Research article

**BDNF mRNA abundance regulated by antidromic action potentials and AP-LTD in hippocampus**

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*Abbreviations: ACSF, artificial cerebral spinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP-LTD, antidromic action potential-induced LTD; (DL)-AP5, 2-amino-5-phosphonopentanoic acid; BDNF, brain-derived neurotrophic factor; CA1, cornus ammonis region 1; CaMKII, calcium/calmodulin kinase II; eEPSP, excitatory postsynaptic potentials; LTD, long-term depression; LTP, long-term potentiation; L-VDCC, L-type voltage-dependent calcium channel; (RS)-MCPC, α-methyl-4-carboxyphenylglycine; NMDA, N-methyl-D-aspartate receptor; RT-PCR, reverse transcription polymerase chain reaction; TBS, theta burst stimulation.

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**HIGHLIGHTS**

- BDNF gene expression in hippocampal CA1 neurons is necessary for LTD induced by antidromic stimulation (AP-LTD).
- BDNF mRNA transcripts are rapidly downregulated during AP-LTD.
- Different BDNF mRNA transcripts respond with distinct temporal profiles to antidromic stimulation.
- In contrast to antidromic stimulation, abundance in BDNF mRNA increases after LTD induced chemically by activation of L-VDCC.

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**ABSTRACT**

Action-potential-induced LTD (AP-LTD) is a form of synaptic plasticity that reduces synaptic strength in CA1 hippocampal neurons firing antidromically during sharp-wave ripples. This firing occurs during slow-wave sleep and quiet moments of wakefulness, which are periods of offline replay of neural sequences learned during encoding sensory information. Here we report that rapid and persistent down-regulation of different mRNA transcripts of the BDNF gene accompanies AP-LTD, and that AP-LTD is abolished in mice with the BDNF gene knocked out in CA1 hippocampal neurons. These findings increase understanding of the mechanism of AP-LTD and the cellular mechanisms of memory consolidation.

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**1. Introduction**

Pyramidal neurons in the CA1 region of hippocampus fire antidromically (from the axon into the cell body and dendrites) during brief bursts of high-frequency oscillations (100–300 Hz) termed sharp wave ripple complexes (SPW-R) [1,2]. Antidromic action potential firing in these neurons in the absence of excitatory synaptic input induces a form of synaptic plasticity that reduces the strength of synapses in a cell-wide manner, termed action potential-induced long-term depression (AP-LTD) [3]. Mechanisms known to promote antidromic firing during SPW-Rs facilitate AP-LTD induction [3]. Although AP-LTD has not yet been studied in vivo, the phenomenon appears relevant to memory consolidation because SPW-Rs occur during slow-wave sleep (SWS) and quiet periods of wakefulness. These behavioral states are characterized by decreased sensory input and offline replay of neural sequences learned during encoding sensory information [4,5], and disrupting SPW-Rs impairs memory retention [6,7]. Compared to other forms of hippocampal synaptic plasticity, the mechanisms of AP-LTD are relatively unexplored. Here the possible involvement of brain-derived neurotrophic factor (BDNF), a protein involved in several forms of synaptic plasticity, is examined in relation to synaptic plasticity induced by antidromic action potentials.
AP-LTD is an unusual form of synaptic plasticity because synaptic activation is not necessary to initiate the depression in synaptic strength, and therefore the plasticity is not synapse-specific. Instead, the mechanism for AP-LTD induction is independent of glutamatergic receptor activation, but it is dependent on ectopic action potential firing via TTX-sensitive sodium channels in axons that are activated by action potential backpropagation into the soma and dendrites. Intracellular calcium signaling is necessary for AP-LTD, as shown by blocking AP-LTD in the presence of an inhibitor of L-type voltage-dependent Ca\(^{2+}\) channels (L-VDCC) during antidromic action potential firing [3].

Our hypothesis was that BDNF mRNA levels would be reduced during AP-LTD in parallel with the reduction in synaptic strength; however, the involvement of BDNF in synaptic plasticity is complex. The BDNF gene contains at least nine differentially regulated promoters and multiple untranslated 5’ exons alternatively spliced to one protein-coding 3’ exon [8,9]. Because multiple transcripts may be obtained from the BDNF gene, with potential subcellular differences and distinct temporal profiles in response to neuronal activity, we measured changes in abundance of the most prominently expressed BDNF transcripts containing exon I, exon II, exon IV and total (full-length) BDNF mRNA [9]. As background support for our hypothesis, previous research has shown that exogenous BDNF promotes long-term potentiation (LTP) in hippocampal slices [10], enhances basal synaptic strength in response to low-frequency stimulation [11], and lowers the threshold of stimulation required for LTP induction [12], while synthetically-induced LTD is prevented by BDNF application [13–15], and blockade of BDNF signaling turns chemically-induced LTP into LTD [16].

The results show that AP-LTD is accompanied by a rapid reduction in BDNF mRNA abundance, with different exons showing different levels of regulation and different temporal responses, and that AP-LTD is impaired in mice with deletion of BDNF restricted to the CA1 region. Together these results indicate that this form of synaptic plasticity, which is associated with SPW-Rs, requires BDNF downregulation.

2. Methods

2.1. Experimental animals

All experiments were conducted in accordance with animal study protocols approved by the National Institutes of Child Health and Human Development Animal Care and Use Committee. For RT-PCR experiments hippocampal slices were prepared from adult (7 to 10-week-old) male Sprague-Dawley rats. For electrophysiological recordings hippocampal slices were prepared from adult male BDNF knockout and wild-type mice (8 to 10-week-old). Floxed BDNF gene mice [17] were used to generate mice with a CA1-restricted deletion of BDNF by crossing with a line expressing Cre recombinase in the CA1 region under the control of the calcium/calmodulin kinase II promoter [18]. The presence of Cre and floxed BDNF alleles was determined as previously described [19].

2.2. Stimulation protocols and tissue collection for mRNA

Hippocampal slices (400\(\mu\)m) were prepared from Sprague-Dawley rats, BDNF KO or BDNF WT mice as described [3]. Field excitatory postsynaptic potentials (EPSPs) in the CA1 stratum radiatum were recorded as described [3,20]. Each slice was examined for its capacity to generate field EPSPs of at least 1 mV at a stimulus intensity of 40–50 \(\mu\)A and slices not meeting this criterion were discarded. Antidromic stimulation was delivered concomitantly by two stimulating electrodes placed in the alveus/stratum oriens in the CA1 region to stimulate the maximal number of cells. The stimulus intensity for the antidromic stimulation was 100–130 \(\mu\)A. Repetitive theta-burst stimulation (TBS) was applied antidromically with the second stimulating electrode to the alveus/stratum oriens in the CA1 in the presence of glutamate receptor blockers: AMPA/kainate receptor antagonist kynurenic acid (3 mM), NMDA receptor antagonist, AP5(50 \(\mu\)M), and group I/group II metabotropic glutamate receptor antagonist (RS)-MCPG (250 \(\mu\)M) for 25–35 min. Stimulation of the alveus may also stimulate cholinergic axons [21] but kynurenic acid at the concentration used also acts as an antagonist of \(\alpha7\) nicotinic acetylcholine receptors [22]. After cessation of stimulation, ACSF containing the drug cocktail was washed away and test stimuli delivered to assess changes in EPSP amplitude. TBS consisted of 10 stimulus bursts delivered at 5 Hz. Each burst consisted of 4 pulses delivered at 100 Hz. The duration of each pulse was 0.2 ms. Three TBSs were applied every 30 s and repeated three times in 5 min intervals.

For experiments presented in Fig. 3, L-VDCC agonist BayK 8644 (10 \(\mu\)M) was bath applied 10 min after start of perfusion with glutamate antagonists to non-stimulated slices. We demonstrated previously induction of long-lasting synaptic depression after application of BayK8644 and blockade of AP-LTD by nifedipine, when applied at the same concentrations [Fig. 3 in [3]].

Hippocampal slices were collected 15, 60 and 180 min after stimulation and snap frozen. To minimize potential masking effects from other hippocampal regions, the CA1 region from each slice was quickly microdissected and stored at −80 °C. Matched control slices, which had not been stimulated, were collected at the same time, from the same recording chamber.

2.3. Semi-quantitative real-time RT-PCR

RNA was extracted from hippocampal slices using TRIzol® reagent (Invitrogen, Carlsbad, CA). Total RNA (2 \(\mu\)g) was reverse transcribed with Superscript II, as previously described [23]. Semi-quantitative, real-time PCR was performed on a Roche LightCycler using the Faststart DNA Master SYBR Green 1 PCR reaction mix (Roche Diagnostics, Indianapolis, IN), essentially as described previously.

Data analysis was performed as described [23]. Data were analyzed by the 2^(-\text{delta delta C(T))} method of analysis of RT-PCR data with respect to an unstimulated control as described by Livak and Schmittgen [24].

The following primer sequences were used:

- BDNF exon 1 FP AAGACACGAGTCTCAGGAC
- BDNF exon 2c FP TATCTCCAGGATCTAGCCAC
- BDNF exon 4 FP AGCAAGCTCGTTAGGTATAC
- BDNF exon 9 FP AGCCAAAGTCCAGGACAAG
- BDNF exon RP TGTCCCGTGAGTGTTCTCGC
- GAPDH FP AATGCAATCTGTCAAGGAC
- GAPDH RP TGGATGCAAGGATGTTCTCG

2.4. Statistical analysis

PCR data were analyzed statistically by the method of 2^(-\text{Delta Delta C(T))} [24], to compare mRNA expression levels relative to an unstimulated control. Control samples were unstimulated in the same recording chamber as slices that received electrical stimulation, and collected at the same time point. Student’s two-tailed, one-sample t-test was used to assess statistical significance between the matched experimental and control slices at the same time point using Sigma Plot 10.0 software (SPSS, Chicago, IL, USA) and Minitab software (version 5, Minitab Inc., State College PA). Values were expressed as percent of control, with variance for the controls estimated from the variance of the experiments. All data points are presented in the graphs and summary data and
3. Results

3.1. BDNF mRNA abundance after AP-LTD

To determine if stimulation that induces AP-LTD alters expression of BDNF mRNA, a semiquantitative RT-PCR assay was used to estimate the abundance of BDNF mRNA in isolated CA1 of stimulated and control unstimulated hippocampal slices. For this purpose, 5′-end forward primers were designed that are specific for BDNF exons I, IIc, IV, IX, and a 3′-end primer was generated that is complementary to sequences in exon IX, that is common to all BDNF transcripts, and used as reverse primer for all transcripts (Fig. 1A).

The results showed that different BDNF transcripts responded differently to antidromic stimulation. In agreement with our preliminary report [20], we found that expression of mRNAs bearing BDNF exon I decreased significantly very rapidly after AP-LTD (Fig. 1). Levels of BDNF exon I were reduced by 40% 15 min after AP-LTD stimulation relative to expression in control slices incubated simultaneously in the same recording chamber but unstimulated (Fig. 1B). A prominent decrease in BDNF transcripts containing exon II was also detected at this time point (Fig. 1C). Similarly to exon I and exon II, a 32% decrease in total BDNF mRNA (exon IX) was observed 15 min after AP-LTD (Fig. 1E). In contrast to other exons, expression of exon IV was not affected significantly by antidromic firing (Fig. 1D). The downregulation of BDNF mRNA transcripts persisted for at least 60 min for exon I and exon II containing transcripts (Fig. 1B, C). However, exon IV-containing BDNF transcript and total BDNF mRNA were not affected 60 and 180 min after stimulation to induce AP-LTD (Fig. 1D, E).

Together these results summarized in Table 1 show that BDNF mRNA transcripts containing exons I and II decrease rapidly, within 15 min after AP-LTD stimulation, and the reduction persists for a long period of time (at least 1 h). Secondly, different BDNF transcripts respond differently to antidromic firing of action potentials. The most prominent effects are on exon I and exon II. Transcripts containing exon IV were not regulated. The reduction in BDNF expression induced by AP-LTD correlates with the decrease in synaptic responses produced by this antidromic stimulation [3].

3.2. Dependence of AP-LTD on BDNF mRNA

Our data showing that mRNA transcripts for several exons of the BDNF gene were decreased during AP-LTD suggests that down
regulation of BDNF mRNA may be necessary for AP-LTD. To further explore the role of BDNF in synaptic changes induced by antidromic stimulation, we used mice in which the genetic deletion of BDNF was restricted only to CA1 neurons [10]. Repeated theta-burst stimulation (TBS) delivered antidromically to alveus in the presence of glutamatergic antagonists evoked robust AP-LTD in CA1 stratum radiatum of wild type mice (54.6 ± 5.2%, p < 0.001, t-test, comparing to pre-stimulated level, N = 7, Fig. 2), consistent with our previous results in rat [3]. However AP-LTD was absent in hippocampal slices prepared from BDNF KO mice (99.3 ± 11.1%, p < 0.01, t-test, comparing to BDNF WT mice, N = 7; Fig. 2). These data demonstrate that BDNF gene expression in CA1 region is necessary for AP-LTD expression.

### Table 1

| BDNF mRNA regulation. |
|------------------------|
| **stimulation** | **time** | **BDNF exon** | **mean ± SEM** | **N** | **t value** | **P value** | **significance** |
| **Antidromic** | 15’ | exon I | -43.5 ± 7.1 | 4 | 6.28 | 0.008 | **”** |
| | 15’ | exon II | -20.4 ± 7.2 | 4 | 2.96 | 0.059 | * |
| | 15’ | exon IV | -8.2 ± 6.1 | 4 | 1.50 | 0.230 |
| | 60’ | exon I | -31.9 ± 12.8 | 4 | 2.58 | 0.082 |
| | 60’ | exon II | -62.7 ± 6.6 | 4 | 9.63 | 0.002 | **”** |
| | 60’ | exon IV | -22.3 ± 12.1 | 4 | 1.92 | 0.151 |
| | 180’ | exon I | -2.0 ± 22.1 | 4 | 0.14 | 0.9 |
| | 180’ | exon II | -17.7 ± 11.0 | 5 | 1.70 | 0.165 |
| | 180’ | exon IV | 4.4 ± 5.0 | 5 | 0.67 | 0.541 |
| | 180’ | exon IX | 7.4 ± 10.5 | 4 | 0.52 | 0.582 |
| **BayK8644** | 15’ | exon I | 7.85 ± 75.3 | 4 | 1.03 | 0.379 |
| | 15’ | exon II | 26.3 ± 35.4 | 4 | 0.71 | 0.526 |
| | 15’ | exon IV | 38.5 ± 7.5 | 4 | 4.96 | 0.016 | **”** |
| | 60’ | exon I | 28.7 ± 41.5 | 4 | 0.67 | 0.553 |
| | 60’ | exon II | 31.3 ± 28.5 | 4 | 1.06 | 0.365 |
| | 60’ | exon IV | 8.6 ± 25.0 | 4 | 0.30 | 0.782 |
| | 60’ | exon IX | 52.2 ± 11.4 | 4 | 4.48 | 0.021 | **”** |
| | 180’ | exon I | 90.5 ± 30.8 | 4 | 2.90 | 0.062 |
| | 180’ | exon II | 42.8 ± 28.0 | 4 | 1.52 | 0.233 |
| | 180’ | exon IV | 46.8 ± 34.8 | 4 | 1.32 | 0.279 |
| | 180’ | exon IX | 39.5 ± 23.6 | 4 | 1.63 | 0.201 |
| | 180’ | exon IX | 55.2 ± 18.2 | 4 | 2.97 | 0.059 | * |

*p < 0.05 and **p < 0.01; t-test, comparing to corresponding control, unstimulated slices. N = 1 represents pooling of experiments performed on 8–10 hippocampal slices combined for analysis of BDNF mRNA and an equal number of paired control slices.

3.3. **BDNF regulation by activation of L-type VDCC**

LTD can be induced chemically by incubation in the L-VDCC agonist Bay K 8644 [3]. Considering other studies showing Ca**2+**-dependent stimulation of BDNF transcription [25], together with our data indicating that AP-LTD also requires L-VDCC activation [3], we tested whether pharmacological activation of L-VDCC in the absence of antidromic stimulation decreased BDNF. BDNF mRNA levels after chemical induction of LTD showed the opposite response to that evoked by antidromic stimulation: a tendency for increased BDNF mRNA levels (Fig. 3). The increase in BDNF mRNA reached statistically significant levels at 15 and 60 min for exon IV (Fig. 3C), and at 180 min for total BDNF (Fig. 3D), but the increasing trends were not significant for exon 1 and II BDNF transcripts (Fig. 3A, B). In summary, these results show that different calcium-dependent signaling mechanisms operate to regulate BDNF and AP-LTD in response to pharmacological stimulation, orthodromic, and antidromic action potential firing.

4. Discussion

The results show that down regulation of BDNF mRNA abundance accompanies AP-LTD, and that expression of BDNF in CA1 of hippocampus is required for this form of synaptic plasticity, which is induced by antidromic firing in the absence of excitatory synaptic input. Given the well-established role of this neurotrophin in increasing synaptic strength, in counteracting LTD, and in promoting synaptogenesis and dendritic complexity, the rapid reduction of BDNF mRNA during AP-LTD could contribute to reducing synaptic strength and promote synaptic remodeling. Also, induction of LTD is impaired during SWS [26,27], and both LTD and loss of dendritic spines are thought to contribute to memory consolidation during sleep [28,29]. Our findings are consistent with the proposal that AP-LTD acts as a mechanism to counteract LTP that develops in hippocampal circuits by synaptic activity during the wakeful state. In this way, AP-LTD would rescale synaptic weights downward in a cell-wide manner and thus prevent saturation of synaptic strength. In so doing, the down-scaling of synaptic strength following AP-LTD would then facilitate assimilation of new information after sleep.
The loss of weaker inputs by AP-LTD would also promote formation of new functional assemblies of neurons that are more sharply tuned to preferred stimuli. The reduction in BDNF mRNA observed following AP-LTD is consistent with regulation of BDNF mRNA during sleep, which has been reported previously [30,31].

The levels and activity of BDNF are modulated by several mechanisms, accounting for the multifaceted effects of this neurotrophin on synaptic plasticity. These mechanisms include activity-dependent effects on BDNF mRNA abundance and protein synthesis [32], secretion of BDNF from neurons [33], BDNF transcript and protein breakdown [32], post-translational modification from pro- to mature forms of BDNF [34], gene polymorphisms [35], epigenetic modifications [36] subcellular compartmentalization [37], interactions between BDNF signaling and other signaling pathways [38,39]. Another mechanism can now be added to this list: down-regulation of BDNF mRNA by antidromic firing. Back-propagation of action potentials has been shown to stimulate secretion of BDNF from dendrites in a calcium-dependent manner, and spontaneous activity alone is not sufficient to do so [40]. The physiological and morphological changes in neurons mediated by antidromic BDNF secretion would be reduced by the reduction in BDNF transcripts shown in these experiments following AP-LTD.

In other studies, as was confirmed here, calcium influx into neurons stimulates transcription of BDNF; but in AP-LTD, the abundance of BDNF mRNA is reduced despite a rise in cytoplasmic calcium that accompanies action potential firing. Although this may appear contradictory, the apparent paradox can be resolved in several ways. The reduction in BDNF mRNA occurs rapidly, within 15 min, suggesting that mechanisms regulating mRNA stability and degradation are responsible for the reduction of BDNF transcripts during AP-LTD. Such mechanisms are likely exon-specific, accounting for the differences in reduction of exon I, II, III, and IV transcripts observed in AP-LTD. For example, miRNAs cause mRNA de-adenylation, which promotes de-capping and more rapid degradation of transcripts [41]. It has been demonstrated that miRNA controlled degradation of mRNA transcripts accounts for most (>84%) of the decreased protein production. Although Ca²⁺ signaling activates BDNF transcription [25]. Ca²⁺ signaling also influences miRNA and mRNA degradation. Thus, the abundance of BDNF mRNA depends upon both transcriptional regulation and mRNA degradation, and different signaling pathways are likely to be activated by orthodromic neuronal activity and antidromic activity.

Homeostatic controls are required to prevent a neural network from becoming incapacitated, and sleep is thought to be an important period for LTD and dendritic remodeling [29]. The present findings demonstrate that downregulation of BDNF mRNA accompanies AP-LTD. Considering the variety of pathological conditions and physiological processes influenced by BDNF mRNA levels, the findings could have broad neurological and pathophysiological significance, and advance understanding of the molecular mechanisms depressing synaptic strength as occurs in memory consolidation during SWS.

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