INTRODUCTION

1. DENDRITE FORMATION AND MORPHOLOGY

During synaptogenesis, growing neurites establish stable connections with competent pre- or postsynaptic partners by ceasing to elongate and/or by forming new branches (Purves and Hume, 1986; Sanes and Lichtman, 1999). Therefore, synapse formation is typically accompanied by a change in neuron morphology (Ziv and Smith, 1996; Colman et al., 1997; Jontes et al., 2000; Niell et al., 2004). The process of synapse formation or reorganization may require either a whole cell response, or a local response when only one axon collateral or dendrite changes its shape. There is growing evidence that the structural plasticity of axons and dendrites is regulated both by neuronal activity and by molecular signals at contact sites. Some of the mechanisms governing dendrite development and synaptogenesis and their possible mutual dependency will be reviewed in more detail below.

1.1. Cellular mechanisms of dendrite growth

The outgrowth of dendrites often occurs after the outgrowth of the axon and, in some cases, the axon may even form connections with its targets before dendritic differentiation has occurred (Fletcher et al., 1991, 1994). In the developing cortex, the apical dendrites of pyramidal neurons develop from the leading process of the migrating neurons while basal dendrites sprout directly from the cell body. Primary basal dendrites emerge directly from the soma, and secondary and higher order dendrites appear by branching of the primary dendrites. Elongation and further maturation of dendrites is dependent on the appearance of growth cones and filopodia. The latter are highly motile, thin, laterally protruding structures on dendrite shafts (Dailey and Smith, 1996). After an encounter with a presynaptic site, the filopodia become less dynamic and eventually retract into the main shaft and may or may not form dendritic spines carrying the acquired synaptic terminal, which may be stabilized into a mature synapse (Fiala et al., 1998). In a low density hippocampal culture, the neuronal and synapse development have been divided into five stages. In stage one, when the cells have attached to a substratum, flattened and motile lamellipodia emerge at multiple sites from the cell membrane. In stage two, the lamellipodia condense at specific points at the cell

membrane, from where short, 'minor' processes emerge. These minor processes are generally four to six in number and have about the same length of 20-30 μm. At this stage, the cells attain a symmetrical appearance and remain unpolarized (a state when neurites have not yet differentiated into axon and dendrites). The minor processes are highly dynamic, extending and retracting over short distances for a duration of 12 to 24 h. At stage three, one of the processes begins to elongate continuously without retracting until it becomes much longer than the other processes. At this stage, polarity is established and the axon can be distinguished from the other processes at an ultrastructural level or by immunocytochemical tests (Goslin et al, 1990; Goslin and Banker, 1990). The axon continues to grow at a rapid rate while the remaining minor processes display no net increase in their length. At stage four (which corresponds to DIV 2-4 in culture), the remaining minor processes begin to elongate and acquire the taper and branching pattern characteristic of dendrites. And at stage five, the neurons become completely mature by events depending on cell interactions.

1.2. The synaptotropic hypothesis of dendrite growth

Dendrite growth and synapse formation are processes which occur in concurrence during development. As a dendrite grows, its branches elongate, retract and get stabilized on encountering a presynaptic site (mostly axons in the case of pyramidal cells) resulting in a synapse. This may also take place independent of the presence of presynaptic terminals. The synaptotropic hypothesis suggests that dendritic branches are formed at synaptic contacts and new branches are stabilized by synapses (Vaughn, 1989; Niell et al., 2004; Fig.1). This infers that dendritic architecture is locally regulated by synaptic activity at each synaptic contact (Maletic-Savatic et al., 1999). For example, in *Xenopus*, light-induced visual activity enhanced the formation of new dendritic branches and stabilized the existing ones in tectal neurons (Sin et al., 2002). Consistently, *in vivo* blockade of neuronal activity with tetanus toxin in neonatal CA1 pyramidal neurons decreased the number of basal dendritic branches (Groc et al., 2002). The dendritic growth and branching of spinal motor neurons was reduced by blockade of NMDA receptors (Kalb, 1994). These studies suggest that afferent synaptic activity plays a role in the maintenance of a mature dendritic architecture.

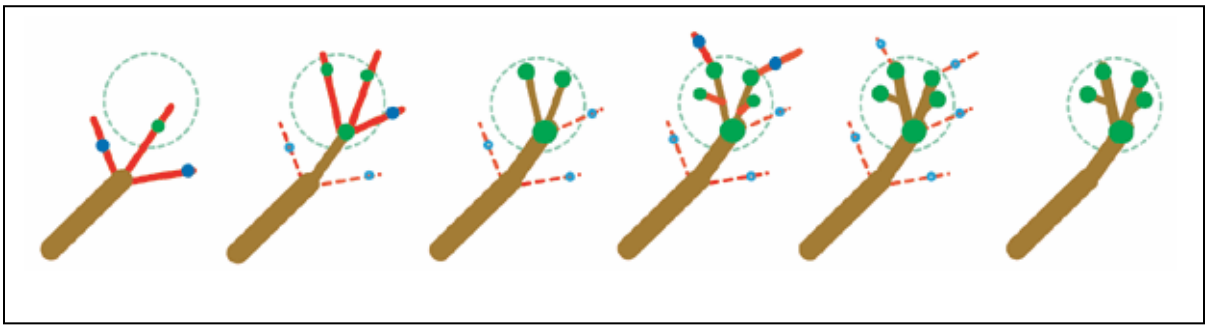

Figure 1. Model of synaptotropic guidance of dendrite growth. A dendritic branch gives rise to a number of filopodia (solid red). Filopodia that encounter their correct partners and form synaptic contacts (green dots) are stabilized as new branches (brown), whereas those that establish inappropriate contacts (blue dots) are retracted (dashed red). Successive rounds of selective stabilization result in arborization within a field of appropriate synaptic connections (dashed green region). From Niell et al., Nature Neuroscience, 7, 254 - 260 (2004).

1.3. Neurotrophinergic control of dendrite development

Neurotrophins (NTs) are the most frequently considered molecules among the factors involved in dendrite morphogenesis. NGF was the first neurotrophin with a demonstrated effect on dendrite morphology. In 1990, Ruit et al., showed that systemic injections of NGF increased the total dendritic length, in sympathetic ganglion cells in mice (Ruit et al., 1990). NT-3 and BDNF were shown to enhance the dendritic complexity in cerebral cortex neurons (McAllister et al., 1997). In particular, BDNF was reported to regulate growth and branching of cortical dendrites (McAllister et al., 1995; Dijkhuizen and Ghosh, 2005) and to control dendritic stability and shape of EGFP-labeled neurons (Horch and Katz, 2002; Gorski et al., 2003). Furthermore, pyramidal neurons that overexpressed BDNF projected a larger number of basal dendrites, dendritic spines and branches (Horch et al., 1999).

1.4. Other molecular factors influencing dendrite growth

Semaphorin 3A (Sema3A), an axon guidance molecule was shown to be a chemorepellant for cortical axons but it also acted as a chemoattractant for apical dendrites of cortical neurons (Polleux et al., 2000).

Cpg15, a member of a group of activity-regulated genes, is expressed in areas undergoing afferent innervation, dendritic growth and synaptogenesis (Nedivi et al., 1998). CPG15

increased the total dendritic branch length of tectal neurons of *Xenopus* and is thought to have mediated these enhancing effects through its local action on neighboring neurons (Nedivi et al., 1998).

Ephrins represent a large family of signaling molecules implicated in the formation of orderly connections during neural development. Two classes of ephrins, ephrin-A and ephrin-B, on interacting with their corresponding receptors EphA or EphB, may regulate dendritic branching in addition to axon guidance (O'Leary and Wilkinson, 1999; Wahl et al., 2000; Murai et al., 2003).

Osteogenic proteins, such as osteogenic protein-1 (OP-1), bone morphogenic protein (BMP)-6 and BMP-2 have been shown to stimulate dendrite growth in rat sympathetic ganglion cells and in cortical neurons in culture (Guo et al., 1998; LeRoux et al., 1999).

Notch, a cell surface protein that functions as a receptor for the membrane-associated ligands Delta 1-3 and Jagged 1-2 (Lindsell et al., 1995; Redmond et al., 2000) was shown to be a negative regulator of dendrite growth. Upregulation of Notch decreased dendrite growth in young cortical neurons in culture (Redmond et al., 2000). The nuclear targets of Notch, the homologues of enhancer-of-split 1 and 5 (Hes1/5), were shown to be involved in NGF-regulated dendritic growth and branching (Salama-Cohen et al., 2005).

Cell adhesion molecules (CAMs) are yet another class of molecular factors important for dendrite development. Mouse models showing null mutation in the L1 gene that encodes for L1, a transmembrane CAM, demonstrated abnormal morphogenesis of cortical dendrites and developmental defects in hippocampus and corpus callosum (Cohen et al., 1997; Demyanenko et al., 1999). Similarly, mice lacking the cell adhesion molecule, contactin, displayed decreased dendritic growth of granule and Golgi cells in the developing cerebellum (Berglund et al., 1999). The neural-CAM (NCAM) mediated neurite outgrowth in cerebellar granule cells via its downstream mediator protein kinase C isoforms (Kolkova et al., 2005). Neuroligin-neurexin interactions have an important role in mediating synaptic assembly, but these interactions may also participate in determining cytoarchitectural changes in

postsynaptic neurons (Grifman et al., 1998). The classic neural (N)-cadherin, non-classic cadherins and β-catenin were shown to stimulate spine morphogenesis and dendrite arborization (Togashi et al., 2002; Yu and Malenka, 2003).

Hormones, like thyroid hormone, gonadal hormones and glucocorticoids also, influenced dendritic branching (Gould et al., 1990; Woolley et al., 1990). Recently, it has been shown that osteonectin, a Schwann cell-secreted factor identified in conditioned medium, promoted neurite outgrowth in postnatal rat retinal ganglion cells (Bampton et al., 2005).

1.5. Intracellular mechanisms of dendrite growth regulation

A majority of intracellular signals that mediate both axonal and dendritic growth are influenced by synaptic activity. This supports the synaptotropic hypothesis i.e., the notion that dendrites are stabilized or otherwise rapidly modified by their afferent input.

Cytoskeletal molecules. The dendritic cytoskeleton is composed of polymeric proteins, microfilaments and microtubules which are in a dynamic state of polymerization and depolymerization. Dendritic shape reflects this balance and is governed by a variety of signals. A lesser phosphorylated state of microtubules-associated proteins (MAPs) is known to cap the growing end of microtubules allowing elongation, whereas a highly phosphorylated state was shown to result in dendritic instability thus inhibiting elongation and inducing branching (Audesirk et al., 1997). Transport of polymerized microtubules from soma to the dendrites and the molecular motors like CHO1/MKLP1 were also regarded as essential for this process (Sharp et al., 1997).

GTPases. The Rho family of small GTPases, including Rho, Rac and Cdc42 are intracellular regulators of signaling pathways to the actin cytoskeleton (Luo et al., 1997). Activation of RhoA caused dendrite retraction while activation of Rac1 and Cdc42 enhanced dendrite growth and branching (Ruchhoeft et al., 1999; Li et al., 2000).

Local protein synthesis. Protein synthesis and post-translational modification at the base of dendritic spines may reflect the interaction between synaptic activity and dendritic remodeling

at individual synapses. The postsynaptic protein synthesizing machinery may help in translating synaptic activity into plastic changes (Gardiol et al., 1999). Some immediate early genes (IEGs) are expressed during rapid activity-dependent remodeling of dendrites. The activity-regulated cytoskeletal-associated protein, Arc, was rapidly induced by both neuronal activity and growth factors and its enrichment was demonstrated in the dendrites (Lyford et al., 1995; Steward and Worley, 2002). Another IEG, Narp, is a secreted molecule induced AMPA receptor clustering concomitant with dendritic growth at postsynaptic sites (O'Brien et al., 1999).

Ca^{2+} signaling. Last, but not least, dendritogenesis is dependent on intracellular calcium signals. In neurons, the calcium levels are regulated by influx through calcium channels and release of calcium from intracellular stores. Two major targets of intracellular calcium are the calcium/calmodulin-dependent protein kinases (CaMKs) and the mitogen-activated kinase (MAPK). Upon calcium entry via voltage-sensitive calcium channels (VSCCs) or transmitter receptor channels, calmodulin binds multiple calcium ions and activates various intracellular effectors, including CaMKs. Among the latter, CaMKII is the most studied calcium target. The two isoforms, CaMKIIα and CaMKIIβ mediate differential effects on dendrite growth. CaMKIIα was shown to stabilize dendritic growth in frog tectal neurons *in vivo* (Wu and Cline, 1998) and mammalian cortical neurons *in vitro* (Redmond et al., 2002). CaMKIIβ exerts a positive effect on the development of fine dendrites and filopodia, by a direct interaction with cytoskeletal actin (Fink et al., 2003). CaMKI has also recently been identified as a positive transducer of growth cone motility and axonal outgrowth (Wayman et al., 2004). CaMKIV was predominantly localized in the nucleus, and mice lacking CaMKIV had defects in cerebellar dendritic development (Ribar et al., 2000; Redmond et al., 2002). MAPKs are also activated by calcium influx, and activity-induced stabilization of MAPK pathway was shown to be prominent in hippocampal dendrite growth (Wu et al., 2001). Phosphorylation of MAP2 by MAPK as well as CaMKII have been implicated in activity-induced dendrite growth of cultured sympathetic neurons (Vaillant et al., 2002). MAPK signaling represented a link between calcium influx and the intracellular pathways mediating dendritic growth in cortical neurons (Redmond et al., 2002). Calcium release from intracellular stores locally

regulated dendritic branch stability (Lohmann et al., 2002), and a rise in local calcium transients regulated filopodial dynamics (Lohmann et al., 2005).

2. SYNAPTOGENESIS

In vertebrates, the formation of synapses occurs over a prolonged period of time beginning in the prenatal life and following into the early postnatal life. Synapse formation also occurs in adults, where it is thought to contribute to learning and memory.

2.1. Pre- and postsynaptic differentiation: a short overview

Synapse formation is a multi-step process which involves the formation of initial contacts between the presynaptic and the postsynaptic membranes. After a contact is stabilized, pre- and postsynaptic differentiation occurs presumably in parallel with each other. N-cadherin and protocadherins, by binding to β-catenin, may ensure that contacts are made at specific locations (Benson and Tanaka, 1998; Huntley and Benson, 1999). The neurexin-neuroligin complex is also known to ensure contact stabilization (Chih et al., 2005). For the formation of the presynaptic active zone, an assembly of elaborate protein complexes is required. These proteins were shown to be carried in clusters of pleiomorphic vesicles that upon fusion with the presynaptic plasma membrane led to rapid formation of new active zones (Zhai et al., 2001; Ziv and Garner, 2004; Nguyen and Sudhof, 1997). The presynaptic active zone proteins, piccolo, bassoon and RIM are thought to provide scaffolding to help SV docking, fusion and neurotransmitter release (tom Dieck, 1998; Zhai et al., 2001).

The postsynaptic surface comprises large multimolecular complexes that are composed of many molecules like receptors, scaffold and adaptor proteins, CAMs, cytoskeleton proteins, protein kinases, protein phosphatases and other signaling molecules. At glutamatergic synapses, the recruitment of scaffolding proteins such as SAP90/PSD95 was observed as one of the earliest events in postsynaptic differentiation (Bresler et al., 2001). Following this, the recruitment of NMDA-type and AMPA-type glutamate receptors was reported to occur (Washbourne et al., 2002; Bredt and Nicoll, 2003). Both receptor classes interact with different sets of PDZ domain-containing proteins, chaperones and cytoskeletal proteins. These interactions regulate the receptor protein intracellular transport and stabilization as well as membrane trafficking at the postsynapse. Accumulation of other proteins like CamKII,

ProSAP/Shank, Homer and cortactin may also contribute to the postsynaptic assembly (Sheng, 2001; Sheng and Sala, 2001). At the inhibitory postsynaptic density, the protein components include neurotransmitter receptors, glycine receptors or $GABA_A$ receptors, the GlyR or $GABA_AR$ anchor protein, gephyrin, Raft1, profilin, collybistin, cdc42, RACK-1, neurologins, actin filaments and microtubules (Moss and Smart, 2001).

Pre- and postsynaptic development entails a prolonged maturation phase at both sides. This includes enlargement of the synaptic structure, as the number of synaptic vesicles per terminals increases with an increase in bouton volume, active zone area, postsynaptic density area and spine head volume (Schikorski and Stevens, 1997). Morphological conversion of filopodial and shaft synapses into more stable spine synapses was also regarded as an important phase of synaptic development (Fiala et al., 1998). Developmental changes in glutamatergic synapses included a decrease in the probability of transmitter release at hippocampal synapses and an increase in the reserve pool of vesicles (Chavis and Westbrook, 2001). Quantal size showed an initial rapid increase and then remained constant (Mohrmann et al., 2003). GABAergic synapse formation was shown to begin already before birth (Meier et al., 2003) and its postnatal development, in the rodent superior colliculus, has been shown to occur in two phases. First, during the initial 2-3 days after birth, and coincidental with the onset of glutamatergic spontaneous activity, the GABA action switched from depolarizing to hyperpolarizing (Grantyn et al., 2004), and the mean release probability decreased. And second, before and during the period of eye opening (P6-P15), and coincidental with the shortening of IPSCs due to a subunit exchange (Grantyn et al., 2004; Henneberger et al., 2005), asynchronous release was down-regulated in favor of stimulus-locked synchronous release (Kirischuk et al., 2005).

2.2. Synapse stabilization and elimination

Neuronal activity is the main determinant of circuit reorganization at later stages of development (Lichtman and Colman, 2000, Groc et al., 2002; Sin et al, 2002; Rao and Craig, 1997; Hua and Smith, 2004) and diffusible growth factors are considered to be dominant players in the activity-dependent regulation of excitatory as well as inhibitory synapse development (see chapter 3 for more details). However, in addition to synapse formation and stabilization, a process called 'synapse pruning' is thought to largely contribute to the activity-

dependent reorganization of neural circuits during development (Lichtman and Colman, 2000). Developmental studies in insects provided direct evidence for activity-dependent synapse elimination. For example, withdrawal of axons from the ipsilateral neuropil innervated by axon branches from filiform hairs was prevented by blocking afferent activity (Pflüger et al., 1994). An expansion of the synaptic territory to strengthen the remaining input may then form the basis of the stabilization of the synapses required for the developing circuits (Walsh and Lichtman, 2003). This view is supported by studies that showed an increase in quantal content during synapse elimination on muscle cells (Colman et al., 1997). Other studies have shown that a regression process occurring at the postsynaptic surface actively contributed to this type of synaptic remodeling (Katz and Shatz, 1996; Duch and Mentel, 2004). Activity-dependent regression of dendrites was concurrent with a reduction in the number of synaptic inputs leading to dismantling of larval circuits during metamorphosis in *Manduca* (Duch and Mentel, 2004).

Membrane-bound factors. Blocking of neural cadherin, N-cadherin, resulted in the formation of exuberant synapses (Inoue and Sanes, 1997). Neuronal activity-regulated pentraxin (Narp) is known to increase AMPA receptor clustering in spinal cord neurons (O'Brien et al., 1999). EphrinB family proteins promote NMDA receptor subunit clustering (Dalva et al., 2000). Neuroligins, the postsynaptic transmembrane proteins interact with presynaptic receptor β-neurexin and induce formation of functional active zones with recycling synaptic vesicles (Dean et al., 2003). SynCAM (synaptic cell-adhesion molecule) is a homophilic CAM expressed on both sides of the synapse. Overexpression of SynCAM1 in cultured neurons increases synapse number by inducing the formation of functional active zones (Sara et al., 2005).

Diffusible target-derived factors. One relevant group of target-derived molecules includes the members of the fibroblast growth factor (FGF) and Wnt families. These proteins are secreted by certain sub-populations of neurons and have been shown to induce regional axon arborization and accumulation of recycling synaptic vesicles in the axonal terminals. Such properties could serve to spatially restrict synaptogenesis (Hall et al., 2000; Umemori et al., 2004). Last, not least, neurotrophins, especially BDNF, have been implied to influence

synapse density by a variety of mechanisms (McAllister et al., 1999; Huang and Reichardt, 2001). These factors may affect excitatory and inhibitory synapses in a differential fashion and thereby change the level of immature and mature neuronal network activity.

2.3. The balance of excitation and inhibition

Normal function of neural networks depends on a delicate balance of excitatory and inhibitory synaptic inputs. Organization of synaptic inputs on the dendritic tree may govern the local balance of a neuronal synaptic output. Some researchers found that in cultured hippocampal neurons inhibitory synapses are distributed in an orderly fashion over the entire dendritic tree to maintain a local balance of excitation and inhibition at any site (Liu, 2004). The E/I balance of individual neurons adjusts to the network (Rittenhouse et al., 1999; Knott et al., 2002; Morales et al., 2002). In this way, the output activity of a neuron can be scaled to remain in an optimal narrow range, despite a rapid change in a major input, for instance during the onset of sensory experience.

Neurodevelopmental disorders such as schizophrenia (Wassef et al., 2003), epilepsy (Li and Prince, 2002), Parkinson's disease (Llinas et al., 1999) and autism (Rubenstein and Merzenich, 2003) have been attributed to changes in the E/I ratio of synaptic input. Suppressed GABAergic inhibition is a frequently considered cause of hyperexcitability, as often observed in autistic patients (Rubenstein and Merzenich, 2003). During epilepsy, the imbalance of excitation and inhibition favors excitation. A decrease in GABA-mediated postsynaptic inhibition is critical to the establishment and spread of partial epileptic seizures, which arise focally at cortical sites. Similarly, a defective GABA-mediated regulation of burst firing in dopaminergic cells has been postulated to underlie schizophrenia (Moore et al., 1999).

Interestingly, pathophysiological studies of these disorders also identified anomalies of dendrite structure, like reduction in dendritic complexity and spine density (Fig. 2; Raymond et al., 1996; Kaufman and Moser, 2000; Armstrong, 2001; Zoghbi, 2003).

The molecular factors which regulate the E/I balance are only recently being identified. Mutations of the X-linked genes encoding two isoforms of neuroligin (NLGN) family, NLGN3 and NLGN4 have been found in autistic individuals (Jamain et al., 2003).

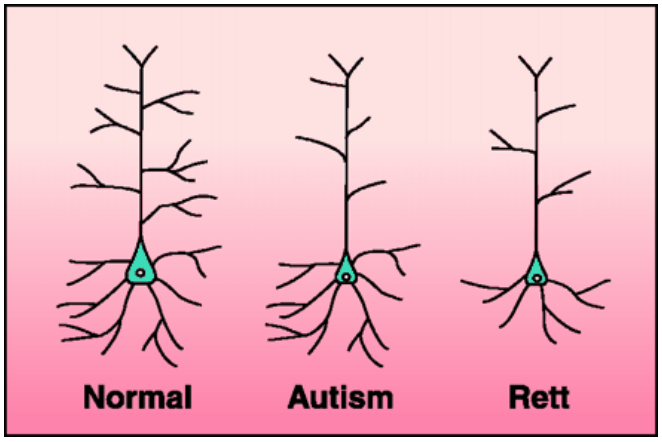

Figure 2. Schematic representation of pyramidal neurons from control, autism, and Rett brains. In autism, the cell body is small and there is reduced dendritic branching. Similar changes occur in Rett brains, along with a reduction in the basilar dendritic branching.
From Zoghbi, Science, 2003, 826-830.

Localization of different pairs of β-neurexins and neuroligins at chemically distinct synapses has generated great interest among researchers studying mechanisms of differential synapse formation. While NLGN1 and NLGN3 are mainly encountered in excitatory synapses, NLGN2 is enriched at the inhibitory synapses (Graf et al., 2004; Chih et al., 2005). Gene suppression of NLGNs resulted in an abnormal E/I balance with greater loss of inhibition than excitation (Chih et al., 2005). Therefore, it will be appropriate to hypothesize that a defect in NLGN3 or NLGN4 may abolish formation, stabilization or recognition of specific synapses essential for the communication processes that are deficient in individuals with autism. Downstream interactions of these synaptically localized molecules to cytoskeleton-associated proteins suggest a possible link between the maintenance of the E/I equilibrium and neuritogenesis (Grifman et al., 1998). Other regulators of the E/I balance are not yet identified, although brain-derived neurotrophic factor (BDNF) has been shown to modulate excitatory and inhibitory synaptic transmission through a variety of mechanisms.

3. CELLULAR AND MOLECULAR MECHANISMS THROUGH THE ACTION OF BDNF

3.1. Neurotrophins: an overview

Neurotrophins (NTs) belong to a family of proteins that have common structural features. Nerve growth factor, NGF, was the first member of this family to be characterized (Levi-Montalchini, 1987). There are four NTs identified in mammals. NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) are derived from a common ancestral gene and are similar in sequence and structure (Hallbook, 1999).

NTs are important in the regulation of neural survival, differentiation, development, function and plasticity (Korsching, 1993; Lewin and Barde, 1996; McAllister et al., 1999). Initially, NTs were solely regarded as neuron survival factors, but later it was hypothesized that this mechanism also contributes to the optimization neuronal circuitry. According to the 'classical' neurotrophic factor hypothesis, developing neurons compete with each other for a limited supply of a neurotrophic factor provided by the target tissue. Successful competitors survive, unsuccessful ones die. This ensures a balance between the size of a target organ and the number of innervating neurons (Levi-Montalcini and Hamburger, 1951; Bothwell, 1995).

The molecular structure of neurotrophins is highly conserved during vertebrate evolution (Gotz et al., 1992). The mature form of human BDNF is about 50% identical to human mature NGF and other neurotrophins. Conserved features among NTs are: 1) a presumptive signal peptide after the initiation codon (Ernfors et al., 1990a; Ip et al, 1992); 2) a pro-region, including an N-linked glycosylation site, and a proteolytic cleavage site for furin-like proteases followed by the mature sequence (Seidah et al., 1996); 3) a distinctive three-dimensional structure formed by two pairs of anti-parallel β-strands and six cysteine residues forming three disulfide bridges called as cysteine knot motif (Ibanez, 1998). Mature forms of neurotrophins function as highly stable non-covalently bound homodimers with a molecular weight of approximately 28kDa. Stable heterodimers of BDNF and NT-3 are also known to exist although with a reduced biological activity (Arakawa et al., 1994; Heymach and Shooter, 1995). Dimerization of the NTs is a pre-requisite for the NT receptor activation (Ibanez, 1998). NTs can bind to two classes of receptors, the common low affinity receptor p75 and the specific high affinity receptor Trk (tropomyosin-related kinase) tyrosine kinase. The p75 receptor, a member of the family of tumor necrosis factor (TNF) receptors, binds all NTs with equal affinity. TrkA binds NGF, TrkB binds BDNF and NT-4/5, and TrkC binds NT-3. NT-3 is also bound to TrkA and TrkB, though with a weak affinity. The three Trk neurotrophin receptors (TrkA, TrkB and TrkC) share a 66-68% sequence homology with each other (Barbacid et al., 1991). They possess a highly similar intracellular catalytic domain and a similar extracellular domain containing three repeats of immunoglobulin-like domains (Schneider and Schweiger, 1991).

Ligand interaction of Trk receptors results in phosphorylation of cytoplasmic tyrosine residues on the cytoplasmic domains of these receptors. Phosphorylation of some of the

tyrosine residues promotes signaling by creating docking sites for adapter proteins containing phosphotyrosine-binding (PTB) or src-homology-2 (SH-2) motifs (Pawson and Nash, 2000). These adapter proteins couple Trk receptors to intracellular signaling cascades, which include the Ras/ERK (extracellular signal-regulated kinase) protein kinase pathway, the phosphatidylinositol-3-kinase (PI-3 kinase)/Akt kinase pathway, and phospholipase C (PLC)-γ1 pathway (Kaplan and Miller, 2000).

Signaling through p75 involves the activation of NFκB, which is a part of an important pathway known to promote neuronal survival by NTs (Hamanoue et al., 1999). Although all NTs bind to p75, only NGF is shown to induce nuclear translocation of NFκB in mouse hippocampal neurons (Salama-Cohen et al., 2005). p75 can mediate apoptosis through activation of Jun-kinase (Bazenet, 1998). NT binding to p75 eliminates RhoA activation which results in a stimulatory effect on axon outgrowth (Yamashita et al., 1999).

3.2. Expression and release of BDNF

In situ hybridization studies showed that the BDNF gene is expressed throughout the gray matter of the brain. Highest levels of BDNF are seen in the hippocampus followed by cerebral cortex, midbrain, cerebellum, hindbrain, olfactory bulb, spinal cord and striatum. Two mRNA transcripts of BDNF are known, a 1.4 kb and a 4.0 kb, both showing similar developmental and regional expression in the brain (Ernfors et al., 1990a). In the mouse hippocampus, BDNF mRNA is first detected at E17, and its level increases with age reaching the highest level at postnatal day 15. The human BDNF gene has been mapped to chromosome 11 and notably to a region involved in a genetic disorder known as the WRAG (Wilm's tumor, aniridia, genitor-urinary abnormalities, mental retardation) syndrome (Rosier et al., 1994) suggesting that abnormality of the BDNF gene may contribute to mental retardation. BDNF and other NTs are synthesized as precursors (proNTs) which are proteolytically cleaved both intra- and extracellularly to generate mature NTs. The mature form of BDNF results from post-translational processing (N-terminal cleavage) of a precursor containing a hydrophobic signal peptide followed by a large pro-region. ProBDNF is a 32 kDa molecule containing a consensus type I cleavage site for furin-like pro-protein convertases which generate the mature 14 kDa protein. The intracellular trafficking of BDNF and other NTs has been studied using immunofluoresence and confocal microscopy (Goodman et al., 1996; Mowla et al.,

1999; Farhadi et al., 2000) and live cell imaging (Adachi et al., 2005). Newly synthesized peptides of BDNF (preproBDNF) carrying the 18 aa signal peptide sequence are imported into the lumen of the ER, where cleavage of the signal peptide gives rise to the proBDNF. Further processing involves N-linked glycosylation and glycosulfation on residues present within the prodomain and then, transport to the Golgi apparatus. On reaching the trans-Golgi network (TGN), a divergence may follow deciding the fate of proBDNF. A cleavage by the membrane-bound proteases (furins in the TGN or proprotein convertases in the secretory granules) may generate the mature BDNF which can take the pathway of either constitutive or regulated secretion (Seidah et al., 1996). Major evidence suggested a regulated release of BDNF (Goodman et al., 1996; Mowla et al., 1999) although release of proBDNF in constitutively secreting fibroblasts and Schwann cells was also reported (Acheson et al., 1991). To this date, most of the studies suggest that proNTs account for a 40%-60% of the total NTs secreted extracellularly, particularly in CNS neurons (Mowla et al., 1991, 2001; Farhadi et al., 2000). This would imply that the regulated release of the pro form of BDNF dominates that of the mature form as it was shown that proBDNF avoids furin cleavage and is sorted to the regulated secretory pathway (Mowla et al., 1999). On comparing the sorting of NGF and NT-3 with that of BDNF using pulse-chase labeling, depolarization-induced release and immunocytochemical localization experiments, it was found that BDNF was the only NT to be packaged in large dense core vesicles (LDCVs) and to follow a regulated route of secretion (Michael et al., 1997; Lu, 2003; Haubensak et al., 1998). However, recently, some authors reported that NGF and NT-3 also sorted to the LDCVs and were released via the regulated pathway in primary rat cortical neurons (Wu et al., 2004). Immunostaining revealed subpopulations of neurons which retrogradely transported BDNF (Sobreviela et al., 1996; Mufson et al., 1999). But, a more direct demonstration of BDNF retrograde transport with live cell imaging is still lacking. BDNF can also be transported in an anterograde direction. In the rat central nucleus of the amygdala, where BDNF mRNA is not expressed, a dense BDNF-immunoreactive fiber plexus was found and lesions of the pontine nuclei eliminated this BDNF-immunoreactive fiber plexus (Conner et al., 1997). Similarly, after seizures, BDNF mRNA expression increased in the granule cells of the dentate gyrus while BDNF-immunoreactivity increased in the mossy fibers which project from the dentate gyrus to the CA3 region of the hippocampus. Lesion of the CA3 layer did not affect the increase in BDNF

protein, as observed in the mossy fibers after seizures indicating an anterograde route for BDNF protein (Smith et al., 1997).

3.3. BDNF expression in neurodegenerative disorders

It has been suggested that individuals affected with Alzheimer disease (AD) have a reduced level of BDNF mRNA and protein in the hippocampal formation (Phillips et al., 1991; Murray et al., 1994), temporal cortex (Connor et al., 1997), and other cortical areas where BDNF-containing neurons showed neurofibrillary tangles. In addition, BDNF protein appeared to contribute to the formation of senile plaques (Ferrer et al., 1999; Murer et al., 1999). Conclusive evidence on an alteration of TrkB expression in the brain of AD patients is not published although preliminary studies reported TrkB reduction in the cerebral cortex and hippocampus of AD-affected individuals (Ferrer et al., 1999). A reduced expression of BDNF protein in the substantia nigra of individuals with Parkinson's disease has also been reported (Parain et al., 1999). Similarly, in Huntington's disease, a reduced expression of BDNF protein has been found (Ferrer et al., 2000). The onset and severity of motor dysfunction in Huntington's disease was regulated by BDNF (Canals et al., 2004). In schizophrenia, the mRNA for BDNF was decreased in the prefrontal cortex of the patients, and it has been found that the level of BDNF mRNA expression was positively correlated with dendritic spine density in the same subjects (Hashimoto et al., 2005).

4. SYNAPSE REARRANGEMENT: ROLE OF PRG-1

4.1. Post-lesion degenerative changes

The changes occurring during post-lesion regenerative growth resemble in many aspects the processes associated with the formation of neural connections during nervous system development. Temporal reversal in the cellular and molecular environment is created by the re-expression of permissive factors for neurite outgrowth and synapse formation. Certain neural components release trophic factors to combat the prime challenge for the lesioned neuron, i.e. survival. For instance, application of glial-derived neurotrophic factor (GDNF) to the transected facial nerve was shown to prevent motoneuronal cell death after axotomy in the neonatal period and adulthood (Yan et al., 1995).

Another major change induced by axotomy is a change in the innervation of axotomized neurons described as synapse detachment (Svensson et al., 1991), synapse degeneration (Sugimoto et al., 1990), central synaptic stripping (Jones et al., 1997), reduction in synapse number (Brannstorm and Kellerth, 1998) and reduction in synaptophysin-immunoreactivity (Gehlert et al., 1997). Therefore, after neuronal survival is ensured, the subsequent task is to restore both the afferent and efferent connections of the lesioned neuron. However, the presence of myelin inhibitors and lipid phosphates in the extracellular space may limit the ongoing repair process (Savaskan et al., 1999; Williams et al., 2005). The neurotrophins, once again, stood out as the first and foremost candidates for initiating repair processes. Recently it was shown that post-lesional fibroblast-mediated delivery of BDNF and NT-3 enabled a partial recovery of function after spinal injury in rats (Mitsui et al., 2005). Together, these studies approved neurotrophins as candidates for pharmacological repair strategies after lesions in the central nervous system, but even better results seems possible when combining neurotrophins with other permissive factors. A recently identified protein product of *plasticity-related gene-1* (*PRG-1*) might suit as one of the molecules supporting regenerative outgrowth of neurites. This is because a marked upregulation of PRG-1 in the denervated hippocampus after entorhinal cortex lesion was shown to occur and its activity overcame the negative effects of extracellular lipid phosphates on neurite outgrowth (Brauer et al., 2003). Interestingly, unpublished results by Savaskan and colleagues at the Charité, Berlin also identify PRG-1 as a candidate molecule in the combat of synaptic stripping of axotomized neurons. After transection of the perforant path, neurons in the entorhinal cortex displayed enhanced expression of PRG-1.

4.2. Plasticity-related gene-1 (PRG-1)

PRG-1 belongs to a family of four newly identified genes known as plasticity-related genes (PRGs). The PRGs show structural similarity to the lipid phosphate phosphatase (LPP) family, and are also known as LPP-related proteins (LPRs). Like LPPs, PRGs possess a transmembrane domain with six membrane-spanning regions, a catalytic extracellular domain and an intracellular domain. Moreover, unlike other members of the LPP and PRG family, PRG-1 and PRG-2 have very long hydrophilic C-terminal tails. PRG-1 encodes a transmembrane protein of 764 aa with the long hydrophilic C-terminal domain of around 400

aa. At the N-terminal, PRG-1 has conserved amino acid residues which have been shown to be essential for ecto-enzyme activity in the LPP class of proteins (Neuwald, 1997; Bräuer et al., 2003). In the rat brain, PRG-1 mRNA was reported to be first expressed at E19 in the subventricular zone and the hippocampal anlage and after birth, it was present in the hippocampus and entorhinal cortex. The LPP-family members are known to dephosphorylate extracellular phospholipids such as lysophosphatidic acid (LPA; 1-acyl-2-hydroxy-s*n*-glycero-3-phosphate), phosphatidic acid (PA) or sphingosine-1-phosphate (S1P). Overexpression of PRG-1 was reported to increase dephosphorylation (inactivation) of exogenous LPA in N1E-115 cells and attenuate LPA-induced neurite retraction (Bräuer et al., 2003).

4.3. Lysophosphatidic acid: a challenge for synapse stabilization?

It has earlier been shown that extracellular phospholipids were important in cortical neurogenesis. Notably, LPA acted as an extracellular signal affecting postmitotic cells (Fukushima, 2002). Lysophosphatidic acid, a simple phospholipid, is a key intermediate in phospholipid biosynthesis. It consists of a glycerol backbone, a phosphate group, a fatty acyl chain and a hydroxyl group (Tokumura et al., 2002). LPA was reported to be secreted by activated platelets, adipocytes, injured corneal tissue and neurons (Moolenaar et al., 1999). LPA is known to mediate numerous biological functions through activation of its G protein-coupled receptors, including LPA1 (EDG2), LPA2 (EDG4) and LPA3 (EDG7) (Fukushima et al., 1998; Moolenaar, 1999; Contos et al., 2000). Recently, an orphan GPCR, p2y9/GPR23, was identified as the fourth LPA receptor, LPA4 (Noguchi et al., 2003). Induction of morphological changes by LPA/S1P has been studied in mouse neuroblastoma cells, PC12 cells, cortical neuroblasts, primary chick neurons and CNS-derived astrocytes. LPA-induced neurite retraction was reported in PC12 cells (Tigyi and Miledi, 1992; Bräuer et al., 2003). In cortical neurons, LPA caused rapid growth cone collapse, neurite retraction and transient cell rounding (Jalink et al., 1994; Saito, 1997). LPA-induced actomyosin contraction was shown in astrocytes (Manning et al., 1998). Given the inhibitory effects on neurite growth and the changes induced in cell shape, LPA is likely to pose a challenge to the maintenance of synaptic contacts with the dendrites of recovering neurons.

AIMS OF THE STUDY

The neurotrophin, BDNF was assigned a central role in sculpting and modifying neuronal connectivity during development. Enhanced expression of BDNF after brain injury highlights the additional importance of BDNF in post-lesional regeneration. PRG-1 is another molecule which undergoes upregulation in the course of circuit development and repair, justifying the attempt to characterize its role in synapse development. In this study, the following tasks were undertaken:

1. To establish an experimental model where effects of postsynaptic BDNF can be characterized in single developing neurons

The aim was to transfect a BDNF::EGFP plasmid construct in a small population of primary hippocampal neurons isolated from each other, in a low density culture. As BDNF is a secreted protein, a low efficiency of transfection could help to set up a localized source of postsynaptic BDNF in the form of individual transfected neurons. The BDNF gradient surrounding solitary neurons expressing BDNF::EGFP in an otherwise BDNF-lacking environment might serve as a chemoattractive or a chemorepulsive cue to the approaching afferents. Postsynaptic BDNF might also play a role in synapse stabilization.

2. To characterize the difference between actions of exogenous BDNF, as opposed to those of local BDNF

According to the classical neurotrophic factor hypothesis, target-derived neurotrophins regulate the survival of outgrowing axons. It is expected that extracellular concentration gradients of neurotrophins facilitate axon guidance and branching. Provided that BDNF is expressed and released from only a small number of neurons and for a short time , one could expect that the resultant effects were local, preferentially directed on the BDNF-deficient neurites of neighboring neurons and on the BDNF-expressing neuron itself. It is expected that these local effects will differ from the diffuse effects obtained with BDNF added to the extracellular medium.

3. To determine whether BDNF alters the E/I ratio of synaptic terminal numbers

The total number of synapses is important for the activity of the developing network. But it has remained unclear as to what extent BDNF can alter the incidence of glutamatergic excitatory (E) as opposed to GABAergic inhibitory (I) synaptic terminals in hippocampal or other neurons of the mammalian brain. By using immunocytochemical methods to visualize the glutamatergic and GABAergic synaptic terminals on EGFP-labeled neurons, it was aimed to clarify the effect of transfected BDNF on the E/I ratio at a stage when the first synapses are being formed in immature neurons. In view of the previously described epileptogenic effect of BDNF we expected a preferential upregulation of glutamatergic synapses.

4. To find out how a change in excitatory synaptic input may affect dendrite development in a BDNF-expressing neuron

Activity shapes not only synaptic connections but also dendritic architecture in the brain. Moreover, there might be a causal relationship between the changes in synaptic input and changes in dendrite growth. The impact of altered glutamate release can be elucidated by studying the relationships between dendritic growth parameters and the number of respective terminals. A second approach would be to apply a mixture of glutamate receptor blockers during the expression time of EGFP or BDNF::EGFP. If a BDNF-induced upregulation of glutamatergic input was the cause of altered dendrite morphology the latter should be abolished by glutamate receptor block.

5. To characterize the possible role of PRG-1 in synapse development or stabilization

The plasticity-related gene-1 (PRG-1) is a newly identified gene. Preliminary results of experiments with perforant path lesion point to a role of PRG-1 in the stabilization of synapses on the axotomized neurons. To characterize this mechanism, it was intended to perform experiments with PRG-1-overexpression and -gene silencing and to quantify the number of E and I synaptic terminals present on the transfected neurons. It was also intended to challenge the neurons by application of lysophosphatidic acid (LPA), a presumptive prohibitive factor, as it was known that LPA reduces mPSC generation in hippocampal neurons. Therefore an LPA-induced decrease in the number of synaptic terminals and a possible protective effect of PRG-1 overexpression in response to LPA was expected.