The physiology of regulated BDNF release

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Abstract
The neurotrophic factor BDNF is an important regulator for the development of brain circuits, for synaptic and neuronal network plasticity, as well as for neuroregeneration and neuroprotection. Up- and downregulations of BDNF levels in human blood and tissue are associated with, e.g., neurodegenerative, neurological, or even cardiovascular diseases. The changes in BDNF concentration are caused by altered dynamics in BDNF expression and release. To understand the relevance of major variations of BDNF levels, detailed knowledge regarding physiological and pathophysiological stimuli affecting intra- and extracellular BDNF concentration is important. Most work addressing the molecular and cellular regulation of BDNF expression and release have been performed in neuronal preparations. Therefore, this review will summarize the stimuli inducing release of BDNF, as well as molecular mechanisms regulating the efficacy of BDNF release, with a focus on cells originating from the brain. Further, we will discuss the current knowledge about the distinct stimuli eliciting regulated release of BDNF under physiological conditions.

Keywords BDNF release • Neurotrophins • Secretion

Introduction

BDNF in health and disease

The neurotrophic factor BDNF plays an important role for the development of brain circuits, the formation and maintenance of neuronal morphology, brain architecture and for synaptic, as well as neuronal network plasticity (Edelmann et al. 2014; Gottmann et al. 2009; Huang and Reichardt 2001; Klein 1994; Lessmann and Brigadski 2009; Park and Poo 2013). Consequently, BDNF crucially regulates learning and memory processes in young and adult mammals (see, e.g., Boschen and Klintsova 2017; Gomez-Pinilla and Vaynman 2005) such that imbalances in BDNF levels and downstream signaling via its cognate TrkB tyrosine kinase receptor are associated with neurodegenerative and psychiatric diseases, like Alzheimer’s disease, major depressive disorder (Castren and Hen 2013), or schizophrenia (Mohammadi et al. 2018). Moreover, BDNF signaling also contributes to physiological functions of the heart and the vasculature and is involved in disorders like coronary artery disease (Ejiri et al. 2005; Jin et al. 2018; Kaess et al. 2015), diabetes mellitus (Eyileten et al. 2017; Suwa et al. 2006), inflammatory diseases such as asthma (Prakash and Martin 2014), different types of cancer (Chopin et al. 2016; Radin and Patel 2017), as well as pain sensation (Deitos et al. 2015; Haase et al. 2010; Laske et al. 2007; Merighi et al. 2008; Sapio et al. 2019). Furthermore, BDNF levels in preterm neonates differ from BDNF levels in full-term neonates (Malamitsi-Puchner et al. 2004), thereby affecting cognitive development in early postnatal life (Chau et al. 2017) and potentially being associated with children’s mental diseases such as autism spectrum disorders (Qin et al. 2016; Zheng et al. 2016).

Measurements of BDNF as a diagnostic marker

In human medical studies, ELISA or Western blot-based measurements of BDNF protein levels in body fluids (e.g., blood
or liquor) or tissue samples are considered as a potential proxy of intact brain (or other organ) function and associated diseases. As a result, great efforts are being made to utilize measurements of the BDNF levels as a potential marker for health status, diagnosis and prognosis of diseases and therapeutic outcome (Baliotti et al. 2018). Variations in BDNF genotype (compare Intracellular sorting of BDNF in neurons section) are considered to be covariate information in such approaches (Shen et al. 2018). In the blood, the secretory protein BDNF is mainly stored in platelets (Fujimura et al. 2002). However, little is known about the original source of circulating BDNF in blood plasma (Dawood et al. 2007; Klein et al. 2011; Krabbe et al. 2007; Rasmussen et al. 2009; Serramillas 2016). BDNF is prominently expressed in the brain, in hippocampus, cortex, amygdala and striatum but also in hypothalamus (reviewed in Edelmann et al. 2014). It is also expressed and released from endothelial cells (Cefis et al. 2020), cells of the immune system (lymphocytes, microglia), megakaryocytes (Chacon-Fernandez et al. 2016; Tamura et al. 2012) and others, like smooth muscle cells (reviewed in Edelmann et al. 2014). Activity-dependent release of BDNF from hypothalamic neurons, which are not shielded from the blood stream by the blood-brain barrier, likely contributes to blood BDNF levels. Transport of BDNF across the blood brain barrier was, although controversially discussed, also described for neurotrophins like BDNF (Pan et al. 1998; Rasmussen et al. 2009; Seifert et al. 2010). Therefore, BDNF released from neurons as a consequence of neural network activity in the brain can act locally by tuning the strength of the neural network but it may also act globally by affecting the function of other organs distant from the brain after diffusion into the bloodstream. Vascular endothelial cells as well as cells of the immune system represent another natural source of BDNF, which likely contribute to the regulation of BDNF levels in the blood (Kerschensteiner et al. 1999; Marie et al. 2018; Nakahashi et al. 2000). However, stimuli-inducing release of BDNF from non-neuronal tissue like platelets and vascular endothelial cells are not well understood.

In the human brain, analysis of post mortem tissue is currently almost the only way to estimate changes in BDNF levels. Decreased levels of BDNF have been detected post mortem in the cortex, hippocampus and nucleus basalis of Meynert in patients suffering from Alzheimer’s disease (Holsinger et al. 2000; Tapia-Arancibia et al. 2008), while region-specific down but also upregulation of BDNF has been shown in major depressive disorder patients (Krishnan et al. 2007; Pandey et al. 2008). However, to understand the relevance of change in BDNF levels in the brain, non-neuronal tissue and blood, a more detailed knowledge of the distinct stimuli-regulating BDNF expression and release in the different cell types is important. Most studies providing information about the molecular and cellular mechanism regulating intracellular and extracellular BDNF levels have thus far been performed in cells originating from the brain. The role of distinct subcellular BDNF release sites as well as release kinetics, e.g., for cell survival or synaptic plasticity is best studied in neurons (reviewed, e.g., in Edelmann et al. 2014). Therefore, this review will discuss recent advancements in cell-type specific activity-dependent release of BDNF, with a focus on BDNF release from cells originating from the brain. We will summarize the stimuli inducing or amplifying the release of BDNF as well as the molecular regulation of BDNF secretion and discuss the role of BDNF secretion in the context of synaptic plasticity and specific diseases.

**Synthesis, processing and sorting of BDNF**

**General aspects of BDNF synthesis**

The secretory protein BDNF is expressed in neuronal and non-neuronal cells (Katoh-Semba et al. 1997; Maisenpierre et al. 1990; Phillips et al. 1990). In neuronal cells, BDNF-immunoreactivity was found in several regions of the central nervous system as well as in the peripheral and enteric nervous system (Barakat-Walter 1996; Conner et al. 1997; Ernfors et al. 1990; Hoehner et al. 1996; Wetmore and Olson 1995; Yan et al. 1997; Zhang et al. 2007; reviewed, e.g., in Lessmann et al. 2003; Lewin and Barde 1996). In nonneuronal tissue, BDNF is synthesized in cells of the immune system, like T cells, B cell and monocytes (Kerschensteiner et al. 1999; Nakahashi et al. 2000), muscle cells (Matthews et al. 2009; Mousavi and Jasmin 2006), the heart (Donovan et al. 2000; Maisenpierre et al. 1990), thymus, liver and spleen (Katoh-Semba et al. 1997). The tissue-specific expression of BDNF is developmentally regulated (Ernfors et al. 1990; Katoh-Semba et al. 1997). Furthermore, physiological as well as pathophysiological conditions and interventions such as exercise, hypoxia, stress, epileptic seizures and ischemia increase expression of BDNF in a tissue-specific manner (Giannopoulou et al. 2018; Lippi et al. 2020; Thomas et al. 2016; Wetmore et al. 1994).

In humans, the BDNF gene is located on chromosome 11p14.1 consisting of 11 exons (I–X) and nine promoters that regulate the developmental and regional expression of multiple alternatively spliced mRNA isoforms (Pruunsild et al. 2007; Timmusk et al. 1993). Only exon IX at the 3’ end of the gene locus contains the major coding sequence for the BDNF precursor protein (Pruunsild et al. 2007). This BDNF coding sequence is translated into a pre-pro-protein, with an N-terminal pre-domain that guides the mRNA to the rough endoplasmic reticulum (rER). The pre-sequence is cleaved of cotranslationally and the immature uncleaved pro-BDNF protein is synthetized into the ER (compare Lessmann and Brigadski 2009). Importantly, transcription of the BDNF gene into a set of distinctively spliced mRNA variants (Aid et al. 2007; Pruunsild...
Bennett et al. 2007) is tightly regulated by electrical activity-induced Ca^{2+} elevation in neurons (e.g., Castrén et al. 1998). The distinct mRNA variants can be transported into dendrites (Baj et al. 2013), where local translation and release of BDNF can be confined to especially active dendritic stretches thereby promoting synaptic plasticity in a BDNF-dependent fashion. This makes BDNF ideally suited to shape developing and fine tune mature synaptic circuits through activity-dependent local synthesis and secretion (compare chapter “Physiological context of regulated release of BDNF section” in this review, see also Johnstone and Mobley 2020).

**Posttranslational processing of BDNF**

Secretory proteins are posttranslationally processed by different enzymes such as endo- or exopeptidases or glycosyltransferases (reviewed in Thomas 2002). The incidence of the diverse posttranslational modifications depends on the entire set of modifying proteins expressed by a specific cell, as well as on the intracellular amino acid composition in cytoplasm, ER and Golgi (Creemers 2002; Gidalevitz et al. 2013). Characteristic features of secreted proteins, such as biological activity, half-life time, or affinity to specific intracellular binding partners are modified by these events. The best-known example for a precursor protein (pro-protein) that undergoes multiple posttranslational modification steps is the hormone prolactin (POMC). POMC is the precursor of a variety of hormones like ACTH, MSH, or beta-endorphin. These diverse peptides are generated after endoproteolytic cleavage of the precursor protein POMC by subtilisine-like prohormone convertases PC1 or PC2. The thereby generated new N- and C-termini are further processed by exopeptidases, acetyltransferases and/or peptidyl-glycine alpha-amidating monooxygenases (compare Lessmann and Brigadski 2009). However, the corresponding processing of BDNF has not been intensively investigated so far. It is known that the 32-kDa precursor proBDNF can be processed endoproteolytically within the pro-domain by the enzyme subtilisin/kexin-isozyme 1 (SKI-1), generating a 28 kDa protein (Seidah et al. 1999). In addition to the 28-kDa cleavage product of the BDNF precursor, the 14-kDa mature BDNF is generated after cleavage of proBDNF by furin-like protein convertases PACE4, PC1, or PC5 (Mowla et al. 2001; Seidah et al. 1996). Inhibition of furin-like enzymes prevents the synthesis of the 14 kDa mature BDNF without any effect on the generation of the 28 kDa cleavage product (Mowla et al. 2001). Furthermore, extracellular processing of proBDNF to the 14 kDa mature BDNF isoform by the tissue-type plasminogen activator (tPA)/plasmin proteolytic system and by matrix metalloproteinases (MMP) 3, 7 and 9 has been described (Gray and Ellis 2008; Lee et al. 2001; Pang et al. 2004). Further modifications like N-glycosylation and glycosulfation within the prodomain of BDNF increase the half-life time of the protein (Mowla et al. 2001). These posttranslational modifications may also be a prerequisite for interactions of BDNF with chaperones or sorting receptors that guide the secretory protein BDNF predominantly to vesicles of the regulated secretory pathway (reviewed in Leßmann and Brigadski 2009).

**Intracellular sorting of BDNF in neurons**

Up to now, two sorting proteins have been identified for BDNF. Both, the chaperone sortilin and the exoproteolytic enzyme carboxypeptidase E, bind to the BDNF pro-domain and play an important role in sorting of BDNF to vesicles of the regulated pathway of secretion (Chen et al. 2005; Lou et al. 2005; Kojima et al. 2020). Interestingly, transfection of neurons with a cDNA construct, in which the pre-pro-domain of BDNF is fused to the mature part of the neurotrophin-4 (NT-4) redirects this BDNF-NT-4 chimera efficiently into vesicles of the regulated secretory pathway, whereas wildtype NT-4 is predominantly located in vesicles of the constitutive secretory pathway (Brigadski et al. 2005). This indicates the importance of the BDNF pro-domain for sorting of the protein to the regulated secretory pathway. The significance of the prodomain for correct sorting of BDNF to secretory granules is also stressed by the commonly observed Val66Met single-nucleotide polymorphism (SNP) located in the BDNF pro-domain. This SNP (carriers in Europe: ~ 60% Val/Val (WT); ~ 35% Val/Met; ~ 5% Met/Met) is associated with reduced sorting of Met-BDNF to vesicles of the regulated secretory pathway, resulting in impaired activity-dependent secretion of BDNF from neuronal cells (Egan et al. 2003). However, this SNP-dependent reduced sorting to secretory granules does not seem to affect all BDNF transcripts to the same extent (Jiang et al. 2009). In addition to the major sorting of BDNF to the regulated secretory pathway (Brigadski et al. 2005), BDNF is also directed to vesicles of the constitutive secretory pathway. However, little is known about the molecular composition of constitutively released vesicles as well as the function of BDNF released in a constitutively manner.

**Physiological context of regulated release of BDNF**

Just as there exist different cell types expressing and releasing BDNF, diverse stimuli are known to induce regulated release of BDNF. The best characterized stimuli are patterns of neuronal electrical activity like prolonged depolarization, high-frequency stimulation (HFS), or theta-burst stimulation (TBS) that trigger BDNF release in developing and mature neurons (Edelmann et al. 2014) (compare Stimuli-triggering BDNF release from neurons section) (Table 1). Release stimuli in electrically non-excitable cells differ, of course, from the
| Site | Ref. | Species / type of preparation | Method | Release stimulus | Pharmacology / molecular mechanism / time course of BDNF release |
|------|------|--------------------------------|--------|-----------------|---------------------------------------------------------------|
| Wilson Horch et al. 1999 | Organotypic slices | Postsynaptic BDNF-myc; TrkB-IgG | Elevated extracellular K⁺ | Postsynaptic BDNF overexpression alters density and stability of spines |
| Hartmann et al. 2001 | Rat; hippocampal cultures | Live cell imaging, BDNF-GFP | HFS | Reduced by: TTX; 0mM Ca²⁺; 2mM Cd²⁺/2mM Ni²⁺; Not affected: APV, DNQX, LY 341495 (mGluR antagonist) |
| Kojima et al. 2001 | Rat; hippocampal cultures | Live cell imaging, BDNF-GFP | Elevated extracellular K⁺ | Reduced by: APV, DNQX |
| Brigadski et al. 2005 | Rat; hippocampal cultures | Live cell imaging BDNF-GFP | Elevated extracellular K⁺ | Dependency of release kinetics on intragranular pH |
| Arancibia et al. 2007 | Adult rat; push pull perfusion + ELISA | ELISA | Intra supraoptic nucleus osmotic stimulation (1M NaCl for 10 min) | In vivo measurement of endogenous BDNF secretion |
| Kolarow et al. 2007 | Rat; hippocampal cultures | Live cell imaging BDNF-GFP | Elevated extracellular K⁺ | Kiss and run fusion events |
| Kuczewski et al. 2008 | Rat; hippocampal cultures | Live cell imaging BDNF-GFP | Depolarizations | Reduced by: GDPβS; Cd²⁺ |
| Dean et al. 2009 | Rat: E18-20; Mice: P1-3; neuronal cultures | Live cell imaging BDNF-pHluorin | Elevated extracellular K⁺ | Increased after syt-IV knockout; Reduced after syt-IV overexpression |
| Fiorentino et al. 2009 | Rat; hippocampal culture | Live cell imaging BDNF-GFP | Baclofen (10μM for 500sec - significant after 5 min application) | Reduced by: CGP55845 (GABA₄ receptor antagonist); Cd²⁺; Not affected by: NBQX, APV, bicuculline |
| Matsuda et al. 2009 | Rat E18-20, neuronal cultures | Live cell imaging BDNF-GFP | HFS field stimulation | Reduced by; CNQX, APV; CNQX; APV; nimodipine; Cd²⁺ |
| Wit et al. 2009 | Mouse; cortical culture | Live cell imaging BDNF-sp-pHluorin | Elevated extracellular K⁺ | Reduced by: nimodipine; Not affected by: CNQX + APV; CNQX; APV |
| Xia et al. 2009 | Rat; Hippocampal Cultures | Live cell imaging BDNF-GFP | Elevated extracellular K⁺ | TBS; Highest efficacy of BDNF release |
|                      |                  |                      |             | Transient and persistent release (deposit) |
|                      |                  |                      |             | Kiss and run fusion events; Release probability in soma is higher than in neurites; Onset of release in soma is delayed in neurites compared to soma |
|                      |                  |                      |             | Blocked by verapamil (L-Type VGCC) |
| Site                  | Ref.          | Species / type of preparation                               | Method                          | Release stimulus                          | Pharmacology / molecular mechanism / time course of BDNF release |
|----------------------|---------------|-------------------------------------------------------------|---------------------------------|-----------------------------------------|-----------------------------------------------------------------|
| Yang et al. 2009     | Xenopus; neuron myocyte coculture | selective knockdown of BDNF                                | Repetitive depolarization of myocyte | Not affected by conotoxin (N/Q-Type); agatoxin (P-type)      | Transsynaptic action of proBDNF ➔ p75 activation ➔ synaptic retraction |
| Jakawich et al. 2010 | Rat; hippocampal cultures         | TrkB-IgG; BDNF knockdown                                    | Inhibition of AMPAR (3h)         | Transcription dependent postsynaptic BDNF release mediates presynaptic increase in mEPSC frequency | Long 3’ UTR controls dendritic localization of BDNF mRNA Reduced by PCP (1 μM for 6h) (NMDAR blocker) ➔ although increased somatic and dendritic accumulation of BDNF Reduced by: TTX |
| Waterhouse et al. 2012 | Mouse; hippocampal cultures | Dendritic myc-IR                                            | Basal secretion                  | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Adachi et al. 2013   | Rat; cortical cultures and acute cortical slices | BDNF-IR ELISA                                                | Glutamate (15 min)               | Jakawich et al. 2010 Rat; hippocampal cultures | Similar release properties of BDNF-GFP in ESC-derived neurons and transiently transfected hippocampal neurons |
| Leschik et al. 2013  | Embryonic stem cell derived neurons | Live cell imaging BDNF-GFP                                  | Elevated extracellular K⁺         | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Petoukhov et al. 2013 | Rat; hippocampal cultures         | Live cell imaging BDNF-GFP                                  | 4-AP                            | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Kolarow et al. 2014  | Rat; hippocampal cultures         | Live cell imaging BDNF-GFP                                  | Elevated extracellular K⁺         | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Lu et al. 2014       | Rat; hippocampal slices; embryonic hippocampal cultures | TrkB-IgG; live cell imaging (BDNF-GFP)                      | Timing: bAPs + iontophoretic glutamate pulses | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Edelmann et al. 2015 | Organotypic slice; hippocampal slices | Live cell imaging                                            | 20 Hz + 8-Br-cAMP                 | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Shimojo et al. 2015  | Cortical cultures                 | Live cell imaging BDNF-pHuorin                               | Elevated extracellular K⁺         | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Wong et al. 2015     | Neuronal culture                  | BDNF-quantum dots                                           | TBS                             | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Baj et al. 2016      | Rat; hippocampal neurons          | ELISA                                                       | Potassium (3h)                   | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Eckenstaler et al. 2016 | Rat; hippocampal cultures; P0-P3, DIV 11-13; BDNF-GFP | Live cell imaging BDNF-GFP                                  | Elevated extracellular K⁺         | Bendix et al. 2013 Mouse; hippocampal cultures | BDNF is localized in progranulin positive vesicles in dendrites and axons Release reduced by: Ca-free solution; CdCl₂ Reduced by SNP (NO-donor) Not affected by: L-NMMA (NOS-inhibitor) |
| Site | Ref. | Species / type of preparation | Method | Release stimulus | Pharmacology / molecular mechanism / time course of BDNF release |
|------|------|-------------------------------|--------|-----------------|---------------------------------------------------------------|
| Harward et al. 2016 | Cortical cultures; organotypic slice | TrkB-IgG; live cell imaging; postsynaptic BDNF deletion | Glutamate uncaging | Reduced by APV; APV + NBQX; CN21 (inhibitor of CAMKII) Not affected by: NBQX |
| Henry et al. 2018 | Rat; hippocampal cultures | BDNF knockdown | Inhibition of AMPAR (3h) | mTor dependent translation of postsynaptic BDNF release mediates presynaptic increase in mEPSC frequency “Postsynaptic release”: LTP maintenance + increase in presynaptic release probability |
| Lin et al. 2018 | Hippocampal slice | Deletion of BDNF in CA1 region | 100 Hz-HFS | Postsynaptic release: LTP maintenance + increase in presynaptic release probability |
| Brigadski et al. 2019 | Rat; hippocampal cultures | Live cell imaging | Elevated extracellular K⁺ | comparable release kinetics in response to high potassium depolarization vs. electrophysiological stimulation |
| Leschik et al. 2019 | BDNF-GFP-knockin mouse, hippocampal cultures, expression regulated by endogenous BDNF promoter | Live cell imaging BDNF-GFP | Elevated extracellular K⁺ | Identical release properties of endogenous BDNF–GFP and overexpressed BDNF-GFP 20 % fusion events 60 % content release Max. of fusion events within 20 s of stimulation; fusion events at 100 s still prominent |
| Persoon et al. 2019 | Neuronal cultures | Live cell imaging | Elevated K⁺ | Rab3a, RIM1/2, Munc13 localized to BDNF-containing granules |
| Kohara et al. 2001 | Mouse, cortical cultures | GFP-IR | Analysis of somatic BDNF-GFP Endocytosis | Increased by: picrotoxin for 48 hours Reduced by: TTX (48 h); TrkB IgG (48 h) |
| Zakharenko et al. 2003 | Hippocampal slice | Deletion of BDNF | 200Hz-HFS | Increased after syt-IV knockout; Reduced after syt-IV overexpression Regulation of mEPSC frequency |
| Dean et al. 2009 | Rat: E18-20 Mice: P1-3 | Live cell imaging; BDNF-pHluorin | Potassium | |
| Matsuda et al. 2009 | Rat, E18-20; neuronal cultures | Live cell imaging; BDNF-pHluorin | TBS 50Hz (3min) or TBS (36 trains) | Induction of net BDNF release |
| Shinoda et al. 2011 | Mouse; hippocampal culture | Live cell imaging; BDNF-pHluorin | Potassium | Analysis of transient fusion events Reduced by: Bafilomycin; Dynasore; Cd²⁺ Not affected by K252a, TrkB-Fc; CNQX, APV; CNQX: APV; nimodipine |
| Sadakata et al. 2012 | Mouse, hippocampal granule cell culture | GFP-IR | Potassium | Analysis of transient fusion events Not affected by: nimodipine; CNQX, APV; CNQX: APV |
| Petoukhov et al. 2013 | Rat; hippocampal cultures | Live cell imaging | 4-AP | Reduced after: CAPS2 with deletion of exon3 |
| Site             | Ref.     | Species / type of preparation | Method                        | Release stimulus | Pharmacology / molecular mechanism / time course of BDNF release |
|------------------|----------|-------------------------------|-------------------------------|------------------|---------------------------------------------------------------|
| Shimoo et al. 2015 | Cortical culture; P1 | Live cell imaging; BDNF-pHluorin | Potassium                | BDNF is localized to progranulin positive granules in dendrites and axons. Release reduced by: Ca-free solution; CdCl |
| Lin et al. 2018 | Hippocampal slice; | Deletion of BDNF in CA3 | 100Hz-HFS                 | Full vesicle collapse is reduced in SNAP 47 kd cultures partial vesicle collapse is reduced in axons of SNAP 47 kd cultures 'presynaptic release': LTP-induction + LTP maintenance + increase in presynaptic release probability |
| Park 2018       | Mouse; corticostriatal slice | BDNF-pHluorin | TBS                      | Reduced by APV (presynaptic NMDAR); CPA (depletion of internal Calcium store); requires GluN1 subunit not affected by dopamine |
| Persoon et al. 2019 | Neuronal culture | Live cell imaging | HFS without Mg<sup>2+</sup> in extracellular solution | Rab3a, RIM1/2, Munc13 localized to BDNF-containing granules |

List of references for somatic and dendritic release of BDNF. 4-AP, 4-amino-pyridine; A2AR, adenosine A2A receptor; Aβ, amyloid-β; ACPD, 1-amino-1,3 dicarboxy cyclopentane; ATP, adenosine triphosphate; Br-cAMP, brom-adenosine 3’5’-cyclic monophosphate; CAMKII, Ca<sup>2+</sup>-calmodulin-dependent protein kinase II; CAPS, calcium-activated protein for secretion; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-APV, D(-)-2-amino-5-phosphonovalerate; DIV, days in vitro; E, embryonic day; ELISA, enzyme-linked immunosorbent assay; EP2, prostaglandin E receptor subtype2; GABABR, g-aminobutyric acid; HFS, high-frequency stimulation; ITI, intertrain-interval; IP3, inositol triphosphate; IR, immunoreactivity; LPS, lipopolysaccharide; NaV, voltage-gated sodium channel; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline; NMDAR, N-methyl d-aspartate receptor; P, postnatal day; P2XR, P2X purino receptor; p38MAPK, p38 mitogen-activated protein kinase; PAR1-AP, protease-activated receptor activating peptide, PCP, phenylcyclidine; PKA, protein kinase A; PKC, protein Kinase C.; PLC, phospholipase C; SpH, super ecliptic pHluorin; synt-JV, synaptotagmin-JV; TBS, theta-burst stimulation; TTX, tetrodotoxin; VGCC, voltage-gated calcium channels; TRPC, transient receptor potential channel.
major release stimuli in neuronal cells. Thus, binding of extracellular nucleotides (Coull et al. 2005; Trang et al. 2009; Ulmann et al. 2008; Vignoli and Canossa 2017), pro-inflammatory factors (Jornot et al. 2007), or neuropeptides (Lopez-Benito et al. 2018) to their corresponding receptors has been shown to induce release of BDNF from astrocytes and microglia (compare BDNF release from astrocytes section and BDNF release from microglia section) (Table 2). Next to the different physiological BDNF-releasing stimuli, knowing the subcellular sites where BDNF secretion can take place is important to understand cell-type and release-site specific functions of BDNF, e.g., during neuronal development, synaptic plasticity processes, or in case of disease (e.g., Hartmann et al. 2001; Matsuda et al. 2009; Xia et al. 2009; reviewed in Edelmann et al. 2014; Leßmann and Brigadski 2009; Park and Poo 2013).

**Stimuli-triggering BDNF release from neurons**

In the central nervous system, BDNF is important for the development of brain circuits and for synaptic as well as for network plasticity processes in the adult brain. These processes require fine-tuning of synaptic activity and structural synaptic rearrangements in an input specific manner. The spatially restricted release of BDNF is ideally suited for this purpose. Since early neuronal networks of immature neurons differ from functionally mature synaptic circuits, it is not surprising that distinct electrical activity patterns are efficient to induce release of BDNF and that the efficacy of releasing BDNF depends on the developmental stage of the neurons as well as on the specific brain area that is investigated.

**Stimuli-triggering BDNF release from developing neurons**

In developing hippocampus, a sequence of three different electrical activity patterns was described to be important to form synaptic circuitry (Crepel et al. 2007; Egorov and Draguhn 2013; Luhmann and Khazipov 2018). First, intrinsically active neurons generate brief L-type voltage-gated Ca\(^{2+}\) channel (VGCC)-mediated spikes at embryonic stage E16-E19, before an ensemble of neurons coupled by gap-junctions generates synchronous non-synaptic, long lasting calcium-plateaus, called synchronous plateau assemblies (SPA). These SPAs are replaced by giant depolarization potentials (GDPs), which represent a spontaneous pattern of network activity via chemical synaptic interactions. GDPs consist of large depolarizations associated with a burst of action potentials. These depolarizations last several hundreds of milliseconds, propagate by synaptic interactions as waves through the developing hippocampus and occur at low-frequency (0.003–0.06 Hz) in rodents during the first week of postnatal life (Egorov and Draguhn 2013; Luhmann and Khazipov 2018). GDPs have also been described for other developing brain areas like the neocortex, thalamus, or spinal cord. Development of cortical networks is known to progress in a similar manner as in hippocampal networks (Egorov and Draguhn 2013). However, the time course of onset of cortical development is shifted to postnatal stages (Hanganu-Opatz 2010; Kilb et al. 2011). Interestingly, the onset of BDNF expression in the rodent hippocampus becomes apparent already at E15.5 and differs from the onset of BDNF expression in cortical neurons, which appears at P4 (Baquet et al. 2004; Katoh-Semba et al. 1997). Furthermore, these electrically activity patterns (intrinsically active neurons, SPAs and GDPs) depend on activation of L-type VGCCs. Consistent with this, the release of BDNF has been reported to depend on Ca\(^{2+}\)-influx via L-type voltage-gated calcium channels (VGCC) in primary cultures of hippocampal neurons from embryonic or newborn rats and mice (Kolarow et al. 2007; Kuczewski et al. 2008; Matsuda et al. 2009; Kojima et al. 2020). Thus, it could be speculated that BDNF release from intrinsically active neurons, which display uncorrelated calcium transients, or BDNF release from gap-junction-coupled neurons, which generate non-synaptic calcium-plateaus might play a role in shaping neuronal networks during embryonic and early postnatal development. However, this has not been extensively investigated so far.

To date, only GDPs were shown to represent robust activity patterns to induce release of BDNF in this context (Kuczewski et al. 2008; Mohajerani et al. 2007). Kuczewski et al. (2008) combined whole-cell recordings and time-lapse fluorescence imaging of transfected hippocampal neurons and demonstrated BDNF-GFP release after repeated depolarization steps that were strongly reminiscent of GDPs (Kuczewski et al. 2008). Furthermore, Mohajerani et al. reported plasticity of CA3-CA1 synapses after pairing of GDPs with Schaffer collateral stimulation in hippocampal slices during the first postnatal week of development (Mohajerani et al. 2007). They described that the depolarizing action of GABA during GDPs induced calcium influx through L-type VGCC, thereby triggering plasticity of CA3-CA1 synapses by endogenously released BDNF (Gubellini et al. 2005; Mohajerani et al. 2007). The depolarizing effects of GABAergic transmission fade out with ongoing postnatal development and this maturation of hippocampal networks requires a shift in the expression of chloride transporters in neurons from K\(^{+}\)-Cl\(^{-}\)-cotransporter (KCC2) to Na\(^{+}\)-K\(^{+}\)-chloride\(^{-}\)-cotransporter (NKCC1), which switches GABA transmission to be hyperpolarizing. Since regulation of KCC2 expression is also mediated by BDNF (Rivera et al. 2002, 2005; reviewed in Fiumelli and Woodin 2007), depolarization-induced release of BDNF is important for proper shaping of hippocampal synaptic circuits.
Stimuli-triggering BDNF release from mature neurons

Depolarizing waves propagating through cortical networks represent on the one hand physiological activity patterns during neural circuit development but on the other hand, they have also been implicated in the pathophysiology of stroke, head trauma, or migraine aura (Hartings et al. 2017; Shen et al. 2016). Transient depolarizations of neurons induced by elevated extracellular K⁺-concentration have been described as a phenomenon called cortical spreading depression (CSD) or spreading depolarizations. CSD is characterized by waves of depolarizations that spread across the cortical surface at a low velocity of 2–5 mm/min. Consistent with activity-dependent regulation of BDNF, increases in BDNF mRNA and protein levels have been observed in response to CSD in rodents (Kawahara et al. 1997; Kokaia et al. 1993). Therefore, a protective role of CSD-induced BDNF expression and release enhancing the tolerance of further injury has been postulated (Kawahara et al. 1997; Shen et al. 2016). Interestingly, a very potent protocol to induce robust neuronal BDNF release is to elevate the extracellular K⁺-concentration, which in turn leads to depolarization and intracellular Ca²⁺-elevation. Such an elevated K⁺-induced release of BDNF was observed, e.g., in embryonic as well as postnatal neuronal cultures, neurons derived from embryonic stem cells and acute and organotypic hippocampal slices (Canossa et al. 1997; Goodman et al. 1996; Griesbeck et al. 1999; Hartmann et al. 2001; Kojima et al. 2001; Leschik et al. 2013; Leschik et al. 2019).

Stimuli-triggering BDNF release in synaptic plasticity

In addition to the development of brain circuits, BDNF is also involved in synaptic and network plasticity processes in mature circuits. Synaptic plasticity (i.e., LTP and LTD) is thought to be a cellular model of learning and memory processes. However, analyzing the activity patterns forming a memory together with the related structural changes of single synapses in a neuronal circuit in vivo is a challenging task. Although it is widely accepted that synaptic changes tune neural circuitry, the contribution of synaptic changes to memory encoding or memory consolidation is not resolved. Furthermore, the physiological correlates of the different activity patterns known to modulate synaptic efficacy, like high or low frequency stimulation, need to be identified. To date, several activity patterns shaping glutamatergic and GABAergic synapses either at pre- and/or postsynaptic sites have been investigated. The efficacy of the different patterns, of course, depends on diverse factors such as the developmental stage of the investigated neuronal circuit. The best-known patterns to induce long-term potentiation (LTP) of synaptic transmission in the hippocampus are high-frequency stimulation (HFS; e.g., repeated 1-s trains at 100 Hz), theta-burst stimulation (TBS; repeated bursts of 4–5 APs at ~ 100 Hz with inter-burst intervals of 0.2 s) or pairing protocols like spike timing-dependent plasticity (STDP; compare, e.g., Gottmann et al. 2009). Protocols that elicit long-term depression (LTD) of synaptic efficacy are characterized by low-frequency synaptic stimulation (LFS at 1 Hz). Both, LTP and LTD, are known to shape synapses at the functional, molecular and structural level. Activity-dependent release of endogenous BDNF or of fluorescently labeled BDNF has been intensively investigated as a cellular mechanism of LTP in brain slices and in neuronal cultures from different brain regions. Release of BDNF induced by HFS was shown, e.g., in PNS-cultured neurons (Balkowiec and Katz 2000), in hippocampal cultures (Balkowiec and Katz 2002; Hartmann et al. 2001; Kojima et al. 2001; Matsuda et al. 2009), amygdala (Meis et al. 2012) and cortico-striatal synapses (Jia et al. 2010), using either BDNF ELISA measurements or live cell imaging of fluorescently labeled BDNF. In contrast, similar patterns of high-frequency stimulation failed to induce efficient release of BDNF in dorsal horn slices from lumbal spinal cord (Lever et al. 2001). However, these authors were among the first to reveal TBS (300 bursts in 75 trains at 100 Hz with an interburst intervals of 0.2 s) as an effective means to induce release of BDNF (compare Balkowiec and Katz 2002). In addition to theta-burst discharges, BDNF release in spinal cord slices could also be elicited by injection of capsaicin, which in turn produces bursting activity reminiscent of theta bursts in the investigated nociceptors (Lever et al. 2001).

Bursting activity, which is repeated at a frequency of the theta rhythm, mimics also electrical activity patterns during hippocampal learning. Accordingly, TBS of Schaffer collateral CA1 synapses in acute brain slices was shown to efficiently release endogenous BDNF, thereby mediating postsynaptically expressed STDP (Edelmann et al. 2015). Correspondingly, TBS was also reported to release fluorescently labeled BDNF from either pre- or from postsynaptic sites in dissociated hippocampal cultures (Bergami et al. 2008; Matsuda et al. 2009) and from cortico-striatal presynaptic terminals (Park 2018). In addition to TBS-induced release of BDNF in hippocampal preparations, many other studies have analyzed the role of endogenously released BDNF in TBS-induced LTP, by either scavenging extracellular BDNF with superfusion of cells with selective BDNF antibodies, or by quantifying released BDNF by ELISA measurements in other brain regions. In this way, BDNF was shown to mediate TBS-LTP, e.g., at glutamatergic inputs to the amygdala (Li et al. 2011) or at retino-optical synapses (Du et al. 2009).

Although the BDNF-dependent LTP-inducing protocols seem to be very similar, the release sites of BDNF as well as the site of BDNF action depend on brain region and context (reviewed in Edelmann et al. 2014; Zagrebelsky et al. 2020). Moreover, even similar patterns of synaptic activity may induce mechanistically distinct types of LTP in the same neuronal circuit and subtle experimental details can determine in
| Site                        | Ref.          | Species/type of preparation | Method   | Release stimulus                                                                 | Pharmacology/molecular mechanism/time course of BDNF release                                      |
|----------------------------|---------------|----------------------------|----------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Saha et al. 2006           |               | Rat, astrocytic cultures   | ELISA    | TNF-alpha                                                                        |                                                                                                   |
| Jean et al. 2008           |               | Rat, basal forebrain       | Western blot, ELISA | Glutamate (100 μM for 10 min)                                                     | Reduced by: U73122 (PLC inhibitor); 2ABP (IP3 inhibitor); BAPTA-AM                                    |
| Baumbauer et al. 2009       |               | Rat                        | TrkB IgG | Tailshock                                                                        |                                                                                                   |
| Hutchinson et al. 2009     |               | Human Astrocytoma cells    | ELISA    | Prostaglandin E₂ (> 0.1 μM for 24 h)                                             | Reduced by: H-89 (PKA inhibitor)                                                                |
|                           |               |                            |          | butaprost (10 μM for 24 h-Ep₂ selective agonant)                                   |                                                                                                   |
|                           |               |                            |          | Forskolin (10 μM for 24 h)                                                        |                                                                                                   |
| Giralt et al. 2010         |               | Mouse, astrocytic cultures | ELISA    | TNF-alpha                                                                        |                                                                                                   |
| Hou et al. 2011            |               | Mouse, astrocytic cultures | ELISA    | Aβ42 oligomers (30 μM for 48 h)                                                   |                                                                                                   |
| Gimenez-Cassina et al. 2012|               | SH-SY5Y neuroblastoma cell | ELISA    | Inhibition of GSK3 (24 h)                                                         |                                                                                                   |
| Su et al. 2012             |               | Rat, cortical primary      | ELISA    | Progestosterone (P4) (0.1 nM for 18 h)                                            | Reduced after siRNA against progesterone receptor membrane component 1 (Pgrmc-1)               |
|                           |               | astrocytes                |          |                                                                                   |                                                                                                   |
| Zhang et al. 2012          |               | Rat, primary astrocytes    | ELISA    | Resveratrol (100 μM, 24 h)                                                        |                                                                                                   |
| Hong et al. 2016           |               | HD mice, primary astrocytes| ELISA    | Elevated K⁺                                                                        | Reduced BDNF release in htt expressing cultures                                                |
|                           |               | brain slices               |          |                                                                                   | Reduced amount of docked BDNF-containing vesicles in htt expressing cultures → rescued after Rab3a overexpression |
|                           |               |                            |          |                                                                                   | Increased BDNF release in Rab3a overexpressing cultures                                          |
|                           |               |                            |          |                                                                                   | Reduced association between Rab3-GAP1 and Rab3a by mHtt                                             |
| Sun et al. 2016            |               | Rat; C6 glioma cells;      | Western blot | Progesterone                                                                      | Increases the ratio of mature to pro-BDNF                                                      |
|                           |               | SH-SY5Y neuroblastoma cell |          |                                                                                   | Reduced after siRNA against progesterone receptor membrane component 1 (Pgrmc-1)               |
|                           |               |                            |          |                                                                                   |                                                                                                   |
| Sen et al. 2017            |               | Human primary astrocytes   | Western blot | ApoE3 + cholesterol (4 h)                                                         | Predominantly release of proBDNF                                                              |
|                           |               |                            |          |                                                                                   |                                                                                                   |
|                           |               |                            |          | ApoE2 + cholesterol (4 h)                                                         | Predominantly release of mBDNF                                                              |
|                           |               |                            |          |                                                                                   |                                                                                                   |
|                           |               |                            |          | Basal release                                                                      | Not affected by ApoE4 + cholesterol or cholesterol                                           |
| Vignoli and Canossa 2017   |               | Cortical astrocytic cultures | ELISA    | Elevated K⁺ Glutamate ATP                                                         |                                                                                                   |
| Datta et al. 2018          |               | Astrocytic culture from    | ELISA    | OHDA                                                                              | Reduced by L-NAME (NO synthase inhibitor)                                                        |
|                           |               | forebrain, midbrain,       |          |                                                                                   |                                                                                                   |
|                           |               | hindbrain                 |          |                                                                                   |                                                                                                   |
| Stahlberg et al. 2018      |               | Astrocytic cultures        | BDNF-mRFP |                                                                                   | Neuronal BDNF-mRFP is endocytosed by astrocytes via TrkB-receptor and is sorted to rab7-positive late endosomal compartment and LAMP1-positive lysosomal compartment |
| Su et al. 2019             |               | Rat, primary culture of    | ELISA    | TNF (3 h)                                                                         | Reduced by 5 BBD (P2x4R antagonist); TNP-ATP (P2x1-4R antagonist)                                |
|                           |               | Schwann cells              |          |                                                                                   | Not affected by PPADS (P2x1,2,3,5,7 antagonist)                                                   |

Release from microglia

| Ref.  | Species/type of preparation | Method | Release stimulus | Pharmacology/molecular mechanism/time course                                      |
|-------|----------------------------|--------|------------------|----------------------------------------------------------------------------------|
| Nakajima et al. 2002 | Rat, primary microglial culture | Western blot | C8-ceramide (16 h) or LPS (16 h) | Reduced by bisindolylmaleimide (PKC inhibitor)                                   |
| Coull et al. 2005   | Rat, primary microglial cultures | ELISA  | ATP (10 μM for 5 h) | Reduced by TNP-ATP (P2X receptor inhibitor)                                      |
| Hutchinson et al. 2009 | Human microglial cells; ELISA |        | Prostaglandin E₂ (> 0.1 μM for 24 h) | Reduced by H-89 (PKA inhibitor)                                                  |
|                   |                               |        |                  | Reduced by H-89 (PKA inhibitor)                                                  |
which way BDNF is involved in this plasticity. For example, spike timing-dependent long-term potentiation (t-LTP) is characterized by nearly coincident pairing of presynaptically induced excitatory postsynaptic potentials (EPSP) with postsynaptic firing of an action potential (AP) (Bi and Poo 1998). Such activity patterns were shown for the first time in the cortex of tadpoles to elicit BDNF release-dependent t-LTP (Mu and Poo 2006). However, slight changes in t-LTP protocols can decide whether t-LTP is mediated by BDNF or other modulators. In this respect, Edelmann et al. (2015) reported that a burst t-LTP protocol with 4 bAP was mediated by endogenously released BDNF, while t-LTP induced by stimulation protocols including only 1 bAP, occurred independent from BDNF release (Edelmann et al. 2015). These slightly different protocols did not occlude each other, indicating the differential physiological relevance of both protocols at the identical synaptic sites. In another study, regional deletion of BDNF either in the CA1 or the CA3 region revealed a role of presynaptically released BDNF in the induction of HFS-LTP, while postsynaptically released BDNF contributed to the maintenance of HFS-LTP (Lin et al. 2018). Complementary to the above-mentioned in vitro studies, Messaoudi et al. investigated the role of released BDNF in HFS-induced LTP in the hippocampus in vivo. Application of exogenous mature BDNF induced LTP (BDNF-LTP) in perforant path-granule cell synapses of the hippocampus, which occluded gene transcription dependent late-LTP but not early-LTP at the same synapses (Messaoudi et al. 2002). Future studies need to elucidate the role of proBDNF- and BDNF-dependent LTP in vivo in other synaptic circuits of the hippocampus and in additional brain areas (see also Zagrebelsky et al. 2020).

Subsequently to the release of BDNF from BDNF-expressing cells, the protein can also be released after endocytosis of the released BDNF (Fig. 5). Interestingly, the endocytosed BDNF can be further modified by the recipient cell before the re-release event. Such a re-exocytosis or recycling of BDNF has been reported for cultures of mature neurons, astrocytes and platelets (Bergami et al. 2008; Huang.

| Site Ref. | Rat, primary microglial culture | Rat, primary microglial culture | Rat, primary microglial culture | Cultured spinal cord slice | BV2 microglial culture | Mouse; primary microglial cultures |
|-----------|---------------------------------|---------------------------------|---------------------------------|--------------------------|------------------------|---------------------------------|
| Trang et al. 2009 | ATP (50 μM for 5–300 min) | ATP (50 μM for 60 min) | ATP (50 μM for 5–300 min) | ATP (60 min) | ATP (120 min) | IL-4 (12 h) |
| Gomes et al. 2013 | Lipopolysaccharide (LPS, 100 ng/mL for 6 h) | CGS21680 (30 nM for 6H-A2AR agonist) | Forskolin (1 μM) | 8-BrcAMP (5 μM) | Chelerythrine (PKC inhibitor) |
| Zhou et al. 2019 | CSF1 (6 h) | Reduced by SB 203580 (p38 MAPK antagonist) | Reduced by 5 BDBD (P2x4A antagonist); SB203580 (p38 MAPK antagonist) | Reduced by HA-TPSO (fusion construct of translocator protein (TPSO); FGIN-1-27 (TPSO agonist) | Reduced by: calcium-free solution; siRNA for P2X4A; TNP-ATP (P2x1-4R blocker); SB203580 (p38MAPK inhibitor) |

List of references for astrocytic and microglial release of BDNF (abbreviations: please see table legend of Table 1)
et al. 2014; Santi et al. 2006; Vignoli and Canossa 2017). In neurons, recycling of BDNF enables, on the one hand, the replenishment of the BDNF pool of releasing cells and re-use of BDNF after further processing steps. On the other hand, it also enables long distance distribution of synthetized BDNF across synaptically connected neuronal circuits and surrounding astrocytes after transcytosis of BDNF, a mechanism which was previously described or postulated for different neurotrophic factors (Bartheld and Johnson 2001; Butowt and Bartheld 2001; Wirth et al. 2005).

Endocytosis or re-uptake of BDNF is also an important mechanism to fill BDNF stores in platelets (Fujimura et al. 2002; Serra-Milàs 2016). These stores may constitute the major source of BDNF that can be released from platelets in response to blood vessel injury. Of note, the expression of BDNF not only in neurons but also in non-neuronal tissue particularly in the immune system and the cardiovascular system raises the interesting question how sorting and release of BDNF is regulated in these cells. Physiological stimuli, like shear stress in blood vessels but also pathophysiological and oncogenic stimuli, might orchestrate BDNF expression and release in these cell types as well as in cell types in which BDNF expression is under debate or even not proven so far. Future research addressing these important questions is critically needed.

**BDNF release from astrocytes**

**Stimuli-triggering BDNF from astrocytes during plasticity processes**

BDNF can be secreted from neuronal cells but also from other cells such as astrocytes. Quantifying BDNF release by ELISA measurements during synaptic plasticity processes or analyzing the contribution of BDNF during LTP by scavenging BDNF from extracellular space (Shelton et al. 1995) do not clarify the original release sites of BDNF. Moreover, a potential permissive function of BDNF for LTP, or acute early or late instructive actions of BDNF during LTP, may depend on BDNF release from different cellular sources. In the central nervous system, astrocytes are closely associated with synapses. One main function of astrocytes is the modulation of synaptic function. It is known that proBDNF is endocytosed by astrocytes in a p75-dependent manner during TBS-LTP in hippocampal or perirhinal cortex slices (Bergami et al. 2008; Vignoli et al. 2016). Extracellular cleavage of proBDNF by plasmin or deletion of glial p75 receptors inhibits endocytosis of proBDNF by astrocytes (Bergami et al. 2008; Vignoli et al. 2016). After recycling of endocytosed proBDNF and intracellular cleavage, the re-released mature BDNF contributes to the maintenance of TBS-LTP in a postsynaptic TrkB-dependent manner (Vignoli et al. 2016). Furthermore, mice that were unable to recycle BDNF failed to recognize familiar from novel objects. Therefore, astrocytic BDNF recycling was shown to be essential for visual recognition memory (Vignoli et al. 2016). Besides the astrocytic recycling of BDNF, neuronal recycling of BDNF has also been shown to contribute to TBS-LTP maintenance in hippocampal slices (Santi et al. 2006). The role of astrocytic recycling has not been addressed in this study. Whether neuronal and astrocytic recylings of BDNF coexist or whether they are successive processes need to be clarified. In contrast to the endocytosis of pro-BDNF, another study suggests that solely the mature form of BDNF is endocytosed by astrocytes via truncated TrkB receptors in dissociated cultures (Stahlberg et al. 2018). Interestingly, inflammatory stimuli like released interferon-gamma increased astrocytic expression of truncated TrkB, which was associated with increased BDNF recycling capacity of astrocytes (Rubio 1997). Additional studies are needed to clarify whether astrocytic recycling of mature BDNF via truncated TrkB-receptors contributes to astrocyte-dependent synaptic plasticity underlying memory formation or whether it is a part of inflammatory processes.

During plasticity processes, different stimuli might induce release of recycled BDNF from astrocytes. Since glutamate is known to induce the release of astrocytic BDNF (Jean et al. 2008; Santi et al. 2006; Vignoli et al. 2016), the prolonged glutamate release from the presynaptic terminal may be important for the release of recycled BDNF from astrocytes during TBS. These authors showed that the effect of glutamate was mimicked by agonists of AMPA or mGluRI/II receptors (Bergami et al. 2008). Interestingly, TBS activity is likely to induce elevated extracellular potassium levels. Consistent with this, BDNF release from astrocytes was also shown to be induced by elevated extracellular potassium concentration (Stahlberg et al. 2018; Vignoli and Canossa 2017). However, HFS was ineffective in releasing recycled BDNF from pure astrocytic cultures (Bergami et al. 2008).

Another stimulus to induce BDNF release was described by Canossa et al., in hippocampal slices and in neuronal cultures (Canossa et al. 1997). There, BDNF-induced BDNF release was observed (Canossa et al. 1997; Nakajima et al. 2008 but compare Kolarow et al. 2007). This mechanism might also occur in astrocytes during synaptic function to refill stores of BDNF and simultaneously to induce release of a readily releasable pool of BDNF vesicles. Taken together, processing and release of BDNF from astrocytes during synaptic plasticity process and memory formation are factors that should not be neglected.

**Stimuli-triggering BDNF from astrocytes in inflammatory or neuroprotective processes**

In addition to synaptic functions, astrocytes have several other functions in the CNS. They are important for the regulation of energy supply and for the homeostasis of neurotransmitters
and ions. They are involved in function and maintenance of the blood brain barrier and they have immunoregulatory functions. Different stimuli are known to activate one or the other effect of astrocytes, respectively. One player that is associated with inflammation, pain, or immune response in astrocytes is prostaglandin E2 (PGE2). PGE2 has been shown to induce release of BDNF from astrocytes (Hutchinson et al. 2009). In addition to PGE2, the proinflammatory cytokine TNF-alpha was also shown to induce BDNF release from astrocytes (Giralt et al. 2010; Saha et al. 2006).

Furthermore, astrocytes are known to mediate neuroprotective functions, which involve BDNF release (Su et al. 2012). However, neuroprotective functions of astrocyte-derived BDNF were shown to be region-specific (Datta et al. 2018). These authors showed that cultured astrocytes from different brain regions exhibit distinct efficacy of BDNF release in response to oxidative stress induced by 6-hydroxydopamine (6-OHDA).

In line with the neuroprotective function, astrocytes are also important in regulating the glymphatic system. This waste clearance system of the brain has been described as a tunnel system around blood vessels formed by astrocytes. It is important for clearing the brain from toxic compounds like Amyloid-β, which is itself a stimulus to induce BDNF release from astrocytes (Hou et al. 2011). However, neuronal release of BDNF was unaffected in transgenic Alzheimer models although transport of BDNF was impaired already after acute application of Amyloid-β (Seifert et al. 2016). In conclusion, regulation of the glymphatic system in response to Amyloid-β might be a BDNF-mediated neuroprotective effect of activated astrocytes in the brain.

**BDNF release from microglia**

Similar to astrocytes, the primary immune cells of the brain, the microglia, also mediate several functions in the CNS, such as neuroprotection and shaping of synaptic spines (Dubbelaar et al. 2018; Stratoulias et al. 2019). Different microglia phenotypes exist, which can be transformed into one another by different chemokines and intercellular interactions, thereby exerting distinct functions in the brain (Orecchioni et al. 2019; Xue et al. 2014). One older simplifying in vitro classification of microglia, the M1/M2 concept, groups the different overlapping activated microglial phenotypes into two extremes: the proinflammatory lipopolysaccharide-(LPS)-induced M1 phenotype and the neuroprotective IL-4-induced M2 phenotype (Werry et al. 2019). BDNF expression and secretion from microglia have been shown for both activated M1 and M2 phenotypes but also for non-activated M0 microglia (Coull et al. 2005; Nakajima et al. 2001; Nakajima et al. 2002; Zhou et al. 2020). For the neuroprotective phenotype, extracellular accumulation of BDNF was shown after polarization of microglial cultures with IL-4 (Zhou et al. 2020). Furthermore, Merlo et al. demonstrated an Amyloid-β 42-induced release of BDNF thereby mediating a neuroprotective effect in cultures of a neuroblastoma cell line (Merlo et al. 2018).

LPS or 8-ceramide-induced polarization of microglia to the proinflammatory phenotype leads to an increase in extracellular mature BDNF that was described to depend on surface expression and activation of adenosine A2a receptor (Gomes et al. 2013; Nakajima et al. 2002). Furthermore, surface expression of P2X4 adenosine receptors (P2X4R) drives microglia polarization towards the pro-inflammatory phenotype. The surface expression of P2X4Rs in microglia was associated with central sensitization like pain hypersensitivity or allodynia (Tsuda et al. 2013). Hypersensitivity in chronic pain was also associated with release of BDNF from microglia (Zhou et al. 2019). The uncovered molecular mechanism underlying BDNF-dependent hypersensitivity in chronic pain involved the small G protein MAPK p38 (Zhou et al. 2019). Activation of this protein is known to be important in P2X4R-mediated release of BDNF from microglia (Coull et al. 2005; Ferrini et al. 2013; Long et al. 2020; Trang et al. 2009). Activation of this purinergic ligand-gated cation channel (P2X4R) by extracellular ATP leads to an increase in intracellular calcium concentration, thereby triggering distinct molecular mechanisms to induce BDNF release from microglia.

**Molecular mechanisms underlying release of BDNF**

**Calcium transients and BDNF secretion**

Fusion of transmitter and peptide vesicles depends on calcium ions, which are essential for triggering membrane fusion and on ATP or GTP supply for energy consuming steps of exocytosis such as priming of vesicles and disassembly of the SNARE complex. In recent years, great efforts have been made to uncover the molecular mechanisms regulating exocytosis of BDNF-containing secretory granules. It is well established that the essential calcium transients to trigger membrane fusion may have extracellular and/or intracellular origin. Activation of NMDA receptors (Hartmann et al. 2001), AMPA receptors (Canossa et al. 2001; Hartmann et al. 2001), L-type, N-Type or P/Q-type voltage-gated calcium channels (Buldyrev et al. 2006; Kolarow et al. 2007), transient receptor potential channels (TRPC) (Vohra et al. 2013), or ATP-gated purinergic receptors (P2XR) (Long et al. 2020; Trang et al. 2009) have been shown to contribute to the calcium transients in different BDNF-releasing cell types (see Tables 1, 2, 3 and Fig. 1). Moreover, calcium release from internal calcium stores crucially contributes to BDNF release (Kolarow et al. 2007) and is of particular importance for BDNF release in response to external stimuli acting on G protein-coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs).
| Ref. | Species/type of preparation | Method | Release induction protocol | Pharmacology/molecular mechanism/time course |
|------|-----------------------------|--------|----------------------------|---------------------------------------------|
| Wetmore et al. 1994 | Rat | Immuno-histochemical staining | Kainat injection | Increase in DNQX dependent transcription of BDNF, irrespective of NMDA activation |
| Goodman et al. 1996 | Rat; E16, hippocampus | Western blot | Potassium | Blocked in At20 cells in calcium-free solution |
| Figurov et al. 1996 | Hippocampal slice | TrkB-IgG | HFS | |
| Kang et al. 1997 | Hippocampal slice | TrkB-IgG | TBS | TrkB-IgG application 0 min and 30 min after LTP induction |
| Canossa et al. 1997 | Rat; hippocampal culture E17, Hippocampal slice | ELISA | NT-4, NT3 | Reduced by: K252; BAPTA-AM, Not affected by: CNQX; BAPTA |
| Goodman et al. 1996 | Hippocampal slice | ELISA | TBS | Potassium (up to 150%) |
| Griesbeck et al. 1999 | Hippocampal slice | TrkB IgG | TBS | Block to ctrl-level in the presence of BAPTA-AM (intracellular calcium scavenging) |
| Balkowiec and Katz 2000 | Rat; cultures from nodose and petrosal ganglia neurons | ELISA | Potassium (72 h) | Accumulation of BDNF |
| Canossa et al. 2001 | Hippocampal slice | ELISA | glutamate, AMPA, t-ACPD, NGF | No release after NMDA application |
| Patterson et al. 2001 | Mouse; hippocampal slice | TrkB-IgG | TBS | Regulated by cAMP (reduction of forskolin-induced phosphorylation of TrkB in the presence of TrkB-IgG) |
| Balkowiec and Katz 2002 | Rat; hippocampal neurons | ELISA | TBS | Blocked by TTX; conotoxin; extracellular Ca-free; dantrolene + thapsigargin; |
| Canossa et al. 2002 | Rat, hippocampal neurons; E17 | ELISA | TBS | Blocked by TTX; conotoxin; extracellular Ca-free; dantrolene + thapsigargin; |
| | | | t-ACPD (activator of mGlur) | Increased by KT5823 (inhibitor of PKG) | |
| Canossa et al. 2002 | Rat, hippocampal neurons; E17 | ELISA | Network activity | Reduction up to 75% in the presence of glutamate receptor antagonists |
| | | | | Increase up to 140% 20 min after L-NAME application |
| | | | | Reduced after application of SNP (NO-donor); NOR3 (NO-donor); YC1 (agonist of sGC); 8 Br-cGMP |
| Ref.               | Species/type of preparation | Method          | Release induction protocol | Pharmacology/molecular mechanism/time course                                                                 |
|-------------------|-----------------------------|-----------------|----------------------------|---------------------------------------------------------------------------------------------------------------|
| Gartner and Staiger 2002 | Rat; hippocampal cultures from E19 | ELISA           | 50 Hz train                | Reduced by: TTX; Thapsigargin + caffeine; IP3R antagonist Not affected by: NMDAR antagonist; extracellular calcium-free solution |
| Egan et al. 2003   | Rat, hippocampal culture E20 transfected with valBDNF or metBDNF | GFP-ELISA       | Potassium                  | Reduced in neurons transfected with metBDNF-GFP                                                                 |
| Aicardi et al. 2004 | Hippocampal slice           | ELISA           | TBS-100 Hz                 | Release within 10 min after TBS Reduced BDNF release after LFS (5 Hz or 1 Hz)                                           |
| Ba et al. 2005     | Brain stem spinal cord preparations E16 | ELISA           | Basal level                | Reduced by TTX; receptor antagonists Increased by elevated potassium                                             |
| Buldyrev et al. 2006 | Rat; Trigeminal neuronal culture | ELISA           | Varying frequency          | Reduced by: extracellular calcium-free solution; N-Type VGCC blocker; L-type blocker; P/Q-type VGCC blocker (reduction to 50% after inhibition of one of the receptor) |
| Guo et al. 2006    | Rat; brainstem/rostral ventromedial medulla slice | TrkB-phosphorylation; TrkB-IgG | TBS                        | Not induced by NMDA application Not induced by NGF Reduced by NO donor NOR3 |
| Santi et al. 2006  | Hippocampal slice incubated with BDNF-YFP | Live cell imaging-release of endo-cytosed BDNF | Basal secretion            | Not induced by NMDA application Not induced by NGF Reduced by NO donor NOR3 |
| Nakajima et al. 2008 | Hippocampal E17 culture    | FRET Cell-based fluorescent indicator for BDNF | BDNF 3.5 nm                 | Not affected by TTX; APV + CNQX |
| Tanaka et al. 2008 | Organotypic hippocampal slice | TrkB-IgG      | Uncaging glutamate + bAP     | Not induced by unpaired stimulus                                                                                     |
| Babu et al. 2009   | Mouse, hippocampal culture P0 | ELISA          | Glycine (60 min)           | Glycine treatment do not induce NT-3 release                                                                     |
| Du et al. 2009     | Xenopus retinotectal system with pre and postsynaptic knockdown | Knockdown of site-specific TrkB expression | TBS |                                                                                                                     |
| Jourdi et al. 2009 | Neuronal cultures           | Western blot    | CX641 (Ampakine)            | Reduced by: CNQX, Ca-free solution; nifedipin; ryanodin                                                          |
| Fritsch et al. 2010 | Motor cortex slice          | TrkB IgG       | Direct current stimulation (DCS)-LTP |                                                                                                                     |
| Hsieh et al. 2010  | Nodose ganglion neuronal culture | ELISA           | 6-Hz stimulation            | Reduced by SNAP (NO donor) Rescued by Tempol (radical scavenger, prevention of S-nitrosylation)                |
| Ref.                | Species/type of preparation                | Method         | Release induction protocol | Pharmacology/molecular mechanism/time course |
|--------------------|--------------------------------------------|----------------|---------------------------|--------------------------------------------|
| Li et al. 2010     | Hippocampal slice                          | TrkB IgG       | TBS                       | MF(IAmpl) reduced in the presence of (glutamate and GABA receptor antagonists) + TTX |
| Li et al. 2011     | Rat; brain slice                           | TrkB-Fc        | TBS                       | LTP in BLA blocked by TrkB-Fc              |
| Porcher et al. 2011| Cortical neurons                           | BDNF-IR        | Muscimol (10 min) Ctrl    | Reduced by: bicuculine (back to control level) Reduced by: TTX: reduction to 50% |
| Meis et al. 2012   | Coronal slices                             | TrkB-IgG       | Pairing protocol: presynaptic 100 Hz + postsynaptic Depolarization | LTP blocked by APV LTP blocked by Pep1-TGL |
| Chen and Russo-Neustadt 2013 | Rat; E18; hippocampal cultures | ELISA          |                            | Norepinephrine                            |
|                   | Significant increase after 5-HT            |                |                           | Significant increase after 10 min          |
| Schildt et al. 2013| Adult mouse; hippocampal slice             | TrkB-Fc        | 50 Hz stimulation         | Reduced MF LTP in the presence of TrkB-Fc |
| Lepack et al. 2014 | Rat; cortical culture; E18                 | ELISA          | Ketamine                  | Reduced by verapamil (inhibitor of L-type VGCC) blocked by NBQ (inhibitor of (AMPAR)) |
| Briz et al. 2015   | Neuronal cultures                          | TrkB-Fc; Western blot Estradiol (1 h) G1 (1h) | G1 = G-protein-coupled estrogen receptor 1 agonist (GPER1) Not affected by ERalpha or ERbeta agonists PPT and DPN |
| Edelmann et al. 2015| Hippocampal slice                          | TrkB-IgG       | Burst t-LTP               | NMDAR-dependent                            |
| Zhao et al. 2015   | Mouse; somatosensory cortex                | TrkB blocking; BDNf rescue | TBS                       | Heterosynaptic facilitation of structural LTP |
| Hedrick et al. 2016| Rodent hippocampal slice                   | TrkB-Fc        | Glutamate-uncaging        | No change in BDNF release after Okadaic acid (8 h) Increase in BDNF level |
| Atasoy et al. 2017 | Rat E16                                   | ELISA          | Okadaic acid (24 h)       | Reduced by TTX; ryanodin                   |
| Lao-Peregrin et al. 2017 | Mouse, hippocampal slice                    | ELISA          | Caffeine (5 min)          | Reduced by verapamil                       |
| Kato et al. 2018   | Rat, primary cortical culture              | Western blot; ELISA GLYX-13 (allosteric modulator of NMDAR) | Reduced by verapamil |
| Lopez-Benito et al. 2018 | DRG and cortical neurons, in vivo, in HD mice | ELISA          | Basal levels Physical activity | Increased by shRNA for ARMS Increased by shRNA for ARMS Increase by shRNA for ARMS Increased by shRNA for ARMS In vivo BDNF release in different regions at different time points |
| Yu et al. 2018     | Cortical neurons of zQ175 mice (HD model) | BDNF-pHluorin; live cell imaging | 50 Hz | Smaller proportion of BDNF-containing vesicles undergoing full release |
| Zimbone et al. 2018| Rat; E15                                   | ELISA          | Basal release Abet1-42 (100 nM, 24 h) IGF (5 ng/ml, 24 h) | Not affected by oligoAbeta Reduced by: selective inhibitor of IGF-IR (picropodophyllin) Not effected by: oligomeric Abet1-42 Transcription dependent increase in BDNF level; increase in BDNF level to 150% Transcription dependent increase in BDNF level, increase in BDNF level to 190% |
the brain area of the investigated neuron (Fig. 2b). Furthermore, BDNF release might depend on the developmental stage and 2008), although the specific type of VGCC contributing to Hartmann et al. 2001; Kolarow et al. 2007; Kuczewski et al. 2008) (Fig. 2a). In these cases, calcium influx was mediated via L-type VGCC (Balkowiec and Katz 2002; Buldyrev et al. 2006; Hartmann et al. 2001; Kolarow et al. 2007; Kuczewski et al. 2008), although the specific type of VGCC contributing to BDNF release might depend on the developmental stage and the brain area of the investigated neuron (Fig. 2b). Furthermore, dendritic elevated potassium-induced release of BDNF in hippocampal cultures was—in contrast to bAP-induced dendritic BDNF release—dependent on calcium influx from internal calcium stores (Griesbeck et al. 1999; Kolarow et al. 2007; Kuczewski et al. 2008) (Fig. 2). This is in line with the role of BDNF during development of neural networks (compare Stimuli-triggering BDNF release from developing neurons section). The maturation of functional synapses might depend on BDNF release triggered by calcium-influx via VGCC and calcium release from internal calcium stores. In contrast, at more mature synapses, the calcium nanodomain defined by calcium influx via only NMDARs and VGCCs seem to be sufficient for bAP-induced site-specific local BDNF release from dendrites. Since calcium influx from internal stores was shown to be important for presynaptic AP-induced NMDAR-mediated BDNF release (Park 2018), calcium nanodomains might differ between dendrites and axons.

**Calcium transients and BDNF secretion in response to electrical stimulation**

In neurons, calcium influx via pre- or postsynaptic NMDA receptors contributes to electrically induced BDNF release at the respective release site (Hartmann et al. 2001; Matsuda et al. 2009; Park 2018). At least for presynaptic terminals of cortico-striatal synapses, calcium influx from internal calcium stores was shown to prevent presynaptic BDNF release mediated via presynaptic NMDA receptors containing the GluN1 subunit (Park 2018) (Fig. 3). At postsynaptic release sites, electrically evoked BDNF release was solely dependent on extracellular calcium influx and not on calcium release from internal calcium stores (Kuczewski et al. 2008) (Fig. 2a). However, elevated potassium-induced BDNF release, depolarization-induced BDNF release and electrically evoked BDNF release in young cultures occur independent from AMPAR and NMDAR activation (Balkowiec and Katz 2002; Hartmann et al. 2001; Kuczewski et al. 2008) (Fig. 2b). In these cases, calcium influx was mediated via L-type VGCC (Balkowiec and Katz 2002; Buldyrev et al. 2006; Hartmann et al. 2001; Kolarow et al. 2007; Kuczewski et al. 2008), although the specific type of VGCC contributing to BDNF release might depend on the developmental stage and the brain area of the investigated neuron (Fig. 2b). Furthermore, dendritic elevated potassium-induced release of BDNF in hippocampal cultures was—in contrast to bAP-induced dendritic BDNF release—dependent on calcium influx from internal calcium stores (Griesbeck et al. 1999; Kolarow et al. 2007; Kuczewski et al. 2008) (Fig. 2). This is in line with the role of BDNF during development of neural networks (compare Stimuli-triggering BDNF release from developing neurons section). The maturation of functional synapses might depend on BDNF release triggered by calcium-influx via VGCC and calcium release from internal calcium stores. In contrast, at more mature synapses, the calcium nanodomain defined by calcium influx via only NMDARs and VGCCs seem to be sufficient for bAP-induced site-specific local BDNF release from dendrites. Since calcium influx from internal stores was shown to be important for presynaptic AP-induced NMDAR-mediated BDNF release (Park 2018), calcium nanodomains might differ between dendrites and axons.

**Calcium transients and BDNF secretion in response to chemical stimulation**

Increased bursting activity of neural networks driven by glutamate or ketamine application or disinhibition of GABAergic transmission by GABA_AR inhibitors represent potent stimuli for the induction of BDNF release (Canossa et al. 1997; Griesbeck et al. 1999; Lepack et al. 2014; Nakajima et al. 2008; Porcher et al. 2011; Santi et al. 2006). Interestingly, in contrast to bAP-induced BDNF release, glutamate-induced BDNF release from brain slices or primary neuronal cultures has been shown to be independent from extracellular calcium but dependent on calcium release from internal calcium stores (Canossa et al. 2001; Griesbeck et al. 1999; Santi et al. 2006) (Fig. 2b). This glutamate-induced BDNF release was also dependent on AMPA receptor or mGluR activation, respectively (Canossa et al. 1997; Canossa et al. 2001; Canossa et al. 2002; Nakajima et al. 2008; Santi et al. 2006), as well as on IP3-signaling that activates calcium release from internal calcium stores (Canossa et al. 2002; Gartner and Staiger 2002) (Fig. 2b). In most of these studies, BDNF levels in the extracellular medium was quantified by ELISA measurements. Therefore, release of BDNF from non-neuronal cells could not be excluded. Ketamine-induced BDNF release was blocked in the presence of AMPA receptor inhibitors but also in the presence of L-type VGCC inhibitors (Lepack et al. 2014). In this study, increased glutamatergic transmission induced by ketamine application was discussed as a reason for induction of BDNF release (Duman et al. 2019; Lepack et al. 2014). However, the molecular mechanisms underlying action of ketamine are not well understood (Lester et al. 2012; Wei et al. 2020).

| Ref.          | Species/type of preparation | Method       | Release induction protocol | Pharmacology/molecular mechanism/time course |
|--------------|-----------------------------|--------------|----------------------------|---------------------------------------------|
| Fogaça et al. 2019 | Primary cortical culture     | ELISA       | Methadone (60 min)          |                                              |
| Fukumoto et al. 2019 | Cortical culture             | ELISA       | Hydroxynor-ketamine (60 min) | Reduced by verapamil (inhibitor of L-type VGCC); NBQX (inhibitor of AMPAR) |
| Lee et al. 2019     | Mouse, cortical neurons, Munc18 ko mice | ELISA | Basal level                | Reduced in x/-munc18 ko culture             |
| Liu et al. 2019     | Rat                         | ELISA       | Conditioned taste aversion memory extinction (12 h) |                                              |

List of references analyzing molecular mechanisms of BDNF release or the contribution of BDNF during LTP in different neuronal preparations. The exact cellular source of BDNF is unknown since BDNF was either quantified by, e.g., ELISA measurements or the significance of BDNF release was shown by scavenging BDNF from extracellular space. (Abbreviations: please see table legend of Table 1)
Therefore, it is possible that the ketamine-induced BDNF release acts via different mechanisms than glutamate-induced release. It is also conceivable that depolarization-induced conformational change of VGCC elicited by glutamate or ketamine-induced increase in bursting activity of the neural network is a prerequisite for BDNF release, which leads to structural reorganization of release sites (Marom et al. 2010) irrespective of calcium influx from the extracellular medium.

Calcium transients and BDNF secretion in response to activation of GPCRs and RTKs

In addition to calcium influx through VGCC or NMDA receptors, calcium release from internal calcium stores is mediated by activation of G protein-coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs). Different GPCRs and RTKs are known to be important for BDNF release. Neurotrophin-induced BDNF release was shown to depend on Trk receptor activation (Lopez-Benito et al. 2018; Nakajima et al. 2008; Santi et al. 2006). Furthermore, neurotrophin-induced BDNF release was solely dependent on calcium release from internal calcium stores (Canossa et al. 1997; Canossa et al. 2001; Lopez-Benito et al. 2018; Nakajima et al. 2008; Santi et al. 2006) (Fig. 2b). Similarly, GPCR-mediated BDNF release was only also dependent on calcium release from internal calcium stores (Buldyrev et al. 2006; Canossa et al. 2001; Lao-Peregrin et al. 2017; Santi et al. 2006). In addition, intracellular application of the non-hydrolysable guanosine diphosphate (GDP) analog GDPβ-S inhibits giant depolarization (GDP)-induced BDNF release (Kuczewski et al. 2008).
neuronal preparations, GPCRs activated by caffeine, glutamate via mGluR, GABA via GABA\(_B\) receptors, or calcitonin-gene-related protein (CGRP) receptor via CGRP have been shown to induce release of BDNF (Balkowiec and Katz 2002; Buldyrev et al. 2006; Canossa et al. 2001; Lao-Peregrin et al. 2017; Santi et al. 2006). Nevertheless, GPCR-mediated release seems to be even more prominent in non-neuronal cells.

Intracellular calcium transients and BDNF secretion from glial cells

Ligands for GPCRs such as prostaglandine E2 or adenosine A2 (A\(_{A2}\)R) induce BDNF release via activation of PKA or PLC in astrocytic and microglial cultures (Gomes et al. 2013; Hsieh et al. 2010; Hutchinson et al. 2009; Jean et al. 2008) (Fig. 4a). In addition to neuronal preparations, glutamate-induced release of BDNF was also observed in astrocytic (Jean et al. 2008) but not in microglial cultures (Trang et al. 2009). Glutamate-induced release of BDNF via mGluR-activation was observed within 10 min of stimulation, while kainate or NMDA application failed to induce release of BDNF in astrocytic cultures. The glutamate-induced release was dependent on activation of phospholipase C (PLC) and inositol-3-phosphate receptor (IP3-R) as well as on calcium release from internal calcium stores (Jean et al. 2008) (Fig. 4a). Besides the glutamate-induced BDNF release, release was also elicited after ATP treatment of astrocytic and microglial cultures (Long et al. 2020; Trang et al. 2009) (Fig. 4b). Trang et al. demonstrated a biphasic release of BDNF with peaks at 5 and 60 min after stimulation with ATP (Trang et al. 2009). siRNA-mediated knockdown or pharmacological inhibition of purinergic P2X4Rs prevented the ATP-induced BDNF release (Long et al. 2020; Trang et al. 2009). Calcium influx from extracellular but not from internal calcium stores was important for BDNF release after ATP treatment (Fig. 4b). Application of TAT-NSF700, which prevents the disassembly activity of NSF, as well as inhibition of p38MAPK phosphorylation, reduced BDNF release from microglia (Long et al. 2020; Trang et al. 2009; Zhou et al. 2019). BDNF release from spinal dorsal horn microglia in response to p38-MAPK activation was associated with chronic pain hypersensitivity (Zhou et al. 2019). In addition to hydrophilic messengers, membrane permeable first messengers like progesterone or C8-ceramide are also effective stimuli to accumulate BDNF in extracellular medium after 16-h incubation time in primary glial cultures (Nakajima et al. 2002; Su et al. 2012).

Contribution of small G proteins, SNARE proteins and signaling pathways to BDNF secretion

Different proteins are involved in the organization of the release site, the transition of BDNF-containing vesicles from the reserve pool to a readily releasable pool, fusion pore opening, as well as disassembly of the SNARE complex. The exact contribution of all the proteins and direct interactions between the proteins with all their multiple binding sites are unknown also for synaptic vesicles. For BDNF-containing granules, some proteins and their binding partners have been identified. Among them are important cytosolic kinases (Gimenez-Cassina et al. 2012; Gomes et al. 2013; Hsieh et al. 2010; Hutchinson et al. 2009; Kolarow et al. 2007; Santi et al. 2006; Trang et al. 2009), small G proteins (Hong et al. 2016; Kato et al. 2018; Persoon et al. 2019), SNARE proteins (Dean et al. 2009; Shimojo et al. 2015; Won et al. 2015) and other priming factors (Eckenstaler et al. 2016; Lee et al. 2019).

Contribution of cytosolic-signaling pathways to BDNF secretion

Important cytosolic kinases, which are also implicated in BDNF release, are the protein kinase A (PKA), calcium calmodulin kinase II (CaMKII), protein kinase C (PKC) via phospholipase C (PLC), as well as protein kinase G (PKG) via the NO/cGMP/PKG-signaling pathway. The NO/cGMP/PKG-signaling pathway was shown to negatively regulate calcium release from internal calcium stores, thereby reducing BDNF secretion (Canossa et al. 2002; Kolarow et al. 2014; Santi et al. 2006). BDNF release was also dependent on activation of the PLC/IP3/IP3R-pathway, thereby mediating calcium release from internal calcium stores (Canossa et al. 2002;
Activation of PKA was shown to be important for BDNF release from neurons and glial cells (Gomes et al. 2013; Hsieh et al. 2010; Hutchinson et al. 2009; Kolarow et al. 2007) although the exact downstream effectors contributing to cAMP/PKA-dependent BDNF release are unknown. Furthermore, inhibition of CAMKII prevented BDNF release from neurons (Kolarow et al. 2007) while chronic inhibition of GSK-3 reduced accumulation of BDNF in culture media of neuroblastoma cells (Gimenez-Cassina et al. 2012). These serine/threonine kinases are known to regulate open channel probability of VGCCs, IP3Rs, the activity of effector proteins important for actin dynamics, as well as the activity of different other proteins, such as small G-proteins.

Contribution of small G-proteins to BDNF secretion

The Ras superfamily of small G proteins is an important regulator of several cellular processes. Based on structural and functional similarities, the superfamily is divided into 5 main subfamilies: Ras, Ran, Rho, Arf and Rab family. While the Ras family controls cell proliferation, differentiation and survival, the Ran family contributes to nucleocytoplasmic transport of RNA and proteins. The Rho family is important for actin dynamics and Rab and Arf subfamilies have been shown to regulate vesicular transport (Winnenberg et al. 2005). Different small G proteins seem to contribute to the release of BDNF. Activation of p38 MAPK is mediated via Ras. Ras/p38-signaling is important for BDNF release in astrocytes and microglia (Long et al. 2020; Trang et al. 2009). The small G-protein rab3a, which is important for vesicular trafficking, is localized on membranes of BDNF-containing granules, in astrocytes. Interactions between huntingtin (htt) and rab3a prevent binding of rab3a GTPase-activating protein1 (rab3a-GAP1) to rab3a, which leads to an impaired attachment of the granules to the plasma membrane (Hong et al. 2016). In synaptic vesicle release, it could be shown that Rab3a interacting protein Rim1alpha directly interacts with the priming factor munc13 thereby recruiting munc13 to the active zone. Inhibition of this interaction reduces the number of readily releasable vesicles (Andrews-Zwilling et al. 2006; Betz et al. 2001; Dulubova et al. 2005). A similar finding has been reported recently for BDNF-containing vesicles. Recruitment of BDNF-containing granules to release sites and thus fusion depends on the interaction of Rab3a-Rim1 and Munc13 (Persoon et al. 2019).

Contribution of regulatory proteins and SNARE proteins to BDNF secretion

The priming protein Munc13 further catalyzes the transition of the closed inhibitory form of the Munc18/syntaxin complex to the opened active form, thus enabling the formation of the SNARE complex. Therefore, Munc-18 is an essential protein for synaptic vesicle priming and stabilizing the core complex by binding to the SNARE protein syntaxin. Accordingly, in...
knockout mice heterozygous for munc-18, BDNF release is negatively affected (Lee et al. 2019).

The $\text{Ca}^{2+}$-dependent activator protein for secretion (CAPS1) is another priming factor during exocytosis of synaptic vesicles and granule exocytosis in neurons (Farina et al. 2015; Jockusch et al. 2007). CAPS1, which is expressed in many brain regions, is localized in axons and dendrite of hippocampal neurons (Eckenstaler et al. 2016; Farina et al. 2015; Sadakata et al. 2006; Speidel et al. 2003). For BDNF-containing granules, a function of CAPS1 in regulating intravesicular pH value could be demonstrated (Eckenstaler et al. 2016). A transient knockdown of CAPS1 significantly increased the pH value in BDNF-containing granules while the pH in the cytosolic compartment was unaffected. Furthermore, knockdown of CAPS1 reduced the number of fusion-competent BDNF-containing vesicles as well as the amount of released BDNF per single vesicle by an unknown mechanism (Eckenstaler et al. 2016).

Furthermore, the contribution of different SNARE proteins to the release of BDNF was analyzed (Shimojo et al. 2015). In cortical cultures, the SNARE proteins involved in BDNF release slightly differ for axonal and dendritic release. While Syb2 and SNAP 25 were important for dendritic and axonal release of BDNF, the SNARE protein SNAP 47, which is irrelevant for synaptic vesicle exocytosis, contributed more to axonal than to dendritic BDNF release (Shimojo et al. 2015). Another protein that interacts with SNARE proteins is synaptotagmin (Syt). Synaptotagmins are localized in the vesicular membrane. One member of this protein family, synaptotagmin-4 (Syt-4), is upregulated by activity and seizure and also localized on BDNF vesicles. Syt-4 reduces depolarization-induced release of BDNF by preventing the fusion step (Dean et al. 2009). However, TBS-induced release of previously endocytosed BDNF was shown to be mediated by syt-6 and not syt-4 at postsynaptic sites in hippocampal neurons (Wong et al. 2015). Knockdown of the SNARE protein syt-6 as well as inhibition of direct interaction of syt-6 with the SNARE complex interacting protein complexin inhibited re-release of BDNF quantum dots. The recycling of BDNF was dependent on extracellular calcium as well as AMPAR or NMDAR activation, respectively (Wong et al. 2015). Wong et al. showed that endogenous and endocytosed BDNFs are both targeted to axons as well as to dendrites in distinct non-overlapping vesicles (Wong et al. 2015). While syt-6 is an important protein for recycling of BDNF in neurons, the release of endogenous post-Golgi BDNF was regulated by the SNARE protein syt-4 (Wong et al. 2015).

Altogether, great effort has been done to uncover the cellular and molecular mechanisms of BDNF release in recent years. A meshwork of proteins such as serine/threonine kinases, small G-proteins and SNARE complex (-interacting) proteins have been demonstrated to participate in regulated release of BDNF, while constitutive release of BDNF is mainly neglected in almost all studies. Since regulated release of synaptic vesicles and BDNF-containing vesicles share multiple proteins during fusion, the regulated release of vesicles seems to be a highly conserved evolutionary process.)

**Conclusion**

BDNF and the other neurotrophins were initially identified as target-derived survival factors that are secreted from non-neuronal tissue to secure survival of innervating neurons. In the early 1990s, the prominent function of BDNF in synaptic plasticity emerged, followed by first insights into activity-dependent release of BDNF from neurons. While sustained firing of action potentials or longer lasting depolarization are keys to neuronal BDNF secretion, the subsequent signaling cascades and the molecular machinery triggering and regulating BDNF vesicle exocytosis have just begun to be elucidated. Importantly, BDNF and potentially also the other neurotrophins can be released from many non-neuronal cell types using similar molecular mechanisms. Therefore, future studies will need to establish the following:

1. What are the physiological and pathophysiological correlates of the diverse BDNF-releasing stimuli in neuronal and non-neuronal cells?
2. How are the molecular pathways triggered in non-neuronal cells that can release BDNF (e.g., microglia, astrocytes, lymphocytes, endothelial cells, muscle cells)?
3. What are the molecular differences between the release machinery for TGN-derived new vesicles vs. recycled vesicles from neuronal and non-neuronal cells, respectively?
4. What are the subcellular release sites of the distinct BDNF vesicles (new or recycled vesicles) in neuronal and non-neuronal cells?
5. What are the exact components of BDNF species (e.g., mBDNF, proBDNF and propeptide) and other cargo proteins (e.g., PCs, other neuropeptides, ATP and pH) in BDNF secretory granules that might help to fine tune the effects of released BDNF in target cells?
6. How do these differences in release sites, release machinery, distinct subsets of BDNF vesicles and components of BDNF species contribute to the distinct physiological and behavioral functions?
7. Last but not least: what is the origin and function of BDNF in the blood stream?

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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