Molecular Mechanisms Underlying Activity-Dependent Regulation of BDNF Expression

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ABSTRACT: Activity-dependent changes in synaptic strength, which appear to underlie cortical plasticity, require long-lasting biochemical changes in the postsynaptic neuron. An inductive event common to several forms of synaptic plasticity is an influx of calcium into the postsynaptic cell. Calcium acts as a second messenger to set into motion a cascade of biochemical signaling events that leads to new gene expression. Brain-derived neurotrophic factor (BDNF) is one such calcium-regulated gene that appears to be involved in activity-dependent cortical plasticity. Studies of the mechanism by which calcium influx induces BDNF expression have revealed that the BDNF promoter is regulated by two calcium response elements. One of the elements appears to be regulated by a novel transcription factor, while the other element is regulated by the previously characterized transcription factor CREB. The calcium signal is propagated to the CREB-mediated component of BDNF expression by CaM kinase IV. This signaling pathway, which links calcium influx to the induction of BDNF via CaM kinase IV and CREB, is likely to be centrally involved in mediating long-term activity-dependent plasticity. J Neurobiol 41: 127–134, 1999

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Much of our knowledge of cortical plasticity has come from studies on the development of ocular dominance columns in the visual cortex (Hubel, 1982; Wiesel, 1982). The segregation of geniculocortical axons into eye specific regions within layer 4 of visual cortex gives rise to ocular dominance columns during development. The pattern of ocular dominance columns can be altered by abnormal visual experience during development, and several lines of evidence suggest that this form of cortical plasticity requires action potential and synaptic activity. For example, intraocular injection of tetrodotoxin *in vivo* inhibits the segregation of lateral geniculate nucleus axons into ocular dominance columns (Stryker and Harris, 1986), and blockade of *N*-methyl-D-aspartate (NMDA) receptors prevents the shift in ocular dominance columns caused by monocular deprivation (Kleinschmidt et al., 1987).

The activity dependence of cortical plasticity suggests that at the cellular level it may be mediated by synaptic plasticity. A widely studied model of synaptic plasticity is long-term potentiation (LTP) (Bliss and Lømo, 1973), which refers to an enhancement of synaptic transmission following high-frequency stimulation. In support of the possibility that activity-dependent synaptic plasticity may underlie cortical plasticity, Kirkwood et al. showed that high-frequency stimulation of cortical slices can induce changes in synaptic efficacy. This form of LTP requires activation of NMDA receptors and demonstrates an age dependence that corresponds to the critical period of cortical plasticity (Kirkwood et al., 1993, 1995).

The induction of LTP requires calcium influx into the postsynaptic neuron and the subsequent activation of the calcium-sensitive kinase, CaM kinase II (Lynch

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et al., 1983; Silva et al., 1992; Pettit et al., 1994; Mayford et al., 1995). Whereas the induction of LTP is a rapid event that involves local biochemical changes in the postsynaptic cell, the maintenance of increased synaptic efficacy appears to requires calcium-induced changes in gene expression, as pharmacological agents that inhibit protein synthesis prevent the late phase of LTP (Nguyen et al., 1994). This has motivated efforts to identify calcium-regulated genes in neurons and to understand their mechanism of induction.

A likely gene target of calcium signaling in neurons is brain-derived neurotrophic factor (BDNF). The possibility that neuronal activity might regulate expression of BDNF was first suggested from the observation that BDNF expression was dramatically increased in the neocortex following experimentally induced seizures (Isackson et al., 1991; Ernfors et al., 1991) Several other groups have also reported regulation of BDNF *in vivo* by sensory stimulation and *in vitro* by neuronal activity (Castren et al., 1992; Bozzi et al., 1995; Lauterborn et al., 1996; Ghosh et al., 1995), suggesting that this is a major mechanism by which levels of BDNF are normally regulated.

Recent observations suggest that BDNF may play an important role in activity-dependent plasticity (reviewed in Ghosh, 1996; Shieh and Ghosh, 1997; Bonhoeffer, 1996). For example, Cabelli et al. (1995, 1997) reported that infusion of either BDNF or trk-B receptor bodies during the critical period prevents the formation of ocular dominance columns, consistent with the possibility that thalamic axons normally compete for limiting amounts of BDNF. Similarly, several studies have demonstrated that LTP can be modulated by BDNF. In the hippocampus, Kang and Schuman (1995) reported that application of extracellular BDNF is sufficient to induce rapid enhancement of synaptic transmission and that application of trkB-immunoglobulin G (IgG) receptor bodies to hippocampal slices prevents theta-burst–dependent potentiation of synaptic transmission (Kang et al., 1997). In the cortex, Akaneya et al. (1997) reported that application of BDNF to the visual cortex is sufficient to enhance synaptic transmission from layer IV neurons to layer III neurons and that bath application of trkB-IgG could prevent the induction of LTP in these slices. Finally, studies on BDNF null mice have demonstrated deficits in basal synaptic transmission and hippocampal LTP which can be can be rescued by BDNF provided either by retroviral infection (Korte et al., 1996) or by bath application of BDNF (Patterson et al., 1996). These studies, taken together, suggest that calcium levels may elevate BDNF expression to effect the mechanisms of long-term synaptic plasticity. Given the critical role of both calcium and BDNF in cortical plasticity, our laboratory became interested in the mechanism of calcium-dependent expression of BDNF, possibly providing a link between calcium influx and long-term changes in synaptic strength.

RESULTS

Identification of Calcium Response Sequences in the BDNF Promoter

To characterize the mechanisms that regulate activity-dependent transcription of BDNF, we began by identifying elements within the promoter that respond to neuronal activity (Shieh et al., 1998). The rat BDNF gene contain five exons, and the first four exons are each flanked with a putative promoter and a splice donor site which all splice to a common splice acceptor site on the fifth exon, which contains the open reading frame (Timmusk et al., 1993). To identify which promoters contribute to calcium-dependent transcription of BDNF, we tested the calcium-dependent induction of a CAT reporter gene under the control of each of the four BDNF promoters and found that promoters sequences of exon III confer over 80% of the calcium responsiveness (see also Tao et al., 1998). Thus, we focused our efforts on characterizing calcium regulation of exon III transcription.

To identify DNA sequences required for calcium-dependent transcription, we generated a series of 5′ deletions of the exon III promoter, fused them to a CAT reporter gene, and tested these constructs in embryonic day (E)18 cortical cultures for inducibility by depolarization (which leads to calcium influx via voltage-sensitive calcium channels (VSCC) [Fig. 1(A)]. Although all constructs containing sequences beyond −72 were all capable of mediating a robust calcium-dependent transactivation of the reporter, −47 and −30 constructs were nonresponsive to VSCC activation [Fig. 1(B)]. Examination of the sequence in this region revealed no known calcium-responsive sequences, suggesting that the 25–base pair DNA sequence between −47 and −72 represents a novel calcium response element required for calcium regulation of BDNF exon III expression. Mutational analysis performed in our laboratory as well as by Tao et al. (1998) suggests that the region between −60 and −72 may represent a critical binding site for a novel calcium-regulated transcription factor. We refer to the calcium-responsive element within this region as calcium-responsive sequence-I (CRS-I), to distinguish it from the previously described calcium

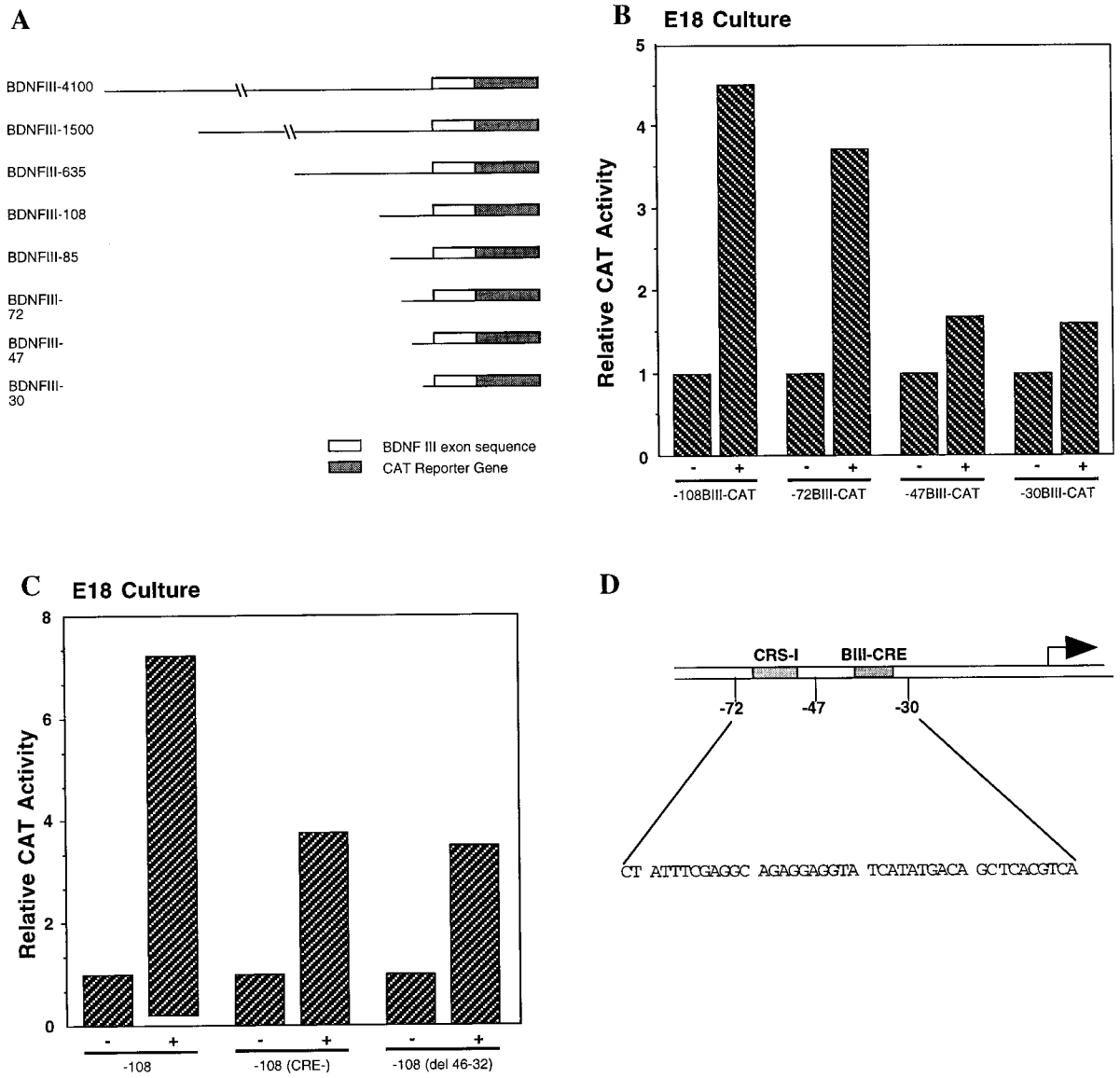

Figure 1 Calcium-dependent activation of BDNF is mediated by two calcium response elements. (A) BDNF reporter constructs used to identify calcium responsive elements with the BDNF exon III promoter. (B) Relative CAT activity in E18 cortical cultures transfected with indicated constructs at 3DIV, left unstimulated (−) or stimulated with 50 m*M* KCl (+) at 5 days *in vitro* (DIV). (C) Relative CAT activity in E18 cortical cultures transfected with −108BIII-CAT or with constructs which have mutations that disrupt BIII-CRE. (C) Sequence of the region of the BDNF exon III promoter that mediates a calcium response. The region between −72 and −47 contains a novel calcium response element (CRS-I). The region between −47 and −30 contains a CRE-like element (BIII-CRE) located between −38 and −31 (TCACGTCA). (Modified from Shieh et al., 1998.)

or cyclic adenosine monophosphate response element (CaRE/CRE) (Montminy and Bilezikjian, 1987; Sheng et al., 1990).

To determine whether the BDNF promoter might contain calcium response elements in addition to CRS-I, we carried out a sequence analysis of the promoter and found a sequence between −38 and −31 that closely matches the consensus for a CaRE/CRE [Fig. 1(D)]. Our deletion analysis suggested, however, that this CRE, which we will refer to as BIII-CRE, was not sufficient to mediate a calcium response since the construct −47BIII-CAT (which includes the BIII-CRE) was unable to mediate calcium-dependent transactivation [Fig. 1(B)]. To deter-

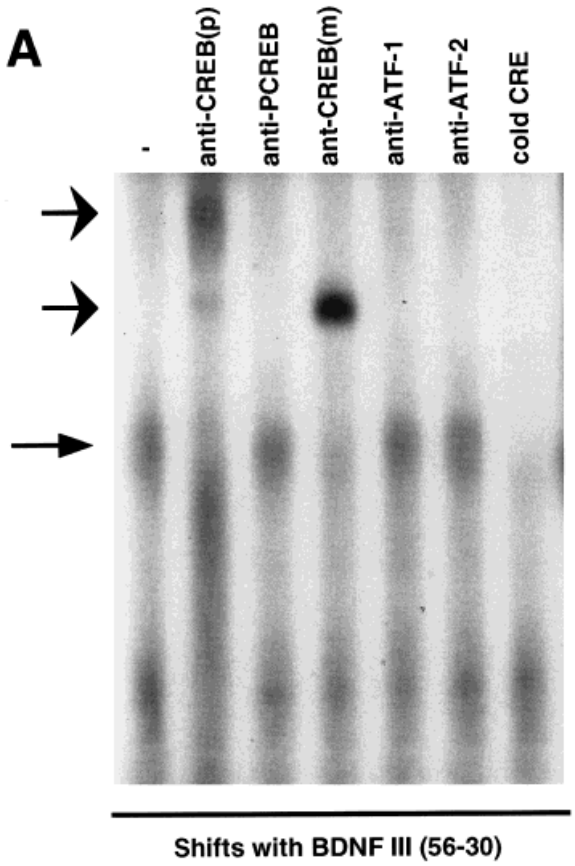

Figure 2 The transcription factor CREB binds to the BDNF promoter and contributes to calcium-dependent transactivation. (A) Antibodies to CREB, but not ATF-1 or ATF-2, can supershift the protein complex from P1 cortical cultures (also present in E18 cultures) that binds to the oligonucleotide BDNF III (56–30) which contains the BIII-CRE sequence. The lower arrow corresponds to the complex that forms at the BIII-CRE. The upper arrows correspond to supershifted bands. The antibodies used for supershift experiments were: anti-CREB(p) = polyclonal antibody to CREB; anti-PCREB = polyclonal antibody to phosphorylated CREB (serine 133); anti-CREB(m) = monoclonal antibody to CREB; anti-ATF-1, anti-ATF-2 = monoclonal antibodies to ATF-1 and ATF-2. (B) Relative CAT activity in E18 cortical cultures transfected with −108BIII-CAT together with a control vector or dominant negative CREB (KCREB). Cells were transfected at 3 DIV and stimulated with 50 m*M* KCl at 5 DIV. (Modified from Shieh et al., 1998.)

mine whether this element was required for calcium-dependent transactivation, we created constructs in which the BIII-CRE was mutated in context within the BDNF promoter and assayed them for their ability to mediate calcium-dependent transactivation. Interestingly, the mutations of the CRE site partially attenuated the calcium response in embryonic cultures and completely prevented it in postnatal cultures [Fig. 1(C)] (Shieh et al., 1998; Tao et al., 1998). These experiments suggest that the BIII-CRE functions as a developmental stage-specific calcium response element within the BDNF promoter, and that it is a critical mediator of calcium responses in postnatal neurons.

Characterization of Protein Complexes That Bind Calcium Responsive Sequences in the BDNF Promoter

The promoter analysis experiments suggest the involvement of two elements in calcium regulation of BDNF expression. To determine whether these two elements bind to distinct DNA-binding proteins, we performed gel mobility shift experiments with three oligonucleotides that spanned the sequence between −72 and −20 (Shieh et al., 1998). Shifts with E18 cortical extracts clearly indicated the existence of two protein complexes at the promoter, one between −72 and −47 and a second between −47 and −20. The results of these gel shift experiments are consistent with the promoter analysis experiments described earlier and suggest that the complex that shifted with 72–47 binds to CRS-I. Shifts performed with oligonucleotides that include the BIII-CRE as well as supershift experiments using antibodies to CREB indicated that the BIII-CRE is bound by the transcription factor CREB [Fig. 2(A)]. The association of CREB with BIII-CRE is functionally relevant since we have found that dominant negative mutants of CREB attenuate calcium dependent transactivation of BIII-CAT reporters [Fig. 2(B)]. In related experiments,

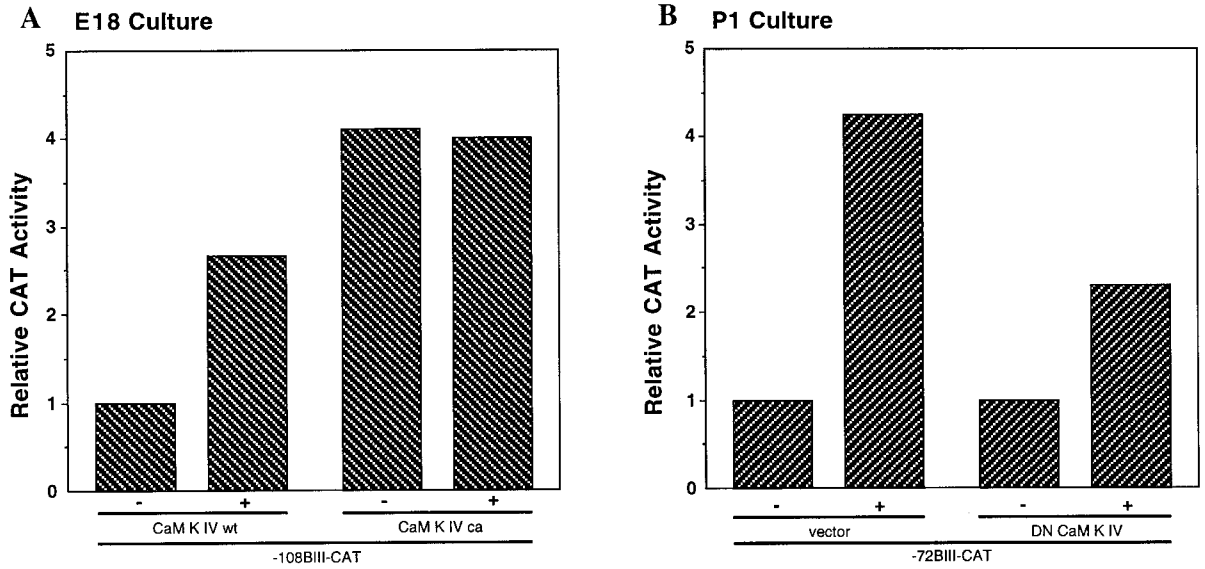

Figure 3 CaM kinase IV is involved in calcium-dependent activation of the BDNF gene. (A) Relative CAT activity in E18 cortical cultures transfected with indicated BDNF reporter constructs together with wild-type (wt) or constitutively active (ca) CaM kinase IV at 3 DIV, and stimulated with 50 m*M* KCl at 5DIV. (B) Relative CAT activity in P1 cortical cultures transfected with −72BIII-CAT together with a control vector or dominant negative mutant of CaM kinase IV at 3 DIV, and stimulated with 50 m*M* KCl at 5 DIV. (Modified from Shieh et al., 1998.)

Tao et al. (1998) showed that transfection of a dominant negative mutant of CREB into E18 cultured neurons attenuates the stimulus-dependent activation of the endogenous BDNF exon III promoter. These experiments indicate that CREB-dependent transcription is important for transactivation of the BDNF gene.

Involvement of CREB-Binding Protein (CBP) in CREB-Mediated Transcription

Since CREB is clearly involved in calcium-dependent induction of BDNF, we decided to study the mechanisms by which calcium influx leads to CREB-mediated transcription in cortical neurons (Hu et al., 1999). To examine CREB-mediated transcription, we transfected cortical neurons with a construct encoding a GAL4-CREB fusion protein together with a CAT reporter construct driven by the GAL4 upstream activating sequence (UAS-CAT). Calcium influx induced by depolarization or NMDA receptor activation leads to a robust increase in CAT activity indicating that, as in other cells, CREB is a target of calcium signaling in cortical neurons. Calcium-induced CREB mediated transcription appears to require the transcriptional coactivator CBP, since inhibition of CBP by E1A constructs inhibits CREB-mediated transcription. Recent experiments suggest that recruitment of CBP to the promoter by phosphorylated CREB is not sufficient for transactivation and appears to require direct activation of CBP by calcium signaling (Chawla et al., 1998; Hu et al., 1999; Hardingham et al., 1999). Therefore, calcium-induced CREB-mediated transcription appears to require two activation events: phosphorylation of CREB at Serine 133 (reviewed in Ghosh and Greenberg, 1995) and an as yet unidentified modification of CBP.

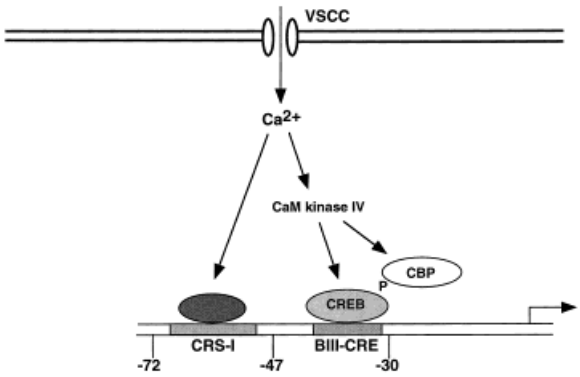

Figure 4 Proposed mechanism by which calcium influx regulates BDNF expression. Transactivation of the BDNF promoter is mediated by two elements, CRS-I and BIII-CRE. The BIII-CRE–dependent component of transactivation is mediated by CREB (and possibly CBP), and can be regulated by CaM kinase IV.

Involvement of CaM Kinase IV in Calcium Signaling to the BDNF Promoter

Previous studies have identified CaM kinases as being involved in conveying calcium signals to the nucleus (Miller and Kennedy, 1986; Matthews et al., 1994; Sun et al., 1994; Braun and Schulman, 1995; Tokumitsu et al., 1995; Bito et al., 1996; Deisseroth et al., 1996; reviewed in Ghosh and Greenberg, 1995). To examine the role of these kinases in the induction of BDNF expression, we cotransfected wild-type (wt) and constitutively active (ca) constructs of CaM kinase II and CaM kinase IV (Sun et al., 1994) with −108BIII-CAT (Shieh et al., 1998). Although calcium-dependent transactivation was preserved in cells expressing wt CaM kinase II, expression of ca CaM kinase II blocked transactivation of the promoter, suggesting that activation of CaM kinase II is unlikely to be directly involved in transactivation of the BDNF promoter. The effects of expressing the CaM kinase IV constructs, however, were quite different. Although the expression of wt CaM kinase IV did not dramatically affect the induction of the reporter constructs, expression of ca CaM kinase IV led to transcription of the BDNF promoter even in the absence of stimulus [Fig. 3(A)]. In fact, expression of constitutively active CaM kinase IV largely occluded calcium-dependent transactivation of the promoter, which is consistent with CaM kinase IV being part of the normal signaling pathway by which calcium influx leads to BDNF expression. Inhibition of CaM kinase IV by transfection of a dominant negative form of CaM kinase IV [CaM kinase IV/Gr(i)] (Ho et al., 1996; Finkbeiner et al., 1997) markedly attenuated calcium-dependent transactivation of the BDNF reporter [Fig. 3(B)]. This observation, taken together with the occlusion effect of constitutively active CaM kinase IV described above, strongly suggests that calcium-dependent activation of the BDNF gene is mediated via activation of CaM kinase IV.

CONCLUSIONS

The observations from our laboratory as well as the work of others has led to the identification of a signaling pathway by which calcium influx regulates BDNF expression (Fig. 4). It appears that this pathway has two components. One component leads to the activation of an as yet unidentified transcription factor that binds to a novel calcium response sequence called CRS-I. The second component involves activation of CaM kinase IV, which in turn leads to activation of a CREB/CBP complex that binds to the BIII-CRE. This pathway by which calcium influx leads to BDNF expression is likely to be centrally involved in converting the acute effects of synaptic stimulation to long-term biochemical and cellular changes that underlie cortical plasticity.

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