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Modulation of dendritic spine development and plasticity by BDNF and vesicular trafficking: fundamental roles in neurodevelopmental disorders associated with mental retardation and autism

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Abstract The process of axonal and dendritic development establishes the synaptic circuitry of the central nervous system (CNS) and is the result of interactions between intrinsic molecular factors and the external environment. One growth factor that has a compelling function in neuronal development is the neurotrophin brain-derived neurotrophic factor (BDNF). BDNF participates in axonal and dendritic differentiation during embryonic stages of neuronal development, as well as in the formation and maturation of dendritic spines during postnatal development. Recent studies have also implicated vesicular trafficking of BDNF via secretory vesicles, and both secretory and endosomal trafficking of vesicles containing synaptic proteins, such as neurotransmitter and neurotrophin receptors, in the regulation of axonal and dendritic differentiation, and in dendritic spine morphogenesis. Several genes that are either mutated or deregulated in neurodevelopmental disorders associated with mental retardation have now been identified, and several mouse models of these disorders have been generated and characterized. Interestingly, abnormalities in dendritic and synaptic structure are consistently observed in human neurodevelopmental disorders associated with mental retardation, and in mouse models of these disorders as well. Abnormalities in dendritic and synaptic differentiation are thought to underlie altered synaptic function and network connectivity, thus contributing to the clinical outcome. Here, we review the roles of BDNF and vesicular trafficking in axonal and dendritic differentiation in the context of dendritic and axonal morphological impairments commonly observed in neurodevelopmental disorders associated with mental retardation.

Keywords Dendritic spine · Mental retardation · Vesicle trafficking · Autism · Rett syndrome · BDNF · Hippocampus · Pyramidal neuron

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General overview: dendritic spines

Cellular models of associative learning and memory have shown that enduring activity-driven changes in the efficacy of synaptic transmission, i.e. synaptic plasticity, initiates changes in neuronal connectivity, which is reflected in the formation of new synapses or the structural remodeling of existing ones. The "hot spot" of this structural plasticity is the dendritic spine. Dendritic spines are small protrusions extending from dendrites that are the main postsynaptic site of excitatory glutamatergic synapses in the brain. Structurally, a spine consists of a spherical head connected by a thin neck to a parent dendrite (Fig. 1). Spines can vary structurally owing to changes in their length, the shape of the head, and the diameter of the neck. Dendritic spines serve as critical compartments in which biochemical (e.g. kinases, phosphatases) and ionic (e.g. Ca^{2+}, Na^{+}) changes occur during excitatory synaptic transmission. In addition,

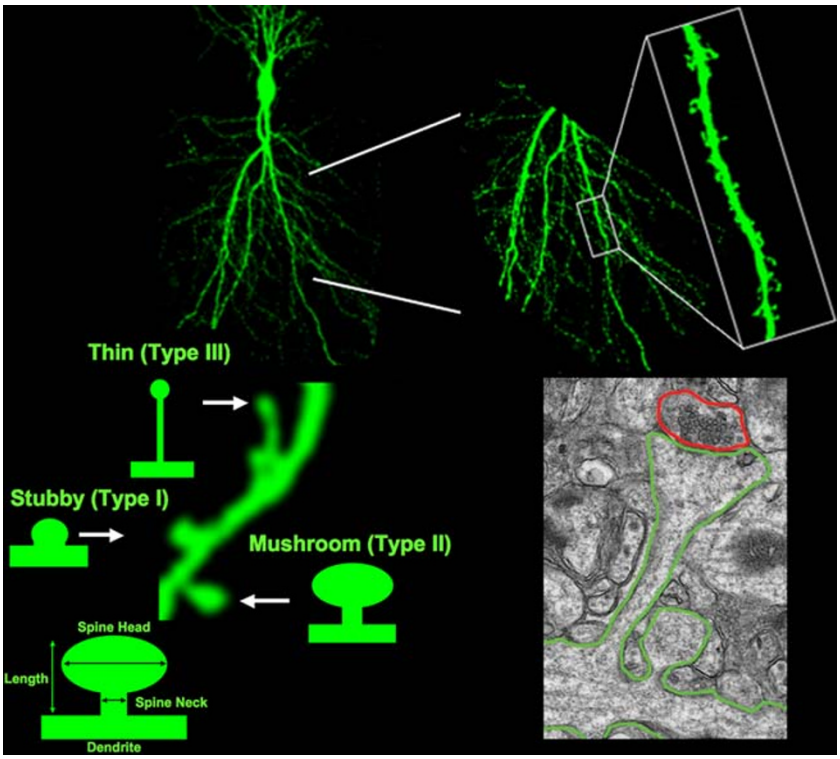

Fig. 1 The structure of dendritic spines of hippocampal pyramidal neurons. Using particle-mediated gene transfer (a.k.a. gene gun), organotypic slice cultures were transfected with cDNA coding for eYFP. *Top panels*: Laser-scanning confocal microscopy images of a pyramidal neuron in area CA1 are shown at different magnifications to illustrate the complexity of their dendritic arbor and the abundance of dendritic spines in secondary and tertiary branches. *Bottom left panel*: A maximum-intensity projection of *z*-stacks shows a dendritic segment studded with the most common spine morphologies, i.e. stubby, mushroom and thin. The cartoon illustrates the geometrical dimensions measured in individual spines to categorize them (adapted from Ref. 32). *Bottom right panel*: A mushroom dendritic spine (outlined in green) forms an asymmetric synapse with a single presynaptic terminal (outlined in red) in *stratum radiatum* of area CA1 in organotypic slice culture

the dynamic and plastic nature of spines observed *in vitro* [1–3] and *in vivo* [4–6] supports the hypothesis that changes in the shape and number of spines contribute to the mechanisms of memory formation and storage [7–9]. Indeed, numerous reports have demonstrated that changes in the morphology or density of spines occur after induction of synaptic plasticity, e.g. long-term potentiation (LTP) and long-term depression (LTD), widely accepted cellular underpinnings of associative learning and memory [10].

The morphology of dendritic spines is thought to play an important role in determining their function. Simple spines are characterized by three major types: stubby (type-I) spines have no obvious neck, mushroom (type-II) spines have a large head that is connected to the parent dendrite by a narrow neck, while thin (type-III) spines have a small head connected to the dendrite by a long neck [11]. Mature spines are thought to originate during development from dendritic filopodia, long thin processes that emanate from the dendrite, which after contact with an axon, initiate spinogenesis and synaptogenesis. Recent work has demonstrated that dendritic spine morphology modulates Ca^{2+} entry into the spine and diffusion through the spine to the parent dendrite [12, 13], synaptic transmission [14], synapse formation [15] and spine stability [16, 17]. Furthermore, *in vivo* evidence in mice and rats demonstrate that in response to environmental manipulations and learning, spine density and morphology are both altered [18]. More recent evidence also indicates that changes in the molecular composition of dendritic spines are initiated during learning. Matsuo *et al*. observed that newly synthesized GluR1 AMPA receptors are recruited directly to mushroom-shaped spines in the hippocampus 24 hrs after fear conditioning [19].

What molecular cue initiates these structural modifications during synaptic plasticity? Given that ionotropic glutamate receptors are highly expressed at the postsynaptic density (PSD) of dendritic spines, it is not surprising that ligands of these receptors modify spine density and morphology [20]. However, the release of glutamate into the synaptic cleft is extremely rapid, so activation of glutamate receptors must also recruit/regulate additional signaling components that can lead to sustained structural modifications of spines that occur for long periods. The chemical signal that would alter the number or structure of

spines would need to cause changes that are prolonged in order for these adjustments to contribute to the process of synaptic plasticity. Thus, this molecular cue would need to be released by some type of plasticity-inducible stimuli, contribute to the process of synaptic plasticity and learning and memory, and be able to modify dendritic spine by itself.

The role of BDNF in dendrite differentiation and dendritic spine formation and plasticity

The mammalian neurotrophins, nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5) have essential roles in neuronal survival and differentiation [21]. BDNF is produced initially as a precursor form, a pro-neurotrophin (30–35 kDa), before it is proteolytically cleaved into a mature neurotrophin form (12–13 kDa). In addition, neurotrophins in general and BDNF in particular, are strong modulators of synaptic transmission and plasticity [18, 22–26]. Anatomically, BDNF levels in the hippocampus, a brain region important for learning and memory, are amongst the highest in the brain [27]. Functionally, long-term exposure to BDNF increases spine density in CA1 pyramidal neurons in rodent hippocampus, an effect that is blocked by the tyrosine kinase inhibitor k-252a, suggesting that the spinogenic effect of BDNF is mediated by the tyrosine kinase activity of the high affinity BDNF receptor, TrkB [28].

BDNF mediate its effect by the binding to one of two different families of receptors: the pan-neurotrophin receptor $p75^{NTR}$, which is a member of the tumor necrosis family of receptors, and a specific tyrosine kinase receptor [29, 30]. Binding of mature BDNF dimers to the TrkB receptor causes receptor dimerization and autophosphorylation in the tyrosine residues that form platforms for adaptor protein binding, leading to the activation of phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK, also ERK) and phospholipase C-γ (PLC-γ) signaling cascades [31]. BDNF-induced spine formation requires activation of at least two of these signaling cascades. Inhibitors of the MEK1, which prevent further signaling through the MAPK/ERK signaling cascade, prevented BDNF-induced spinogenesis [32], while functional TRPC channels, which are activated by the PLC-γ cascade [26, 33] and cAMP signaling, are also necessary for BDNF to enhance dendritic spine density [34, 35]. In contrast, the effect of BDNF-induced modulation of dendritic spines by $p75^{NTR}$ remains under intense investigation in several labs [36, 37].

In addition to its spinogenic effects, BDNF also increases the proportion of mature and stable stubby spines under conditions of both action potential-dependent and -independent synaptic transmission [38]. In contrast, when SNARE-dependent vesicular synaptic transmission is abolished with *Botulinum* neurotoxin C, BDNF increases the proportion of the highly unstable and immature thin type spines [38]. These data suggest that not only does long-term exposure to BDNF induce new spine formation regardless of neuronal activity levels, but BDNF also works together with neurotransmitter release [39] to modulate spine morphology. The role for BDNF in activity-dependent functional plasticity of excitatory glutamatergic synapses is further supported by the observation that release of endogenous (native) mature BDNF occurs in response to afferent stimulus patterns known to be effective for neuropeptide release from dense-core vesicles and to induce synaptic plasticity [40–43]. It should be noted that whether and under what circumstances the BDNF precursor, proBDNF, is secreted by neurons remain unclear [44]; but see [45, 46]. A differential modulation of dendritic spine density and morphology by proBDNF *vs*. mature BDNF, as proposed for synaptic plasticity [47], will certainly expand the already extensive repertoire of this multifaceted neurotrophin.

It has been suggested that thin spines are "learning spines" because they are constantly changing in response to activity, while mushroom spines are "memory spines" because they are highly stable structures [48]. Intriguingly, hippocampal slice cultures maintained in serum-containing media, which has a lower $p75^{NTR}$-to-TrkB expression levels than hippocampal slice cultures maintained in the previously published serum-free media [38], BDNF not only increased spine density, but also shifted the proportions of spines towards the "learning" (thin) and "memory" (mushroom) shaped spines [49]. Together with observations in $p75^{NTR}$ knockout mice [37], these results suggest a potential opposite effect of TrkB and $p75^{NTR}$ signaling on the morphology of dendritic spines, as another example of the "Yin-Yang" of neurotrophin receptor signaling [47]. Furthermore, the enlargement of spine head volume and "spine twitching" caused by repetitive pairing of two-photon glutamate uncaging onto single spines and postsynaptic action potentials is mediated by release of endogenous BDNF [50]. Though it is still unknown whether it mediates activity-dependent dendritic spine plasticity during learning and memory *in vivo*, BDNF is a strong candidate as an inducible factor that structurally prepares excitatory synapses for consolidation of hippocampal-dependent learning [24]. Does BDNF-induced structural plasticity of dendritic spines have functional consequences? At least for intracellular Ca^{2+} signaling, the shift towards a higher proportion of mature shaped spines after BDNF exposure promotes supralinear Ca^{2+} elevations in oblique dendrites of CA1 pyramidal neurons during coincident pre and postsynaptic activity [51].

In summary, BDNF is one of the strongest candidates to serve as a critical molecular cue that contributes to activity-dependent structural plasticity of dendritic spines [18]. BDNF-induced modifications in dendritic architecture including spine density and morphology, along with its modulation of presynaptic neurotransmitter release [39, 52], likely underlie the role of BDNF in the establishment and connectivity of the neuronal network required for synaptic plasticity and hippocampal-dependent learning and memory [24, 26, 53].

Dendritic pathologies in neurodevelopmental disorders associated with mental retardation

Neurodevelopmental disorders associated with mental retardation are characterized by a prevalent deficit in cognitive function and behavioral adaptations that range in severity and are often accompanied with symptoms specific to each disorder. Mental retardation-associated disorders that have an environmental or genetic origin have long been associated with morphological pathologies of dendrites and spines [54, 55]. Pioneering studies by Huttenlocher, Marin-Padilla, and Purpura published in the 1970's described abnormalities in the dendritic morphology of cortical neurons obtained from postmortem brain samples [56–61]. The abnormalities in dendritic structure included an overall reduction in dendritic spine numbers or the prevalence of long and thin spines (sometimes called tortuous spines), a cellular neuropathology termed "spine dysgenesis" [59]. While the results between reports varied as to the exact morphologically aberrations detected, the results consistently demonstrated abnormal dendritic structure.

These findings provide a morphological basis for the proposed synaptic deficiencies thought to underlie mental retardation, whereby smaller spine head sizes and lower spine density results in a reduction in postsynaptic surface area, leading to impaired excitatory neurotransmission and activity-dependent Ca^{2+} influx. Since those initial observations, reduced dendritic complexity, as well as significant differences in dendritic spine numbers and morphological spine types, have been described in several mental retardation-associated disorders, ranging from environmental (e.g. fetal alcohol syndrome or lead exposure), to autosomal genetic (e.g. Down syndrome), to X chromosome linked origins (e.g. Rett syndrome, fragile-X syndrome) [54, 55, 62]. Identifying the specific dendritic pathologies in every mental retardation syndromes will provide a deeper understanding of the underlying structural and molecular dysfunction causing these disorders. Below, we will describe the spine and synaptic abnormalities reported for Rett syndrome, a neurodevelopmental disorder associated with mental retardation. While this disease will be specifically discussed, it should be noted that many other neurological diseases and neurodevelopmental disorders have been shown to have altered dendritic spine structure [55].

Rett syndrome

A neurodevelopmental disorder associated with mental retardation and presenting with "spine dysgenesis" in cortical neurons is Rett syndrome (RTT). RTT is an X chromosome-linked disorder that affects approximately 1:15,000 females worldwide, without predisposition to any particular racial or ethnic group. Birth and the normal milestones of early development (e.g. growth patterns, motor, language and social skills) appear uneventful in individuals with RTT until approximately 6–18 months. Furthermore, features demonstrating signs and symptoms of autism, have also be observed in some individuals afflicted with RTT [63].

Mutations in *MECP2*, the gene encoding methyl-CpG-binding protein-2, have been identified in >80% of RTT individuals [64, 65]. MeCP2 is a DNA-binding protein with high affinity for A/T rich sites in close proximity to methylated CpG islands, recruiting co-repressors and histone deacetylase complexes, thereby altering the structure of genomic DNA and repressing the transcription of specific target genes [66–69]. The brain pathology of RTT includes reduced neuronal size and increased cell density in several brain regions including the cerebral cortex, hypothalamus and the hippocampal formation [70, 71]. Reduced dendritic tree size and complexity in pyramidal cells was observed in the frontal and motor cortices and in the subiculum, the main output region of the hippocampal formation [72]. Furthermore, reduced levels of microtubule-associated protein-2 (MAP-2), a protein involved in microtubule stabilization, were found throughout the neocortex of RTT autopsy material [73–75]. Lastly, reduced dendritic spine density and expression of cyclooxygenase, a protein enriched in dendritic spines, was reported in the cortex of RTT individuals [74, 76, 77]. These observations support the hypothesis that RTT is caused by impaired development that altered activity-dependent refinement of synaptic connections [78–80].

Observations regarding dendritic and synaptic pathologies in experimental animal models of Rett syndrome have produced varied results. However, impairments in excitatory synaptic transmission and common neurological phenotypes that are reminiscent of several RTT symptoms are consistent across the three mouse models of RTT based on MeCP2 loss-of-function. Two of the RTT models are full deletions of *Mecp2* ([81] exon 3

deletion, a.k.a. Jaenisch null mice; [82] exons 3 and 4 deletions, a.k.a. Bird null mice), and one mouse model contains a premature stop codon after codon 308 that yields a non-functional truncated protein (*Mecp2*^{308} [83]). Though the genetic backgrounds and extent of MeCP2 deficiencies in these mouse lines are different, they all show delayed onset of symptoms (approximately 5 weeks of age), which included motor impairment and abnormal gait. *Mecp2* null mice have hind-limb impairments, while *Mecp2*^{308} mice show forelimb impairments [83, 84]. In addition, excitatory (but not inhibitory) synaptic transmission onto cortical pyramidal neurons is impaired in *Mecp2* null mice [85], as well as between cultured *Mecp2*-deficient hippocampal neurons [86].

Consistent with impairments of glutamatergic synaptic transmission, deficits in hippocampal synaptic plasticity and hippocampal-dependent learning and memory were observed in *Mecp2* null mice [87] and *Mecp2*^{308} mice [88]. Intriguingly, overexpression of MeCP2 also led to neurological abnormalities. Transgenic mice expressing one copy of the human *MECP2* gene with all regulatory elements (which approximately doubled MeCP2 protein levels), initially showed a higher learning performance and enhanced hippocampal LTP, though seizures and other symptoms developed after 20 weeks of age and death occurred shortly thereafter [89]. Micro-island cell cultures of hippocampal neurons from *Mecp2* overexpressing mice formed more excitatory autapses compared to wildtype neurons, while the opposite was observed in cultures from *Mecp2*-deficient mice [90]. In contrast, a five-fold increase in MeCP2 levels did not affect dendritic spine density in hippocampal pyramidal neurons maintained in slice culture, but did show a significant increase in dendritic spine length [91]. Recent evidence gives more precedent that altered expression of MeCP2 lead to a reduction in dendritic spine number. Dendritic spine density is reduced throughout the brain of *Mecp2* knockout mice, with a great deficiency observed in the CA1 region of the hippocampus [92, 93] a phenotype also observed in humans individuals with RTT [77]. Cortical and hippocampal pyramidal neurons from *Mecp2*^{308} mice did not show any dendritic or synaptic morphological pathology, despite significant impairments in hippocampal-dependent learning and memory, as well as hippocampal synaptic plasticity [88]. In contrast, *Mecp2* knockout mice have smaller and less complex pyramidal neurons in cortical layers II/III, while no differences in spine density were reported at 8 weeks of age [94]. In the somatosensory cortex of *Mecp2* null mice, pyramidal neurons showed lower spine density and reduced dendritic branching by 6 weeks of age [95].

It has been suggested that these reduced numbers of excitatory synapses reflect delayed neuronal maturation, since newly born neurons in the adult dentate gyrus have lower dendritic spine density than their mature neighboring neurons [96]. Consistent with such role in neuronal differentiation, NGF-induced neurite outgrowth in PC12 cells was inhibited by *Mecp2* knockdown with antisense oligonucleotides [97]. On the other hand, *Mecp2* overexpression increased the complexity and length of axons and dendrites in primary cortical neurons, while overexpressing *Mecp2* with a truncation at the C-terminal (*Mecp*^{293}) increased axonal and dendritic branching without affecting their overall length [98]. New evidence suggests that the abnormalities in dendritic structure in *Mecp2* deficient neurons (cultured from knockout mice) are a result of the release of toxicity substances from neighboring *Mecp2* deficient astrocytic cells [99, 100]. Taken altogether, observations in *Mecp2*-based mouse models, as well as overexpression and knockdown experiments in cultured neurons and brain slices support the hypothesis that Rett syndrome is caused by impaired growth and activity-dependent maturation of pyramidal neuron dendrites, axons and their excitatory synapses, leading to deranged synaptic transmission and plasticity. Thus, specific neurological symptoms arising from impaired functioning of improperly wired neuronal networks in specific brain regions, likely cause defects in activity-dependent synaptic strengthening and pruning during postnatal development.

Four experimental approaches have been shown to reverse severe impairments in symptomatic *Mecp2* null mice, two based on gene expression manipulations, and two by pharmacological treatments. The overexpression of *Bdnf* in postnatal forebrain neurons under control of the CaMKII promoter extended the lifespan, rescued a locomotor defect, and reversed an electrophysiological deficit observed in *Mecp2* null mice [101]. The overexpression of the *Bdnf* gene in primary hippocampal cultures fully rescued the dendritic atrophy caused initiated by endogenous *Mecp2* knockdown [102]. However, when *Bdnf* was overexpressed in neurons that were transfected with RTT-associated *MECP2* mutants, only a partial rescue of the dendritic phenotype occurred [102]. The potential target of BDNF expression as a therapeutic approach to alleviate RTT symptoms is further supported by the reversal of breathing pattern irregularities in *Mecp2* null mice by treatment with an AMPAkine [103], a family of allosteric modulators of AMPA-type glutamate receptors known to enhance BDNF mRNA and protein levels [104, 105]. Supporting the potential use of trophic factors to reverse the RTT-like impairments in *Mecp2* null mice, an active peptide fragment of Insulin-like Growth Factor 1 (IGF-1) extended the lifespan, improved locomotor function, ameliorated breathing patterns, reduced heart rate irregularity, and increased brain weight. Furthermore, IGF-1 partially restored dendritic spine density and excitatory synaptic current amplitude, the expression of the synaptic scaffold-

ing protein PSD-95, and stabilized cortical plasticity in *Mecp2* null mice to wild-type levels [106]. Finally, in a proof-of-concept experiment that demonstrates that severe impairments in symptomatic *Mecp2* null mice can be reversed, the re-expression of the *Mecp2* gene under control of its endogenous promoter extended the lifespan and prevented the advanced neurological symptoms [107]. Altogether, these successful therapeutic approaches that reversed RTT-like symptoms in *Mecp2* null mice provide further support to the potential pharmacological reversal of neurodevelopmental disorders in adults [108].

The elusive link between MeCP2 and BDNF in the pathogenesis of Rett syndrome

The molecular pathway(s) contributing to the pathogenesis of Rett syndrome (RTT) remain unclear. Since MeCP2 is a transcriptional regulator, identifying the genes under its control will clarify the pathogenesis of RTT and have a profound impact for the development of therapies. While many genes have been shown to be regulated by MeCP2 [109], their contributions to the manifestation of the RTT remains unknown [110–112]. Using gene-target approaches, two studies identified *Bdnf* as a target of MeCP2 transcriptional control [113, 114]. BDNF mRNA levels are diminished in *Mecp2* null mice [101]. Similarly, BDNF mRNA levels are lower in brain samples from RTT patients [115, 116]. However, while nerve growth factor (NGF) was found to be reduced in either blood serum or cerebral spinal fluid from RTT patients, differences in BDNF levels were not detected [117–120].

The Chen *et al*. and Martinowich *et al*. studies [113, 114] that proposed a mechanistic link between MeCP2 and BDNF, demonstrated that MeCP2 binds to and represses the transcription of mouse *Bdnf* promoter IV, which is activated by neuronal activity and Ca^{2+} influx [121]. Cortical neurons cultured in the absence of neuronal activity (i.e. in the presence of TTX) from *Mecp2* null mice showed a 2-fold higher level of *Bdnf* exon IV transcript compared to neurons from wildtype mice [113]. This result may predict that BDNF levels should be elevated when Mecp2 is missing or mutated. However, BDNF protein levels were found to be lower in the brains of *Mecp2* knockout mice at 6–8 weeks of age compared to wildtype littermates [101]. Furthermore, conditional deletion of the *Bdnf* in the forebrain of *Mecp2* null mice exacerbated the onset of the RTT-associated phenotypes of the *Mecp2* null animals. And consistently, overexpression of *Bdnf* in the forebrain slowed the disease progression phenotype in *Mecp2* null mice [101].

The link between MeCP2 and BDNF appears to be more complex than originally described. A recent study shows that *Mecp2* overexpression in cultured neurons increases *Bdnf* mRNA levels through a homeostatic mechanism involving miR132, a BDNF-inducible microRNA that inhibits *Mecp2* expression [122]. Despite the identification of this intriguing microRNA feedback loop, the specific underlying mechanisms and whether a deregulation of such mechanisms contribute to the disease pathology of RTT remain unclear. Furthermore, the relationship between MeCP2 and BDNF may vary in different brain regions. A recent microarray study comparing hypothalamic samples from *Mecp2* null and *Mecp2* overexpressing mice found that BDNF mRNA levels were lower in the absence of *Mecp2* and higher when MeCP2 levels were doubled [123]. The challenge ahead is to identify specific clinical symptoms with affected brain regions, paving the way to the reversal of life-threatening impairments by region-specific manipulations of MeCP2 target genes.

Vesicle trafficking, BDNF and Rett syndrome

The proper secretory trafficking of BDNF appears to be critical for its function in neuronal development and synaptic plasticity. A common single nucleotide polymorphism in the *BDNF* gene, resulting in a valine to methionine substitution in codon 66 of the proBDNF domain (Val66Met), is associated with reduced hippocampal volume, memory impairment and susceptibility to psychiatric disorders in humans who are heterozygous for this variant *BDNF* gene [124]. The substitution of valine to methionine in the *BDNF* gene impairs the intracellular trafficking of the protein and the regulated secretion of BDNF from hippocampal neurons in culture [125]. Consistently, Val66Met knockin mice have reduced dendritic branching in dentate granule cells, suggesting that maintaining the trafficking of BDNF is necessary for the establishment of dendritic branching [126]. The importance of BDNF in RTT severity has come into question recently by two observations that have been described in individuals afflicted with RTT in addition to carrying the polymorphisms of the BDNF Val66Met gene. It has been described by clinical studies that the Val66Met polymorphisms might be neuroprotective in RTT, where girls with RTT and carrying the wildtype BDNF gene demonstrated seizures earlier in life than girls who with the Val66Met polymorphism [127]. While this polymorphism might be neuroprotective in terms seizure onset, individuals with the Val66Met polymorphism tended to possess severe phenotypic characteristics of the RTT compared to individuals without the polymorphism [128].

The endosomal trafficking pathway is involved in delivery of proteins to intracellular compartments and internalization, recycling and degradation of plasma mem-

brane proteins [129–131]. It is through this mechanism neurons internalize BDNF bound to its receptor in a clathrin-dependent manner. Proteins delivered to the endosomal pathway from the secretory pathway or via endocytosis from the plasma membrane can take three different route of signaling. They can be either sorted in the early endosome, recycled, or sent for lysosomal degradation via the late endosome. In neurons, recycling endosomes and early endosomes, which have been identified in dendritic spines, are proposed to allow membrane proteins to recycle locally within the spine [132, 133]. Recently it was reported that collapse of the recycling endosome results in a decrease in spine density in an activity-dependent manner [133]. Recent reports have demonstrated that proteins involved in endosomal trafficking as candidate gene products in some patients with autism. For example, a haploinsufficiency of *RAB11FIP5* was described in one patient. Rab11FIP5 is a Rab effector involved in protein trafficking from the recycling endosome to the plasma membrane and in neurotransmitter release and receptor recycling [134].

In the secretory pathway membrane proteins and secreted cargo are synthesized in the endoplasmic reticulum (ER), trafficked through the Golgi, and are packaged into vesicles at the trans-Golgi network (TGN) [131, 135]. Vesicles traffic to endosomes, lysosomes, or the plasma membrane, where they undergo constitutive or calcium-dependent, regulated secretion. In neurons regulated secretory vesicles, called secretory granules (SGs) or dense core vesicles (DCVs), are involved in the packaging, processing and release neuropeptides, neurotrophins, and biogenic amines [135–138]. Neuropeptides and neurotrophins like BDNF, are packaged and processed from pro-forms in immature secretory granules (ISGs) via proteolytic cleavage by peptidases, to generate their active mature forms, which undergo maturation to mature secretory granules (MSGs) that can undergo exocytosis in response to increases in intracellular Ca^{2+} concentration [139–142]. The *Mecp2* knockout mouse model of Rett syndrome demonstrate abnormal secretory granule exocytosis, with increased catecholamine release at low frequency stimulation and exacerbated release at high frequency, suggesting a larger readily releasable pool of catecholamine containing secretory granules [143].

Understanding the unknown: a link to autism spectrum disorders

Many neurodevelopmental disorders associated with mental retardation, such as RTT and fragile X, show comorbidity with autism spectrum disorders (ASD). ASDs, which include autism, Asperger syndrome, and pervasive developmental disorders not otherwise specified (PDDNOS), are characterized by deficits in social interaction and communication. ASDs are thought to involve the interaction of multiple gene variants with environmental factors that contribute to disruption of normal brain development. Of the genes implicated in autism, those most pertinent to this review are *BDNF* and components of the BDNF signaling cascade [144, 145]. Indeed, alterations in BDNF levels have been reported in autism [145, 146]. Downstream of BDNF, enzymes that regulate the synthesis and degradation of the signaling phospholipid phosphoinositide-3,4,5-P_3 (PIP_3) have been implicated in autism, including the genes encoding the PI3K catalytic p110 subunit *PIK3CG*, the Ras-GAP *NF1* (Ras is an activator of p110), the inositol phosphate phosphatase *INPP1*, and the PIP3 3' phosphatase *PTEN*. Human genetic studies have identified polymorphisms in the *PTEN* locus that are associated with macrocephaly and autistic behaviors [147]. Moreover, mice created with conditional *Pten* knockout in the cortex and dentate gyrus, resulted in reduced social interactions, increased activity in novel environments, impaired sensorimotor gating, in addition to macrocephaly and alterations in spine density and morphology [147].

Several genes downstream of BDNF in the PI3K cascade, such as *TSC1/2* and Centaurin gamma-2 (*CENG2*), have also been identified as autism susceptibility genes. Mutations in *TSC1* and *TSC2* in humans cause tuberous sclerosis, a syndrome associated with an increased incidence of autism. TSC1 and TSC2 are phosphorylated and regulated by the protein kinase Akt, which is regulated by PIP3. In a mouse model of tuberous sclerosis, conditional loss of *Tsc1* resulted in enhanced cortical excitability, enlarged neurons in the cortex and hippocampus, and seizures [148].

Sheffield *et al*. identified three subjects with ASD with a deletion in the region of chromosome 2q37.3 where the *CENG2* is located [149]. Analysis of another cohort of autism subjects revealed several variants of the *CENG2* gene, including a variant in the Arf-GAP domain predicted to lead to a loss of GAP activity. In a recent study, 10% of autism patients showed copy number variations, as estimated by comparative genomic hybridization on genomic DNA of ~100 patients; two of them were identified with 2q37.3 deletions [150]. *CENG2* has emerged as an intriguing candidate among the deleted genes because of its brain mRNA expression pattern and potential role in regulation of endosomal trafficking and dendritic spine density (Larimore *et al*. submitted).

Finally, it is intriguing in the context of BDNF and vesicle trafficking, that deletion of *Caps2*, the Ca^{2+}-dependent activator protein for secretion, results in autistic-like behavioral features [151]. CAPS2 mediates the exocytosis of dense core vesicles in neurons. Overexpression of *Caps2p* enhances NT-3 and BDNF release

from PC12 cells and cultured granule cells [152]. Conversely BDNF release is impaired in *Caps2* knockout mouse [153], consistent with the possibility that deregulated trafficking and release of BDNF-containing DCVs may contribute to autism. Indeed, altered levels of BDNF have been consistently found in serum of individuals with autism [145, 154–157]. In summary, studies support links between the deregulation of intracellular vesicular trafficking and signaling pathways downstream of BDNF to neurodevelopmental disorders associated with mental retardation and autistic-like behaviors.

Final considerations

Here, we have reviewed the evidence that proper axonal and dendritic development is a fundamental process for the establishment of synaptic circuitry, and that it results from complex interactions between intrinsic molecular factors and the external environment. Among those molecular factors relevant for activity-dependent neuronal development, BDNF stands out as critical player, not only for its role in normal development but also for the multiple links to neurodevelopmental disorders associated with mental retardation and autism spectrum disorders. Indeed, deregulation of any step in BDNF synthesis and release (i.e. transcription, translation, vesicular packaging, processing and trafficking, Ca^{2+}-dependent regulated release, and signaling) may result in improper axonal, dendritic and synaptic development, as well as impaired activity-dependent refinement of synaptic connections during brain development. Likewise, altered synaptic plasticity in cortical and limbic regions (e.g. hippocampus, amygdala) may also underlie the cognitive and behavioral adaptation deficits observed in neurodevelopmental disorders associated with mental retardation and autism. Since specific neurological symptoms arise from impaired functioning of improperly wired neuronal networks in specific brain regions (likely caused by defective activity-dependent synapse strengthening and pruning during postnatal development), the challenge ahead is to develop rational therapeutic approaches for the reversal of life-threatening impairments by region-specific manipulations of specific intracellular signaling cascades. Indeed, such pharmacological reversal of neurodevelopmental disorders in adults has been demonstrated in several animal models [108].

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