

CROSSTALK BETWEEN BRAIN-DERIVED NEUROTROPHIC FACTOR AND N-METHYL-D-ASPARTATE RECEPTOR SIGNALING IN NEURONS

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Glutamate is the major excitatory neurotransmitter in brain exerting prosurvival effect on neurons via N-methyl-D-aspartate receptor (NMDAR) signaling under physiological conditions. However in pathological circumstances such as ischemia, NMDARs might have proapoptotic excitotoxic activity. In contrast brain-derived neurotrophic factor (BDNF) signaling via tropomyosin-related receptor kinase B (TrkB) has been largely considered to promote neuronal differentiation, plasticity and survival during normal development, and protect neurons in pathophysiological conditions antagonizing the NMDAR-mediated excitotoxic cell death. In this review we summarize recent evidence for the existent crosstalk and positive feedback loops between the BDNF and NMDAR signaling and point out some of the important specific features of each signaling pathway.

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Key words: NMDA receptor, BDNF, neurotrophins

INTRODUCTION

Neurotransmitters and growth factors promote growth, survival and differentiation of neurons in central nervous system. Moreover, in adult brain they participate in synaptic signaling and synaptic remodeling, which underlie learning and memory formation (1). While N-methyl-D-aspartate receptor (NMDAR) signaling is physiologically essential for neuronal survival (2), it might have excitotoxic proapoptotic effect under pathophysiological conditions such as ischemia (3-5). In contrast brain-derived neurotrophic factor (BDNF) is considered to have predominantly prosurvival activity both in health and disease and its tropomyosin-related receptor kinase B or tyrosine receptor kinase B (TrkB) signaling is a major

protective mechanism against ischemic injury (6). In this review we focus on the recent progress made in elucidating the mechanisms for crosstalk between BDNF and NMDAR prosurvival signaling pathways.

CALCIUM AS A SECOND MESSENGER

NMDAR-mediated Ca²⁺ increase

Glutamate-mediated trophic effects in the brain depend on the type of receptors, intensity of the signal, and the signaling context (7-10). Undoubtedly one of the most intricate signaling pathways involves the NMDARs, which are excitatory heterotetramer channels highly permeable for Ca²⁺ ions, require the coagonist glycine for proper function and are subject to

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voltage-dependent block by physiological Mg^{2+} concentrations. Blockade of normal NMDAR function induces apoptotic cell death of cortical and thalamic neurons (11-13). Currently there are seven NMDAR subunits known: NR1 (ζ 1), NR2A-D (ϵ 1-4) and NR3A-B (14,15). For five of the subunits (NR1, NR2B, NR2C, NR2D and NR3A) are reported splice variants. Most native NMDARs are assembled of two glycine-binding NR1 and two glutamate-binding NR2 subunits. NMDARs are therefore NR2-containing receptors in which NR1/NR2 het-

erodimers (Fig. 1) appear to be the functional unit (16). NR3 subunit containing NMDARs are expressed at late postnatal stages of CNS neuronal development (17) and could be either NR1/NR2/NR3 heteromeric channels with decreased Ca^{2+} permeability (18), or excitatory NR1/NR3 glycine receptors, which are insensitive for glutamate or NMDA (19). Activation of NMDARs leads to direct Ca^{2+} influx, and subsequent activation of Ca^{2+} -dependent protein kinases or protein phosphatases, which act as intracellular messengers.

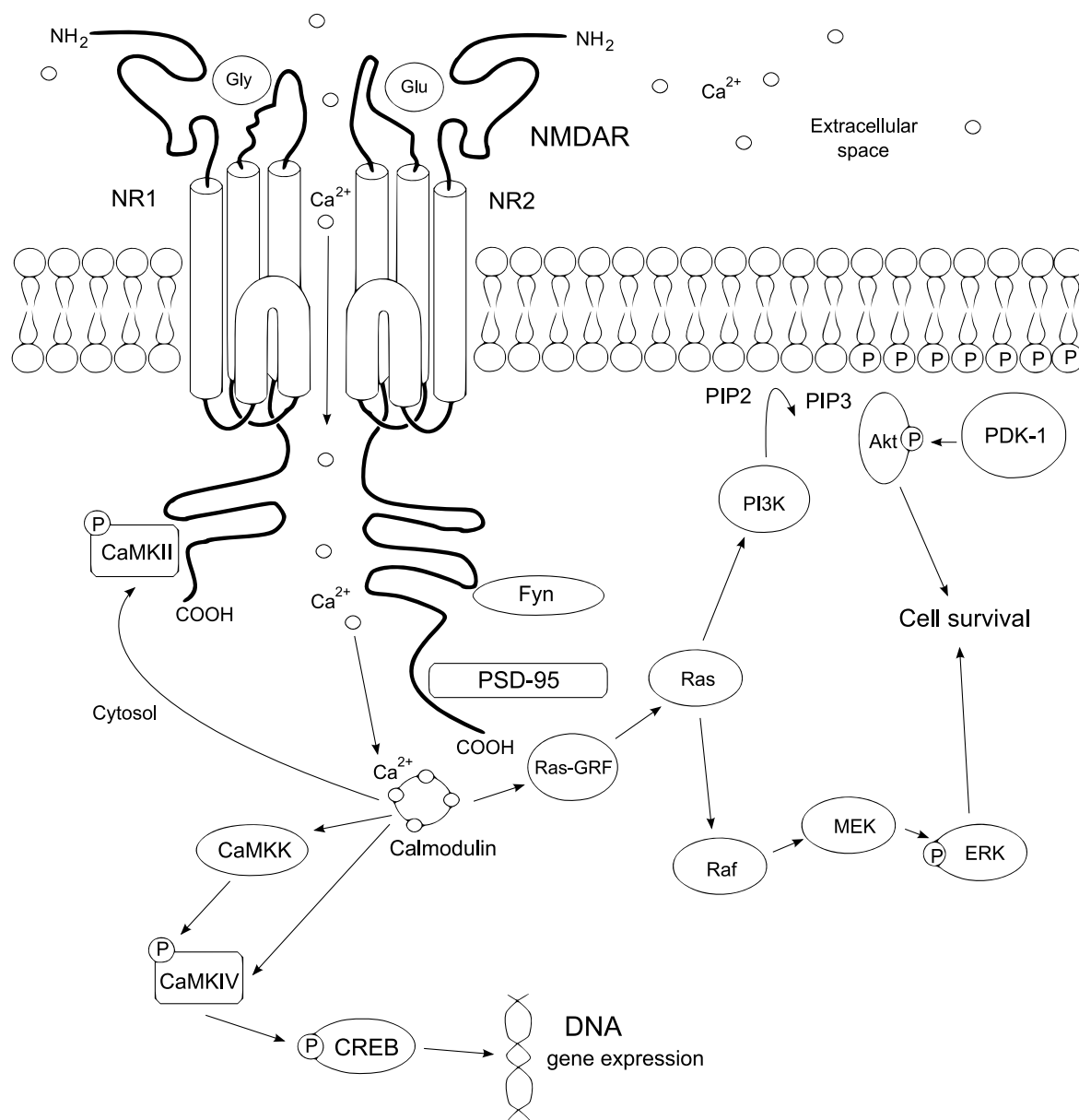


Figure 1. NMDAR signaling - triggering of Ca^{2+} /CaMK, PI3K/Akt and Ras/MEK/ERK pathways. Abbreviations: ERK, extra-cellular signal-regulated kinase; NMDAR, N-methyl-D-aspartate receptor; pCREB, phosphorylated cAMP response element-binding protein; PDK-1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol-3 kinase; PIP2, phosphatidylinositol 4,5 bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate.

TrkB-mediated Ca²⁺ increase

BDNF exerts its effect through TrkB that has tyrosine kinase activity triggering several distinct biochemical pathways, amongst which is the activation of γ -isoform of phospholipase C (PLC- γ). PLC- γ further cleaves phosphatidylinositol-4,5-bisphosphate (PIP₂) to produce diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP₃), the latter binding to IP₃ receptors (IP₃Rs) releasing Ca²⁺ from the endoplasmic reticulum. However, TrkB receptor activation also potentiates the Ca²⁺ influx through NMDARs via NR1 subunit phosphorylation (20) or NR2B subunit phosphorylation (21). It has been also shown that TrkB, Fyn and NR2B subunit form a multimeric protein complex and the extent of TrkB-Fyn-NR2B protein interaction increases in behavioral tests for spatial learning (22).

THE CALMODULIN/CAMK PATHWAY

NMDAR-mediated calmodulin/CaMK activation

Glutamate binding to NMDARs in the presence of the coagonist glycine directly opens the ion channel pore leading to Ca²⁺ influx from the extracellular space. Ca²⁺ ions then activate calmodulin, which is a small, acidic protein with a molecular mass ~17 kDa. It contains four EF-hand motifs, each of which binds a Ca²⁺ ion. Ca²⁺/calmodulin complex further activates Ca²⁺/calmodulin-dependent protein kinase kinase (CaMKK), which phosphorylates Ca²⁺/calmodulin-dependent protein kinases I and IV (CaMKI/IV), but not CaMKII, which is regulated by autophosphorylation. Though it did not seem clear why both the enzyme (CaMKK) and its substrates (CaMKs) both require binding of Ca²⁺/calmodulin (23), now there is an evidence that CaMKK phosphorylates and activates protein kinase B (PKB) triggering an alternative cascade pathway, and by this, has an anti-apoptotic effect on the cell (24). CaMK has several isoforms, of which CaMKIV is predominantly nuclear, while CaMKII may exist in the cytoplasm or nucleus, depending on the isoform mix of the heteromultimerized holoenzyme (25). In postsynaptic space CaMKII mediates long-term potentiation (LTP), which underlies memory storage and formation. Interestingly, CaMKII activity is regulated via docking to NR1 and NR2B subunits of NMDARs, where it might be locked in an active conformation (26,27). Auto-phosphorylated CaMKII α binds directly to NMDARs in the postsynaptic density, and phosphorylates NR2B subunits at S¹³⁰³, which leads to NR2B NMDAR desensitization (28). In cultured rat cerebellar granule neurons the pharmacological intervention either with CaMKII/IV inhibitor KN62 (29) or calmodulin inhibitor W13 (30) blocked the protective effects

of NMDA against exposure to low level K⁺ medium. Both CaMKII and CaMKIV might phosphorylate CREB at S¹³³ thus enhancing target gene expression such as BDNF (31). Moreover, it has been suggested that BDNF expression via pCREB is due to synaptic NMDAR activation, and not to extrasynaptic NMDAR function (32). Although initially it has been proposed that extrasynaptic NMDARs are NR2B, while synaptic are NR2A, and this subunit composition difference might explain the different roles of synaptic and extrasynaptic NMDARs in cell survival (32,33), currently there is evidence that NR2B receptors in synapses have prosurvival function, while NR2B receptors in extrasynaptic locations mediate cell death. This has been shown in rat primary hippocampal neuronal cultures, at an early developmental stage where the immature neurons express NR2B NMDARs both in synaptic and extrasynaptic locations, but do not express NR2A (8). Interestingly, it was shown that 65% of synaptic NMDARs are exchanged with extrasynaptic ones in less than 7 min via lateral diffusion process (34,35), which implies that the supramolecular organization and recruitment of specific transduction pathways to synaptic NMDARs is critical for NMDA-mediated survival.

TrkB-mediated calmodulin/CaMK activation

BDNF is a peptide with a molecular mass ~13kDa, which forms stable homodimers that are secreted in both constitutive and regulated pathways. Binding of BDNF homodimers to TrkB receptors triggers ligand-induced receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain (Fig.2), and at the Y⁵¹⁵-Shc and Y⁸¹⁶-PLC γ sites (36). Activation of PLC γ leads to IP₃-dependent release of Ca²⁺ from the internal stores with subsequent calmodulin and CaMKII or CaMKIV activation.

THE PI3K/AKT PATHWAY

NMDAR-mediated PI3K/Akt activation

Ca²⁺ influx through the NMDAR receptor leads to activation of the GTP/GDP exchange factor RasGRF via calmodulin (37), with subsequent activation of Ras having diverse intracellular effects amongst which is activation of PI3K (38,39). Phosphatidylinositol-3 kinase (PI3K) phosphorylates phosphatidylinositols of the cell membrane, thus generating phosphatidylinositol-3,4,5-trisphosphate (PIP₃) from phosphatidylinositol-4,5-bisphosphate (PIP₂). PIP₃ at the cell membrane recruits protein kinases such as PKB (known also as Akt kinase) and phosphoinositide-dependent kinase-1 (PDK-1), which bind with their pleckstrin homology (PH) domain to

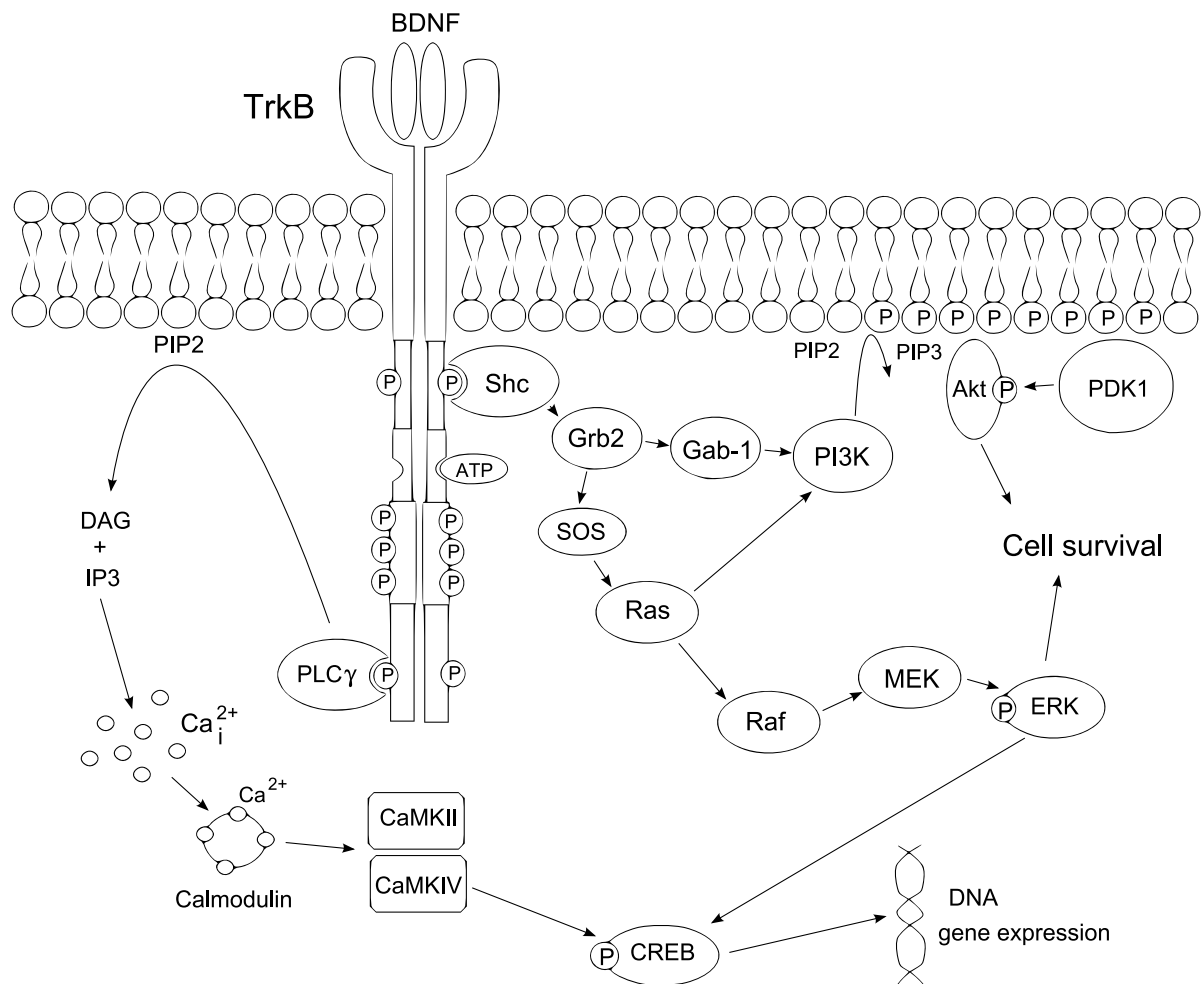


Figure 2. BDNF/TrkB signaling - triggering of Ca^{2+} /CaMK, PI3K/Akt and Ras/MEK/ERK pathways. Abbreviations: BDNF, brain-derived neurotrophic factor; DAG, diacylglycerol; Grb2, growth factor receptor bound protein-2; IP3, inositol-1,4,5-trisphosphate; PLC, phospholipase C.

PIP3 (40). Activation of PKB/Akt requires the phosphorylation by PDK-1, which is regulated by the conformation of PKB/Akt. Specifically, the engagement of the PH domain on the membrane by binding PIP3 relieves autoinhibition of the active site, allowing PDK-1 to access T³⁰⁸ on the PKB/Akt activation loop (41). Though PDK-1 is a major activator of PKB/Akt, there is a crosstalk with the calmodulin/CaMK pathway since both CaMK and CaMKK phosphorylate PKB/Akt (42). Active PKB then promotes growth and protein translation via phosphorylation of mTOR (43), and it was shown that NMDAR mediated neuroprotection requires both transcription activation and synthesis of new proteins (44). Moreover, PKB/Akt suppresses apoptosis via triggering phosphorylation of Bad, caspase 9, GSK-3 β (45) or dephosphorylation of MKL3,

JNK and c-Jun (46). The importance of PI3K/Akt signaling in NMDAR pro-survival activity has been shown in cultured rat cerebellar granule neurons, where PI3K inhibition with either LY294002 (47) or wortmanin (48) reduced NMDA protection against apoptosis in low level K⁺ medium (49).

TrkB-mediated PI3K/Akt activation

BDNF activation of TrkB, leads to phosphorylation of the Y⁵¹⁵-Shc receptor site that recruits Shc protein and subsequent activation of Grb2, Gab-1 and PI3K (50). PI3K then might mediate activation of PKB/Akt, which activates the kinase mTOR (Fig. 3). mTOR further phosphorylates eIF4E-binding proteins (4E-BPs), which leads to eukaryotic initiation factor 4E (eIF4E) liberation and local spine translation of CaMKII,

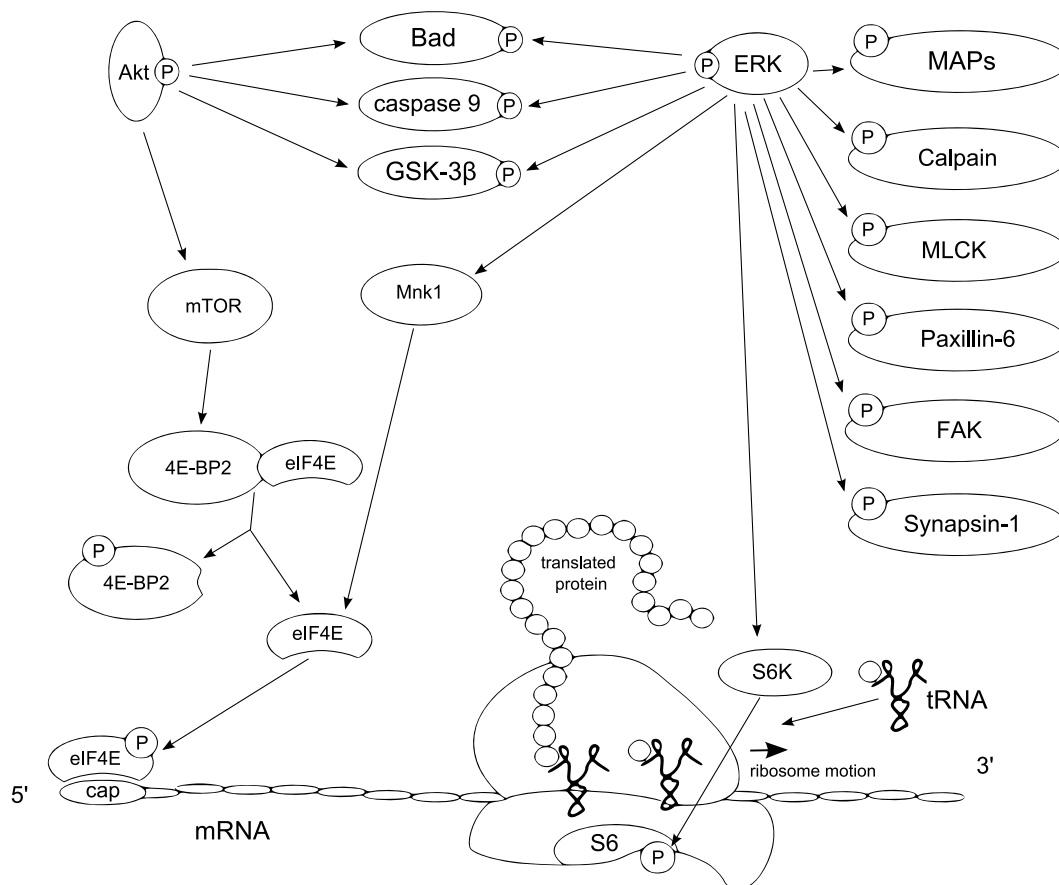


Figure 3. Akt/ERK control of protein translation and cellular activities (cytoskeletal dynamics, releasable pool of synaptic vesicles, apoptosis, etc.). Abbreviations: 4E-BPs, eIF4E-binding proteins; eIF4E, eukaryotic initiation factor 4E; FAK, focal adhesion kinase; MAPs, microtubule-associated proteins; MLCK, myosin light polypeptide kinase; mRNA, messenger RNA; tRNA, transport RNA.

Arc (activity-regulated cytoskeletal-associated protein), LIMK1 and GluR1, mediating the BDNF trophic effect on dendritic spines (51). Additional bifurcation of the biochemical pathway occurs at Grb2, which might activate SOS, followed by Ras, Raf, MEK, and ERK-1/2 activation (50). Mutations in TrkB Y⁵¹⁵-Shc receptor site impair both PI3K and MEK signaling pathways, thereby compromising neuronal survival and local axon growth (52).

THE ERK-1/2 PATHWAY

NMDAR-mediated ERK activation

NMDAR activation activates Ras via Ca²⁺/calmodulin-dependent activation of Ras-GRF (39) or enhancement of nitric oxide (NO) production by neuronal NO synthase (nNOS), which

is anchored to NMDARs via PSD-95 (53). Ras has a critical cysteine group which is nitrosylated and thus mediates the NO mediated activation (54). Except for activation of PI3K, Ras further activates Raf (also known as mitogen-activated protein kinase kinase kinase, MAPKKK), MEK-1/2 (also known as mitogen-activated protein kinase kinase, MAPKK), and ERK-1/2 (also known as mitogen-activated protein kinase, MAPK) (55). Recent evidence shows however that NMDAR-mediated ERK activation requires a specific coupling of NMDAR, Src and ERK, via caveolin-1, which helps the assembly of the signaling cascade within the neuronal lipid rafts (56). The organization of supramolecular complexes for NMDAR/ERK signal transduction explains the interesting observation that synaptic NMDARs activate ERK, while extrasynaptic

NMDARs trigger ERK shut off pathway (57,58). Activation of ERK-1/2 leads to CREB phosphorylation, expression of pro-survival/anti-apoptotic target genes (59) and activation of DNA repair enzymes (60). Other important intraneuronal targets phosphorylated by ERK-1/2 are the microtubule-associated proteins (MAPs), which participate in building the neuronal microtubule cytoskeleton (61). ERK-1/2 also phosphorylates myosin light polypeptide kinase (MLCK), calpain, paxillin-6 and focal adhesion kinase (FAK) that play important role in cytoskeletal rearrangement. Protein synthesis is controlled by ERK-1/2 via direct phosphorylation of 40S ribosomal protein S6 kinase (S6K), which further phosphorylates the ribosomal protein S6 and stimulates the cap-dependent translation (62). Moreover, ERK-1/2 enhances the release of neurotransmitters via phosphorylation of synapsin-1, which docks the synaptic vesicles in the presynaptic space (63). ERK-1/2 also mediates anti-apoptotic effects via phosphorylation of Bad (64), caspase 9 (65), and GSK-3 β (42).

TrkB-mediated ERK activation

BDNF activation of TrkB receptor also triggers ERK-1/2 pathway, via Ras, Raf and MEK. ERK-1/2 activity further mediates Arc gene expression and Arc mRNA transport to dendritic spines. Local Arc protein synthesis leads to formation of Arc/cofilin complexes, which reduce cofilin activity and thus promote actin polymerization and growth of dendritic spines (66). Cofilin phosphorylation at S³ also inhibits cofilin activity and enhances actin polymerization. The growth of the F-actin cytoskeleton transforms the active spines into big mushroom spines, which underlies the formation of long-term synaptic memory. In addition to the BDNF triggered PKB/Akt activation of mTOR, which regulates the availability of eIF4E, ERK-1/2 activates Mnk1 kinase, which phosphorylates eIF4E at S²⁰⁹ (51). The latter phosphorylation is considered to be a rate limiting step in protein translation. Therefore BDNF controls both the availability and activity of translation factors via bifurcation of the biochemical cascades at Grb2 or Ras.

BDNF-NMDAR POSITIVE FEEDBACK LOOPS

NMDAR mediated pro-survival activity in many cases is believed to be result from autocrine BDNF secretion and subsequent activation of ERK-1/2 and PKB/Akt pathways (67). NMDAR activation at postsynaptic sites leads to activity dependent BDNF release from secretory granules in the postsynaptic neurons, mediating retrograde BDNF signaling in the synapse (68). However, electric activity also stimulates

BDNF transport along the axon, and subsequent release from the presynaptic terminals of cortical neurons (69). Both cited studies used overexpression of BDNF-GFP fusion protein and both studies reported co-existent axonal and dendritic localization of the BDNF secretory granules, therefore it is essential to assume that BDNF acts both as anterograde and retrograde synaptic messenger. Secreted BDNF acts both on presynaptic and postsynaptic TrkB receptors, either to facilitate neuromediator release presynaptically (63) or to contribute to postsynaptic LTP mediated by enhancement of NMDAR and L-type VGCC function (70). It is interesting to note however that NMDAR function does not only regulate the autocrine secretion of BDNF, it has been shown that basal level of intracellular Ca²⁺ gates the TrkB receptor function and the subsequent activation of the PI3K/Akt pathway via calmodulin (71). Conversely, BDNF rapidly augments glutamatergic synaptic transmission via enhanced activity of NR2B NMDARs (72). BDNF-activated TrkB receptors form macromolecular complexes with Fyn kinase and NR2B NMDARs, leading to NR2B phosphorylation and potentiation of the NR1/NR2B NMDAR-mediated Ca²⁺ influx. Fyn kinase attaches with its SH2 domain to the C-terminal tail of NR2B NMDARs and phosphorylates tyrosine residues at three different sites - Y¹²⁵², Y¹³³⁶ and Y¹⁴⁷² (21,73,74). Additionally, BDNF enhances NMDAR ionic currents by stabilization of microtubules and promotion of microtubule-dependent transport of NR2B subunits with their subsequent incorporation in the dendritic membrane (75). This completes a fast positive feedback loop between NMDAR and BDNF signaling (Fig.4) – NMDAR function leads to activity-dependent BDNF release from secretory granules and Ca²⁺-dependent gating of TrkB function, while BDNF-mediated TrkB activation potentiates NMDAR currents and controls the NMDAR insertion in the dendritic membrane.

There is a second much slower positive feedback loop at the level of gene transcription. Particularly it has been shown that synaptic NMDAR activity leads to enhanced CREB phosphorylation and BDNF gene expression (32,76). NMDAR-mediated Ca²⁺ entry leads to decreased BDNF gene methylation (77) and upregulation of BDNF promoter III, via binding of pCREB, USF, and the novel Ca²⁺-responsive transcription factor CaRF (78,79) and derepresses the BDNF promoter IV activity, which is negatively regulated by BHLHB2 (80). NMDAR-activity regulated gene expression depends also on the NR1 subunit splice variants, and particularly on the inclusion of exon 21 encoding for the C1 cassette (81).

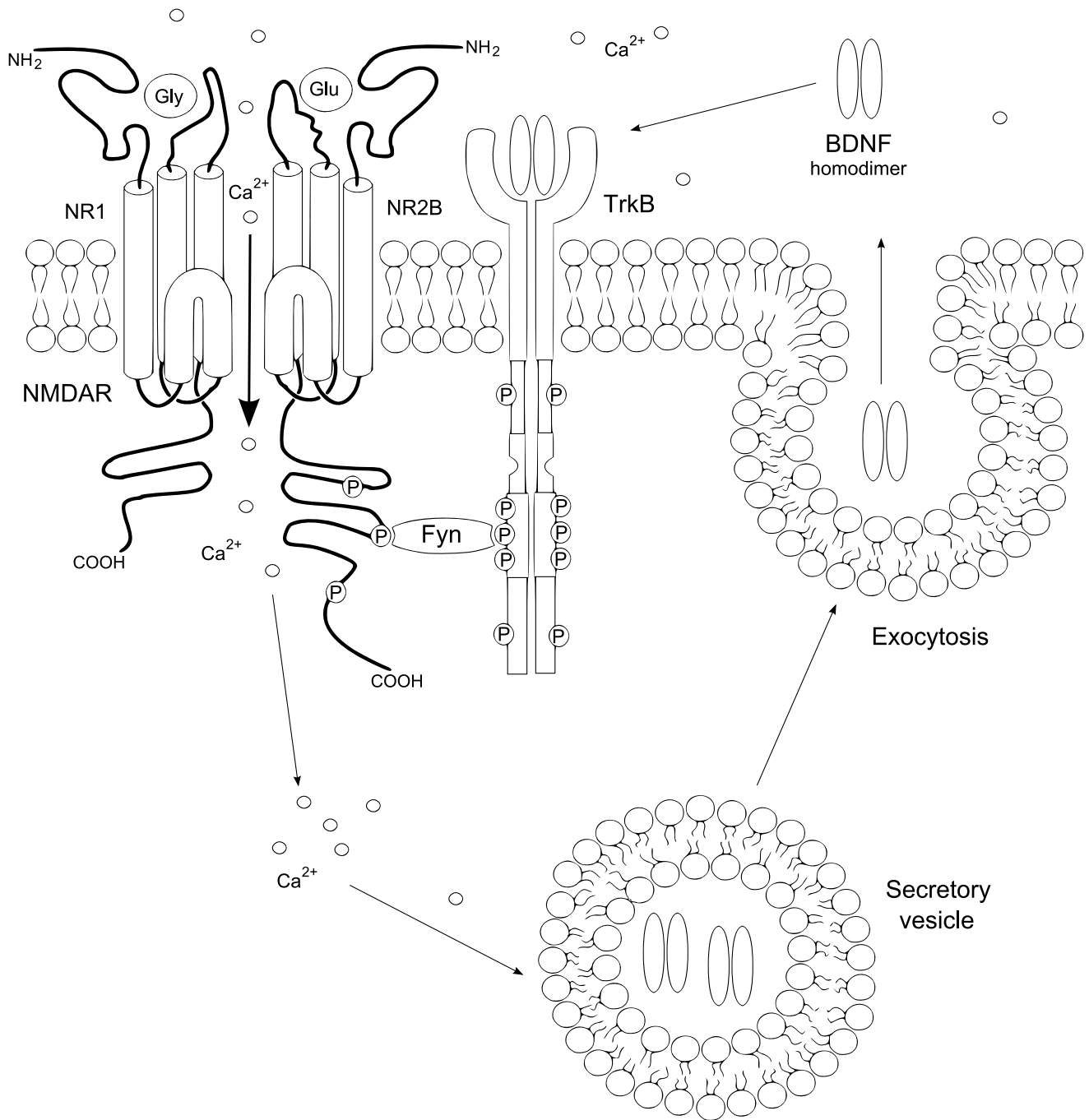


Figure 4. NMDAR-BDNF fast positive feedback loop. NMDAR activation leads to BDNF secretion from postsynaptic neurons, while BDNF enhances postsynaptic NMDAR currents via TrkB/Fyn/NR2B phosphorylation.

Moreover, it has been shown that NMDAR activity leads to transport and accumulation of mRNA^{BDNF} in distal dendrites, which accounts for local BDNF translation (82). Conversely, BDNF seems to be essential for NR2A but not NR2B subunit expression in cortex (83,84), and interestingly in cerebellum BDNF promotes the replacement of NR2B with NR2C subunits during neuronal maturation (85).

CONCLUDING REMARKS

The existence of fast and slow positive feedback loops as well as existence of multiple crosstalk possibilities at all major biochemical pathways triggered by NMDA or BDNF makes it extremely difficult to delineate the relative contributions of classical neurotransmitters (glutamate) and neurotrophins (BDNF) in prosurvival signaling. Recent data suggests that neurotransmitters such as glutamate might have 'trophic' effects in central nervous system, while neurotrophins might act as 'neurotransmitters' having fast modulating effects on synaptic function. Thus neurotransmitters and neurotrophins operate in concert and have overlapping functions *in vivo*, and the smearing of boundaries between these two groups of signaling molecules reflects the rapid progress in neurosciences made in the past decade.

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