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REVIEW

BDNF isoforms: a round trip ticket between neurogenesis and serotonin?

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Abstract

The brain-derived neurotrophic factor, BDNF, was discovered more than 30 years ago and, like other members of the neurotrophin family, this neuropeptide is synthetized as a proneurotrophin, the pro-BDNF, which is further cleaved to yield mature BDNF. The myriad of actions of these two BDNF isoforms in the central nervous system is constantly increasing and requires the development of sophisticated tools and animal models to refine our understanding. This review is focused on BDNF isoforms, their participation in the process of neurogenesis taking place in the hippocampus of adult mammals, and the modulation of their expression by serotonergic agents. Interestingly, around this triumvirate of BDNF, serotonin, and neurogenesis, a series of recent research has emerged with apparently counterintuitive results. This calls for an exhaustive analysis of the data published so far and encourages thorough work in the quest for new hypotheses in the field.

Keywords: adult hippocampal neurogenesis, BDNF isoforms, rodents, serotonergic system, SSRI antidepressants.

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> If one is master of one thing and understands one thing well, one has at the same time, insight into and understanding of many things Vincent van Gogh (1853–1890).

Neurotrophic factors are a family of highly conserved proteins in mammals, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Neurotrophins play key roles in several biological processes in both the developing and adult nervous system, though they can be synthesized in other peripheral organs. The first neurotrophin was discovered in the middle of the 20th century: works carried out by Rita Levi-Montalcini, Victor Hamburger and Stanley Cohen first described the NGF (Hamburger and Levi-Montalcini 1949; Cohen *et al.* 1954; Levi-Montalcini 1966). Indeed, Levi-Montalcini and Cohen earned the Nobel Prize in Physiology or Medicine in 1986 for this paramount discovery. The main action originally described for NGF pointed to a pro-survival effect on subpopulations of sensory neurons. The particularly low levels of neurotrophins in the mature brain has greatly complicated attempts to reliably identify the other trophic factors. Nevertheless, in 1982, the search for growth factors with effects on other neurons populations made possible the discovery of BDNF (Barde *et al.* 1982). This original study was performed on pig brain extracts, and subsequent studies in other mammals' brains also reported a pro-survival effect for BDNF (for a revision, see Bibel and Barde 2000) as well as effects on neuronal processes growth (McAllister *et al.* 1995; Rabacchi *et al.* 1999; Tanaka *et al.* 2000; Tucker *et al.* 2001). Nevertheless, prompted by a better comprehension of the biology of BDNF, it was later

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Abbreviations used: 5-HT, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; ADORA2A, adenosine receptor; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DG, dentate gyrus; eGFP, enhanced green fluorescence protein; f.l.TrkB, full-length TrkB; GPCR, G-protein-coupled receptor; JNK, c-Jun N-terminal kinase; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; m-BDNF, mature BDNF; MDD, major depressive disorder; MMP, matrix metalloprotease; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; p75^{NTR}, 75 kDa neurotrophin receptor; PCPA, p-chlorophenylalanine; PI3K, phosphatidylinositol 3-kinase; PLC-γ, phospholipase C-γ; PTrkB, phosphoTrkB; SERT, serotonin transporter; SSRI, serotonin-selective reuptake inhibitor; SVZ, subventricular zone; tPA, tissue plasminogen activator; Tph2, tryptophan hydroxylase 2; Trk, tyrosine protein kinase; VMAT, vesicular monoamine transporter; WB, western blotting.

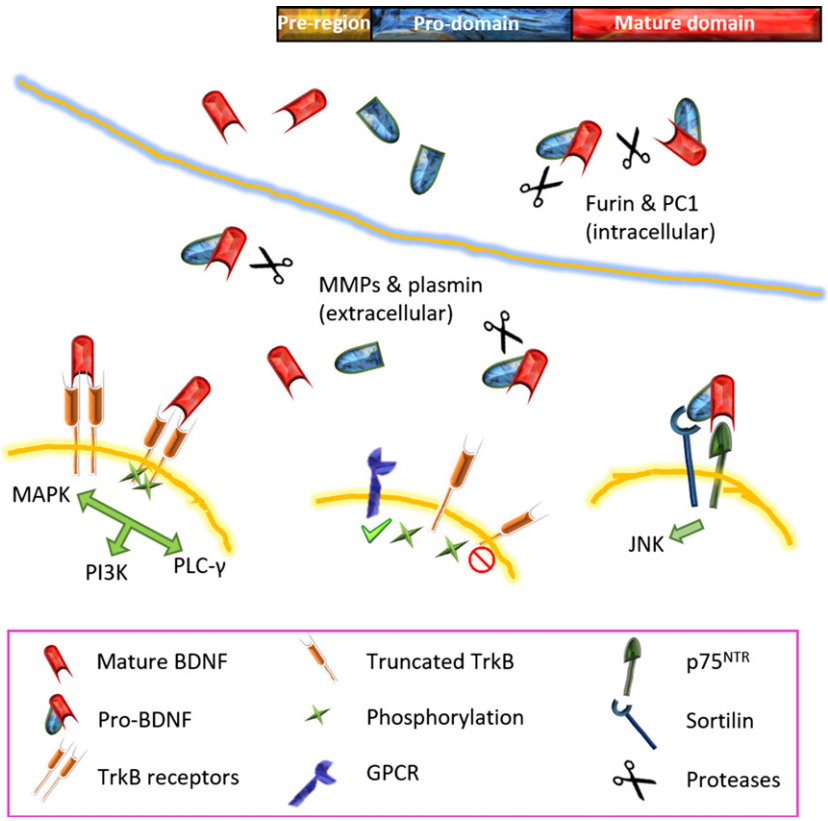

Fig. 1 Cellular processing of the brain-derived neurotrophic factor (BDNF). BDNF is synthetized as a pre-proneurotrophin. After removal of the pre-region, pro-BDNF can be cleaved intracellularly by furin or by extracellular proteases like matrix metalloproteases (MMP-9, MMP-2), or plasmin. In the synaptic cleft, mature BDNF (mBDNF) can bind TrkB receptors, promoting their homodimerization and phosphorylation of intracellular tyrosine residues. Phosphorylated-TrkB can activate three different signaling pathways: MAPK, PI3K & PLC-γ cascades to promote neurogenic, synaptogenic, and other actions. Whereas G-protein-coupled receptors (GPCRs) can transactivate TrkB receptors, truncated forms (with a short intracellular domain) can act as dominant negative inhibitor of mBDNF signaling. ProBDNF binds $p75^{NTR}$ by its mature domain, whereas the pro-region binds co-receptors like sortilin, leaving to activation of c-Jun N-terminal kinase pathway to induce programmed cell death.

observed that this neurotrophin can also cause programmed cell death (Bamji *et al.* 1998; Lee *et al.* 2001). All in all, the global function of BDNF appeared to be more linked to the regulation of neuronal populations, a critical process for brain development that requires a 'correct' number of cells. This regulation is also crucial for the neuronal homeostasis in the adult mammalian brain, particularly in the hippocampus. Since the first link reported between adult hippocampal neurogenesis and BDNF (Nibuya *et al.* 1995), a great interest in better understanding the role of BDNF still exists. The history of findings around neurotrophins has followed two compasses: a tandem *in vitro-in vivo*, as it is the case for many other biological molecules, as well as another inner rhythm, given by initial discoveries around the NGF, later verified for BDNF. This review will try to reflect both rhythms, although with more knowledge being produced in the field of neurotrophins, BDNF has acquired its own relevance, 'getting rid' of its elder brother.

This review deals with biological aspects and functions of endogenous BDNF isoforms, their role in adult hippocampal neurogenesis, as well as their association with pro-neurogenic interventions. Although there are certain references to the developmental period, it is mostly focused on the adult brain, and most of the results discussed come from studies performed in rodents. More detailed discussions covering current advances on neurotrophins have already been published and they are cited herein in case a more thorough revision is required.

Cellular processing and biological functions of neurotrophins

The four members of the neurotrophin family are initially synthesized as 31–35 kDa precursors or proneurotrophins containing at their N-termini, signal peptides followed by the pro-region that, after cleavage, yields the mature neurotrophin form, with a molecular size ranging 13.2–15.9 kDa (Seidah *et al.* 1996). The cellular processing of neurotrophic factors has been extensively reviewed (Lu *et al.* 2005; Lessmann and Brigadski 2009). Briefly, the proneurotrophins are synthesized in the endoplasmic reticulum, transported to the Golgi to be folded and finally, to the secretory vesicles.

Specifically, BDNF is initially synthesized as a precursor form, the pre-pro-BDNF (Fig. 1). The pre-region sequence is normally removed when translocated across the Golgi membrane, yielding the 32 kDa pro-BDNF. Small amounts of a 28 kDa, truncated pro-BDNF can also be formed in the endoplasmic reticulum without interfering with final levels of mature-BDNF (m-BDNF) (Mowla *et al.* 2001). The N-terminal pro-domain of BDNF facilitates intracellular trafficking and regulated secretion (Egan *et al.* 2003). After cleavage of

the N-terminal region, the C-terminal mature form, m-BDNF, is released. Interestingly, both the N- and C-terminal domains are relevant for BDNF sorting by, respectively, interacting with sortilin and carboxypeptidase E (Reichardt 2006). *In vivo*, intracellular pro-BDNF can have three different fates: (i) intracellular cleavage and release of m-BDNF, (ii) secretion as pro-BDNF and extracellular cleavage, or (iii) secretion as pro-BDNF with no further modifications. In fact, although difficult to verify *in vivo*, pro-BDNF is apparently the main form secreted (Mowla *et al.* 2001; Dieni *et al.* 2012). The regulated equilibrium between pro-BDNF and m-BDNF becomes decisive for physiological as well as pathological conditions. Unlike NGF and NT-3, which only uses the constitutive secretory pathway (Mowla *et al.* 1999; Farhadi *et al.* 2000), BDNF secretion can be constitutive, both in non-neuronal cells and neurons, or regulated, exclusively in neurons (Seidah *et al.* 1996; Mowla *et al.* 2001). Thus, among other growth factors, BDNF has a unique mechanism of secretion: activity-dependent or regulated secretion.

The subcellular distribution of BDNF were thoroughly studied with different tools and both pro- and m-BDNF were shown to be stored in pre-synaptic large dense core vesicles supporting their anterograde transport to axons (Zhou *et al.* 2004; Dieni *et al.* 2012), as well as activity-dependent transfer to post-synaptic neurons (Kohara *et al.* 2001).

Intracellular proteolytic cleavage of pro-BDNF can take place either in the trans-Golgi by furin, to be constitutively released, or within granules by convertases in the regulated secretory pathway (Lu *et al.* 2005). Pro-BDNF can also be extracellularly cleaved by the serine protease plasmin (Pang *et al.* 2004) and matrix metalloproteases (MMP) (Hwang *et al.* 2005). Plasmin is the active form obtained through plasminogen cleavage by the tissue plasminogen activator (tPA). Plasminogen is present in the synaptic cleft, where tPa is secreted by axonal terminals to the extracellular space (Tsirka *et al.* 1997). Moreover, *in vitro* studies has shown that plasminogen, tPA and pro-BDNF are co-packaged in dense core granules and co-transported along dendrites of hippocampal cultured neurons (Lochner *et al.* 2008). On the other hand, efficient conversion of pro-BDNF to m-BDNF by several MMP was also demonstrated in cell culture (Lee *et al.* 2001; Hwang *et al.* 2005). Interestingly, MMPs have a well-characterized role in synaptic plasticity and long-term potentiation (see review in Ethell and Ethell 2007; Vafadari *et al.* 2016), and more importantly, MMP-9 has been shown to be responsible for conversion of pro-BDNF to m-BDNF in the hippocampus of mice (Mizoguchi *et al.* 2011). As described, the processing of proBDNF has several levels, and expression or activation of the involved proteases represent candidate targets to regulate the balance of BDNF isoforms.

Neurotrophin receptors

The two BDNF isoforms preferentially bind different receptors, as it is the case for all the members of the neurotrophin family (reviewed in Reichardt 2006). The first study that analyzed the binding characteristics of BDNF to its receptors, performed in embryonic chick sensory neurons, showed two classes of receptors: high affinity, with a $K_d = 1.7 \times 10^{-11}$ M, and low affinity, with a $K_d = 1.3 \times 10^{-9}$ M (Rodriguez-Tébar and Barde 1988). Later, the 'low affinity' receptor, a 75 kDa glycoprotein member of the tumor necrosis factor receptor family, was found to be a common target for all the neurotrophins, and thus named $p75^{NTR}$ receptor (Chao and Hempstead 1995). On the other hand, the high-affinity receptor was shown to be part of the tyrosine protein kinase (Trk) family, which involves the TrkA, TrkB, and TrkC receptors, for which the main ligands are NGF, BDNF/NT-4 and NT-3, respectively (Ebendal 1992; Chao and Hempstead 1995). Since these first studies elucidating interactions between neurotrophins and their receptors, much work has been devoted to better comprehend the biological aspects of these contacts.

Mature neurotrophins and Trk receptors

Mature neurotrophins are soluble proteins that form non-covalently linked homodimers. Although the Trk receptors were originally characterized as 'high-affinity receptors', it was later demonstrated that the binding of BDNF to TrkB is of low affinity, and it can be increased by TrkB homodimerization or association with $p75^{NTR}$ (reviewed in Chao 2003). BDNF binding to TrkB triggers its dimerization and subsequent autophosphorylation of intracellular tyrosine residues (phosphoTrkB) (Kaplan and Miller 2000), resulting in recruitment of adaptor proteins to activate a triumvirate of intracellular signaling pathways: MAPK, phosphatidylinositol 3-kinase, and phospholipase C-γ (PLC-γ) (Fig. 1). The intracellular domain of the vertebrate Trk receptors contains 10 tyrosines that are substrates for phosphorylation (Reichardt 2006). Particularly, phosphorylation of tyrosine at position 816 induces the binding to PLC-γ, whereas the activation of MAPK and phosphatidylinositol 3-kinase pathways results from recruitment of Shc protein after phosphorylation of tyrosine at position 515 (Huang and Reichardt 2001).

Interestingly, G-protein-coupled receptors (GPCRs) are able to regulate the tyrosine kinase cascade by increasing tyrosine phosphorylation of adaptor proteins (reviewed in Luttrell *et al.* 1999). Consistent with this signaling mechanism, activation of the GPCR ADORA2A by adenosine is able to promote transactivation of TrkB receptors in hippocampal neurons deprived of BDNF (Lee and Chao 2001). This crosstalk effect was also demonstrated for other GPCR agonists, like the pituitary adenylate cyclase-activating polypeptide (Lee *et al.* 2002a), norepinephrine (Chen *et al.* 2007) and dopamine (Swift *et al.* 2011). Furthermore, serotonin added to SH-SY5Y cells was able to transactivate TrkB receptors through low concentrations of reactive oxygen species (Kruk *et al.* 2013). Together, these *in vitro*

results raise the possibility of eliciting neurotrophic effects by administration of GPCRs agonists, without involvement of neurotrophins.

Besides the full-length (f.l.) TrkB receptor, a C-terminally truncated form lacking the intracellular catalytic portion has also been described by alternative splicing in mice, rats (Klein *et al.* 1990; Middlemas *et al.* 1991) and later in humans (Stoilov *et al.* 2002). The molecular weight of f.l.TrkB and truncated TrkB is 145 and 95 kDa, respectively, and since their extracellular and transmembrane domains are identical, both isoforms are able to bind m-BDNF. Two isoforms of truncated TrkB were described: T1 and T2 in rats (Middlemas *et al.* 1991); T1 and T-shc in humans (Stoilov *et al.* 2002). By heterodimerization with f.l.TrkB, T1, the truncated form predominantly expressed in the adult brain, can act as a dominant negative inhibitor of BDNF signaling (Eide *et al.* 1996; Haapasalo *et al.* 2002), becoming a candidate molecule to modulate BDNF synaptic effects. Nevertheless, given that the distribution of T1 is abundant in the brain, highly regulated, region specific and increases with age, it was proposed that this form cannot act only as a dominant negative inhibitor, but has its own active signaling pathway (Fenner 2012). Indeed, truncated TrkB T1 and T2 have a short intracellular portion capable of inducing signaling transduction by themselves, as shown in cell lines transfected with the truncated forms (Baxter *et al.* 1997). Interestingly, the expression of truncated TrkB receptors was observed in both cortical neurons and immature astroglia, but as astrocytes differentiate, a decrease in f.l.TrkB mRNA was observed, whereas the truncated form becomes the predominant receptor in differentiating and confluent astrocytes (Climent *et al.* 2000). Previous *in vivo* reports had already shown expression of truncated TrkB receptors in glial cells (Frisén *et al.* 1993; Rudge *et al.* 1994). Furthermore, increased levels of the truncated form were reported in the glial scar after injury (Frisén *et al.* 1993). The intriguing links between the expression of truncated TrkB receptors and cell populations in the brain were further studied *in vivo*. A critical role for gliogenesis was suggested for the truncated TrkB receptor in the cortex, where expression of this form promotes the differentiation of neural stem cells to glial progenitors and astrocytes, while inhibiting neurogenesis (Cheng *et al.* 2007). All in all, mBDNF appears to be able to promote proliferation of neurons or glial cells depending on the form of TrkB receptor expressed by different cell populations.

Biological functions of mBDNF–TrkB pathway

Neuronal activity regulates not only the secretion of BDNF as mentioned before, but also TrkB trafficking and signaling as well as endocytosis of the BDNF-TrkB complex (reviewed in Nagappan and Lu 2005), contributing to globally increase the response to BDNF. For example, BDNF binding to TrkB receptor promotes the translocation of the activated receptor toward membrane microdomains rich in cholesterol and sphingolipids, known as lipid rafts (Suzuki *et al.* 2004), resulting in better BDNF signaling at synapses. Certainly, short-term modulation of synaptic transmission and plasticity is one of the major functions described for m-BDNF in the nervous system (Lu 2003), but several other effects have been attributed to TrkB receptors activation by m-BDNF. The spectrum of BDNF actions involves regulation of neuritic growth shown first by *in vitro* studies (Avila *et al.* 1993; Lefebvre *et al.* 1994; McAllister *et al.* 1995), and further confirmed *in vivo* (Lom and Cohen-Cory 1999; Horch and Katz 2002). Among different brain structures, BDNF is mainly expressed in the adult hippocampus (Aid *et al.* 2007). It is not surprising thus, that BDNF/TrkB signaling has been associated with learning (reviewed in Musumeci and Minichiello 2011) and memory (reviewed in Bekinschtein *et al.* 2014).

One of the best characterized roles of m-BDNF is linked to the physiological mechanism of long-term potentiation (LTP). Indeed, LTP is impaired in mice deficient in BDNF (Korte *et al.* 1995), whereas addition of BDNF is sufficient to rescue normal LTP in hippocampal slices of BDNF knockout mice (Patterson *et al.* 1996). The participation of the TrkB receptor in this phenomenon was further confirmed in mice with a targeted mutation in the PLC-γ docking site of TrkB, showing that this particular signaling cascade is required for LTP (Minichiello *et al.* 2002).

Since this is a trophic factor highly expressed in the hippocampus (Aid *et al.* 2007), it is not surprising that researchers focused their attention on finding a role for BDNF in the neurogenic process that takes place in the hippocampus of adult mammals. Prompted by the knowledge that reduced hippocampal BDNF levels are associated with impaired memory performance, Kato and colleagues, proved that an increase in BDNF endogenous levels by means of riluzole, a neuroprotective agent, is sufficient to induce neural progenitor proliferation in the rat hippocampus (Katoh-Semba *et al.* 2002). Simultaneously, genetic and physiological manipulation of BDNF levels in mice, extends the influence of this neurotrophin to the survival of newly generated neurons in the adult hippocampus (Lee *et al.* 2002b). More evidence continue to associate BDNF with neurogenesis and, particularly, the neurogenic response to physical exercise (Russo-Neustadt *et al.* 1999) or enriched environment (Rossi *et al.* 2006). A case in point is the pivotal discovery in the field of antidepressants: increased neurogenesis is responsible for the therapeutic effects of chronic treatment with these drugs (Malberg *et al.* 2000). After this paramount finding, several lines of evidence have implicated BDNF in cell proliferation (Larsen *et al.* 2007) or survival (Sairanen *et al.* 2005) induced by antidepressants. Indeed, a decade earlier, the laboratory of Duman had already demonstrated an increase in BDNF mRNA in the hippocampus of rats chronically treated with different

antidepressants, suggesting that the induction of this neurotrophin could promote neuronal survival to overcome the damaging effects of stress (Nibuya *et al.* 1995).

Proneurotrophins and p75^{NTR}

Unlike the Trk receptors, the physiological roles of the p75^{NTR} receptor have been more difficult to elucidate. Two main types of actions can be assigned to p75^{NTR}: (i) a functional collaboration with Trk receptors to modulate its activation through neurotrophins, and (ii) activation of signaling cascades driving apoptosis (see reviewed in Barker 1998). The first provocative reports suggesting the involvement of p75^{NTR} in programmed cell death came from *in vitro* studies performed in different cell types (Rabizadeh *et al.* 1993; Zheng *et al.* 1995; Casaccia-Bonnefil *et al.* 1996). Accordingly, it was originally proposed that p75^{NTR}-mediatied apoptosis takes place in developing neurons not expressing Trk receptors (Casaccia-Bonnefil *et al.* 1998). Based on these *in vitro* reports, Bartlett and colleagues, reduced the expression of p75^{NTR} by means of antisense oligonucleotides in dorsal root sensory neurons of rats, and verified *in vivo* that this receptor can also function as a death signal (Cheema *et al.* 1996). On the other hand, developmental neural death in the superior cervical ganglion occurs during the first 3 weeks after birth, both in rats and mice. Thus, using 1–21-day-old mice models in which the BDNF or p75^{NTR} genes are knocked-out, it was proposed that BDNF interacts with p75^{NTR} to mediate developmental apoptosis of sympathetic neurons by activation of the c-Jun N-terminal kinase pathway (Bamji *et al.* 1998). The interpretation of these results indicated that p75^{NTR} is an instrument to ensure rapid cell clearance when a neuron is unable to compete for adequate amounts of the appropriate neurotrophin. This mechanism becomes relevant during CNS development and, indeed, p75^{NTR} is widely expressed in many neuronal populations in the developing brain (reviewed in Barker 1998). Moreover, p75^{NTR} is highly expressed after damage in several neuronal populations, specifically in the apoptotic neurons (Martínez-Murillo *et al.* 1993; Roux *et al.* 1999), suggesting a role for this receptor in controlled cell elimination after injury. Nevertheless, at this point, the intriguing question remained how the same neurotrophin, either NGF or BDNF, could promote such opposite actions when binding the Trk receptor or the p75^{NTR}, and how this balance allows for a fine-tuned regulation leading to the appropriate number of neuronal populations. This riddle began to be solved by the elegant series of studies performed by Hempstead and colleagues demonstrating that proneurotrophins are high-affinity ligands for 75^{NTR}. Indeed, they confirmed that pro-NGF is able to selectively bind p75^{NTR} and mediate apoptosis in cultured neurons (Lee *et al.* 2001) and also in corticospinal neurons after adult brain injury (Harrington *et al.* 2004). Therefore, by demonstrating that proneurotrophins have high affinity for p75^{NTR}, pro-survival and pro-apoptotic antagonistic actions of these growth factors could be finally reconciled. Importantly, these highly relevant discoveries revealed that some of the studies conducted before could contain misinterpretations, attributing effects to the activation of Trk receptors instead of p75^{NTR}.

Although apoptotic effects mediated by p75^{NTR} are the best characterized, many other actions have been attributed to this receptor, such as regulation of axonal growth cones (Yamashita *et al.* 1999; Gehler *et al.* 2004), promotion of BDNF-induced myelin formation by Schwann cells (Cosgaya *et al.* 2002), and facilitation of hippocampal long-term depression (LTD) (Woo *et al.* 2005).

While the extracellular portion of p75^{NTR} contains tandem arrays of cysteine-rich domains, it lacks an intracellular kinase domain, but can cooperate with different proteins to induce biological responses. The first partners reported were the Trk receptors: p75^{NTR} enhances the ability of Trk receptors to bind neurotrophins and to discriminate their preferred neurotrophin (see a reviewed in Barker 2004). In addition, several co-receptors were described to form heteromeric receptor complexes with p75^{NTR}, like sortilin (reviewed in Lu *et al.* 2005). Particularly, pro-NGF-mediated apoptosis requires the formation of a complex between p75^{NTR} and sortilin, where the pro-domain directly binds sortilin and the mature portion most likely interacts with the p75^{NTR} (Nykjaer *et al.* 2004). Following the classical compass developed thus far in the field of neurotrophins, the *in vitro* role of the sortilin-p75^{NTR} complex in pro-NGF-mediated neuronal apoptosis was later confirmed for the cell-programmed death promoted by pro-BDNF (Teng *et al.* 2005).

The BDNF pro-domain

A thorough study conducted by the laboratory of Dr. Barde (Dieni *et al.* 2012) described the presence of the 17 kDa BDNF 'pro-peptide' in hippocampal cells, after glutaraldehyde fixation of the transfer membranes. The level of expression of this BDNF pro-domain was 10-fold higher than pro-BDNF, raising questions about its potential functionality. In previous studies, a single nucleotide polymorphism has been identified in the BDNF human gene, corresponding to a valine to methionine substitution at codon 66 (Val66Met), in the middle of the pro-domain. Studies in neurons transfected with this mutated met-BDNF prompted a better comprehension of the role of the pro-region, which is apparently involved in intracellular trafficking and regulated secretion of BDNF (Egan *et al.* 2003; Chen *et al.* 2004). Thus, it was hypothesized through indirect evidence, that the region containing the Val66Met substitution is key for the interaction of the pro-domain with sortilin (Chen *et al.* 2005). Interestingly, the Val66Met polymorphism is observed in around 20–25% of the human population and has been mainly associated with memory alterations (Egan *et al.* 2003; Hariri *et al.* 2003) but also with

other neuropsychiatric disorders like depression, anxiety, and schizophrenia (Bath and Lee 2006; Hashimoto 2007; Ignácio *et al.* 2014; Notaras *et al.* 2015). Indeed, the 66Met pro-domain has been recently proposed as a new bioactive ligand capable of inducing growth cone retraction depending on the expression of $p75^{NTR}$ plus the sortilin-related receptor, SorCS2 (Anastasia *et al.* 2013; Hempstead 2015).

Structure and expression of the BDNF gene

BDNF gene structure and expression in rodents was revisited almost a decade ago to propose a new numbering system for BDNF exons (Aid *et al.* 2007). Although only one pro-BDNF protein is synthetized, the BDNF gene contains multiple promoters and can undergo alternative splicing, bearing transcriptional regulation and yielding different mRNAs. The BDNF gene consists of eight 5′ non-coding exons, named I to VIII, plus a common 3′ exon, IX, encoding the pro-BDNF sequence and two poly-adenylation sites. Each BDNF transcript contains only one of the eight 5′ untranslated exons spliced to the 3′ protein coding exon plus either a long or a short 3′UTR (Aid *et al.* 2007). mRNAs with a long 3′UTR are located in spine dendrites and translated upon neuronal activation, whereas the short 3′UTR promotes a constitutive translation of the protein in the soma (An *et al.* 2008; Lau *et al.* 2010). Interestingly, the antisense-BDNF transcripts that have been described in the human BDNF gene (Liu *et al.* 2005) are not expressed in mouse or rat BDNF gene (Aid *et al.* 2007). In addition, BDNF expression is tissue- and age-specific, being the different promoter regions responsible for this spatial and temporal determination. All in all, the existence of different BDNF transcripts with distinct functional properties could be associated to specific neuropsychiatric disorders and thus, become attractive candidates for developing potential new pharmacotherapies. For a thorough revision on this topic, see Martínez-Levy and Cruz-Fuentes (2014).

Research tools to study the biology of BDNF

Expression of neurotrophins has been classically studied by usual methods like *in situ* hybridization and quantitative PCRs. Nevertheless, the subnanomolar quantities of BDNF protein that are physiologically produced in the brain reveal discrepancies between mRNA levels and results obtained by western blot (WB) or ELISA. Moreover, most commercial antibodies yield non-specific staining and display sensitivity limitations given that the sequence corresponding to the m-BDNF region is highly conserved (Dieni *et al.* 2012; Yang *et al.* 2014). Therefore, tissue identification and/or quantification by immunohistochemistry or immunofluorescence has proven difficult and sometimes unreliable. Also, most commercial ELISA kits have limited specificity and are unable to discriminate BDNF isoforms (Hartmann *et al.* 2012; Niitsu *et al.* 2014), contributing to the heterogeneity of results. A recent study claims that a commercial kit with different antibodies is able to recognize human pro-, m-, and truncated-BDNF (Tongiorgi *et al.* 2012). Also, it is sometimes probable that, although certain antibodies are able to recognize both isoforms, experimental conditions may not allow effective detection of pro-BDNF. Finally, four different possible domains mentioned above can be revealed in a classical WB membrane: the 32 kDa pro-BDNF, the 28 kDa truncated pro-BDNF, the 17 kDa pro-region and the 13.2–15.9 kDa mBDNF, yielding a sometimes confusing multi-band pattern. Readers may find a more detailed description of the latest approaches available to study the transport and activity-dependent secretion of BDNF in Hartmann *et al.* (2012).

Considering the physiological importance of both BDNF isoforms, measuring individual levels of pro-BDNF and m-BDNF is critical. Given the difficulties exposed regarding precise determination of BDNF protein, information about each isoform can be indirectly obtained by analyzing their specific receptor signaling pathways as well as several aspects of the proteases responsible for pro-BDNF cleavage. In addition, genetically modified animals are valuable tools to circumvent some technical problems. The first BDNF knockout mice were generated to evaluate the effects of a constitutive lack of this neurotrophin (Ernfors *et al.* 1994). Nevertheless, BDNF null mutant mice ($BDNF^{-/-}$) do not survive more than 2–4 weeks after birth, and therefore, heterozygous mice, with BDNF brain protein levels reduced by a half, are mostly employed. Also, genetically modified models, including mice lacking the TrkB receptor (Saarelainen *et al.* 2003), over-expressing the truncated TrkB (Sairanen *et al.* 2005) or BDNF conditional knock-out mice (Rios *et al.* 2001) have greatly helped to challenge hypotheses about the role of BDNF isoforms and its receptors (see a reviewed in Hashimoto 2010). By removing the BDNF gene, either constitutively or conditionally, the lack of both pro- and m-BDNF renders difficult the task of identifying their specific actions. It is thus tempting to speculate that mice models in which TrkB, p75, their downstream signaling molecules or even proteases genes are deleted can be more useful to elucidate the roles of BDNF isoforms.

Dissecting the role of bdnf isoforms in physiological and induced neurogenesis

The confirmation of the dichotomic roles of either BDNF isoforms underscores the notion that a dissection of the BDNF pathway is required to understand the details of its participation in physiologic and pathologic conditions. Therefore, research on the field should be conducted considering that references to ‘total BDNF effects’ can be very general and of doubtful value. Accordingly, in this section, studies in which BDNF isoforms are discriminated in light of their participation in the process of adult hippocampal neurogenesis are summarized.

Adult hippocampal neurogenesis in rodents

One of the most amazing discoveries of the 20th century in the field of neurosciences concerns the description of neurogenesis in the adult rodent brain (Altman and Das 1965). Physiological neurogenesis occurs in the adult nervous system in two specific areas: the subventricular zone and the dentate gyrus (DG) of the hippocampus (Gage 2000). In the latter region, stem cells located in the subgranular zone divide and proliferate to give rise to progenitor cells that differentiate into immature and later, mature neurons. Ironically, around 70–85% of the immature newborn neurons die in the maturation process, during the two-first weeks of their live, before getting integrated into the hippocampal neural circuit (Kempermann *et al.* 2003; Snyder *et al.* 2009). The surviving new-generated neurons become granule cells, extend their axons to the hilus and CA3 along the mossy fiber tract, and receive connections from the entorhinal cortex through the dendrites in the molecular layer. Thus, neurogenesis in the adult DG is a complex multistep process that can be divided into four events: (i) proliferation of neural stem/progenitor cells, (ii) neuronal differentiation, (iii) neuritic growth, synaptogenesis, and migration, and (iv) functional integration (Kempermann *et al.* 2015). Most regulation events occur during the early survival and maturation phases and, as a result, the majority of newborn cells are eliminated. Fueled by the potential benefits of the generation of newborn neurons in the adult brain, an emerging field of research has appeared concerning how to regulate this multistep process, potentially useful for the treatment of certain neuropsychiatric and neurodegenerative diseases.

Positive and negative modulation of adult neurogenesis

A bunch of studies demonstrated how neurogenesis can be increased by factors like environmental enrichment (Kempermann *et al.* 1997), hippocampus-dependent learning (Gould 1999), running (van Praag *et al.* 1999), and chronic administration of antidepressants (Malberg *et al.* 2000; Encinas *et al.* 2006), or serotonergic agonists of different receptors like 5-HT_{1A} (Santarelli *et al.* 2003; Banasr *et al.* 2004), 5-HT_{2B} (Diaz *et al.* 2012) and 5-HT_4 (Mendez-David *et al.* 2014). Some of these receptors are expressed not only in serotonergic neurons, but also in DG neural progenitors and interneurons (Klempin *et al.* 2010; Diaz *et al.* 2012), suggesting that the consequence of their stimulation could be complex and result from direct and indirect actions. Physiological neurogenesis can also be attenuated by glucocorticoid hormones (Gould *et al.* 1992), aging (Kuhn *et al.* 1996), and drugs of abuse like opiates (Eisch *et al.* 2000), alcohol (Nixon and Crews 2002), and cocaine (Domínguez-Escribà *et al.* 2006). Taking together the relations demonstrated between neurogenesis, antidepressants and glucocorticoids, a detrimental effect of stress on neurogenesis might be suspected. Indeed, seminal studies from the laboratories of McEwen and Gould have shown that chronic stress suppresses neurogenesis in the adult DG (see review from pioneer McEwen 1999; Gould and Tanapat 1999). Nevertheless, the picture is less clear when the question refers to the role of neurogenesis on the etiology of depression. A recent and exhaustive revision covering diverse behavioral tests classically used to evaluate a depressive-like phenotype in rodents, clearly demonstrates that solely decreasing neurogenesis is not enough to induce a depressive-like state (Petrik *et al.* 2012). Thus, whereas intact adult hippocampal neurogenesis is required for antidepressants effects, a causative role for neurogenesis in depression is more difficult to confirm. Remarkably, an analogous picture has been described for BDNF. For example, exposure of rats to chronic unpredictable stress did not correlate with any regulation of hippocampal BDNF mRNA, whereas chronic antidepressant treatment in the same model induced a significant increase in BDNF expression in the DG (Larsen *et al.* 2010). All in all, while changes in BDNF levels may not directly be involved in the pathophysiology of depression, this neurotrophin could be critical for antidepressant effects (see a reviewed in Martinowich *et al.* 2007; Castrén and Rantamäki 2010).

As mentioned above, it was shown early on that BDNF and TrkB mRNA increase in the hippocampus of rats in response to chronic electroconvulsive seizure as well as to chronic (and not acute) administration of antidepressant drugs, including the serotonin-selective reuptake inhibitor (SSRI) sertraline, the noradrenergic desipramine, the monoamine oxidase inhibitor tranylcypromine, and the atypical mianserin (Nibuya *et al.* 1995). After this novel inspiring study, the expression of BDNF and its receptors in brain regions of rodents treated with antidepressants has been widely analyzed. In an extensive and complete revision on this topic by Tardito *et al.* (2006), it is not surprising that out of the 15 articles compiled in the Table 1 reporting effects of antidepressants on BDNF expression, none of them discriminates pro- and m-BDNF levels. Given the scope of the present review, a compilation of articles studying the changes of pro- and m-BDNF in response to pro-neurogenic treatments is presented in Table 1.

Indirect avenues to evaluate participation of BDNF isoforms on neurogenesis

Given the complex biology already described for BDNF, reliable measures of its low extracellular levels are difficult to obtain (Lu 2003). Furthermore, the values obtained by WB of either isoform usually reflect the intracellular plus extracellular content. It becomes, therefore, motivating to explore other components involved in the pathway. A plausible alternative is to analyze the phosphorylation of TrkB, as an indirect indicator of BDNF release from neurons (Aloyz *et al.* 1999). A series of studies conducted in mice at the laboratory of Castren have demonstrated that chronic

Table 1 Effect of pro-neurogenic interventions on BDNF expression and BDNF isoforms.

References	Animal model	Pro-neurogenic intervention	Brain region and technique	Outcome
Wyneken *et al.* 2006	SD rats	Flx 0.65–0.75 mg/kg, 15 days, oral	HC & Cx post-synaptic densities. WB	Decreased TrkB from PND 15 to 42. n.c. in $p75^{NTR}$
			HC & Cx homog. WB	Increased TrkB from PND 12 to 42. Increased BDNF levels from PND 9 to 42
		Reboxetine 126 μg/kg, 5 days, i.m.	HC & Cx post-synaptic densities. WB	Decreased TrkB from PND 6 to 15
			HC & Cx homog. WB	Increased TrkB receptors at PND 15. Increased BDNF levels from PND 12 to 15
Rantamäki *et al.* 2007	BALB/c & C57BL/6 mice	Acute Flx 30 mg/kg & several others antidepressants, i.p.	HC. WB-Immunoprec	n.c. in TrkB protein levels. Increased TrkB autophosphorylation at Y705/6 site, but not at Y515, 1 h after acute antidepressant
		Acute Flx 20 mg/kg		Increased TrkB autophosphorylation at Y705/6. n.c. in PLC-γ1-TrkB association
		Flx 0.08 mg/kg (drinking sol.), 21 days		n.c. in TrkB or PLC-γ1 protein levels. Increased PLC-γ1-TrkB association. n.c. in TrkB-Shc interaction
Calabrese *et al.* 2007	SD rats	Duloxetine 10 mg/kg, 21 days	Cx. WB	Increased m-BDNF& n.c. in pro-BDNF in synaptosomal fraction Decreased pro- and m-BDNF in cytosolic fraction
			HC. WB	n.c. in pro- or m-BDNF
		Flx 10 mg/kg, 21 days	Cx & HC. WB	n.c. in pro- or m-BDNF
Musazzi *et al.* 2009	SD rats	Flx 10 mg/kg, 7 days, 14 days or 21 days; drinking water	HC. RT-qPCR	Increased BDNF mRNA started at PND 14 and peaked at PND 21
			HC. WB	Increased pro-BDNF at PND 21 and m-BDNF from PND 7 to 21
			Cx. RT-qPCR	Idem HC
			Cx. WB	Increased pro-BDNF from PND7 to 14. n.c. in mBDNF at any time point
		Reboxetine 10 mg/kg, 7, 14 or 21 days; drinking water	HC. RT-qPCR	Increased BDNF mRNA started at PND 7 and peaked at PND 21
			HC. WB	Increased pro-BDNF from PND 14 to 21 and m-BDNF from PND 7 to 21
			Cx. RT-qPCR	Idem HC
			Cx. WB	Increased pro-BDNF and m-BDNF from PND 7 to 21
Molteni *et al.* 2009	SD rats	Duloxetine 10 mg/kg × 21 days + 5 min (acute) stress the day 21	HC homog. WB	n.c. in pro- or m-BDNF
			HC synaptosomic fraction. WB	n.c. in pro-BDNF. Increased m-BDNF after 21 days duloxetine and acute stress
Griesbach *et al.* 2009	SD rats	Running wheel, 7 days	HC. WB	Increased m-BDNF, n.c. in pro-BDNF induced by exercise
Sartori *et al.* 2011		Running wheel, 28 days	HC. WB	Increased m-BDNF, n.c. in pro-BDNF or truncated-BDNF induced by exercise Increased mRNA of tPA and p11 (BDNF cleavage related gene)
Ding *et al.* 2011		Running wheel, 7 days	HC. WB & enzyme activity	Increased pro-BDNF, mBDNF, tPA activity and PTrkB
Yeh *et al.* 2012		Offspring from dams subjected to prenatal stress	CA1 region. WB	Increased pro-BDNF, decreased mBDNF and t-PA in 3- & 5-week-old mice (normalization at 8-week old)
			CA1. Activity & Zimography	Decrease in tPA, and no change in MMP-2 & MMP-9 in 3- & 5-week-old mice (normalization at 8-week old)
Costa *et al.* 2012		Treadmill running, 8w, 0, 1, 3, & 7 days/week	HC. WB	Increased pro- and m-BDNF in young rats training 1 day/week. Decreased pro-BDNF in middle-aged rats training 7 days/week. Decreased TrkB in young rats at all frequencies

(continued)

Table 1. (continued)

References	Animal model	Pro-neurogenic intervention	Brain region and technique	Outcome
Segawa *et al.* 2013	SD rats	Electroconvulsive seizure: 1 daily shock, 10 days	HC. RT-qPCR	Increased BDNF, furin, and t-PA mRNA, n.c. in PC1 between 1 & 2 h post shock
			HC. WB & immunoprec	Increased pro- and m-BDNF between 1 & 24 h post shock
			HC. Zimography	Increased t-PA activity between 1 & 24 h post shock
		Imipramine 20 mg/kg, 14 days, i.p.	HC. WB	Increased m-BDNF, n.c. in pro-BDNF, furin, PC1 or t-PA
			HC. Zimography	n.c. in t-PA activity
Réus *et al.* 2014	Rats spp?	Ketamine 15 mg/kg i.p. 3 injections	HC. WB	Increased pro-BDNF
Cao *et al.* 2014	Wistar rats	Enriched environment from PND 21–70	HC. RT-qPCR	n.c. in BDNF mRNA
			HC synaptosomic fraction. WB	Increased m-BDNF and MMP-9. n.c. in pro-BDNF or tPA

SD, Sprague–Dawley; Flx, fluoxetine; i.p., intraperitoneal, i.m., intramuscular; PND, postnatal day; HC, hippocampus; Cx, cortex; n.c., no change; MMP, matrix metalloprotease; PLC-γ, phospholipase C-γ; tPA, tissue plasminogen activator.

administration of antidepressants from different pharmacological groups induces the activation of the TrkB-PLC-γ1 pathway (Saarelainen *et al.* 2003; Rantamäki *et al.* 2007). Adding force to the participation of the TrkB signaling pathway in hippocampal neurogenesis, transgenic mice models in which TrkB receptors are specifically lacking in DG neural progenitor cells, but not in differentiated DG neurons, display a reduction in physiological as well as antidepressant- or exercise-induced neurogenesis (Li *et al.* 2008). Although the sets of studies described would advocate m-BDNF as required to stimulate the TrkB pathway signaling, the possibility that autophosphorylation of TrkB receptor is induced by GPCR transactivation, as already described, or by another low-affinity TrkB ligand, such as NT-4, cannot be disregarded. Intriguingly, it has been demonstrated that the responsiveness of TrkB to BDNF decreases during postnatal development (Di Lieto *et al.* 2012): from the second postnatal week, which marks the transition between developmental and adult neurogenesis, the TrkB receptor becomes weakly activated by BDNF, but clearly phosphorylated by antidepressants. This observation suggests that m-BDNF might be dispensable for activation of TrkB signaling in the adult hippocampus. Again, a reliable measure of BDNF isoforms could be a helpful complement to disentangle this dilemma.

The conditions under which BDNF isoforms are secreted through the regulated pathway were studied by means of refined and selective tools in cultured hippocampal neurons free of glial cells (Nagappan *et al.* 2009). Whereas pro-BDNF was the isoform predominantly secreted following low-frequency stimulation, m-BDNF was mostly secreted under high-frequency stimulation. Interestingly, low- and high-frequency stimulation facilitates LTD and LTP, respectively. Likewise, tPA was only secreted after high frequency stimulation, explaining the higher levels of m-BDNF obtained in these *in vitro* conditions. These results suggest that the ratio pro-BDNF/m-BDNF can be controlled by the frequency of neuronal activity, but this hypothesis is difficult to challenge *in vivo*.

As already mentioned, physical exercise has pro-neurogenic properties, apparently mediated by BDNF, like antidepressant drugs (Russo-Neustadt *et al.* 1999). It is, thus, not unexpected that after 4 weeks of physical exercise, increased levels of m-BDNF were detected in the hippocampus of mice (Sartori *et al.* 2011). Moreover, whereas pro-BDNF levels were unaltered in these animals, mRNA increases of the pro-BDNF cleavage related genes tPA and p11 were reported. In line with these findings, other studies in the hippocampus of mice and rats chronically treated with antidepressants, also reported increases in the mature form rather than the precursor (Molteni *et al.* 2009; Musazzi *et al.* 2009), supporting the hypothesis that antidepressant drugs act more rapidly on pro-BDNF processing by extracellular proteases.

The dissection of the biosynthesis and processing of BDNF in the hippocampus of rats receiving chronic electroconvulsive stimulation has been recently published (Segawa *et al.* 2013), showing increased BDNF mRNA levels in the hippocampus after 14 days of electroconvulsive stimulation accompanied by increases in pro- and m-BDNF proteins. Moreover, the levels and activity of intracellular furin and extracellular t-PA were also increased after the chronic electroconvulsive stimulation. Despite *in vivo* neuronal release of pro-BDNF is technically difficult to put in evidence, results obtained using synaptosomes from electroconvulsive stimulation-treated rats allow the authors to propose secretion of pro-BDNF and tPA, leading to extracellular production of m-BDNF. Unfortunately, a somewhat

short chronic imipramine treatment of 2 weeks was employed in the same article. Therefore, the lack of change in levels of BDNF or convertases in these conditions may not be conclusive, and further studies should be conducted with antidepressant drugs administered for at least 3 weeks, the period of time required to induce neurogenic effects (Santarelli *et al.* 2003).

Effects of pro-BDNF binding to $p75^{NTR}$ have been usually deduced after acute delivery of recombinant pro-BDNF. However, it is difficult to know how similar they can be to physiologically elicited effects. To evaluate the actions of pro-BDNF under the control of its endogenous promotors, knock-in mice have been generated in which the cleavage site of pro-BDNF is mutated (Yang *et al.* 2014). To avoid early lethality, mice expressed only one allele of cleavage-resistant pro-BDNF, keeping one allele, which can be cleaved ($BDNF^{+/-}$) and allowing comparisons with heterozygous $BNDF^{+/-}$mice. This sophisticated tool enable the authors to confirm that pro-BDNF levels increase from birth, peak at postnatal day 15 and then, low but sustained levels are detected during adulthood. These observations suggest an important role of pro-BDNF in early postnatal brain development, but still the developmental period for the DG. Moreover, the authors demonstrated that endogenous expressed pro-BDNF reduces dendritic arborization and dendritic spine densities of DG granule cells. Finally, impairment in synaptic plasticity and enhanced LTD was also reported in this mice model.

BDNF as a potential biomarker of major depressive disorder

Major depressive disorder (MDD) is one of the most prevalent psychological disorder in the population worldwide, with difficult diagnosis and yet, more difficult effective treatment. A way to ameliorate its diagnosis would be to validate biological markers that allow an early detection in patients. Postmortem studies in the hippocampus of patients receiving antidepressant treatments revealed a higher BDNF immunoreactivity compared to non-treated individuals (Chen *et al.* 2001). Likewise, decreased mRNA and protein levels of BDNF and TrkB receptors were reported in the hippocampus of suicidal subjects (Dwivedi *et al.* 2003). Considering the links already described between BDNF, antidepressants and even depression, the potential value of this neurotrophin as MDD biomarker has been exhaustively evaluated (reviewed by Hashimoto 2010). Although more highly concentrated in the brain, BDNF levels are also detectable in the blood, where it is mainly stored in platelets (Fujimura *et al.* 2002). Moreover, BDNF serum levels appear to reflect brain BDNF levels (Karege *et al.* 2002). Fueled by these antecedents and others, several studies have gone deeper into the search and reported decreased BDNF serum levels in depressed or suicidal individuals, as well as increased BDNF levels in antidepressant-medicated patients (see two meta-analysis studies by Sen *et al.* 2008; Brunoni *et al.* 2008). In late-onset geriatric depression, reduced BDNF plasma levels have also been accompanied by a reduction in plasma tPA levels (Shi *et al.* 2010). Interestingly, a pool of recent studies has refined the search and taken advantage of new commercial ELISA kits able to detect either human BDNF isoforms. Decreased serum levels of mBDNF in MDD patients were reported by three different laboratories (Yoshida *et al.* 2012; Zhou *et al.* 2013; Yoshimura *et al.* 2014). In contrast, whereas two of them did not find any change in the pro-BDNF levels of depressed patients (Yoshida *et al.* 2012; Yoshimura *et al.* 2014) the third indicated a significant increase in pro-BDNF levels of MDD patients compared to healthy controls (Zhou *et al.* 2013). Further studies are ensured to refine the methodology and validate the measurement of blood pro-BDNF/m-BDNF levels as biomarkers in patients with MDD.

A powerful triumvirate: BDNF, serotonin, and neurogenesis

Triangulation between adult neurogenesis, the serotonergic system, and BDNF signaling is supported by several findings (see a reviewed in Gardier *et al.* 2009). Indeed, neurogenic effects are induced after 3–4 weeks of chronic antidepressants treatment, a delay proposed to be because of the time required for neurotrophins to promote cell proliferation, and which is related to the 'neurotrophic hypothesis of depression' (Duman 2004). Studies conducted in genetically modified mice mostly suggest that impairment of the BDNF/TrkB signaling pathway do not affect depressive-like behaviors in mice but hinder responses to chronic antidepressant treatment (Wang *et al.* 2008). Neurogenic effects induced by SSRI antidepressants were classically supposed to be mediated by an increase in serotonergic transmission, as this is the main effect of SSRIs. These findings lead to the prediction that a decrease in serotonin levels would determine a decline in hippocampal adult neurogenesis. However, destruction of serotonergic neurons by local injection of a serotonin toxin into the raphe nucleus of rats induced discordant results on DG cell survival: whereas a decrease was reported by Brezun and Daszuta (1999), no change was detected by others (Ueda *et al.* 2005; Jha *et al.* 2006). Models with constitutive serotonin depletion may help to explain the discrepancies revealed by these studies. We took advantage of these tools and demonstrated a counterintuitive increase in DG cell survival in three different mice models with profound reductions of serotonin neurotransmission (Diaz *et al.* 2013). Mutant mice in which serotonergic raphe cells fail to differentiate because of the lack of the transcription factor Pet1, $Pet1^{-/-}$ mice, or lacking the vesicular monoamine transporter specifically in serotonergic neurons, $VMAT2^{f/f}$;$SERT^{cre/+}$ mice, have lifelong depletion of 80% and 95%, respectively, of brain serotonin. Both mice models displayed enhanced cell survival with increased amounts of new neurons, 1 and 4 weeks after BrdU injections, but normal rates of cell proliferation. Furthermore,

a similar phenotype was displayed by wild-type mice with serotonin synthesis blocked at adult age by chronic administration of p-chlorophenylalanine, an inhibitor of tryptophan hydroxylase 2 (Tph2), the rate-limiting enzyme for the synthesis of central serotonin (Diaz *et al.* 2013). These observations have been replicated in another model of hyposerotonergy, the Tph2 knock-in mice on a mix C57BL/6J-129S6/SvEv background, in which an increased survival of DG cells is described without significant changes in cell proliferation (Sachs *et al.* 2013). Likewise, normal neural progenitor proliferation was also seen in $Tph2^{-/-}$ mice on a pure C57BL/6N background (Klempin *et al.* 2013) confirming that serotonin is not required for physiological DG cell proliferation. On the contrary, serotonin was necessary for exercise-induced proliferation as demonstrated by the lack of increase in immature cells (types 2a, 2b & 3) after 1 week of running wheel in the same mouse model (Klempin *et al.* 2013). The evidence accumulated supports the concept that physiological levels of serotonin are essential for normal survival of adult-born neurons. A detailed description of the serotonergic neuronal circuitry was conducted in knock-in mice in which Tph2 is replaced by the enhanced green fluorescence protein reporter (Migliarini *et al.* 2013). This exhaustive study revealed that the lack of central serotonin induces a patent serotonergic hyperinnervation in the hippocampus coincident with up-regulated BDNF expression exclusively in this brain region. This matching is not only spatial, but also temporal, as both features appear around postnatal day 10. Confirming this phenotype, the content of total BDNF protein is increased in the hippocampus of $Tph2^{-/-}$ mice (Kronenberg *et al.* 2016), a finding in line with the increased DG cell survival described in different hyposerotonergic mice models. Supporting these results, a preliminary screening from our laboratory reveals a tendency to increased activation of TrkB receptor in the hippocampus of hyposerotonergic mice (unpublished experiments), but more thorough studies are needed to be conclusive. Also, it would be clarifying to discriminate BDNF isoforms in future studies on hyposerotonergic mice models.

To further explore the role of the serotonin system on physiological neurogenesis, we found that 7 day-stimulation of 5-HT_{1A} receptors is sufficient to normalize the altered survival phenotype reported in hyposerotonergic mice models (Diaz *et al.* 2013). We propose a working model in which this rescue could be attained by either direct or indirect mechanisms (Fig. 2), given that 5-HT_{1A} receptors are expressed by both proliferating neural progenitors (type 1 and 2 cells), and GABAergic interneurons in the DG (Klempin *et al.* 2010). Particularly, parvalbumin-expressing GABAergic interneurons can participate in the differentiation and maturation of DG neural precursors through the BDNF/TrkB signaling pathway (Waterhouse *et al.* 2012). Interestingly, a novel mechanism has been recently uncovered by which parvalbumin-positive interneurons are able to suppress proliferation and differentiation by activating $GABA_A$ receptors on type 1 cells (Song *et al.* 2012). Therefore, the interactions revealed between parvalbumin-expressing GABAergic interneurons and neural precursors appear attractive targets to unravel the BDNF-serotonin interplay on the fate of adult-born hippocampal neurons.

Serotonin can act through either excitatory or inhibitory serotonin receptors. Serotonin receptors are subdivided into seven families and fourteen subtypes, entitling a myriad of actions. With the exception of the ligand-gated ion channel 5-HT_3 receptor, all other serotonin receptors are GPCRs. Several of them have been related to the pro-neurogenic actions of SSRI antidepressants and in some cases, associations with BDNF have been established (Table 2). For example, classical behavioral and neurogenic effects of SSRIs were not observed in mice with genetic or pharmacological ablation of the 5-HT_{2B} receptor (Diaz and Maroteaux 2011; Diaz *et al.* 2012). Even though basal levels of cell proliferation and survival in the adult hippocampus were normal, chronic treatment with fluoxetine or paroxetine failed to induce neurogenic actions in these mice. Remarkably, we have just reported increased basal expression of BDNF in the DG of mice knock-out for 5-

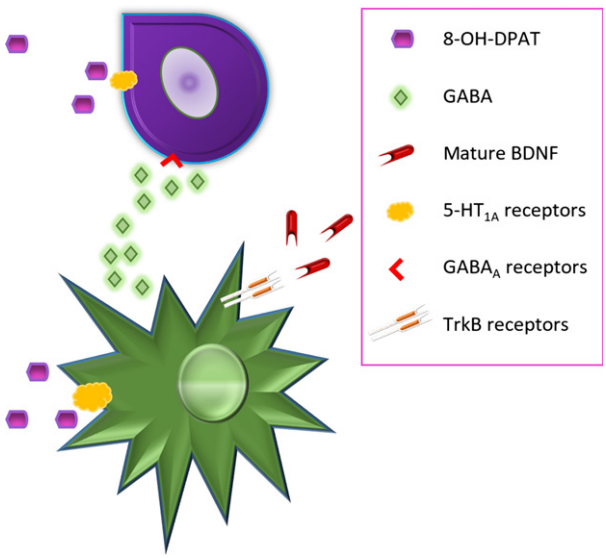

Fig. 2 Working model to explain 5-HT_{1A} agonist actions on Dentate gyrus (DG) cell survival. Administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT for 7 days is sufficient to normalize altered DG cell survival phenotype displayed by hyposerotonergic mice (Diaz *et al.* 2013; see text). We hypothesized that rescue of the phenotype can be attained either directly or indirectly. On one hand, 8-OH-DPAT might stimulate 5-HT_{1A} receptors expressed by DG neural progenitors (top). On the other hand, 8-OH-DPAT could act on 5-HT_{1A} receptors present on the membrane of parvalbumin-expressing GABAergic interneurons (bottom) to induce release of GABA, and in turn, this neurotransmitter can bind $GABA_A$ receptors expressed by neural progenitors. Interestingly GABAergic interneurons can promote differentiation and maturation of DG newborn neurons through activation of TrkB receptors.

Table 2 Effects on DG neurogenesis and BDNF expression induced by modulation of serotonin receptors.

Receptor subtype	Pro-neurogenic effects	Interaction with BDNF	Reference
5-HT_{1A}	Impaired SSRIs neurogenic effects in 5-HT_{1A} KO mice. Increased DG cell proliferation & survival in WT mice after 28 days treatment with the 5-HT_{1A} agonist 8-OH-DPAT		Santarelli *et al.* 2003
	n.c. in DG cell proliferation or survival in WT mice after 7 days treatment with the 5-HT_{1A} agonist 8-OH-DPAT		Klempin *et al.* 2010 Diaz *et al.* 2013
		Decreased mBDNF in ventral HC of 5-HT_{1A} KO mice (only in females). Decreased TrkB activation in ventral HC of 5-HT_{1A} KO mice	Wu *et al.* 2012
5-HT_{2B}	Impaired SSRIs & 5-HT_{1A} agonist pro-neurogenic effects in 5-HT_{2B} KO mice. Increased DG cell proliferation in WT mice after 28 days treatment with the 5-HT_{2B} agonist BW723C86		Diaz *et al.* 2012
		Increased mRNA BDNF & n.c. in TrkB or $p75^{NTR}$ in the HC of 5-HT_{2B} KO mice	Diaz *et al.* 2016
5-HT_{2C}	Increased DG cell proliferation in WT rats after 21 days treatment with the 5-HT_{2C} antagonist S32006	Increased BDNF mRNA in the HC of WT rats after 14 days treatment with the 5-HT_{2C} antagonist S32006	Dekeyne *et al.* 2008
		n.c. in mRNA BDNF and pro-BDNF, but decreased mBDNF in the HC of 5-HT_{2C} KO mice	Hill *et al.* 2011
5-HT_3		Increased 5-HT_3 receptors mRNA in the HC of mice with deletion of BDNF gene in CA3 pyramidal cells	Huang and Morozov 2011;
5-HT_4	Increased DG cell proliferation in WT mice after 3 days treatment with the 5-HT_4 agonist RS67333	Increases BDNF mRNA in the HC of WT mice after 3 days treatment with the 5-HT_4 agonist RS67333	Imoto *et al.* 2015
	n.c. of pro-neurogenic effects of 21 days fluoxetine 22 mg/kg in 5-HT_4 KO mice		Lucas *et al.* 2007
5-HT_6	Increased DG cell survival in WT rats after 4 days but not 14 days treatment with the 5-HT_6 agonist LY586713	Increased BDNF mRNA in CA1 & CA3, but not in DG of WT rats after 4 days but not 14 days treatment with the 5-HT_6 agonist LY586713	de Foubert *et al.* 2013
5-HT_7	Normal basal cell proliferation in the DG of 5-HT_7 receptors KO mice		Sarkisyan and Hedlund 2009

KO, knock-out; DG, dentate gyrus; n.c; no change; HC, hippocampus; BDNF, brain-derived neurotrophic factor; SSRI, serotonin-selective reuptake inhibitor.

HT_{2B} receptors (Diaz *et al.* 2016). In our eyes, since an intact BDNF pathway is required for antidepressants effects, the reported altered expression of BDNF could be at least partially responsible for the lack of SSRIs effect in these mutant mice. Although the role of the different serotonin receptors on the neurogenic effects of antidepressants has been comprehensively studied, less is known about the relationship between serotonergic receptors and hippocampal BDNF levels (Table 2), assuring future studies in this field.

The links between BDNF and serotonergic interventions have been deeply explored. Constitutive decrease in BDNF levels, as displayed by $BDNF^{+/-}$ mice, results in an elevation of basal extracellular serotonin in the ventral hippocampus (Guiard *et al.* 2008). On the other hand, local intra-hippocampal injection of BDNF reduced extracellular serotonin levels, an effect that was eliminated by perfusion of the Trk receptor inhibitor K252a (Deltheil *et al.* 2008). These complementary results suggest the challenging idea that decreased contents of extracellular serotonin are linked to increased BDNF levels, whereas high levels of this neurotrophin have been extensively associated with antidepressant properties. How can these data be reconciled? A thorough analysis of hippocampal extracellular serotonin levels has been conducted by Gardier and colleagues, demonstrating that, the well-known increase in serotonin levels after acute administration of SSRIs, is attenuated after chronic administration (Popa *et al.* 2010). Indeed by the time neurogenic effects occur, extracellular serotonin levels are normalized in the hippocampus of chronically fluoxetine-treated mice. Thus, SSRIs neurogenic effects occur in the

absence of increased serotonin hippocampal levels but in the presence of increased BDNF expression. Remarkably, it has been reported that intrahippocampal infusion of BDNF in rats induces antidepressant effects in a behavioral model of depression, like learned-helplessness, and in the forced-swimming test (Shirayama *et al.* 2002). Conversely, deletion of BDNF in the forebrain of mice attenuated antidepressant effect of desipramine in the forced-swimming test (Monteggia *et al.* 2004). More precisely, the same lack of response after the non-serotonergic desipramine or citalopram was seen when BDNF was absent in the DG of mice but not in the CA1 (Adachi *et al.* 2008). All together, these results would indicate that BDNF, probably through the TrkB signaling pathway, collaborates with serotonin homeostasis, facilitating pro-neurogenic effects of SSRIs antidepressant in the DG.

Concluding remarks

Since the discovery of BDNF more than 30 years ago, a lot of research has been devoted to elucidate its role in physiological and pathological conditions. The complexity of the BDNF system is given by multiple factors, i.e., the heterogeneous population of mRNAs, two functional isoforms, two different receptors with their multiple signaling pathways, requiring thus more precise and fine-tuned methodological tools to make advancements in the field. The modulation of the BDNF signaling pathway by the serotonergic system, and their imbricated interactions operating on adult hippocampal neurogenesis opens up an exciting area of research that undoubtedly, will impress our imagination for a long time.

Acknowledgments and conflict of interest disclosure

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All experiments were conducted in compliance with the ARRIVE guidelines.

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