I. INTRODUCTION

The development of the nervous system is guided by a balanced action of intrinsic factors defined by the genetic program and of epigenetic factors characterized by cell-cell interactions via contact-dependent or secreted signals. It is well established that the interplay of neurons and glial cells is highly relevant for many aspects of nervous system development.

1.1 Importance of glia neuron interaction for brain development

The nervous system consists mainly of two types of cells, neurons and glial cells or neuroglia. Glial cells are further divided into three different classes (Fields & Stevens-Graham, 2002): Schwann cells and oligodendrocytes are the myelin forming cells of the peripheral nervous system (PNS) and central nervous system (CNS), respectively. These cells wrap layers of myelin membrane around axons to allow for fast impulse conduction. Second, there are astrocytes, which are closely associated with neurons in the brain but do not form myelin. The name refers to their stellate form observed in histological preparations, but their morphology varies widely. Astrocytes form endfeet feeds which connect to blood capillaries and wrap synapses. These interconnections allow them to regulate extracellular concentrations of ions, metabolites and neurotransmitters and to provide neurons. Microglia make up the third category of glial cells in the brain. In contrast to oligodendrocytes and astrocytes, which derive from ectodermal precursors within the nervous system, microglia derive from bone marrow monocyte precursors (Kaur et al., 2001). Like their counterparts in the hematopoietic system, microglia respond to injury or disease by engulfing cellular debris and triggering inflammatory responses.

For a long time, glial cells were regarded as somewhat passive companions to neurons. Today, however, more than a century after their description by Virchow (1856), there is increasing evidence that neurons and glia cells have an intimate and plastic morphological and functional relationship (Pfrieger & Barres, 1996). So intimate is the association between astrocytes and neurons, for example, that monitoring activity of these nonneuronal cells is a reliable surrogate for measuring neural activity (Dani et al., 1992; Porter & McCarthy, 1996; Rochon et al., 2001).

Neuron-glia interactions control several processes of brain development such as neurogenesis (Lim & Alvarez-Buylla 1999), myelination (Girault & Peles, 2002; Bhat, 2003), synapse formation (Slezak & Pfrieger, 2003; Ullian et al., 2004), neuronal
migration (Nadarajah & Parnavelas, 2002), proliferation (Gomes et al., 1999) and differentiation (Garcia-Abreu et al., 1995). Several soluble factors secreted by either glial or neuronal cells, such as neurotransmitters, hormones and growth factors, have been implicated in nervous system morphogenesis (Gomes, 2001).

In the following, I will describe axon pathfinding at the optic chiasm and the differentiation of nodes of Ranvier as examples of well established neuron-glia interactions during development.

1.1.1 Axon pathfinding at the optic chiasm

The correct wiring of the nervous system relies on the ability of axons and dendrites to locate and recognize their appropriate synaptic partners. Axons are guided along specific pathways by attractive and repulsive cues in the extracellular environment. In the mammalian visual system, for example, retinal ganglion cell (RGC) axons form the optic nerve. Axons from each eye grow towards one another to meet at the ventral midline of the diencephalon where they establish an X-shaped intersection called the optic chiasm (Fig. 1). During mouse development, the formation of the optic chiasm appears to occur in two separate phases. In the first phase, early generated RGC axons originating from dorsal-central retina reach the developing ventral diencephalon at embryonic day E12-E12.5 and grow across the ventral midline to establish the correct position of the X-shaped optic chiasm (Colello & Guillery, 1990; Godement et al., 1990; Sretavan, 1990). A number of these early axons, instead of crossing the midline, project into the ipsilateral side of the brain, forming a transient ipsilateral projection (Fig. 1). RGC axons from more peripheral parts of the retina enter the chiasm later, at E13-E14, and make specific pathfinding choices such that the adult-like pattern of chiasmatic axon routing into the ipsilateral and contralateral optic tracts is established by E15-E16 (Sretavan & Reichardt, 1993; Marcus et al., 1995).

One of the cellular specializations localized to the site at which the chiasm will form is a palisade of radial glia draped along either side of the midline, occupying the midline zone at which retinal axons diverge (Fig. 1) (Marcus et al., 1995; Reese et al., 1994; Marcus & Mason, 1995). RGC axons segregate into the ipsilateral and contralateral components during the time when their growth cones contact the midline radial glia (Marcus et al., 1995), suggesting that midline glia could provide important guidance information. Such an interaction has been shown in the Drosophila ventral nerve cord and the vertebrate spinal
cord, where midline glia mediate differential axon guidance (Kaprielian et al., 2001). To control axons crossing at the ventral midline, in both systems, midline glia release Netrins, acting as attractants (Harris et al., 1996; Mitchell et al., 1996; Serafini et al., 1996), and Slits, acting as repellents (Kidd et al., 1999; Brose & Tessier-Lavigne, 2000). Axons modulate their responsive to these two signals during midline crossing. Crossing axons are initially attracted by Netrin and insensitive to Slit, which draws them to the midline. After reaching the midline they become insensitive to Netrin and upregulate the Slit receptor Robo in axons, which propels them out of the midline (Kidd et al., 1998). This acquired sensitivity to Slit also prevents later recrossing. Other axons, not destined to cross, are sensitive to Slit from the beginning and so never reach the midline. However, to guide RGC axons through the optic chiasm, Netrin is expressed highly at the optic nerve head and acts as an attractant (Deiner et al., 1997). Whereas Slit 1 and 2 are expressed by cells surrounding the chiasm and repel ipsilateral and contralateral axons alike (Erskine et al., 2000; Plump et al., 2002). This has led to the idea that Slit-expressing cells form a repulsive corridor to guide all RGC axons through the chiasm. This model is supported by genetic experiments that disrupt Slit/Robo signaling in fish and mice. Zebrafish carrying a mutation in the astray/robo2 gene have profound defects in retinal axon pathfinding (Fricke et al., 2001). Double mutant mice for Slit 1 and 2 genes show a large additional chiasm developed anterior to the true chiasm (Plump et al., 2002). Recently, other glia-derived axon guiding signals at the optic chiasm have been described. Williams et al., (2003) could show that the axonal decision about crossing the midline to project contralaterally or uncrossing the midline to form ipsilateral projections is mediated by Ephrin-B2. They found that Ephrin-B2 is expressed in the midline radial glia exactly during the period of ipsilateral projections, and that blocking Ephrin-B2 function eliminates the ipsilateral projection. On the other side, they found that the expression of the Ephrin-B2 receptor EphB1 was restricted to a small number of ganglion cells located exclusively in the ventrotemporal retina. This expression pattern suggests that EphB1 may be present exclusively on ipsilateral axons (Fig. 1).

These data show that interactions between glial cells and neurons are essential for the correct wiring of the nervous system. Glia cells set important landmarks and actively guide axons to their appropriate synaptic partners.
**Figure 1**: Model of axon sorting at the mouse optic chiasm

(A) Zic2-expressing RGCs give rise to ipsilaterally projecting axons, which express EphB1, allowing them to sense ephrin-B2 at the optic chiasm and turn into the ipsilateral tract. Axons that do not express EphB1 cross at the optic chiasm, joining the contralateral optic tract. D, dorsal; V, ventral; N, nasal; T, temporal. (B) Timeline. RGCs are generated from E11.5 until birth. The first RGCs are born in the dorsocentral (DC) retina. Most project contralaterally, but a few make transient ipsilateral projections. The permanent ipsilateral RGCs arise from the ventrotemporal (VT) retina and are generated from E14.5 to E17.5. These axons express Zic2 and EphB1. Ephrin-B2 is found at the chiasm from E13.5 to E17.5. From Rasband et al., (2003) based on the findings of Williams et al., (2003) and Herrera et al., (2003).
1.1.2 Differentiation of nodes of Ranvier

The development of myelinated axons represents one of the most complex interactions among glia cells and neurons. Many vertebrate axons are surrounded by a myelin sheath allowing rapid and efficient saltatory propagation of action potentials. In myelinated fibers, the contacts between neurons and oligodendrocytes or Schwann cells display a very high level of spatial and temporal organization. This organization requires a tight developmental control and the formation of a variety of specialized zones of contact between different areas of the myelinating cell membrane, and between the myelinating glia cell and the axon (Scherer & Arroyo, 2002). At regular intervals, myelinated axons show gaps in the sheathing myelin, called nodes of Ranvier. These sites interrupt the high-resistance, low-capacitance barrier of myelin and are responsible for the rapid transduction of action potentials by voltage-dependent Na$^+$ (Na$_v$) and potassium channels. Each node of Ranvier is flanked by paranodal regions where heliocoidally wrapped glial loops are attached to the axonal membrane by septate-like junctions. The segment between nodes of Ranvier is termed the internode. Its outermost part, in contact with paranodes, is referred to as the juxtaparanodal region (Fig. 2) (Arroyo & Scherer, 2000).

The initial physical contact of the myelinating glial cells with the axon triggers dynamic changes in the distribution and localization of Na$^+$ and K$^+$ channels. Prior to the glial cell contact, these channels are uniformly distributed along the axon. Contact of oligodendrocytes or Schwann cells cause an enrichment of Na$_v$ channels at the node of Ranvier, which reach an estimated density of 1500/µm$^2$ compared to less than 100/µm$^2$ in the adjacent non-nodal regions (Rosenbluth, 1999). These channels display a unique molecular composition, in that they are composed of an α-subunit which is responsible for ion pore formation, and two β-subunits which mediate interactions with the extracellular and intracellular components (Isom, 2002). During development, the Na$_v$ channel subtypes expressed at the node undergo a transition from Na$_v$1.2 to Na$_v$1.6, such that only Na$_v$1.6 channels are seen at the adult nodes of Ranvier (Boiko et al., 2001; Rios et al., 2003). In the unmyelinated proximal segment of the rat optic nerve, Na$_v$1.2 stays further on diffusely expressed (Boiko et al., 2001). Thus Na$_v$ channel subtypes are differentially targeted within the same axon in a manner regulated by contact with myelinating glia. Two publications show that oligodendrocytes secrete so far unidentified soluble factors sufficient to trigger regulatory spaced axonal clustering of Na$_v$1.2 channels in cultured RGCs, but not of Na$_v$1.6 channels (Kaplan et al., 1997 and 2001).
Figure 2: Organizational structure of nodes of Ranvier
(A) Electron micrograph of a longitudinal section through the node of Ranvier in the spinal dorsal root of rat. (B) Four specific domains are defined by axon-glial interactions at the node of Ranvier: the Na\(^+\) channel-enriched node of Ranvier, the adjacent paranode (PN), the juxtaparanodal region (JP), which contains delayed rectifier K\(^+\) channels, and the internode (IN). At the paranode, the transmembrane protein Caspr is found on the axon surface in association with the glycosyl-phosphatidyl-innositol anchored cell adhesion molecule, contactin (Cont). This molecular complex interacts with the glial cell adhesion molecule, neurofascin 155 (NF) and anchors the intercellular junction to the axonal cytoskeleton through the actin-associated protein 4.1B, which binds to the cytoplasmic domain of Caspr. From Fields & Stevens-Graham, (2002).
The requirement of glial cells for Na\textsubscript{v} channel clustering was also demonstrated by the early postnatal selective ablation of oligodendrocytes in transgenic mice (Mathis et al., 2000). In these mice, no Na\textsubscript{v} channel clustering was visible. Nodal Na\textsubscript{v} channels appear to be part of multimolecular complexes including several intracellular [ankyrin G, \(\beta\)IV spectrin and syntenin-1 (Kordeli et al., 1995; Berghs et al., 2000; Lambert et al., 1997; Koroll et al., 2001)] and transmembrane proteins [NrCAM and neurofascin 186 kDa (Davis et al., 1996)]. These complexes have been implicated in the clustering or localization of Na\textsubscript{v} channels to the node (Berghs et al., 2000; Komada & Soriano, 2002) and possibly interact with Schwann cell microvillus in the PNS or astrocyte endfeeds in the CNS (Isom, 2002; Salzer, 2003).

On either side of the node of Ranvier, the compact myelin lamellae open up into a series of cytoplasmic loops that spiral around, closely appose and form a series of septate like junctions with the axon. These paranodal junctions have been proposed to perform several functions at this axo-glial interface, including anchoring of the glial myelin loops to the axon, creating an ionic diffusion barrier into the periaxonal space and serving as a fence to maintain the axonal domains and preventing lateral diffusion of the various membrane protein complexes (Bhat, 2003). The paranodal loops of oligodendrocytes and Schwann cells contain the 155 kDa splice isoform of neurofascin (NF155) (Tait et al., 2000), that binds to the Caspr-contactin complex on the axonal side (Charles et al., 2002). Since these three molecules are interdependent, it is very likely that Caspr/paranodin, contactin/F11 and NF155 form the core of the axoglial cell adhesion apparatus (Girault & Peles, 2002) (Fig. 2). Caspr/paranodin (Menegoz et al., 1997; Einheber et al., 1997) and contactin/F11 (Rios et al., 2000) are highly enriched in the paranodal axolemma. The association between Caspr/paranodin and contactin/F11 is necessary to address Caspr/paranodin to the plasma membrane in transfected cells (Faivre-Sarrailh et al., 2000) and its targeting to the axon \textit{in vivo} (Rios et al., 2000). Knockout mice lacking Caspr/paranodin or contactin/F11 display ataxia, motor deficits and a dramatically reduced nerve conduction velocity (Bhat et al., 2001; Boyle et al., 2001). In these mutants the ultrastructure of the paranodes is severely altered: the glial paranodal loops are disorganized and the gap between glial and axonal membranes is increased (Bhat et al., 2001; Boyle et al., 2001).

Altogether these data show that the domain organization of nodes of Ranvier including accumulation of nodal and paranodal markers in the axolemma are all dependent on glia cells. The process of node formation is regulated by soluble signals from myelinating glia.
as well as direct contact and interactions between proteins expressed on the surface of axons and oligodendrocytes or Schwann cells.

1.1.3 Influence of glia on synaptogenesis

As described above, there is good evidence that glial cells play a profound role at specific steps during neuronal differentiation. The hypothesis that astrocytes play a role in synapse formation stems from a temporal correlation between synaptogenesis and the differentiation of this glial cell type (Pfrieger & Barres, 1996). Most CNS neurons innervate their target areas at least one week before they form most of their synapses. Interestingly, synaptogenesis is delayed until about the same time as astrocytes are generated. This correlation was described for different brain areas (Jacobson, 1991; Parnavelas et al., 1983; Skoff, 1990; Rakic et al., 1986). Figure 3 illustrates the temporal correlation between synaptogenesis and glial differentiation, exemplified by the rodent retinocollicular pathway.

![Figure 3: Temporal correlation between synaptogenesis and glial differentiation, exemplified by the rodent retinocollicular pathway. RGC axons reach their target in the superior colliculus (SC) between embryonic day 16 and the day of birth, postnatal day 0. But the majority of synapses are formed between the postnatal day 10 and 20 (Lund & Lund, 1972; Warton & McCart, 1989). Precisely during this delay period, between target innervation and synapse formation, astrocytes are born and proliferate in the superior colliculus. From Slezak & Pfrieger (2003).]
Several glial factors with influence on synapse differentiation have been found within the last five years. Beattie et al., (2002), have shown that glia-derived tumor necrosis factor α (TNFα) raises the surface expression of glutamate receptors in hippocampal neurons from postnatal rats in vitro and in acute slices. Another study has shown that activity-dependent neurotrophic factor, which is released by astrocytes, acts as maturation signal for synapses (Gozes & Brenneman, 2000; Blondel et al., 2000). A glia-derived signal that controls the expression of specific transmitter receptors has been detected in the chick retina (Belmonte et al., 2000). Cultured Müller glia secrete a protein, termed muscarinic acetylcholine receptor-inducing activity (MARIA), which induces the expression of a specific subtype (M2) of muscarinic receptors in retinal neurons of chick embryos in ovo. Finally, there is a link between astrocytes and the most prominent synaptogenic factor agrin, a motoneuron-derived signal that is essential for the formation of neuromuscular junctions (NMJs) (Sanes & Lichtman, 1999) and that may play a role in synaptogenesis in the CNS (Böse et al., 2000). Contact with mouse glia reduced mRNA encoding for agrin in cultured rat hippocampal neurons, while soluble glial factors halved the expression of a specific isoform, but left the total level unaffected (Lesuisse et al., 2000). Notably, a very interesting study showed recently that neurotrophins support survival and growth of cultured frog spinal cord neurons, but inhibit agrin synthesis and thus NMJ formation, whereas soluble Schwann cell-derived factors override this inhibition and switch motoneurons to a synaptogenic state (Peng et al., 2003). An astrocyte-induced increase in synaptogenesis has been reported in different culture preparations including neuronal cell lines (Hartley et al., 1999), hippocampal neurons derived from stem cells of adult rats (Toda et al., 2000; Song et al., 2002) and neurons from spinal cord (Li et al., 1999), cortex (van den Pol & Spencer, 2000), hippocampus (den Pol & Spencer, 2000; Verderio et al., 1999) and hypothalamus (den Pol & Spencer, 2000) from embryonic or perinatal rats. It should be noted, however, that these studies did not exclude that astrocytes increased synapse numbers indirectly by enhancing neuronal survival or neuritic growth.

Direct evidence that astrocytes enhance synapse formation has been obtained by a series of studies on highly purified RGCs (Pfrieger & Barres, 1997; Mauch et al., 2001; Nägler et al., 2001; Ullian et al., 2001). These studies became possible by the establishment of a glia-free preparation of postnatal RGCs (Barres et al., 1988) and the opportunity to culture these cells for several weeks under serum- and glia-free conditions (Meyer-Franke et al., 1995). First, Pfrieger & Barres, (1997), showed that cultured RGCs form ultrastructurally defined synapses in the absence of glia. Thus, initial synapse formation
appears to be an intrinsic property of these neurons that does not require external signals. However, RGCs cultured in the absence of astrocytes, even for several weeks, exhibited very little spontaneous synaptic activity. In contrast, RGCs exhibited high levels of synaptic activity when they were cultured in the presence of a feeding layer of astrocytes or in glia-conditioned medium (GCM) (Pfrieger & Barres, 1997). Even when RGCs were co-cultured with purified collicular neurons, their normal targets, little synaptic activity was observed unless astrocytes were present. Importantly, the culture medium contained several neurotrophic factors that ensured equally high neuronal survival rates and extensive neuritic growth regardless of the presence of astrocytes. Two subsequent studies examined in parallel the effects of astrocytes on the number of synapses that formed between RGCs \textit{in vitro}. As measured by immunostaining with pre- and postsynaptic markers, as well as by electron microscopy, astrocytes were found to induce a 7-fold increase in synapse number between RGCs (Nägler et al., 2001; Ullian et al., 2001). These synapses were presynaptically functional as shown by FM1-43 imaging, a measure of vesicular recycling. They were also postsynaptically functional, as shown by the amplitudes of mini- excitatory postsynaptic currents. Shortly after, at least one synaptogenic factor contained in GCM was identified as cholesterol (Mauch et al., 2001). This somewhat surprising finding provoked the hypothesis that cholesterol is an astrocyte-derived factor that limits the extent of synaptogenesis (Görziet al., 2002; Pfrieger, 2003). Recently, Ullian et al., (2003) reported that, similar as described for RGCs, Schwann cells induce the formation of functional glutamatergic synapses between spinal motor neurons in culture. This study also showed that soluble astrocyte-derived factors could mimic the synapse-inducing effect of Schwann cells on motoneurons and conversely that Schwann cell-derived factors induce synapse formation in cultured RGCs. However, the nature of these synapse inducing factors remains unclear.

1.1.3.1 Role of cholesterol in synapse formation

The brain contains five to ten times more cholesterol than any other organ and this sterol represents 2-3% of the total weight and 20-30% of all lipids in the brain. Cholesterol is an essential component of biological membranes that determines their biophysical properties by its unique structure. The polar hydroxyl group on one end and the long hydrophobic tail on the other anchor its orientation in the phospholipid monolayer, and its flat shape allows for a neat fit between the hydrophobic tails of fatty acid chains. Cholesterol lowers the
permeability of membranes, possibly by compacting phospholipids, and regulates their fluidity in a temperature-dependent manner by changing the order of fatty acyl chains.

How can cholesterol promote synapse formation? There are three possible explanations: First, cholesterol may serve as a precursor for steroids, which have been shown to promote synaptogenesis (Sakamoto et al., 2001). Second, cholesterol may serve as a building material for different synaptic components. And finally, cholesterol may act by determining the functional properties of membrane-resident proteins like ion channels and neurotransmitter receptors due to creation of microdomains (Bastiaanse et al., 1997; Burger et al., 2000; Bruses et al., 2001; Suzuki et al., 2001).

1.1.3.1.1 Neurosteroids
Steroids, which are synthesized in the central or peripheral nervous system, are called neurosteroids. Neurosteroids are synthesized by oligodendrocytes, Schwann cells, astrocytes and neurons (Zwain & Yen, 1999), either de novo from cholesterol or from steroidal precursors imported from peripheral sources (Baulieu, 1998). They include steroids like pregnenolone and dehydroepiandrosterone (DHEA), their sulfates, and reduced metabolites as well as progesterone (Baulieu, 1998). These compounds can act as allosteric modulators of neurotransmitter receptors, such as GABA\(_A\) (Majewska et al., 1986), NMDA (Wu et al., 1991) and sigma receptors (Monnet et al., 1995) or via members of the nuclear hormone receptor superfamily. Nuclear hormone receptors are ligand-inducible transcription factors that bind to hormone responsive elements in DNA to activate or repress the expression of specific genes (Aranda & Pascual, 2001).

It has been shown by electron microscopy that estrogens increases dendritic spine density on CA1 pyramidal neurons, and in parallel increases synapse density on spines without any decrease in shaft synapses (Woolley & McEwen, 1992), implying that new spine synapses are formed. Confocal microscopic imaging showed that estrogen treatment up-regulates immunoreactivity for the largest NMDA receptor subunit, NR1, on dendrites and cell bodies of CA1 pyramidal neurons (Gazzaley et al., 1996). Murphy & Segal (1996) revealed that estrogen induces spines on dendrites of dissociated hippocampal neurons in culture by a process that is blocked by an NMDA receptor antagonist and not by an AMPA/kainate receptor blocker. It has been supposed that these effects are mediated by nuclear and extranuclear estrogen receptors (McEwen et al., 2001). Ultrastructural studies have revealed non-nuclear estrogen receptor \(\alpha\) immunoreactivity on dendritic spines, axon terminals and glial processes within hippocampal principal cells (Milner et al., 2001).
These receptors are postulated to mediate rapid non-genomic effects (McEwen et al., 2001) via coupled second messenger systems (Levin, 1999; Kelly & Wagner, 1999; Simoncini et al., 2000). Interestingly, in vivo and in vitro studies using administration of progesterone to pups and cultured cerebellar slices of newborn rats, respectively, showed that progesterone promotes dendritic growth and spine formation of Purkinje cells (Sakamoto et al., 2001). Further analysis by electron microscopy revealed that progesterone induces an increase of synapse density on Purkinje cells, which have been shown to express nuclear progesterone receptors (Sakamoto et al., 2001). This suggests that progesterone promotes both dendritic outgrowth and synaptogenesis in Purkinje cells through nuclear receptor-mediated mechanisms.

1.1.3.1.2 Building material

The simplest explanation of how cholesterol promotes synapse formation would be that it is an essential component of the synaptic machinery and that its availability limits the assembly of synaptic structures. Due to its effects on the biophysical properties of membranes (Yeagle, 1985), cholesterol affects the function of membrane-resident signaling components including ion channels, transporters and receptors (Spector & Yorek, 1985; Yeagle, 1989; Barrantes, 1993; Bastiaanse et al., 1997; Burger et al., 2000). Measurement of cholesterol transbilayer distribution in brain synaptosomes by leaflet-selective quenching of fluorescent cholesterol analoga, has shown that the inner leaflet of synaptic membranes contains a eight-fold higher cholesterol concentration than its outer counterpart and that this distribution is affected by disease (Wood et al., 1990) and aging (Igbavboa et al., 1996). Moreover, ApoE and the LDL receptor influence the transbilayer distribution of cholesterol in synaptic membranes (Igbavboa et al., 1997; Hayashi et al., 2002). Another approach to visualize cholesterol distribution relies on the fact that the polyene antibiotic filipin forms complexes with sterols carrying a hydroxyl group at the third carbon atom. These complexes appear as protuberances in freeze-fractured membranes visualized by electron microscopy (Severs & Robenek, 1983). In frog NMJs, sterol-filipin complexes are localized in the transmitter release zone, but absent from the adjacent area (Nakajima & Bridgman, 1981; Ko & Propst, 1986). A similar distribution was observed at ribbon-type synapses between photoreceptors and bipolar neurons of chick retina (Cooper & McLaughlin, 1984). Notably, Surchev et al. (1995) showed that the density of sterol complexes in presynaptic membranes increases during postnatal development, using freeze-fracture electron microscopy. Egea et al. (1989) reported that potassium-induced acetylcholine release enhances the density of sterol-filipin complexes in
freeze-fracture replicas of synaptosomes from Torpedo electric organ. This effect was abolished in low calcium and by botulinus toxin indicating that the rearrangement was induced by the release process itself rather than by depolarization or calcium influx. Studies on isolated synaptic vesicles from rodent brain or Torpedo electric organ revealed that their cholesterol to phospholipid ratios range from 0.4 to 0.6 (Breckenridge et al., 1973; Nagy et al., 1976; Wagner et al., 1978; Deutsch & Kelly, 1981) showing that synaptic vesicle membranes contain more cholesterol than other intracellular organelles including mitochondria or the endoplasmatic reticulum (Yeagle, 1985; Schmitz & Orso, 2001). The high cholesterol content in the synaptic vesicle membrane suggests a link between vesicle biogenesis (Hannah et al., 1999) and the cellular cholesterol level. This is supported by a study that aimed to identify cholesterol-binding proteins using a new photoaffinity-probe (Thiele et al., 2000). The authors compared patterns of cholesterol-binding proteins in two PC12 lines that differed in their neurosecretory competence and identified synaptophysin as cholesterol-binding component of synaptic vesicles. Importantly, they could establish that the cholesterol level controls the availability of secretory vesicles in PC12 cells: lowering the cellular cholesterol content by methyl-β-cyclodextrin diminished the steady-state pool of synaptic-like microvesicles and their rate of biogenesis, but did not affect endocytosis in general.

1.1.3.1.3 Microdomains/rafts
The above mentioned studies indicate that cholesterol is not equally distributed along membranes and that certain molecules bind to it. An interesting hypothesis extends these observations and claims that biological membranes contain a number of small microdomains, enriched in cholesterol and sphingolipid content, which are essential for various cell functions (Simons & Ikonen, 1997). These cholesterol-rich domains (rafts) have been implicated in numerous cellular processes including signal transduction, membrane trafficking, cell adhesion and molecular sorting (Paratcha & Ibanez, 2002; Simons & Toomre, 2000; Brown & London, 1998; Ikonen, 2001). The fundamental principle, by which lipid rafts may exert their functions, is to separate or concentrate specific membrane proteins and lipids in microdomains (Harder et al., 1998). Two studies provide direct evidence for a role of rafts and cholesterol in exocytosis (Lang et al., 2001; Chamberlain et al., 2001). Lang et al. (2001) observed in the neuron-like cell line PC12 that docking and fusion of secretory granules occur at syntaxin- and synaptosomal-associated protein of 25 kDa (SNAP25)-positive clusters that are sprinkled across the plasma membrane. Lowering
the plasmalemal cholesterol content by methyl-β-cyclodextrin dispersed syntaxin clusters and inhibited KCl-induced release of dopamine and of green fluorescent protein-labeled neuropeptide Y, as monitored by amperometry and fluorescence microscopy, respectively. The relevance of cholesterol for exocytosis has been confirmed by Chamberlain et al. (2001), who observed as well that reduction of the cellular cholesterol level reduces dopamine release from PC12 cells. Together, both studies show that SNARE-dependent exocytosis occurs at cholesterol-rich domains in the plasma membrane (Lang et al., 2001; Chamberlain et al., 2001). Several studies indicate that neurotransmitter receptors and other postsynaptic components are associated with rafts. Nicotinic acetylcholine receptors from cultured chick ciliary ganglion neurons were found in raft-like microdomains, which are cholera toxin-positive and detergent resistant. Moreover, receptor clusters were dispersed by methyl-β-cyclodextrin-dependent cholesterol depletion, but not by actin depolymerization (Bruses et al., 2001). Another study shows detection of AMPA-type glutamate receptors in detergent-insoluble rafts from rat brain synaptosomes (Suzuki et al., 2001). The intercellular adhesion, necessary for the stability of synapses, may also depend on cholesterol considering evidence that different types of cell adhesion molecules are localized in rafts. For example, glycoprophosphatidylinositol-linked proteins (Buttiglione et al., 1998; Lang et al., 1998), NCAM (He & Meiri, 2002; Niethammer et al., 2002), integrins (Leitinger & Hogg, 2002), and cadherins (Crossin & Krushel, 2000; Angst et al., 2001; Tsui-Pierchala et al., 2002) have been shown to anchor in rafts. Another interesting aspect has been raised by the observation that the kinesin-mediated transport of vesicles along microtubule requires cholesterol and sphingomyelin-rich rafts in the vesicular membrane in vitro (Klopfenstein et al., 2002).

These results indicate possibilities how glia-derived cholesterol affect synapse formation and function and underline the importance of cholesterol for neurotransmission (Pfrieger, 2003).

### 1.2 My project

The aim of my project was to investigate how cholesterol supports synapse formation and function and whether other glia-derived factors are involved in synaptogenesis in cultured RGCs. Further, I was searching for neuronal target genes involved in glia-dependent postnatal differentiation of rat RGCs.