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 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

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. $\text{Ca}^{2+}$, $\text{Na}^+$) changes occur during excitatory synaptic transmission. In addition,
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 spineogenesis and synaptogenesis. Recent work has demonstrated that dendritic spine morphology modulates Ca²⁺ 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

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**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.
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 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 p75NTR, 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 p75NTR 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 p75NTR-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 p75NTR knockout mice [37], these results suggest a potential opposite effect of TrkB and p75NTR 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 Ca²⁺ signaling, the shift towards a higher proportion of mature shaped spines after BDNF exposure promotes supralinear Ca²⁺ 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 been 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<sup>308</sup> [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<sup>308</sup> 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<sup>308</sup> 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<sup>308</sup> 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 mouse 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 over-expression increased the complexity and length of axons and dendrites in primary cortical neurons, while over-expressing Mecp2 with a truncation at the C-terminal (Mecp2<sup>290</sup>) 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 AMPAkin [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-
BDNF protein levels were found to be lower in the brains of mice showing a 2-fold higher level of Bdnf transcript compared to neurons from wildtype mice [113]. 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.

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 Ca2+ 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
clothrin-dependent manner. Proteins delivered to the endo-
somal pathway from the secretory pathway or via endocy-
tosis 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 secre-
tory 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 Ca2+ 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 secre-
tory 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 devel-
opmental disorders not otherwise specified (PDDNOS), are
characterized by deficits in social interaction and commu-
nication. 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-P3
(PIP3) 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 polymor-
phisms in the PTEN locus that are associated with macro-
cephaly 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 senso-
rimotor 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
tumor 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 gene 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 esti-
mated 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 Ca2+-
dependent activator protein for secretion, results in
autistic-like behavioral features [151]. CAPS2 mediates
the exocytosis of dense core vesicles in neurons. Over-
expression 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 a 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, Ca2+-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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References

1. Ziv NE, Smith SJ. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron. 1996;17:91–102.
2. Fischer M, Kaech S, Knutti D, Matus A. Rapid actin-based plasticity in dendritic spines. Neuron. 1998;20:847–54.
3. Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R. Developmental regulation of spine motility in the mammalian central nervous system. Proc Natl Acad Sci USA. 1999;96:13438–43.
4. Gruetzendler J, Kasthuri N, Gan WB. Long-term dendritic spine stability in the adult cortex. Nature. 2002;420:812–6.
5. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature. 2002;420:788–94.
6. Mizrahi A, Crowley JC, Shtoyerman E, Katz LC. High-resolution in vivo imaging of hippocampal dendrites and spines. J Neurosci. 2004;24:3147–51.
7. Yuste R, Bonhoeffer T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci. 2001;24:1071–89.
8. Nimchinsky EA, Sabatini BL, Svoboda K. Structure and function of dendritic spines. Annu Rev Physiol. 2002;64:313–53.
9. Ethell JM, Pasquale EA. Molecular mechanisms of dendritic spine development and remodeling. Prog Neurobiol. 2005;75:161–205.
10. Segal M. Dendritic spines and long-term plasticity. Nat Rev Neurosci. 2005;6:277–84.
11. Peters A, Kaiserman-Abramof I. The small pyramidal neuron of the rat cerebral cortex. The perikarion, dendrites and spines. J Anat. 1970;127:321–56.
12. Korkotian E, Holeman D, Segal M. Dynamic regulation of spine-dendrite coupling in cultured hippocampal neurons. Eur J Neurosci. 2004;20:2649–63.
13. Noguchi J, Matsuizaki M, Ellis-Davies GC, Kasai H. Spine-neck geometry determines NMDA receptor-dependent Ca2+ signaling in dendrites. Neuron. 2005;46:609–22.
14. Matsuizaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci. 2001;4:1086–92.
15. Knott GW, Holtmaat A, Wilbrecht L, Welker E, Svoboda K. Spine growth precedes synapse formation in the adult neocortex in vivo. Nat Neurosci. 2006;9:1117–24.
16. Parnass Z, Tashiro A, Yuste R. Analysis of spine morphological plasticity in developing hippocampal pyramidal neurons. Hippocampus. 2000;10:561–8.
17. Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, et al. Transient and persistent dendritic spines in the neocortex in vivo. Neuron. 2005;45:279–91.
18. Chapleau CA, Pozzo-Miller L. Activity-dependent structural plasticity of dendritic spines. In: Byrne J, editor. Concise learning and memory: the editor's selection. Oxford: Elsevier; 2007. p. 281–305.
19. Matsuo N, Reijmers L, Mayford M. Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. Science. 2008;319:1104–7.
20. McKinney RA, Capogna M, Durr R, Gahwiler BH, Thompson SM. Miniature synaptic events maintain dendritic spines via AMPA receptor activation. Nat Neurosci. 1999;2:44–9.
21. Lewin GR, Barde YA. Physiology of the neurotrophins. Annu Rev Neurosci. 1996;19:289–317.
41. Gartner A, Staiger V. Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. Proc Natl Acad Sci USA. 2002;99:5091–99.

42. Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, et al. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. Proc Natl Acad Sci USA. 2004;101:15788–92.

43. Brigadski T, Hartmann M, Lessmann V. Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. J Neurosci. 2005;25:7601–14.

44. Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R, Korte M, et al. Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci. 2008;11:131–3.

45. Nagappan G, Zaitsev E, Senatorov VV Jr, Yang J, Hempstead BL, Lu B. Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci USA. 2009;106:1267–72.

46. Yang J, Siao CJ, Nagappan G, Marinic T, Jind G, McGrath K, et al. Neuronal release of proBDNF. Nat Neurosci. 2009;12:113–5.

47. Lu B, Pang PT, Woo NH. The yin and yang of neurotrophin action. Nat Rev Neurosci. 2005;6:603–14.

48. Bourne J, Harris KM. Do thin spines learn to be mushroom spines that remember? Curr Opin Neurobiol. 2007;17:381–6.

49. Chapleau CA, Carlo ME, Larimore JL, Pozzo-Miller L. The actions of BDNF on dendritic spine density and morphology in organotypic slice cultures depend on the presence of serum in culture media. J Neurosci. 2008;169:182–90.

50. Tanaka J, Horike Y, Matusuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H. Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science. 2008;319:1683–7.

51. Pozzo-Miller L. BDNF enhances dendritic Ca2+ signals evoked by coincident EPSPs and back-propagating action potentials in CA1 pyramidal neurons. Brain Res. 2006;1104:45–54.

52. Tyler WJ, Zhang XL, Hartman K, Winterer J, Muller W, Stanton PK, et al. BDNF increases release probability and the size of a rapidly recycling vesicle pool within rat hippocampal excitatory synapses. J Physiol. 2006;574:787–803.

53. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol. 2005;76:99–125.

54. Kaufmann WE, Moser HW. Dendritic anomalies in disorders associated with mental retardation. Cereb Cortex. 2000;10:981–91.

55. Fiala JC, Spacek J, Harris KM. Dendritic spine pathology: cause or consequence of neurological disorders? Brain Res Rev. 2002;39:29–54.

56. Huttonlocher PR. Dendritic development and mental defect. J Neurosci. 1997;17:2038–41.

57. Marin-Padilla M. Structural abnormalities of the cerebral cortex in human chromosomal aberrations: a Golgi study. Brain Res. 1974;44:625–9.

58. Huttonlocher PR. Dendritic development in neocortex of children with mental defect and infantile spasms. Neurology. 1974;24:203–10.

59. Purpura DP. Dendritic spine "dysgenesis" and mental retardation. Science. 1974;186:1126–8.

60. Purpura DP. Normal and aberrant neuronal development in the cerebral cortex of human fetus and young infant. UCLA Forum Med Sci 1975; 141-169.

61. Marin-Padilla M. Pyramidal cell abnormalities in the motor cortex of a child with Down's syndrome: A Golgi study. J Comp Neurol. 1976;168:203–41.

62. Newey SE, Velamoor V, Govek EE, Van Aelst L. Rho GTPases, dendritic structure, and mental retardation. J Neurobiol. 2005;64:58–74.

63. Schanen NC. Epigenetics of autism spectrum disorders. Hum Mol Genet 2006; 15 Spec No 2:R138–50.

64. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet. 1999;23:185–8.
65. Percy AK, Lane JB. Rett syndrome: clinical and molecular update. Curr Opin Pediatr. 2004;16:670–7.
66. Nan X, Campoy FJ, Bird A. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell. 1997;88:471–81.
67. Nan X, Cross S, Bird A. Gene silencing by methyl-CpG-binding proteins. Novartis Found Symp. 1998;214:6–16. discussion 16–21, 46–50.
68. Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landberger N, et al. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet. 1998;19:187–91.
69. Klose RJ, Sarraf SA, Schmiedeberg L, McDermott SM, Stancheva I, Bird AP. DNA binding selectivity of MeCP2 due to a requirement for A/T sequences adjacent to methyl-CpG. Mol Cell. 2005;19:667–78.
70. Bauman ML, Kemper TL, Arin DM. Pervasive neuroanatomic abnormalities of the brain in three cases of Rett's syndrome. Neurology. 1995;45:1581–6.
71. Bauman ML, Kemper TL, Arin DM. Microscopic observations of the brain in Rett syndrome. Neuropediatrics. 1995;26:105–8.
72. Armstrong D, Dunn JK, Antalffy B, Trivedi R. Selective dendritic alterations in the cortex of Rett syndrome. J Neuropathol Exp Neurol. 1995;54:195–201.
73. Kaufmann WE, Naidu S, Budden S. Abnormal expression of microtubule-associated protein 2 (MAP-2) in neocortex in Rett syndrome. Neuropediatrics. 1995;26:109–13.
74. Kaufmann WE, Taylor CV, Hohmann CF, Sanwal JB, Naidu S. Abnormalities in neuronal maturation in Rett syndrome neocortex: preliminary molecular correlates. Eur Child Adolesc Psychiatry. 1997;6(Suppl 1):75–7.
75. Kaufmann WE, MacDonald SM, Altamura CR. Dendritic cytoskeletal protein expression in mental retardation: an immunohistochemical study of the neocortex in Rett syndrome. Cereb Cortex. 2000;10:992–1004.
76. Belichenko PV, Oldfors A, Hagberg B, Dahlstrom A. Rett syndrome: 3-D confocal microscopy of cortical pyramidal dendrites and afferents. Neuroreport. 1994;5:1509–13.
77. Chapleau CA, Califa GD, Lane MC, Albertson AJ, Larimore JL, Kudo S, Armstrong DL, Percy AK, Pozzo-Miller L. Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. Neurobiol Dis. 2009;35:219–33.
78. Naidu S. Rett syndrome: a disorder affecting early brain growth. Ann Neurol. 1997;42:3–10.
79. Kaufmann WE, Johnston MV, Blue ME. MeCP2 expression and function during brain development: implications for Rett syndrome's pathogenesis and clinical evolution. Brain Dev. 2005;27(Suppl 1):S77–87.
80. Chahroul M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. Neurobiol. 2007;56:422–37.
81. Chen RZ, Akbarian S, Tudor M, Jaenisch R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nat Genet. 2001;27:327–31.
82. Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse MeCP2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat Genet. 2001;27:322–6.
83. Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, et al. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. Neuron. 2002;35:243–54.
84. Armstrong DD. Neuropathology of Rett syndrome. J Child Neurol. 2005;20:747–53.
85. Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. Proc Natl Acad Sci USA. 2005;102:12560–5.
86. Nelson ED, Kavalali ET, Monteggia LM. MeCP2-dependent transcriptional regulation regulates excitatory neurotransmission. Curr Biol. 2006;16:710–6.
87. Asaka Y, Jugloff DG, Zhang L, Eubanks JH, Fitzsimonds RM. Hippocampal synaptic plasticity is impaired in the Mecep2-null mouse model of Rett syndrome. Neurobiol Dis. 2006;21:217–27.
88. Moretti P, Levenson JM, Battaglia F, Atkinson R, Teague R, Antalffy B, et al. Learning and memory and synaptic plasticity are impaired in a mouse model of Rett syndrome. J Neurosci. 2006;26:319–27.
89. Collins AL, Levenson JM, Vilaythong AP, Richman R, Armstrong DL, Noebels JL, et al. Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. Hum Mol Genet. 2004;13:2679–89.
90. Chao HT, Zoghbi HY, Rosenmund C. MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron. 2007;56:58–65.
91. Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, et al. Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neurosci. 2006;52:255–69.
92. Belichenko NP, Belichenko PV, Mobley WC. Evidence for both neuronal cell autonomous and nonautonomous effects of methyl-CpG-binding protein 2 in the cerebral cortex of female mice with Mecep2 mutation. Neurobiol Dis. 2009;34:71–7.
93. Belichenko PV, Wright EE, Belichenko NP, Masliah E, Li HH, Mobley WC, et al. Widespread changes in dendritic and axonal morphology in Mecep2-mutant mouse models of Rett syndrome: evidence for disruption of neuronal networks. J Comp Neurol. 2009;514:240–58.
94. Kishi N, Macklis JD. MeCP2 is progressively expressed in post-migratory neurons and is involved in neuronal maturation rather than cell fate decisions. Mol Cell Neurosci. 2004;27:306–21.
95. Fukuda T, Itoh M, Ichikawa T, Washiyama K, Goto Y. Delayed maturation of neuronal architecture and synaptogenesis in cerebral cortex of Mecep2-deficient mice. J Neuropathol Exp Neurol. 2005;64:537–44.
96. Smrt RD, Eaves-Egenes J, Barkho BZ, Santisteven NJ, Zhao C, Aimone JB, et al. MeCP2 deficiency leads to delayed maturation and gene expression in hippocampal neurons. Neurobiol Dis. 2007;27:77–89.
97. Cusack SM, Rohn TT, Medeck RJ, Irwin KM, Brown RJ, Mercer LM, et al. Suppression of MeCP2beta expression inhibits neurite extension in PC12 cells. Exp Cell Res. 2004;299:442–53.
98. Jugloff DG, Jung BP, Purushotham D, Logan R, Eubanks JH. Increased dendritic complexity and axonal length in cultured mouse cortical neurons overexpressing methyl-CpG-binding protein MeCP2. Neurobiol Dis. 2005;19:18–27.
99. Ballas N, Liyo DT, Grunseich C, Mandel G. Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. Nat Neurosci. 2009;12:311–7.
100. Maezawa I, Swanger S, Harvey D, LaSalle JM, Jin LW. Rett syndrome astrocytes are abnormal and spread MeCP2 deficiency through gap junctions. J Neurosci. 2009;29:5051–61.
101. Chang Q, Khare G, Dani V, Nelson S, Jaenisch R. The disease progression of Mecep2 mutant mice is affected by the level of Bdnf expression. Neurosci. 2006;49:341–8.
102. Larimore JL, Chapleau CA, Kudo S, Theibert A, Percy AK, Pozzo-Miller L. Bdnf overexpression in hippocampal neurons prevents dendritic atrophy caused by Rett-associated MECP2 mutations. Neurobiol Dis. 2009;34:199–211.
103. Oiger M, Wang H, Hong E, Wang Q, Greenberg ME, Katz DM. Brain-derived neurotrophic factor expression and respiratory...
function improve after ampakine treatment in a mouse model of Rett syndrome. J Neurosci. 2007;27:10912–7.

104. Lauterborn JC, Lynch G, Vanderklish P, Arai A, Gall CM. Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. J Neurosci. 2000;20:8–21.

105. Lynch G, Gall CM. Ampakines and the threefold path to cognitive enhancement. Trends Neurosci. 2006;29:554–62.

106. Tropea D, Giaconetti E, Wilson NR, Beard C, McCurry C, Fu DD, et al. Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. Proc Natl Acad Sci USA. 2009;106:2029–34.

107. Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. Science. 2007;315:1143–7.

108. Ehninger D, Li W, Fox K, Stryker MP, Silva AJ. Reversing neurodevelopmental disorders in adults. Neuron. 2008;60:950–60.

109. Bienvenu T, Chelly J. Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. Nat Rev Genet. 2006;7:415–26.

110. Ballestar E, Ropero S, Alaminos M, Armstrong J, Setien F, Delgado IJ, Kim DS, Thatcher KN, Lasalle JM, Van den Veyver IB. Expression profiling of clonal lymphocyte cell cultures from Rett syndrome patients. Hum Mol Genet. 2005;14:2247–56.

111. Nuber UA, Kriaucionis S, Roloff TC, Guy J, Selfridge J, Steinhoff C, et al. Up-regulation of glucocorticoid-regulated genes in a mouse model of Rett syndrome. Hum Mol Genet. 2005;116:91–104.

112. Vanhala R, Korhonen L, Mikelsaar M, Lindholm D, Riikonen R. Levels of cerebrospinal fluid nerve-growth factor differ in infantile autism and Rett syndrome. J Child Neurol. 1999;13:429–33.

113. Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, et al. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science. 2003;302:885–9.

114. Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, et al. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science. 2003;302:890–3.

115. Abuhatzira L, Makedonski K, Kaufman Y, Razin A, Shemer R, Abuhatzira L, Makedonski K, Kaufman Y, Razin A, Shemer R. FXYD1 is an MeCP2 target gene overexpressed in the MeCP2 mutant mice. Proc Natl Acad Sci USA. 2009;106:2029–34.

116. Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, et al. Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. Proc Natl Acad Sci USA. 2009;106:2029–34.

117. Lappalainen R, Lindholm D, Riikonen R. Low levels of nerve-growth factor in cerebrospinal fluid of children with Rett syndrome. J Child Neurol. 1996;11:296–300.

118. Steinhoff C, et al. Up-regulation of glucocorticoid-regulated genes in a mouse model of Rett syndrome. Hum Mol Genet. 2007;16:640–9.

119. Riikonen R, Vanhala R. Levels of cerebrospinal fluid nerve-growth factor differ in infantile autism and Rett syndrome. Dev Med Child Neurol. 1999;41:148–52.

120. Riikonen R. Neurotrophic factors in the pathogenesis of Rett syndrome. J Child Neurol. 2003;18:693–7.

121. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron. 1998;20:709–26.

122. Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat Neurosci. 2007;10:1513–4.
143. Wang H, Chan SA, Ogier M, Hellard D, Wang Q, Smith C, et al. Dysregulation of brain-derived neurotrophic factor expression and neurosecretory function in Mecp2 null mice. J Neurosci. 2006;26:10911–5.

144. Campbell DB, Sutcliffe JS, Ebert PJ, Militeri R, Bravaccio C, Trillo S, et al. A genetic variant that disrupts MET transcription is associated with autism. Proc Natl Acad Sci USA. 2006;103:16834–9.

145. Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, et al. Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog Neuro-Psychopharmacol Biol Psych. 2006;30:1529–31.

146. Katoh-Semba R, Tsuzuki M, Miyazaki N, Matsuda M, Nakagawa C, Ichisaka S, et al. A phase advance of the light-dark cycle stimulates production of BDNF, but not of other neurotrophins, in the adult rat cerebral cortex: association with the activation of CREB. J Neurochem. 2008;106:2131–42.

147. Kwon C-H, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, et al. Pten regulates neuronal arborization and social interaction in mice. Neuron. 2006;50:377–88.

148. Meikle L, Talos DM, Onda H, Pollizzi K, Rotenberg A, Sahin M, et al. A mouse model of tuberous sclerosis: neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. J Neurosci. 2007;27:5546–58.

149. Wassink TH, Piven J, Vieland VJ, Jenkins L, Frantz R, Bartlett CW, et al. Evaluation of the chromosome 2q37.3 gene CNTG2 as an autism susceptibility gene. Am J Med Gen Part B: Neuropsych Gen. 2005;136B:36–44.

150. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, et al. Strong association of de novo copy number mutations with autism. Science. 2007;316:445–9.

151. Sadakata T, Washida M, Iwayama Y, Shoji S, Sato Y, Ohkura T, et al. Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. J Clin Invest. 2007;117:931–43.

152. Sadakata T, Mizoguchi A, Sato Y, Katoh-Semba R, Fukuda M, Mikoshiba K, et al. The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. J Neurosci. 2004;24:43–52.

153. Nelson KB, Grether JK, Croen LA, Dambrosia JM, Dickens BF, Jelliffe LL, et al. Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. Ann Nutr. 2001;49:597–606.

154. Miyazaki K, Narita N, Sakuta R, Miyahara T, Naruse H, Okado N, et al. Serum neurotrophin concentrations in autism and mental retardation: a pilot study. Brain Dev. 2004;26:292–5.

155. Connolly AM, Chez M, Streif EM, Keeling RM, Golumbek PT, Kwon JM, et al. Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy. Biol Psychiatry. 2006;59:354–63.

156. Katoh-Semba R, Wakako R, Komori T, Shigemi H, Miyazaki N, Ito H, et al. Age-related changes in BDNF protein levels in human serum: differences between autism cases and normal controls. Int J Dev Neurosci. 2007;25:367–72.