courtesy of Journal of Neuroscience.

4. Where are BDNF transcripts targeted to?

Under basal conditions, BDNF mRNA is constitutively localized to the proximal dendritic compartment (on average about 30 μm from the cell soma with maximal distances of about 80 μm for CA1, 160 μm for CA3 and 40 μm for DG neurons). However, it extends into distal dendrites (farther than 40 μm, with maximal distances of about 280 μm for CA1, 400 μm for CA3, and 100 μm for DG neurons) in response to status epilepticus *in vivo*. Similar results were obtained in response to tetanic electrical activity *in vitro* (compare Fig. 1E and F with Fig. 2C and D and indicated references).

The analysis of three animal models of epilepsy provided evidence that epileptogenic seizures induces BDNF mRNA to localize selectively to the most activated sets of synapses (Tongiorgi et al., 2004). For example, BDNF mRNA localizes to laminae that correspond to the terminal fields of the mossy fibers in CA3 or of the associational-commissural projection system in DG after pilocarpine-induced seizures. The neurons that give rise to the mossy fibers (granule cells) and associational-commissural projections (neurons in the hilus of the dentate gyrus) are highly activated during epileptogenic seizures; therefore, the selective targeting of BDNF mRNA to the sites of their terminations coincides with the set of synapses that are most active during epilepsy. In agreement with this conclusion, dendritic accumulation of BDNF mRNA at specific synaptic contacts also occurs after kainate-induced seizures in the CA1 stratum radiatum and at the early stages of kindling development in the CA3 stratum lucidum and stratum radiatum (Tongiorgi et al., 2004; Chiaruttini et al., 2008).

After having established that BDNF mRNA is targeted to hippocampal laminae containing the most active synapses, a further fundamental question is whether the final destination of BDNF mRNA coincides with the sites where the translational machinery is located or whether BDNF transcripts are temporarily stored in specific structures awaiting further

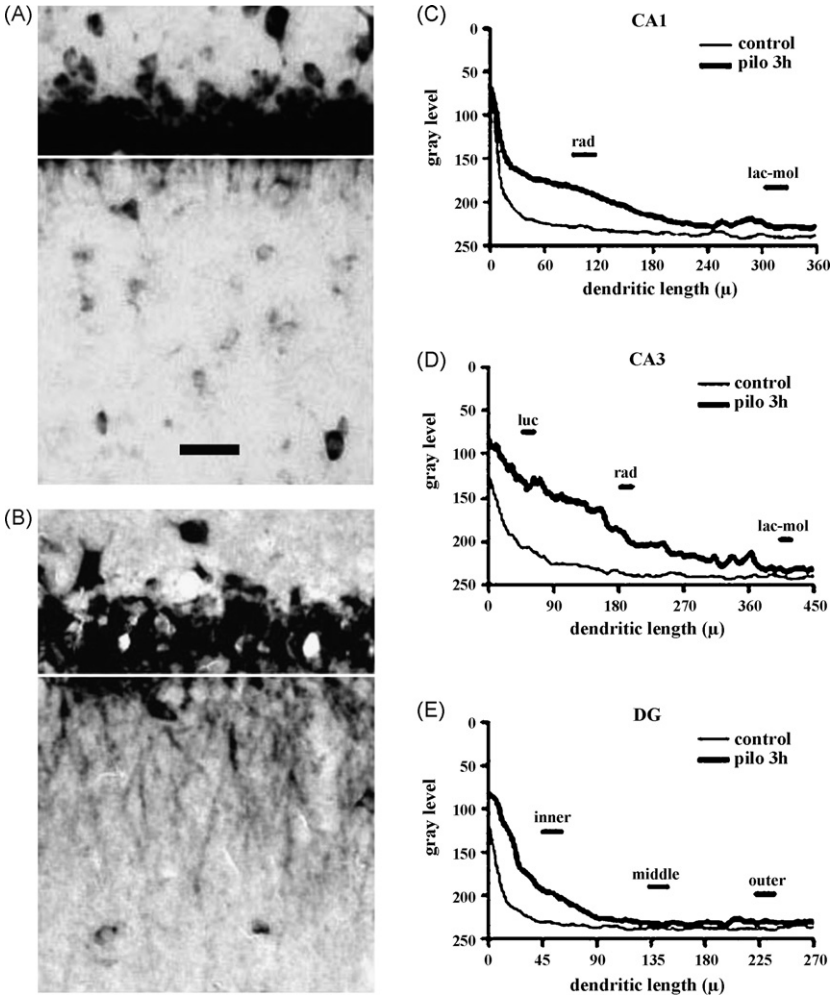

Fig. 2. Densitometric analysis of effects of pilocarpine seizures in rat hippocampus *in vivo*. (A) High magnification of the CA1 region of a control animal showing proximal dendritic *in situ* staining for BDNF mRNA in pyramidal neurons and in interneurons within the stratum radiatum, (B) CA1 region of a pilocarpine-treated adult rat (3 h) showing dramatic enhancement of BDNF mRNA *in situ* staining. Several strongly labelled dendrites are visible in the stratum radiatum but not in the stratum oriens. The white lines in (A) and (B) indicate the distance 0 from the cell soma used for densitometric analysis. The left panels in (C)–(E) show the densitometric analysis of dendritic staining, expressed as pixel intensity (0–255 gray levels; 255 = white; 0 = black) as a function of the distance from the cell soma (in μm). Data are the means ± S.E. of four to six animals per group: control (thin line) and 3 h pilocarpine treatment (pilo 3 h; thick line). Note that, under control conditions, the average gray level in CA1 and CA3 reaches background levels between 50 and 100 μm from the cell somas, whereas in the dentate gyrus it decreases to a background level at 40 μm from the cell somas. Calibration bar in A = 50 μm. Lac-mol = stratum lacunosum-moleculare; luc = stratum lucidum; ML = molecular layer; rad = stratum radiatum. Reprinted from Tongiorgi et al., 2004, courtesy of Journal of Neuroscience.

signals for the final delivery to synapses. *In situ* hybridization carried out on thousands of cultured hippocampal and cortical neurons showed that the mRNA for BDNF has always a discontinuous distribution in dendrites and is often accumulated at varicosities and branching points (Tongiorgi et al., 1997; Pattabiraman et al., 2005). Importantly, BDNF mRNA is targeted not only to primary but also to secondary and tertiary dendrites (Tongiorgi et al., 1997; Baj, Leone, Chao and Tongiorgi, personal communication). Furthermore, by high-resolution *in situ* hybridization at the electron microscopy level, BDNF mRNA can be detected in association with polyribosomes located both in the dendritic shaft and spines bearing excitatory synapses (Tongiorgi et al., 2004). However, the available data including those on the similar distribution of BDNF mRNA and protein (Fig. 3), still do not allow to discriminate between the two hypothesis that BDNF mRNA is targeted to the sites where it is translated or, in alternative, is targeted in a dormant state to non-synaptic sites of storage, and subsequently mobilized to sites of active translation. Further studies are required to clarify this issue.

5. What are the mechanisms of activity-dependent targeting of BDNF mRNA?

Dendritic targeting of BDNF can be triggered by stimuli as diverse as KCl (Tongiorgi et al., 1997), BDNF (Righi et al., 2000), agonists of muscarinic acetylcholine receptors (pilocarpine), and kainic acid (Tongiorgi et al., 2004), which likely share a common Ca^{2+} signalling pathway. Initial studies showed that BDNF mRNA accumulation in distal dendrites requires external Ca^{2+} flow through voltage-gated L-type calcium channels and activation of glutamate receptors

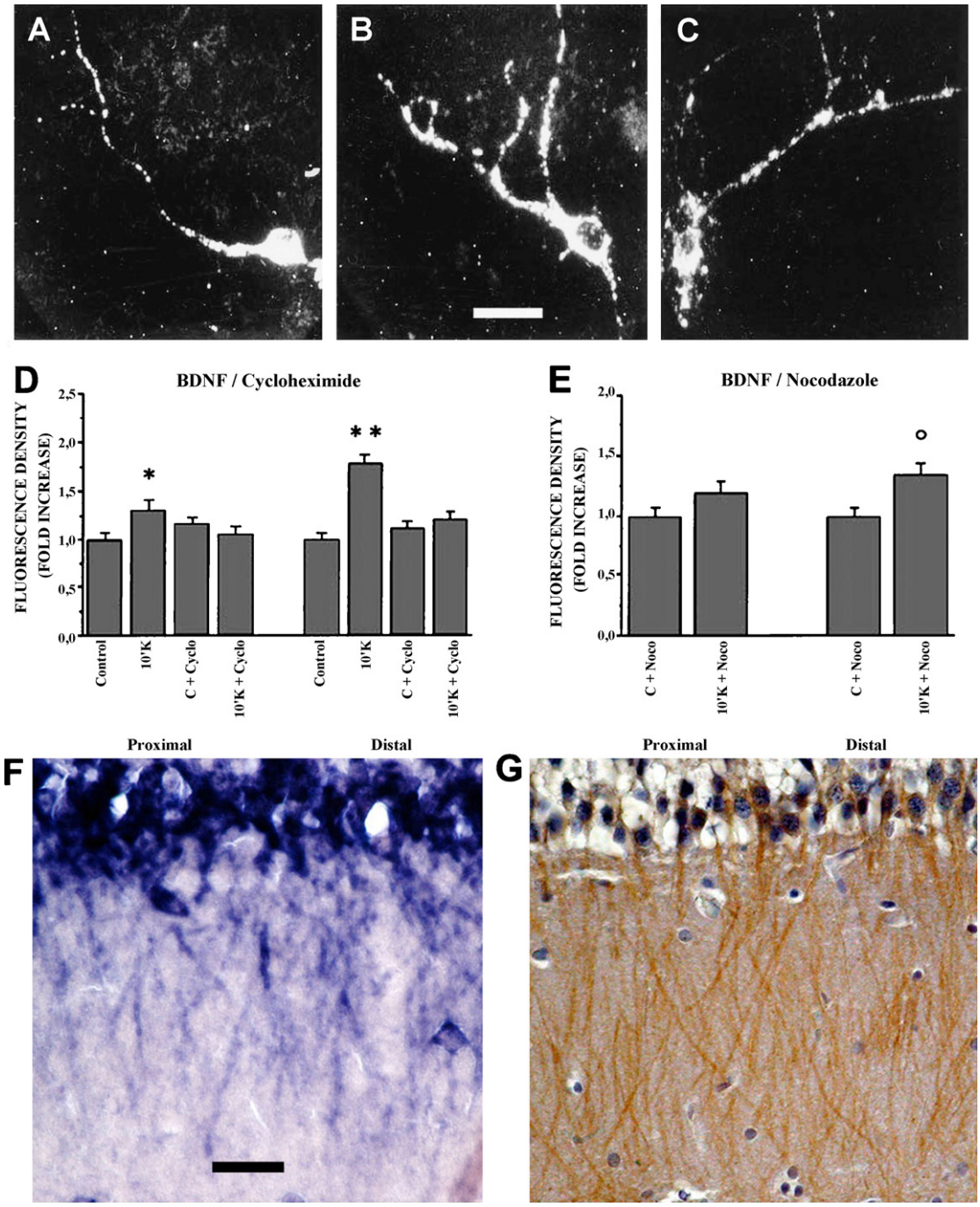

Fig. 3. Immunohistochemistry for BDNF in hippocampal neurons. Immunofluorescence for BDNF (chicken anti-BDNF antibody from Promega; similar images were also obtained using the anti-BDNF antibody from Amgen) was acquired using five confocal sections and integrated in a single projection, under control conditions (A), after 10 min depolarization with 10 mM KCl (B), or in the presence of 10 mM KCl and the microtubule inhibitor nocodazole (C). Immunofluorescence intensity of BDNF (D) and (E) was determined in proximal (30 μm from the base of dendrites) and distal (30–90 μm from the base of dendrites) regions. Bars represent the mean fold increase in the fluorescence intensity of 45 dendrites, with respect to the controls (=1.0). Error bars represent S.E. (A) Incubation of cells in 10 mM KCl for 10 min leads to a significant increase in BDNF immunofluorescence intensity in both proximal and distal regions (10′K). Incubation of control cells with the protein synthesis inhibitor cycloheximide has no effect (C +Cyclo). Cycloheximide completely inhibits the increase in immunofluorescence intensity induced by the 10 mM KCl stimulus (10′K + Cyclo). Blockade of microtubule-dependent transport from the soma with nocodazole does not prevent the increase in BDNF immunofluorescence intensity in distal dendrites, suggesting the presence of local protein synthesis (E). Significance with respect to controls: o = $p < 0.05$; *$p < 0.01$; **$p < 0.001$. Comparison of localization of BDNF mRNA (F) and protein (G) in the CA1 layer of the hippocampus from adult rats treated with pilocarpine for 3 h (*in situ*) or 6 h (immunohistochemistry with the Santa-Cruz sc-546 anti-BDNF antibody). Both mRNA and protein have a discontinuous distribution. Calibration bar in $F = 50$ μm. (A)–(E) reprinted from Tongiorgi et al., 1997, courtesy of Journal of Neuroscience. (F) and (G) Original images.

(Tongiorgi et al., 1997). Subsequent experiments demonstrated that activation of NMDAR but not AMPA glutamate receptors is required for activity-dependent targeting of BDNF mRNA into distal dendrites *in vivo* (Tongiorgi et al., 2004). Taken together, dendritic targeting of BDNF transcripts likely depends on external Ca^{2+} flow through both ionotropic glutamate receptors and L-type calcium channels, and even BDNF release may contribute to an increase in intracellular Ca^{2+} level (Sakai et al., 1997). It is still unclear, however, whether the different Ca^{2+} entry routes may affect BDNF mRNA localization through different molecular mechanisms. Concerning BDNF-induced BDNF mRNA trafficking in dendrites, it was demonstrated that activation of the PI-3 kinase signalling pathway is required (Righi et al., 2000). Although, the ultimate targets of this signalling cascade, are still not identified, the overall mechanisms that control BDNF mRNA transport are beginning to be clarified. In a recent set of experiments, we found that the RNA-binding protein translin mediates a constitutively active dendritic targeting signal (Chiaruttini, Vicario, Baj, Li, Baraban and Tongiorgi, personal communication).

6. Biosynthesis of BDNF in dendrites

According to the current literature, in dendrites, protein biosynthetic compartments including rough endoplasmic reticulum (RER), endoplasmic reticulum-to-Golgi intermediate compartment (ERGIC), Golgi apparatus, and trans-Golgi Network (TGN) are clustered together at dendritic branchings and varicosities (Gardiol et al., 1999; Pierce et al., 2000, 2001; Tang et al., 2002; Horton and Ehlers, 2003). A recent study, showed that BDNF-GFP is targeted to dendritic "Golgi outposts", located in the dendritic shaft. These Golgi structures "were most common in the proximal dendrites, but were also present more distally in secondary and tertiary dendrites" (Horton and Ehlers, 2003). Another study, by electron microscopy, showed that Golgi cisternae that are too small to be easily visible under a light or confocal microscope, are also present within the head of dendritic spines (Pierce et al., 2001). Taken together, these studies indicate that secretory proteins like BDNF, can be translated both within the dendritic shaft as well as within spine heads.

The scheme shown in Fig. 4 summarizes the current model of the distribution of protein biosynthetic compartments based upon the most prominent labeling densities for marker proteins within distal dendritic spines and dendrites and 3D reconstruction of electron microscopy data (Gardiol et al., 1999; Pierce et al., 2000, 2001; Horton and Ehlers, 2003; Aridor et al., 2004; Kolarow et al., 2007; Sytnyk et al., 2004). According to this model, early secretory pathway compartments (RER and ERGIC) are found throughout dendrites and at basis or within spine neck; middle compartments (Golgi outposts and Golgi cisternae) are predominately found in spine heads and dendritic shafts, whereas late compartments (later Golgi and TGN) are predominately found in dendrites, at the basis of spines, and occasionally, in spine heads (Fig. 4). This distribution can however be remodelled by plasticity. Indeed, trans-Golgi

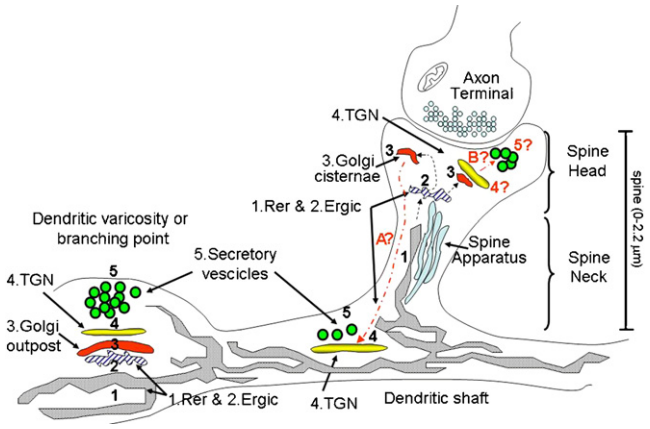

Fig. 4. Current model of distribution of protein biosynthetic compartments in dendrites and spines. The translation apparatus located in the dendritic shaft, at dendritic branchings and varicosities is generally larger than that located in the spines and likely has a larger biosynthetic capacity. In addition, the different biosynthetic compartments are closely located within the dendritic varicosities and branchings, suggesting a "ready to go" translational machinery. In spines, the single components are less clustered but spatial rearrangements of the biosynthetic compartments are known to occur following stimulation suggesting "on demand" assembly of the translational machinery in a subset of activated synapses. Early secretory pathway compartments are found throughout dendrites and within dendritic spines, in particular endoplasmic reticulum (1 = ER) forms a network in the dendrite and occasionally, enters into head and neck of spines mostly, of large mushroom type (Cooney et al., 2002). In analogy to the ER, vescicles of the endoplasmic reticulum-to-Golgi intermediate compartment (2 = ERGIC), characterized by ERGIC53/58 and Rab1b proteins expression, are distributed in spine heads and necks and in association with large diameter membranes in dendritic shafts (Pierce et al., 2001; Aridor et al., 2004) but result highly mobile in both anterograde and retrograde directions, with a bias towards retrograde transport (Horton and Ehlers, 2003). Middle compartments (3 = Golgi cisternae), positive for alpha-Mann II, giantin or GM 130 proteins, are predominately found in spine heads or dendritic shafts (Pierce et al., 2001; Horton and Ehlers, 2003), whereas late compartments (4 = later Golgi and TGN), identified as positive for TGN38 and Rab6, or alpha-adaptin and beta-COP proteins, are predominately found in association with spine heads, large diameter membranes in dendritic shafts (Pierce et al., 2001; Horton and Ehlers, 2003) and synapses (Sytnyk et al., 2004). Secretory vescicles (5) are accumulated at the base of the spine, in the spine heads or in dendritic branchings and varicosities (Kolarow et al., 2007). Dashed arrows indicate the possible synthetic route in spines. At present, BDNF synthetic routes A? and B? are still undemonstrated.

network markers have been shown to redistribute within dendrites during synaptogenesis and become associated with sites of axon-to-dendrites contacts (Sytnyk et al., 2004), and synaptic stimulation induces translocation of ribosomes and the endoplasmic reticulum into the spines (Ostroff et al., 2002; Toresson and Grant, 2005).

Although a detailed description of the actual extrasomatic sites where the BDNF protein is synthesized is still lacking, it is possible to hypothesize that BDNF is initially synthesized in the endoplasmic reticulum (1, Fig. 4) in close proximity with or within activated spines, then BDNF-containing early secretory cargos (2, Fig. 4) undergo a short-range transport to Golgi outposts or Golgi cisternae (3, Fig. 4) located either in the dendritic shaft or within the spine head, respectively; thereafter, BDNF is transferred into TGN (4, Fig. 4) located either in the spine head or in the dendritic shaft and then, secretory vescicles are formed (5, Fig. 4). Thus, the entire biosynthesis of BDNF

and other membrane-bound or secretory proteins might well occur within membranes tightly clustered in dendritic shafts (especially at branching points) or spines, without requiring organelles exchange between two distant clusters (compare Fig. 4, and the model proposed by Lu et al. (2008)).

7. What is the functional significance of BDNF mRNA dendritic targeting?

While the role of BDNF in LTP has been extensively discussed in other reviews (Lu, 2003; Bramham and Messaoudi, 2005; Lu et al., 2008), it is worth discussing here what is the functional significance of BDNF mRNA dendritic targeting with respect to synaptic plasticity. Logically, the main purpose of BDNF mRNA targeting to dendrites is to ensure the local translation of BDNF in the dendritic district, and previous studies on cultured neurons strongly suggest that this is indeed the case (Fig. 3). More recently, analysis of the subcellular localization of the principal BDNF transcripts in hippocampal and cortical neurons has suggested that differential targeting of these transcripts may provide a sophisticated mechanism of regulating BDNF availability at distinct cellular locations (Tongiorgi et al., 2006; Chiaruttini et al., 2008). Transcription of *bdnf* gene produces 11 primary transcripts in rodents (Aid et al., 2007) and 17 in humans (Pruunsild et al., 2007) each containing one of the 5′ untranslated (UTR) exons alternatively spliced to a common 3′ exon coding for the protein and 3′ UTR. Because the 3′UTR contains two termination sites, for each transcript two forms are generated, one with the short and the other the long 3′ UTR, producing 22 (in rodents) or 34 (in humans) possible transcripts. In two different studies, we analysed the subcellular localization of the principal BDNF transcripts in neurons from the rat visual cortex and hippocampus (Pattabiraman et al., 2005; Chiaruttini et al., 2008). We found that, following injection of pro-convulsant agents such as kainic acid or pilocarpine, a subset of transcripts are localized in the cell soma (exons 1 and 3), whereas others have a somatodendritic distribution (exons 2B, 2C, and 6). Since pyramidal neurons of cortical layer 5 in the visual cortex as well as hippocampal neurons, receive segregated synaptic inputs from GABAergic interneurons on the cell soma and glutamatergic inputs on dendrites, these results suggest that different BDNF transcripts may be recruited to modulate specifically different types of synaptic contacts (reviewed in Tongiorgi et al., 2006). In conclusion, the selective local expression of BDNF transcripts appears to be a general mechanism used throughout the nervous system in which BDNF transcripts represent a spatial code for the delivery of BDNF mRNA and protein to specific subsets of synapses (Chiaruttini et al., 2008).

One example of such transcript-specific regulation is provided by studies on LTP in dentate gyrus. Stimulation of the entorhinal cortex perforant path with HFS administered for 2 h of continuous stimulation at 400 Hz to induce LTP, failed to induce translocation of BDNF mRNA in the dendrites of dentate gyrus granule cells (Tongiorgi et al., 2004). Notably, the very same stimulation induces targeting of mRNA encoding activity-regulated cytoskeleton-associated protein (Arc) in dendrites of granule cells at laminae (inner molecular layer, or medial molecular layer) corresponding to the synaptic contacts with the stimulated perforant path (Steward et al., 1998). In another study, LTP was induced in dentate gyrus either by HFS of perforant path (three sessions of eight pulses at 400 Hz, repeated four times, at 10-s intervals given at intervals of 5 min) or with infusion of BDNF (2 μg in 2 μl PBS) for 25 min directly into the dentate gyrus. Both stimulations increase BDNF expression in granule cells but BDNF mRNA does not accumulate in dendrites (Wibrand et al., 2006). This finding can be explained by the fact, also shown in the same study, that the main splice variant upregulated in dentate gyrus during these forms of LTP, is the one containing exon 4 (formerly named exon III in Timmusk et al., 1993) which was recently demonstrated to have a somatic localization even after status epilepticus (Chiaruttini et al., 2008). It is conceivable that in dentate gyrus, transport of newly synthesized BDNF protein from the somatic and proximal dendritic compartments may represent a dominant mechanism in these forms of LTP with respect to local translation.

The possible involvement of the local synthesis of BDNF in long-lasting changes of synaptic activity has already been discussed in the introductory section of this review. It is worth remarking here, that release of BDNF during the first 15–30 min following the initial stimulus is a necessary step to establish a long-lasting LTP at least in hippocampal neurons from the CA1 area. Under these conditions, synapses likely undergo a depletion of their reserves of BDNF secreting vesicles within minutes. It has been shown that recycling of BDNF through endocytosis and resecretion may help maintain the size of the activity-dependent releasable pool of BDNF (Santi et al., 2006). Nevertheless, the current view is that synaptic potentiation is certainly dependent on secretion of newly synthesized BDNF for an initial supply. However, at present, the time course of requirement for BDNF synthesis in LTP is only indirectly derived from studies using protein synthesis inhibitors (reviewed in Lu et al., 2008). The exact proportion of newly synthesized and recycled BDNF is presently unknown and a formal proof by immunohistochemistry that BDNF protein synthesis actually occurs near or at the synapses undergoing LTP is still lacking.

Another aspect that deserves particular attention is the role of dendritic BDNF in the regulation of the architecture of dendrites and spines.

8. Dendritic translation of BDNF and the local regulation of spines

Several studies demonstrated the capability of BDNF to act as a modifying factor for the shape and number of dendritic spines (Horch et al., 1999; Matsutani and Yamamoto, 2004; Tyler and Pozzo-Miller, 2003). As this morphogenic effect is spatially localized and involves only a subset of synapses, it can be explained by assuming that BDNF is transported anterogradely in axons or locally translated in dendrites. Regarding this scenario, it must be added that BDNF released from both

axonal or dendritic terminals (Altar et al., 1997), by binding to trkB, may also regulate its own production through a positive feedback signalling. Accumulating evidence suggests that this BDNF autoregulatory loop may indeed be crucial for controlling spine development, growth, form, and density in hippocampal neurons by acting in two different and somehow opposite ways (Murphy et al., 1998). First, when the new spines formed are not needed as permanent connections, they may be readsorbed to balance the excitatory drive. Second, in the new spines able to develop a permanent connection, BDNF is available to facilitate transmission at these newly formed synapses, helping regulation of synaptic protein levels and ensuring spine maturation (Stoop and Poo, 1996; Takei et al., 2004). It should be emphasized here that the local regulation of protein synthesis is crucial for the correct development and function of dendritic spines and that accumulation of polyribosomal rosettes at the base or within spine heads is associated with morphological changes of spines occurring during LTP (Ostroff et al., 2002; Harris et al., 2003). On the basis of recent results on the differential localization of different transcripts encoding BDNF (Pattabiraman et al., 2005; Chiaruttini et al., 2008), it is possible to predict that only BDNF splice variants targeted to dendrites are crucial for establishing the number and morphology of the dendritic spines. Accordingly, it can be predicted that the functional integrity of dendritic spines is likely affected by pathological or physiological conditions leading to decreased expression levels of dendritic BDNF splice variants.

An example of aberration that destroys the physiological structure and functionality of hippocampal dendritic spines is Fragile X syndrome. In the pathogenesis of this syndrome, the RNA-binding protein FMRP is severely deregulated as a consequence of transcriptional gene silencing (Oberle et al., 1991). This protein has at least three different RNA-binding domains and as a consequence of its misexpression, important mRNAs are no longer transported in dendrites. Moreover, the absence or the strong reduction of this protein causes a lack of inhibition of local translation. The phenotypical effect, studied using postmortem samples from patients, indicates that dendritic spines fail to have a normal mature shape and size and that there is a marked increase in the number of immature spines on dendrites (Greenough et al., 2001). FMRP is therefore involved in the functional maturation of neurons and their higher cognitive functions, similarly to the neurotrophin BDNF. Interestingly, as demonstrated by Castren et al. (2002), BDNF plays its role in activity-dependent synaptic plasticity also by decreasing the global levels of FMRP protein and mRNA, thus enhancing the translation of proteins involved in synaptic potentiation. Future studies will be required to determine whether FMRP-related proteins can regulate BDNF mRNA trafficking and translation in dendrites.

9. Conclusive remarks

The finding 10 years ago that BDNF mRNA is targeted into the dendrites in an activity-dependent manner (Tongiorgi et al., 1997) has assumed a broader significance since the local translation of BDNF has been implicated in LTP, long-term memory and spine remodeling. Given that the exact time course and the sites of translation of BDNF during LTP are as yet unknown, there is still a conceptually challenging problem on how locally synthesized BDNF in dendrites can ensure synapse-specific modulation of LTP. Further insights into the molecular mechanisms of mRNA trafficking in dendrites and final protein delivery to activated synapses will clarify this fascinating problem.

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