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EXPERIMENTAL STRATEGIES TO PROMOTE AXONAL REGENERATION AFTER TRAUMATIC CENTRAL NERVOUS SYSTEM INJURY

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Abstract—A damage or pathological process that destroys the continuity of axons in the mature central nervous system (CNS) has devastating consequences and produces lasting functional deficits. One of the major challenges in this field is to stimulate the regrowth of severed axons and reconstruction of pathways. Recent progress in molecular and cell biology has resulted in an explosion of knowledge on factors in the adult CNS being nonsupportive or even actively inhibitory to axonal regrowth. The new findings have a strong impact on the development of new therapeutic concepts directed to stimulate axonal regeneration. They give rise to cautious optimism, showing that under some circumstances repair of a CNS lesion is possible. In this review the authors summarize the current knowledge on the factors and mechanisms involved in regeneration failure and provide an overview of the current therapeutic approaches that may enable effective CNS regeneration in the future. © 1998 Elsevier Science Ltd. All rights reserved

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ABBREVIATIONS

BDNF	Brain-derived neurotrophic factor	MBP	Myelin basic protein
CNS	Central nervous system	MHC	Major histocompatibility complex
CNTF	Ciliary neurotrophic factor	NCAM	Neural cell adhesion molecule
CSPG	Chondroitin sulphate proteoglycan	NGF	Nerve growth factor
ECM	Extracellular matrix	NI35/250	Neurite growth inhibitor 35/250 kDa
FGF	Fibroblast growth factor	NT	Neurotrophin
GABA	γ -Aminobutyric acid	NTF	Neurotrophic factor
GAP-43	Growth associated protein 43 kDa	PDGF	Platelet-derived growth factor
GFAP	Glial fibrillary acidic protein	PN	Peripheral nerve
HSPG	Heparan sulphate proteoglycan	PNS	Peripheral nervous system
IGF	Insulin-like growth factor	TEN	Tenascin C
KSPG	Keratan sulphate proteoglycan	TGF	Transforming growth factor
MAG	Myelin-associated glycoprotein	WGA-HRP	Wheat germ agglutinin horseradish peroxidase

1. INTRODUCTION

Mechanical injury to adult mammalian central nervous system (CNS) may result in widespread functional losses and in permanent neurological deficits. Spinal cord injury is one of the prominent examples of such a traumatic insult leading to permanent disability with loss of sensory, motor and reflex activities [for reviews see Adams and Victor (1985) and Schwab and Bartholdi (1996)]. The severe clinical consequences are due to the fact that, in contrast to most other tissues, the CNS lacks the ability to reconstitute itself by neuronal cell proliferation and fails to regrow severed axons. Axotomized nerve fibers within the CNS show an initial growth response but after a short distance this regrowth ceases. The poor axonal regeneration in mammalian CNS stands in sharp contrast with the situation observed in fish, amphibia or mammalian peripheral nervous system (PNS) and immature mammalian CNS. The latter systems possess significant regenerative capacities and are able to restore neuronal function after lesion (Ramón y Cajal, 1928; Gaze, 1970; Kiernan, 1979; Cohen *et al.*, 1988; Stürmer *et al.*, 1992; Sharma *et al.*, 1993; Davis and McCellan, 1994; Lang *et al.*, 1995; Li *et al.*, 1995).

At the beginning of this century, the regeneration failure of CNS was a dogma and differentiated mammalian CNS neurons were considered inherently incapable of regrowth. However, since the pioneering studies of Ramón y Cajal (1928) and those of Aguayo and colleagues (David and Aguayo, 1981; Duncan *et al.*, 1981) it has been well established that CNS neurons have the capacity to regrow axons given a suitable environment. The latter authors have shown that lesioned CNS axons grow successfully into a peripheral nerve (PN) implant but cease growing as they enter the CNS tissue. These PNS–CNS grafting experiments have been instrumental in reversing the previous dogma. A more optimistic outlook began to evolve during the last two decades and a number of studies were performed to identify inhibiting factors responsible for axonal regeneration failure and to develop effective experimental strategies to stimulate structural and functional repair of lesioned CNS.

The present review briefly summarizes fundamental molecular and cellular reactions to traumatic injury and their putative involvement in regener-

ation failure, reviews recent progress in the development of regeneration promoting strategies and discusses their possible therapeutic application.

2. MOLECULAR AND CELLULAR CHANGES AT THE LESION SITE

2.1. Molecular Changes

Traumatic injuries of the CNS initiate a complex sequence of pathophysiological responses at the lesion site [for reviews see Hall (1989); Hilton (1994); McIntosh (1994); Schwab and Bartholdi (1996); Tymianski and Tator (1996)]. The first gross observation immediately after injury is the extravasation of blood into the lesion site due to disruption of blood vessels. The lesion site becomes filled with a plug of haematogenous material, including monocytes, lymphocytes and inflammatory mediators. As a result of vascular spasm and disruption there is local ischemia, hypoxemia and hypoglycaemia. Vasogenic edema becomes evident and contributes to necrosis of cells. Likewise at this very early time after lesion a profound change in the composition of the extracellular fluid is observed. Excitatory amino acids like glutamate and aspartate are secreted; iron is released from intracellular storage proteins and catalyzes the generation of oxygen radicals; excess of calcium and potassium appears and growth factors as well as proteases accumulate within the wound. All these factors form an interactive reaction cascade leading to the death of neurons and glial cells and the degeneration of axons at the site of injury.

Besides these acute changes in the extracellular milieu the lesion-induced transition of glial cells from the resting to the activated state (see later) is associated with the expression of new molecules as well as the upregulation of factors that are found at lower levels in normal brain [for reviews see Brodkey *et al.* (1993) and Eddleston and Mucke (1993)]. Astrocytes are particularly very active early after injury, and among the first molecular changes are the increase in glial fibrillary acidic protein (GFAP) (Schiffer *et al.*, 1986; Condorelli *et al.*, 1990) and the induction of major histocompatibility complex (MHC) antigens (Lee *et al.*, 1990; Neumann *et al.*, 1996). Further, astrocytic molecules

expressed after injury comprise diffusible polypeptide factors, membrane-associated macromolecules, extracellular adhesion molecules and membrane proteins. Prominent examples of such lesion-induced molecules [for review see Brodkey *et al.* (1993)] are the glycoproteins laminin (Liesi *et al.*, 1984; Giftchristos and David, 1988; Sosale *et al.*, 1988) and fibronectin (Egan and Vijayan, 1991), different types of collagen (Feringa *et al.*, 1980; Maxwell *et al.*, 1984), proteoglycans (Giftchristos and David, 1988; Bovolenta *et al.*, 1993; Lips *et al.*, 1995; Stichel *et al.*, 1995a; Levine, 1996), tenascin C (TEN-C) (Chuong and Chen, 1991; Laywell *et al.*, 1992; Ajemian *et al.*, 1994; Lips *et al.*, 1995; Zhang *et al.*, 1995) and neurotrophic factors (Nieto-Sampedro *et al.*, 1983; Needels *et al.*, 1986; Asada *et al.*, 1996). These molecules differ largely in their extracellular distribution. Laminin and collagen type IV, which are generally considered to provide a particularly attractive substratum for axonal elongation (Carbonetto *et al.*, 1983; Edgar *et al.*, 1984), are mainly organized as basal lamina (basement membrane) (Timpl and Brown, 1996) around lesion-induced blood vessels (Giftchristos and David, 1988; Hagg *et al.*, 1989; Shigematsu *et al.*, 1989; Stichel and Müller, 1994b) and as part of the glia limitans (Risling *et al.*, 1993) in the wound, while TEN-C and proteoglycans are widespread in the extracellular matrix (ECM) and within astrocytes (Bartsch *et al.*, 1992; Laywell *et al.*, 1992; Pindzola *et al.*, 1993; Ajemian *et al.*, 1994; Mitrovic *et al.*, 1994; Lips *et al.*, 1995). Following lesion astrocytes also remodel the composition and architecture of their cellular membrane. The expression of the neural cell adhesion molecule (NCAM) (Chuong and Chen, 1991; Nait Oumesmar *et al.*, 1995) and the number of orthogonal arrays and gap junctions (Wolburg and Kästner, 1984), that are determinants of cell-cell communication, are significantly increased.

Besides astrocytes, activated microglia and monocytes/macrophages also exhibit strong secretory properties. They secrete a variety of cytokines, degradative enzymes, such as proteases, and small bioactive molecules, such as leukotrienes and superoxide [for reviews see Nathan (1987); Perry and Gordon (1988); Auger and Ross (1992) and Banati *et al.* (1993)].

The molecular response to injury is complex and results in a complete reorganization of the ECM. The composition of the ECM is highly dynamic, depends on the type of injury and changes continuously. Whether and how the ECM components contribute to the neuronal response has to be evaluated and will add to our understanding of lesion-induced neuronal degeneration and regeneration processes.

2.2. Responses of Nonneuronal Cells

The acute phase of molecular changes of the extracellular environment is accompanied by a rapid activation of neuroglial and other nonneural cell types. Among the neuroglial cells, astrocytes and microglia/macrophages have received considerable attention in recent years. Both glial cell types exhibit a vigorous response to traumatic injury with marked

hypertrophy, proliferation, migration and transition to a different morphological phenotype [for reviews see Maxwell *et al.* (1990a); Hatten *et al.* (1991); Landis (1994); Norenberg (1994); Moore and Thanos (1996); Ridet *et al.* (1997)]. Reactive gliosis is the result of this very early response to injury and is characterized by a dense web of interlacing glial processes that forms within a lesioned area. It is a hallmark of CNS lesion and a central element of the lesion scar which includes, in addition, invading fibroblasts, endothelial cells, oligodendrocytes, neutrophil granulocytes, lymphocytes and various ECM proteins (Lindsay, 1986; Reier and Houle, 1988). Several features of the postlesion glial responses are common to different lesion types and independent of the site of CNS lesion, such as:

1. the rapidity of the glial response (Maxwell *et al.*, 1990a,b; Matsumoto *et al.*, 1992; Stichel and Müller, 1994a);
2. the spatial distribution (Takamiya *et al.*, 1988; Schmidt-Kastner *et al.*, 1990; Berry *et al.*, 1996b);
3. the sequential activation of microglia/macrophages and subsequently astrocytes (Maxwell *et al.*, 1990a; Schmidt-Kastner *et al.*, 1990; Matsumoto *et al.*, 1992; Stichel and Müller, 1994a); and
4. the transient absence of activated astrocytes in the perilesion area (Dusart and Schwab, 1994; Stichel and Müller, 1994a,b; Frank and Wolburg, 1996).

The first structural responses of endogenous glial cells have been already observed between 5 and 20 min postlesion (Phillips and Turner, 1991). This activation is a dynamic process and spreads from the epicenter of the lesion into the surrounding neuropil. Glial reactions peak at *ca* 2–3 weeks postlesion, but macrophages as well as prominent phenotypic astrocytic changes are found up to 2 years after lesion (Stoll *et al.*, 1989; Miklossy and Van der Loos, 1991; Stichel and Müller, 1994a,b). Such a long-term presence of activated glial cells indicates the persistence of metabolic changes, that might, in turn, influence the physiological properties of the affected brain area.

It is important to mention that besides these similarities in gliotic responses formation there are differences in the composition and molecular profile that vary according to the site of injury and the age of the animal [Wilkin *et al.* (1990); Hatten *et al.* (1991); Milligan *et al.* (1991); Alonso and Privat (1993); Fernaud-Espinosa *et al.* (1993); Hill *et al.* (1996); for review see Ridet *et al.* (1997)]. Dense gliosis is usually seen in areas of white matter, while gliosis in gray matter tends to be variable. Another interesting observation is that astroglial response to injury is much more moderate or even absent in neonates (Gearhart *et al.*, 1979; Maxwell *et al.*, 1990b; Trimmer and Wunderlich, 1990). Thus, the gliotic response to CNS lesion is surprisingly diverse. Even within the same gliotic reaction the individual responses of reactive astrocytes, such as the expression of glycoproteins or proteoglycans, vary according to the relative position of the cell to the lesion center (Giftchristos and David, 1988; Bartsch *et al.*, 1992; Laywell *et al.*, 1992; Stichel and

Müller, 1994a,b; Lips *et al.*, 1995; Stichel *et al.*, 1995a; Ridet *et al.*, 1997).

Brain endothelial cells also take part in the cellular response to lesion and start to migrate actively and to proliferate (Shigematsu *et al.*, 1989; Landis, 1994). Numerous blood capillaries with increased diameter and surrounded by basal laminae appear within the wound and constitute a dense vascular network (Sosale *et al.*, 1988; Hagg *et al.*, 1989; Blight, 1991; Stichel and Müller, 1994a,b). As a result blood circulation within the lesioned area is re-established. Vascular repair coincides with a permanent increase in the number of capillary profiles in the lesion area and with long-term changes of the blood-brain barrier including reduced astrocyte-endothelial cell association, expansion of perivascular space and prominent ECM deposits (Jaeger and Blight, 1997).

2.3. Response of Neurons

Lesions directly affecting gray matter may cause neuronal death either by direct physical insult (primary cell death) or indirectly by changes in the local environment (secondary cell death) and axonal lesion close to the cell body (retrograde cell death), respectively. However, if the lesion affects the white matter and occurs at some distances from the cell body most adult neurons survive axotomy. Since the present review focuses on strategies to promote axonal regeneration, we summarize the principle features of pathophysiological axon reactions (see Fig. 1).

When axons are completely severed or simply compressed (crush injury) they are essentially divided into two cytoplasmic compartments; the proximal and distal segment. The cut ends retract after injury leaving an intervening gap. Between 1 and 8 days after lesion the axoplasm begins to assume a more granular and dark appearance due to breakdown of cytoskeletal components. Shrinkage of axons, distension with multivesicular bodies as well as myelin retraction and disintegration are common pathological features after axonal lesion [Ramón y Cajal (1928); Steward *et al.* (1973); Richardson *et al.* (1982) and Maxwell *et al.* (1990a); for reviews see Povlishock *et al.* (1992) and Povlishock and Christman (1995)]. These axonal changes spread in a centrifugal fashion into antero- and retrograde directions. The remaining axonal and myelin debris is phagocytosed by macrophages, activated microglia and astrocytes (Bignami and Eng, 1973; Bignami *et al.*, 1981; Noske *et al.*, 1982; Vinore and Herman, 1993), but normally removal of debris in the CNS is much slower than in the PNS (Bignami *et al.*, 1981; Perry *et al.*, 1987; Avellino *et al.*, 1995). While the distal axonal stump disappears gradually, the proximal end exhibits morphological changes that lead to the emergence of growth cones. Within the first three weeks after lesion adult mammalian CNS axons show an initial growth response. However, sprouting axons extend only for very short distances, *ca* 1 mm, before they cease growing and either degenerate (Richardson *et al.*, 1982) or end locally at the lesion border (Reier *et al.*, 1983; Stichel and Müller,

1994a; Anders and Hurlock, 1996). Although there are some exceptions of the rule (Wendt and Ayyad, 1987; Frisén *et al.*, 1993), most sprouting axons in the adult mammalian CNS fail to enter the lesion site. However, from the latter observations it is clear that the lesion area itself is of prime importance in the context of regeneration failure.

3. HYPOTHESES TO EXPLAIN FAILURE OF AXONAL REGENERATION IN THE ADULT MAMMALIAN CNS

The key question of regeneration research since many years is for the mechanisms that are responsible for regeneration failure in adult mammalian CNS. Studies by Tello (1911) have already demonstrated the intrinsic capacity of mature CNS neurons to sustain long-distance axonal regeneration provided that their growth cones are placed in a favorable environment. Indeed, later investigations succeeded in identification of extrinsic factors and/or structures that might arrest axonal regrowth. However, besides these extrinsic influences on regeneration it has become increasingly clear in the last years that intrinsic growth properties of the neurons are likely to be as important [for reviews see Fawcett (1992) and Caroni (1997)]. There is now evidence for differences in growth potentials of various neuronal types (Rossi *et al.*, 1995) and a decline in regenerative axonal response with neuronal age (Chen *et al.*, 1995; Li *et al.*, 1995; Dusart *et al.*,

Fig. 1. Pathological changes of lesioned axons in adult mammalian CNS. This figure summarizes the lesion-induced changes after axonal disruption in the adult mammalian CNS. Axons first degenerate in the antero- and retrograde direction. Following, axons of the proximal stump spontaneously sprout up to the lesion site, where they stop abruptly. They never succeed in crossing or bypassing the lesion site but persist at the lesion border or degenerate secondarily.

1997). While the characterization of determinants for regeneration failure is still not brought to an end, the concept emerges that axonal regeneration depends on the interplay between extrinsic cues and intrinsic properties of the lesioned neuron. Whether or not a mature neuron re-extends an axon depends on:

1. the ability to reexpress growth-related genes;
2. the availability of neurotrophic factors and substrate molecules to which growing neurites may attach and extend;
3. the presence of growth-inhibiting molecules; and/or
4. the formation of a glial scar.

3.1. Intrinsic Determinants

Neurons undergo changes in gene expression following axonal injury. Several studies have shown that the period of regrowth in axonal regeneration is characterized by the selective expression of a set of growth-associated proteins, immediate early genes and transcription factors, such as *c-fos*, *c-jun* and KROX 24 (Jenkins *et al.*, 1993; Robinson, 1994; Hayes *et al.*, 1995; Honkaniemi *et al.*, 1995; Wu, 1996; Herdegen *et al.*, 1997), tubulin and actin (Bisby and Tetzlaff, 1992; Fournier and McKerracher, 1995) and the growth-associated protein GAP-43 (B-50/pp46/P-57) (Skene, 1989; Benowitz and Routtenberg, 1997). Clearly, nerve regrowth is controlled by the expression of specific proteins and implicates that the regulation of the corresponding genes might provide a basis for the stimulation of axonal regeneration. Especially, three proteins that are known to be upregulated after injury and/or downregulated at the end of the regeneration-permissive period have been considered to play a prominent competence role in neurite outgrowth, GAP-43 [Doster *et al.* (1991); Schaden *et al.* (1994); Aigner *et al.* (1995); Vaudano *et al.* (1995); for review see Benowitz and Routtenberg (1997)], a transcription factor of the immediate early gene family *c-jun* (Hüll and Bähr, 1994; for review see Herdegen *et al.*, 1997) and the proto-oncogene *bcl-2* [Chen *et al.* (1997); for review see Kroemer (1997)]. Overexpression of these genes supports regeneration of CNS axons at least during early perinatal period (Aigner *et al.*, 1995; Chen *et al.*, 1997), suggesting a function as intrinsic determinants for neurite regrowth. However, since neurons with *bcl-2* overexpression or sustained *c-jun* expression may fail to exhibit axonal regeneration in mature CNS (Hüll and Bähr, 1994; Schaden *et al.*, 1994; Chen *et al.*, 1997) and certain populations of regenerating axons express little or no GAP-43 (Doster *et al.*, 1991; Schaden *et al.*, 1994), the expression of these growth-related genes is either not sufficient or not a prerequisite for axonal regeneration in the adult CNS. The latter observations might be indicative for additional extrinsic determinants of regeneration.

3.2. Extrinsic Determinants

Over the years several extrinsic conditions have been proposed to be adverse to CNS regeneration.

These can be divided into three basic features of the lesion-induced environment, that are, by no means, exclusive:

1. the absence of appropriate neurotrophic/neurite-growth promoting factors required for the maintenance of axonal regrowth;
2. the presence of growth-inhibitory components; and
3. the scar acting as a physical impediment to axon elongation.

Considering the neurotrophic and neurite-promoting properties of Schwann cells in regenerating PNS, the inability of CNS neurons to regenerate has been attributed to the lack of neuritogenic/neurite-growth promoting molecules after CNS lesion (Tello, 1911; Ramón y Cajal, 1928; Liesi, 1985). However, Nieto-Sampedro and others (Nieto-Sampedro *et al.*, 1983; Needels *et al.*, 1986; Asada *et al.*, 1996) noted increased neurotrophic activity at CNS lesion sites, as well as a pronounced upregulation of fibroblast growth factor (FGF) (Frautschy *et al.*, 1991; Logan *et al.*, 1992) and ciliary neurotrophic factor (CNTF) (Ip *et al.*, 1993b). Thus, the 'lack of trophic factors hypothesis' in the lesioned CNS may reflect an insufficient upregulation rather than the absence of those factors.

The observation that cultured neurons are unable to extend neurites into optic nerve explants even in the presence of optimal trophic factor conditions (Schwab and Thoenen, 1985) have led to the recent concept, that the inability of adult mammalian CNS neurons to regenerate is a result of the predominance of neurite growth-inhibiting molecules. There is now a substantial list of molecules that exert either repulsive or inhibitory effects on growing axons. The most prominent members of this group are the myelin and oligodendrocyte-associated inhibitor of neurite growth (NI 35/250), the myelin associated glycoprotein (MAG), the glycoprotein tenascin-C (TEN-C) and several proteoglycans such as chondroitin sulphate (CSPG), heparan sulphate (HSPG) and keratan sulphate proteoglycan (KSPG).

The myelin protein NI35/250 has been demonstrated to restrict axonal elongation after spinal cord lesion (Schnell and Schwab, 1990, 1993; Schnell *et al.*, 1994) but is not a repellent component of the impermeable scar at the lesion site (Schnell and Schwab, 1993; Stichel *et al.*, 1995b). Similarly, the myelin protein MAG strongly inhibits neurite outgrowth *in vitro* (McKerracher *et al.*, 1994; Mukhopadhyay *et al.*, 1994) but *in vivo* axons grow for long distances along or across myelinated fiber tracts or through degenerating myelin (Victorin *et al.*, 1992; Davies *et al.*, 1993; Berry *et al.*, 1996a; Stichel *et al.*, 1996). Furthermore, mice deficient in MAG show no improved axonal regeneration (Bartsch, 1996). Thus, the myelin hypothesis of axon growth inhibition is ambiguous and does not offer a conclusive explanation for the regeneration failure in adult CNS.

Another potential candidate with growth-inhibitory properties is TEN-C, which is reexpressed and secreted by astrocytes after lesion (Laywell *et al.*, 1992; Ajemian *et al.*, 1994; Lips *et al.*, 1995; Zhang

et al., 1995). TEN-C is a member of the Ig superfamily of proteins and appears as a multifunctional glycoprotein with protein domains involved in neuron-binding, cell migration, neuron-repulsion and in the control of neurite growth [Götz *et al.* (1994); for review see Brodkey *et al.* (1993); Faissner and Steindler (1995)]. The effects of TEN-C depend on the form in which it is presented (Lochter *et al.*, 1991; Taylor *et al.*, 1993), as well as on the interactions with other ECM molecules (Marton *et al.*, 1989; Faissner *et al.*, 1990). Since TEN-C is also upregulated in regenerating PN (Martini *et al.*, 1990) and is not expressed at the site of growth arrest in a CNS lesion (Lips *et al.*, 1995), there is no clear-cut interpretation of growth-inhibitory vs growth-promoting effects and the contribution of this molecule to axon regeneration is still unresolved.

Proteoglycans comprise a heterogeneous group of molecules which are all composed of one or more glycosaminoglycan chains covalently linked to a core protein. Proteoglycans are widespread components of the ECM and a variety of these molecules are also expressed in the CNS [for reviews see Margolis and Margolis (1989); Kjellén and Lindahl (1991); Hardingham and Fosang (1992); Müller (1993) and Müller *et al.* (1996)]. In particular their inhibitory influences on neurite extension (Snow *et al.*, 1990; Cole and McCabe, 1991; Snow and Letourneau, 1992; McKeon *et al.*, 1995) as well as their involvement in boundary formation (Crossin *et al.*, 1989; Flaccus *et al.*, 1991; Katoh-Semba *et al.*, 1995; Watanabe *et al.*, 1995), ECM organization (Gallo and Bertolotto, 1990; Iozzo, 1997) their upregulation following brain injury (Giftchristos and David, 1988; McKeon *et al.*, 1991; Bovolenta *et al.*, 1993; De Witt *et al.*, 1994; Lips *et al.*, 1995; Stichel *et al.*, 1995a; Szele *et al.*, 1995; Levine, 1996) and deposition in regions of growth arrest (Davies *et al.*, 1997) make them potential candidates for interactions with growing axons. There are many different proteoglycans of which some are conducive to neurite outgrowth and some are inhibitory. Moreover, similar to TEN-C the effects of proteoglycans depend on concentration, the timing and distribution of expression and the molecular interactions with a particular environment (Christner *et al.*, 1980; Johnson-Green *et al.*, 1992; Kresse *et al.*, 1994; McKeon *et al.*, 1995). While the lesion-induced re-expression of the distinct types of proteoglycans varies considerably and may thus lead to diverse actions on neurite growth, experiments that could establish a causal link between the presence of these constituents and neurite growth inhibition are lacking.

The third important extrinsic constraint that could limit regrowth of lesioned axons in the adult CNS is the formation of a prominent glial scar in the lesioned area. In almost all instances where CNS regeneration has been described, regenerating nerve fibers bypass or stop at the neuroglial scar (Reier *et al.*, 1983; Schnell and Schwab, 1990, 1993; Lips *et al.*, 1995; Stichel *et al.*, 1995b; Anders and Hurlock, 1996) suggesting that the scar matrix is either improper in relation texture, surface and trophic or tropic factors to maintain axonal growth and/or may act

as a mechanical barrier to axonal regeneration. However, there are conflicting reports regarding the mechanism responsible for the growth inhibition exerted by the glial scar. The above mentioned hypothesis of a mechanical inhibitory function of the scar has been questioned by the observations that:

1. in some brain regions and under certain conditions regrowing axons enter the scar and persist there for a long time (Frisén *et al.*, 1993, 1995; Li and Raisman, 1995; Berry *et al.*, 1996a);
2. the composition and influence of the scar depends on the type of insult and the region of the CNS that is affected [Alonso and Privat (1993); Garcia-Abreu *et al.* (1996); Hill *et al.* (1996); for review see Wilkin *et al.* (1990); Hatten *et al.* (1991) and Ridet *et al.* (1997)];
3. the age and differentiation stage of astrocytes determine their capacity to either inhibit or support axonal growth (Smith *et al.*, 1986; Smith *et al.*, 1990); and
4. astrogliosis, a central component of glial scar, does not inhibit the advance of regrowing axons (Stichel and Müller, 1994b; Li and Raisman, 1995).

Nonetheless, regeneration is prevented by the lesion scar. While it is unlikely that the gliotic scar forms a physical barrier, changes in the molecular properties of the cells present in the scar, the absence of conducive factors in this region or the deposition of another impermeable scar component, the basal lamina, may account for the nonpermissive environment. Studies analysing the lesion-induced basal membrane formation, have proven that there is a clear spatio-temporal correlation between axonal growth arrest and the deposition of a dense collagenous basal membrane (Feringa *et al.*, 1985; Stichel *et al.*, 1998).

It has become clear that a plethora of lesion-induced extrinsic molecular factors may contribute to an inhospitable environment for axonal regeneration. Unfortunately, the direct influence of these factors on postlesion axonal regrowth is largely unclear and consequently their role in determining the success or failure of axonal regeneration remains speculative.

4. REQUIREMENTS FOR THE STRUCTURAL AND FUNCTIONAL RECOVERY OF INJURED AXONS

The precise regeneration of a lesioned projection in the CNS depends on a series of specific events, that can be viewed as a recapitulation of steps normally taken during development. Assuming that neurons survive the transection of their axons regeneration processes include:

1. regrowth (spontaneous sprouting) of the damaged axon;
2. passage through the lesion site;
3. elongation in the correct direction;
4. topographic reinnervation of the normal target; and
5. restoration of former electrophysiological properties [for reviews see Freed *et al.* (1985); Bähr and

Fig. 2. Requirements for structural and functional repair of injured axons. Schematic summary of the cascade of cellular reactions necessary to restore structure and function of CNS neurons following axonal disruption.

Bonhoeffer (1994); Aubert *et al.* (1995) and Yen and Kalb (1995)] (Fig. 2).

Damage to axons results first in the complete degeneration of the distal stump and partial degeneration or retraction of the proximal axons [Fig. 3(A)] that is followed by an initial regeneration response. The proximal cut ends form growth cones, that advance towards the lesion site. However, these terminal sprouts emitted by the lesioned axons are transient and never elongate for >1 mm into the lesion area (Ramón y Cajal, 1928; Richardson *et al.*, 1982; Borgens *et al.*, 1986; Schnell and Schwab, 1993). These sprouts are later retracted and usually the proximal stump of the cut axon dies back. Exceptions of this 'dying-back' phenomenon are the persisting terminal sprouts of corticospinal axons (Bernstein and Bernstein, 1971; Li and Raisman, 1995) and of transected postcommissural fornix axons (Stichel and Müller, 1994a; Stichel *et al.*, 1995b) [Fig. 3(B)]. The latter axons persist at the lesion site for at least up to 2 years after lesion. The spontaneous terminal sprouting has to be distinguished from another type of sprouting, the collateral sprouting [Li *et al.* (1994); for review see Frotscher *et al.* (1997)]. Collateral sprouting refers to the growth of processes from an intact axon. While both types of sprouting may occur in parallel after a CNS lesion, most regrowing sprouts observed after CNS lesion are probably collateral sprouts of nonlesioned afferent projections to a partially denervated CNS region (Raisman and Field, 1973; McMahon and Kett-White, 1991; Rossi *et al.*, 1991; Li *et al.*, 1994; Schauwecker and McNeill, 1995). Interestingly collateral and terminal sprouting takes only place in regions with little or no myelin at all (Kapfhammer and Schwab, 1994; Stichel *et al.*, 1995a,b).

Spontaneous terminal sprouting of damaged axons indicates the initial attempt and the potential of mature CNS neurons to regenerate. However, the axon sprouts stop growing after a short distance. While this lack of elongation might suggest an inherent switch into a growth arrest state, the most favored hypothesis suggests extrinsic growth-inhibitory factors in the immediate environment of the growth cone (see Section 3.2). The universality of the abortive axonal sprouting and the susceptibility of axonal elongation to extrinsic factors support the concept that sprouting and elongation are differentially regulated processes (Ramón y Cajal, 1928; Schnell *et al.*, 1994). Schwab and coworkers (1994) have shown that sprouting of severed corticospinal axons is regulated by neurotrophic factors (NTFs), while elongation of the same axons is influenced by lesion-induced extrinsic determinants.

One major extrinsic constraint for regrowing axons is the scar within the lesion site (see Section 3.2). In most cases where axon regeneration was stimulated (see Section 5) the fibers grew around the

Fig. 3. Transected postcommissural fornix axons fail to regenerate. (A) Within 1 week after mechanical transection fornix axons degenerate up to $500\ \mu\text{m}$ in the antero- and retrograde direction (arrow, lesion site). (B) During the following week the axons spontaneously sprout up to the lesion site (arrow). They reach but fail to elongate across the lesion border. d, Distal; p, proximal. Scale bars $100\ \mu\text{m}$.

lesion site rather than through the center. This implicates misrouting of axons with a high risk of inappropriate pathway choices and failure to innervate the normal target as well as possible troublesome interference with surrounding projections. Therefore, a crucial element of successful axonal regeneration is a continuous pathway through the former lesion site.

Once the axons have overcome the scar barrier, they encounter the problem of choosing the right pathway to their former target and the correct target neurons. Perhaps the clearest suggestion for these problems comes from studies of normal nervous system development. It was shown that process outgrowth depends on the molecular composition of the surrounding substrate. Growth cones encounter a variety of guidance molecules, attractive or repulsive, that are either short-range (immobilized) or long-range (diffusible) signals [for reviews see Dodd and Schuchardt (1995); Keynes and Cook (1995) and Goodman (1996)]. Interestingly, some of the immobilized molecules are re-expressed or constitutively present along lesioned pathways. The enhanced expression of the guidance molecules TEN-C and proteoglycans along lesioned pathways has been reported (see Section 3.2) and the myelin growth inhibitors persist in unlesioned myelinated neuropil bordering the lesion site (see Section 3.2). Of par-

ticular importance is the ECM molecule laminin, which promotes and directs the growth of processes extending from a broad range of neurons (Salonen *et al.*, 1987; Kuffler and Luethi, 1993; Kuhn *et al.*, 1995). Alternatively, it is accepted that growth cones can be steered by diffusible gradients of ions and transmitters (Kater *et al.*, 1988; Kater and Lipton, 1995; Kuffler, 1996) and it is very likely that diffusible growth factors secreted from the denervated target can attract regrowing axons to appropriate locations (McFarlane and Holt, 1997). Recently, it was demonstrated that a spatially restricted distribution of nerve growth factor (NGF) in the hippocampal formation dictates what regions are reinnervated by sprouting axons (Hagg and Varon, 1993; Conner and Varon, 1994; Conner and Varon, 1995). Very similarly, in the spinal cord NGF promotes the reentry of sensory fibers into nonpermissive white matter (Oueda and Hagg, 1996). These studies suggest that NGF is tropic and may provide directional, target-dependent information to ensure appropriate axon guidance. ECM molecules and NGF might act in concert to guide axons along a given pathway. Such interactions should allow the use of a limited number of factors in a large number of combinations, each of which provides guidance specificity to different populations of axons.

Table 1. Experimental approaches to promote axonal regeneration in adult mammalian CNS

Experimental approach	Methods	Region of treatment
Overexpression of neurite-growth associated proteins	<i>In vivo</i> gene transfer Transgenic mice	Olfactory bulb, cortex, hippocampus
Delivery of neurotrophic substances	Intraventricular infusion Polymer matrix Transplanted genetically modified cells <i>In vivo</i> gene transfer Activation of intrinsic growth factors	Striatum Spinal cord, ventricle, subst. nigra Sympathetic ganglia
<i>Implantation of growth-promoting cells</i>		
Fetal CNS tissue	Tissue blocks Suspension graft	Spinal cord Spinal cord, hippocampus
Schwann cells	Peripheral nerve graft Coupled to prostheses/matrices Suspension graft	Spinal cord, optic nerve, dorsal root Spinal cord, optic nerve Spinal cord, thalamus, ventricle, fornix
Astroblasts	Coupled to prostheses/matrices Suspension graft	Spinal cord, corp. callosum, dorsal root Optic nerve, cortex, fornix
Olfactory bulb ensheathing cells	Coupled to prostheses/matrices Suspension graft	Dorsal root, fimbria fornix Spinal cord
Microglial cells	Suspension graft	Spinal cord
Neutralization of neurite growth inhibitors	Antibodies against NI35/250 Transglutaminase	Spinal cord, optic nerve, fimbria fornix Optic nerve
Reduction of scarring	Piromen Excision of scar Trypsin, elastase, collagenase X-irradiation Glucocorticoids Antibodies against TGF β Antibodies against collagen IV Prolylhydroxylase inhibitor	Spinal cord Spinal cord Spinal cord Spinal cord Spinal cord, cortex Cortex Fornix Fornix
Electric field	Direct current field	Spinal cord

Within the target 'stop- or docking-signals' are necessary to arrest the homecoming axons at the appropriate target neurons. A well-studied example of such a docking mechanism is the retinotectal projection, in which molecules that specify the anterior-posterior tectal axis for fibers of the temporal retina are reexpressed upon denervation in the adult superior colliculus (Holt and Harris, 1993; Wizenmann *et al.*, 1993). These molecules, ELF-1 (ephrin-A2) and RAGS (ephrin-A5, AL-1), are ligands for Eph-related receptor tyrosine kinases and are repellent for temporal or temporal and nasal retinal axons, respectively (Nakamoto *et al.*, 1996; Monschau *et al.*, 1997). The elongation of ganglion cell axons is determined by a concentration gradient of ELF-1 and the retinal fibers stop when facing a critical change in the gradient that is 5% or more across the diameter of the growth cone (Baier and Bonhoeffer, 1992). Neurotransmitters and *N*-methyl-D-aspartate (NMDA) receptors are other classes of molecules that comprise stop signals for axon growth and thereby may contribute to target cell selection (Kater and Mills, 1991; Baird *et al.*, 1996).

In the final stage regrowing axons must regain synaptic contact with the target neurons and normal functional properties, for example, conduction velocity and action potential amplitude. The latter parameters are determined by the diameter and the course of the axon and the presence of a myelin sheath.

A cascade of molecular and cellular reactions is necessary to restore structure and function of lesioned CNS projections. The striking similarity of these steps to developmental events indicates that the understanding of the cellular and molecular biology of development holds the key to restoration of CNS projections after lesion.

5. EXPERIMENTAL APPROACHES TO PROMOTE AXONAL REGENERATION

The rapid progress of molecular and biochemical techniques and the knowledge of the mechanisms underlying neurodegeneration and regeneration encouraged several attempts to manipulate the regrowth response of lesioned neurons. These efforts concentrate on two main strategies:

1. the *activation of intrinsic neuronal capacities* by overexpression of regeneration-promoting genes (see Section 5.1) or by the delivery of neurotrophic substances in the vicinity of lesioned axons or perikarya of injured neurons (see Section 5.2); and
2. the *modification of the nonpermissive environment* in the lesioned CNS tissue by the implantation of acellular guiding prostheses (see Section 5.3.1), fetal neural tissue (Section 5.3.2) and glial cells (see Sections 5.3.3, 5.3.4, 5.3.5 and 5.3.6) or the neutralization of growth inhibitory molecules (see Section 5.4) and the permeabilization of the lesion scar (see Section 5.5), respectively.

An additional strategy is the application of an electrical field across lesion sites (see Section 5.7).

Table 1 provides an overview of the different experimental approaches to stimulate axon regeneration following CNS lesioning.

5.1. Overexpression of Neurite-Growth Associated Proteins

Several lines of evidence have indicated that GAP-43, Bcl-2 and c-Jun play important roles in determining the inherent growth properties of CNS neurons (see Section 3.1). However, only certain populations of neurons continue to express constitutively high levels of these genes. Interestingly, these neurons have been associated with a high degree of plasticity [for review see Benowitz and Routtenberg (1997) and Herdegen *et al.* (1997)]. Transgenic mice provide additional compelling evidence for the central role of such genes in axon regeneration (Aigner *et al.*, 1995; Chen *et al.*, 1997). Upregulation of their expression in affected neurons might be a useful approach to promote axonal regeneration. In this respect, an efficient potential therapy is on the way to be established using gene transfer methods. A first attempt to direct overexpression of GAP-43 *in vivo* was performed by Verhaagen and coworkers (Holtmaat *et al.*, 1996; Hermens *et al.*, 1997). Adenoviral vector-mediated GAP-43 expression induced alterations of membrane organization in olfactory axon terminals (Holtmaat *et al.*, 1997), which underline the influence of this protein on morphological plasticity of neurons.

Gene therapy directed to enhance expression of growth-associated proteins in the CNS could provide a new strategy for treatment of traumatic injury.

5.2. Delivery of Neurotrophic Substances

Neurotrophic factors are molecules that, by definition, are capable of supporting the survival, growth and differentiation of neurons and of regulating synaptic plasticity during development and in the mature nervous system. In nervous tissue NTFs are available only in limited quantities and induce cellular changes over hours to days through signaling pathways that induce gene transcription and protein synthesis. The list of identified NTFs is large and there is considerable more complexity and diversity than initially anticipated (Table 2). NGF was the first NTF discovered (Levi-Montalcini, 1987) and is often viewed as the prototype for all NTFs. Other members of the neurotrophin family are the brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) (Bothwell, 1995; Lewin and Barde, 1996). Besides this NGF family several NTFs belonging to different protein families were identified, such as the FGF, the platelet-derived growth factor (PDGF), the transforming growth factor (TGF) and the insulin-like growth factor (IGF) families. NTFs are target-derived molecules, while autocrine and nontarget-derived paracrine modes of presentation have also been described. They are synthesized by astrocytes, oligodendrocytes and neurons [for review see Müller *et al.* (1996)] and may act on both neurons and glial cells. Interestingly, individual popu-

Table 2. Partial list of neurotrophic factors and their neuronal specificities in CNS

Name	Responsive CNS neurons	References
NGF	Basal forebrain cholinergic neurons Striatal cholinergic neurons	Hefti (1986); Holtzman <i>et al.</i> (1995)
BDNF	Spinal motor neurons Cortical motor neurons Basal forebrain cholinergic neurons Basal forebrain GABAergic neurons Mesencephalic GABAergic neurons Nigral dopaminergic neurons Cerebellar granular neurons Hippocampal neurons Retinal ganglion neurons	Sendtner <i>et al.</i> (1992); Yan <i>et al.</i> (1997) Yan <i>et al.</i> (1997) Alderson <i>et al.</i> (1990); Hyman <i>et al.</i> (1991) Koliatsos <i>et al.</i> (1994) Hyman <i>et al.</i> (1994) Hyman <i>et al.</i> (1991); Hyman <i>et al.</i> (1994) Nonomura <i>et al.</i> (1996); Coffey <i>et al.</i> (1997) Zafra <i>et al.</i> (1991); Patterson <i>et al.</i> (1992) Barde (1990); Sawai <i>et al.</i> (1996)
NT-3	Spinal motor neurons Cortical motor neurons Mesencephalic GABAergic neurons Nigral dopaminergic neurons Hippocampal neurons Locus coeruleus neurons Cerebellar granule cells	Maisonpierre <i>et al.</i> (1990) Zhou and Rush (1994); Grill <i>et al.</i> (1997) Hyman <i>et al.</i> (1994) Hyman <i>et al.</i> (1994) Zafra <i>et al.</i> (1991); Patterson <i>et al.</i> (1992) Senut <i>et al.</i> (1995) Nonomura <i>et al.</i> (1996)
NT-4/5	Cortical motor neurons Basal forebrain cholinergic neurons Mesencephalic GABAergic neurons Nigral dopaminergic neurons Cerebellar granular cells Hippocampal neurons Striatal neurons Locus coeruleus neurons Retinal ganglion neurons	Junger and Varon (1997); Sendtner <i>et al.</i> (1996) Koliatsos <i>et al.</i> (1994); Alderson <i>et al.</i> (1996) Hyman <i>et al.</i> (1994) Hyman <i>et al.</i> (1994) Huber and Chao (1995) Ip <i>et al.</i> (1993a); Lessmann <i>et al.</i> (1994) Hynes <i>et al.</i> (1994) Friedman <i>et al.</i> (1993) Cohen <i>et al.</i> (1994); Sawai <i>et al.</i> (1996)
FGF	Cortical motor neurons Spinal cord neurons Hippocampal neurons Thalamic neurons Septal neurons Retinal ganglion neurons	Grothe and Wewetzer (1996) Gouin <i>et al.</i> (1996) Ferhat <i>et al.</i> (1997) Ferguson and Johnson (1991) Lindholm <i>et al.</i> (1994) Lipton <i>et al.</i> (1988); Bähr <i>et al.</i> (1989)

lations of neurons may respond to a variety of NTFs and, conversely, a single factor may act on several distinct neuronal populations.

Besides their potent effects on the survival of neurons, *in vitro* studies also demonstrate an important role of NTFs in stimulating axon regrowth and branching (Lindsay, 1988; Cohen *et al.*, 1994; Ahmed *et al.*, 1995). With these pronounced effects on neurite outgrowth in mind it seems reasonable to ask whether the absence of trophic factors may lead to regeneration failure and whether the postlesion application of NTFs may promote axon regrowth. Recently, studies analysing the effects of postlesion applied NTF on morphological and functional recovery of lesioned CNS projections have been initiated. These studies indicate that BDNF and NT-4/5 promote the regrowth of injured retinal ganglion cell axons (Mansour-Robaey *et al.*, 1994; Sawai *et al.*, 1996), NT-3 stimulates sprouting of corticospinal projections (Schnell *et al.*, 1994; Grill *et al.*, 1997), BDNF and NT-3 infusion enhances axonal regeneration of propriospinal and brain stem axons (Xu *et al.*, 1995a) and sensory as well as motor and brain stem axons grow toward a source of NGF in lesioned spinal cord (Tuszynski *et al.*, 1996).

The existence of a blood-brain barrier that blocks the entry of systemically circulating proteins into the

nervous system and the need for high concentrations of these substances have required invasive techniques as the main method for NTF delivery. In experimental animals, NTFs have been injected intraventricularly through chronically implanted cannulas with/without the help of osmotic minipumps. Despite unknown degradation kinetics of the neurotrophic substances in the cerebrospinal fluid and the brain parenchyma and the short biological half-life of some of the neurotrophins, infused NTFs have been shown to exert beneficial effects on axon regeneration. However, since NTFs rarely influence only a single neuronal target population the broad delivery of NTFs via the intraventricular application may result in side-effects on multiple nontargeted cells. For example, intracerebroventricular infusions of NGF not only protect cholinergic neurons from degeneration but also induce sprouting of sympathetic neurites that invest cerebral blood vessels (Saffran *et al.*, 1989), cause weight loss (Williams, 1991), and promote sprouting of spinal cord sensory neurites (Tuszynski *et al.*, 1994). These diverse NGF effects, a consequence of the broad delivery of NGF after infusion into the ventricular system of the brain, could limit clinical application. Another drawback using NTFs to promote axonal regeneration is their limited tissue pen-

etration (Lapchak *et al.*, 1993; Emmett *et al.*, 1996). Thus direct application of NTFs to the lesion site and maintenance of elevated trophic levels over several weeks seems to be required and make repeated injections or refilling of pump reservoirs necessary. Ideally, neurotrophin delivery into the nervous system should be target-specific, regionally restricted, well-tolerated and of sufficient concentration to elicit a response.

Alternatives to NTF infusion have been developed such as slow releasing intracerebral implants that contain the active protein embedded in a biodegradable polymer matrix (Powell *et al.*, 1990) and genetically modified cells that secrete the desired NTF [for reviews see Doering (1996); Verhaagen *et al.* (1996) and Snyder and Senut (1997)]. The most often used technique for gene transfer is the *ex vivo* approach, wherein genetically modified cultured cells are transplanted into the CNS. Numerous studies have employed fibroblasts and viral vectors for the *in vitro* gene transfer of NTFs (Rosenberg *et al.*, 1988; Lucidi-Phillipi *et al.*, 1995; Ray *et al.*, 1995; Senut *et al.*, 1995; Grill *et al.*, 1997). However, one major obstacle that arises using this approach is the continued growth of fibroblasts after implantation leading to varying degrees of tumor formation (Hoffman *et al.*, 1993; Tuszyński *et al.*, 1994). Polymer encapsulation may help to overcome this problem since it prevents the inherent risk of tumor formation and protects the genetically modified cells from immune destruction, while permitting the diffusion of molecules into the surrounding tissue for >1 year after implantation in adult rat ventricles (Emerich *et al.*, 1994; Winn *et al.*, 1996). Recently, X-ray treated genetically modified Schwannoma cells stimulated axonal regeneration in the nigrostriatal pathway (Brecknell *et al.*, 1996), genetically modified rat astrocytes have been shown to survive well after transplantation and express the desired protein (Lundberg *et al.*, 1996) human astrocytes have been efficiently infected with a retrovirus harbouring the NGF gene (Lin *et al.*, 1997) and rat Schwann cells genetically modified to secrete BDNF increased axonal regeneration in spinal cord (Menei *et al.*, 1998). The latter cell type may be a useful gene carriers, as astrocytes are natural hosts of the CNS and can be expanded *in vitro*. Another strategy to express NTF in lesioned CNS areas is the *in vivo* viral vector-mediated gene transfer. Numerous vectors have been shown to be effective for gene transfer in the CNS, including those derived from herpes viruses, adenoviruses, adenoassociated viruses and human immunodeficiency viruses (Le Gal La Salle *et al.*, 1993; Kaplitt *et al.*, 1994; Naldini *et al.*, 1996). Viral vectors carrying neurotrophin genes have been successfully used to locally express neurotrophic molecules in the lesioned nervous system (Fedoroff *et al.*, 1992; Geschwind *et al.*, 1994; Wang *et al.*, 1995b). However, the injected viruses have been shown to exert immune responses indicating the cytotoxicity of the current viral generations (Byrnes *et al.*, 1995; Hodgson, 1995). This risk and the occurrence of replication competent viral particles in viral vector stocks are severe problems that need to be resolved before the great potential of

these viruses for neurotrophic strategies may be used in neuroregeneration studies.

Another feasible approach to influence neurotrophic actions may be the stimulation of synthesis, release and activity of endogenous growth factors. It is known that hormones, corticosteroids, thyroid hormone and retinoic acid are involved in the regulation of NTF synthesis (Hefti *et al.*, 1986; Haskell *et al.*, 1987; Lindholm *et al.*, 1990) and thereby may provide tools for the pharmacological manipulation of NTF expression. Thus, the alkaloid-like compounds K-252a and K-252b, directly and selectively inhibit the neurotrophin receptors and at lower concentrations, selectively potentiate the actions of NT-3 (Koizumi *et al.*, 1988; Knusel and Hefti, 1991), suggesting that it may be possible to develop highly selective neurotrophin agonists and antagonists. Moreover, glucocorticoids (Chao and McEwen, 1994; Kononen *et al.*, 1994; Schaaf *et al.*, 1997), the ganglioside GM1 (Rabin and Mocchetti, 1995; Ferrari and Greene, 1996) and the glutamate antagonists MK-801 and NBQX (Lindvall *et al.*, 1992; Rocamora *et al.*, 1994; Timmusk and Metsis, 1994) mimic and potentiate many activities of NTFs including stimulation of neurite outgrowth *in vitro* as well as *in vivo*.

5.3. Grafting

One of the most intriguing possibilities for promoting the regeneration of damaged CNS is through the implantation of growth supportive material in the immediate environment of the lesioned neurons. Historically this strategy is one of the earliest attempts to induce repair of the CNS and has become widely used in the regeneration field. Depending on the material that is grafted different aspects of the regeneration process are addressed. Using fetal CNS tissue, transplantation may be primarily a useful tool to replace lost cells in a lesioned area. On the other hand, the latter as well as acellular prostheses and glial implants may be viewed as modifiers of the lesion area, which either interfere with the process of scar formation by physically preventing the infiltration of cellular elements that promote the development of a glial scar (Reier and Houle, 1988) resulting in looser scarring and/or provide a bridge across the impermeable lesion scar by reestablishment of a growth supportive framework (Li and Raisman, 1997). Finally, cellular implants may be used as a source for trophic factors for axonal growth.

5.3.1. Acellular Bridges

In the intensive search for methods to improve the regenerative capabilities of CNS tissues, many different artificial bridging models have been attempted. All of these approaches aim to provide:

1. oriented paths of low physical resistance which guide the regrowing axons through the dense glial-connective tissue scar; and
2. support frames for glial and vascular reorganization in lesioned CNS tissue.

In this respect, various matrices have been used, like collagen, hydrogels, nitrocellulose membranes, car-

bon filaments, glass filaments, gelfoam and guidance tubes of different polymers (Gelder, 1990; Marchand and Woerly, 1990; Khan *et al.*, 1991; Schnell and Schwab, 1993; Joosten *et al.*, 1995; Montgomery *et al.*, 1996; Borgens and Bohnert, 1997), with varying but generally unsatisfactory, degrees of success. In some of these conduits limited axon growth was reported, but these axons never succeeded in reentering into the host CNS tissue. Addition of neuroactive substances like laminin to a collagen matrix bridge, however, enhanced the axonal ingrowth but allowed only single axons to cross the bridge and to enter the distal spinal cord stump (Goldsmith and de la Torre, 1992). Although the artificial bridges may provide a favorable milieu for axons and a new avenue to modulate scar formation, in most cases the axonal regrowth into the former pathway is halted by properties of the distal graft–host interface and structural repair of a lesioned projection is never achieved.

On the other hand, numerous studies have confirmed the usefulness of these matrices when used as vehicles for growth-promoting glial cells (Kliot *et al.*, 1990; Lustgarten *et al.*, 1991; Paino and Bunge, 1991; Plant *et al.*, 1995; Xu *et al.*, 1995b) and for growth factor application (Powell *et al.*, 1990; Houle and Ziegler, 1994; Wells *et al.*, 1997). The advantages of such a matrix/glial cell or matrix/NTF combination are the fixation and accumulation of cells in the implantation site, the provision of a defined environment and the controlled and slow release of neuroactive substances (Powell *et al.*, 1990).

5.3.2. Fetal CNS Tissue

One of the most intriguing possibilities for promoting the reconstitution of damaged CNS parts is through the implantation of fetal tissue grafts. Many experiments in the past several years have shown that transplantation of fetal CNS tissue can serve as an important approach in studies of CNS regeneration. One of the exciting findings to emerge from this field is that there are essentially two main avenues through which fetal grafts can achieve their beneficial effects.

First, regenerative potential can be improved by replacing damaged or lost adult neurons with fetal ones. Transplants of developing CNS tissue have revealed a remarkable capacity to develop in the new environment, to integrate with the host, to form synapses on host cells and to receive synapses from host neurons [Isacson *et al.* (1995); Pundt *et al.* (1996); for reviews see Barker *et al.* (1993); Brundin and Wictorin (1993) and Isacson and Deacon (1997)]. This technique has now been used in experimental clinical trials in many patients with neurodegenerative diseases and the results are promising [for reviews see Sladek and Shoulson (1988); Freed (1991); Lindvall (1991); Kupsch *et al.* (1995); Olanow *et al.* (1996) and Wichmann *et al.* (1996)].

Second, and even more important for axonal regeneration, fetal grafts may serve as a tissue bridge or a relay station that could facilitate axonal elongation across a lesion site. Moreover, fetal transplants may be viewed as a modifier of the scar matrix and since such grafts also introduce imma-

ture glial cells, they are also a source of trophic and tropic factors for axonal growth (Bregman and Reier, 1986). These potential beneficial effects for regenerating systems have been illustrated by intraspinal grafting studies. In neonatal rats Bregman and colleagues have observed that fetal spinal cord can be used in the injured spinal cords as a bridge for axons that are either in their normal developmental growth phase or are undergoing actual regrowth. More recently, the enhanced ability of embryonic neurons to grow and reach distant targets has been used experimentally to show that both ends of a foetal spinal cord graft will connect and restore function across a complete spinal cord lesion (Iwashita *et al.*, 1994). On the other hand, no evidence has yet emerged for fetal grafts serving as bridges in adult spinal cord [for reviews see Horvat (1992) and Schwab and Bartholdi (1996)]. Thus far the only transplantation study in adult mammals is that of Kromer *et al.* (1981), who showed that transplants of fetal hippocampus serve as bridges that facilitate the regrowth of septal cholinergic fibers and their reinnervation of the host hippocampus.

5.3.3. Peripheral Nerve/Schwann Cells

Tello performed one of the earliest recorded PNS to CNS implantation experiments (Tello, 1911). He implanted predegenerated grafts of sciatic nerve into the mammalian cerebrum and after *ca* 2 weeks, he noted extensive growth of central fibers into the graft. Following this initial trial a number of studies have confirmed that PNS grafts constitute a sufficient stimulus for axonal regeneration and that fully mature CNS axons have the capacity to regenerate (Richardson *et al.*, 1982; Thanos and Vanselow, 1989; Houle, 1991; Thanos, 1992; Carter and Jhaveri, 1997; Thanos *et al.*, 1997; Watanabe and Fukuda, 1997). Of particular relevance here are the studies of Aguayo and his colleagues. In a series of very elegant experiments, they showed that a proportion of lesioned adult mammalian retinal axons that regenerated into PN autografts for at least 2 cm grew out from the distal end of the implants and formed long-lasting, functionally effective synapses with tectal neurons (David and Aguayo, 1981; So and Aguayo, 1985; Vidal-Sanz *et al.*, 1987; Keirstead *et al.*, 1989; Bray *et al.*, 1992; Carter *et al.*, 1994). Recently, a revival of this technique with more refined surgical procedures was applied in the spinal cord. The elegant and technically difficult study of Cheng *et al.* (1996) demonstrated for the first time functional regeneration in the completely transected spinal cord by using several PNs stabilized by FGF-impregnated fibrin glue to bridge individual spinal axon bundles to the gray matter of the distal stump. This study is an important step towards the goal of efficiently restoring axonal regeneration in the damaged CNS. While in the above mentioned studies the PN grafts were placed directly into the lesion site, for example, in close proximity to the degenerating axon segments, in a recent study the effect of a PN graft placed in the vicinity of severed cell somata was analyzed. Berry *et al.* (1996a) showed that PN tissue introduced into the vitreous body promotes the regeneration of crushed optic

nerve axons without the prerequisite for the suppression of growth inhibitory molecules. However, the PN-implantation studies also showed a number of limitations to this approach. Apart from the inevitable mechanical damage caused by the physical insertion of the nerves, the most favorable grafts pick up only a proportion of the host fibers (Cheng *et al.*, 1996), the axons leaving the grafts show only restricted penetration of the central target tissues (Vidal-Sanz *et al.*, 1987; Cheng *et al.*, 1996) and in some cases abnormal connections may be formed (Zwimpfer *et al.*, 1992).

There is evidence that the growth-promoting effects of transplanted PNs is dependent on the presence of viable Schwann cells in the nerve (Friede and Samorajski, 1968). Schwann cells express and secrete a variety of NTFs (Bandtlow *et al.*, 1987; Acheson *et al.*, 1991; Rende *et al.*, 1992), ECM molecules (Bunge, 1993) and surface adhesion molecules (Bandtlow *et al.*, 1990; Schachner, 1990; Martini, 1994) that stimulate axonal growth. Moreover, their abilities to organize into PN-like aligned arrays (Montgomery and Robson, 1993) and to modify the lesion-induced, dense astrocytosis (Li and Raisman, 1997) may promote axonal regeneration. In fact, Schwann cell-free PN grafts fail to support CNS regeneration (Berry *et al.*, 1988; Smith and Stevenson, 1988) as do Schwann cell-free collagen membranes (Kromer and Cornbrooks, 1985; Paino *et al.*, 1994). A number of implantation studies have demonstrated that Schwann cells represent a very suitable and effective substrate for stimulating axonal regeneration (Chen *et al.*, 1991; Martin *et al.*, 1991; Montero-Menei *et al.*, 1992; Neuberger *et al.*, 1992; Montgomery and Robson, 1993; Raisman *et al.*, 1993; Brook *et al.*, 1994; Paino *et al.*, 1994; Harvey *et al.*, 1995; Plant *et al.*, 1995; Xu *et al.*, 1995b; Chen *et al.*, 1996). Besides their growth-promoting effects, Schwann cells are also able to myelinate CNS axons (Blakemore, 1977) and thus have the capacity to myelinate regenerated axons (Chen *et al.*, 1991; Felts and Smith, 1992; Guénard *et al.*, 1993; Paino *et al.*, 1994; Honmou *et al.*, 1996; Li and Raisman, 1997). Different approaches have been used to introduce the Schwann cells into lesioned CNS. Initial attempts used tubes made of permeable gels (Levy *et al.*, 1988; Cohen *et al.*, 1990), synthetic polymers (Montgomery and Robson, 1993; Harvey *et al.*, 1994; Xu *et al.*, 1995b) or basement membrane components (Neuberger *et al.*, 1992; Paino *et al.*, 1994). Besides the fact that such prostheses are needed in cases in which a wide gap needs to be bridged (Guénard *et al.*, 1991; Harvey *et al.*, 1991) or the geometry of the proximal and distal stumps is complex (Shine *et al.*, 1985; Aebischer *et al.*, 1988), their use raises fundamental difficulties. Such solid large grafts themselves cause extensive damage to the host tissue and their size and the need for exact positioning restrict their application to more superficial lesion areas. In an attempt to minimize these complications a microtransplantation method for the controlled injection of cell suspensions has been developed (Schmidt *et al.*, 1981; Emmett *et al.*, 1990). Using this approach several studies have been successful in transplanting highly purified Schwann cell suspensions into the adult rat brain (Montero-

Menei *et al.*, 1992; Brook *et al.*, 1993; Brook *et al.*, 1994; Li and Raisman, 1994; Pizzorusso *et al.*, 1994; Fagioli *et al.*, 1997; Li and Raisman, 1997).

In our own work we have used this minimal invasive microtransplantation approach to introduce a Schwann cell suspension into the transected post-commissural fornix of the adult rat (Stichel *et al.*, 1996) (Fig. 4). We could show that the injected Schwann cells, which are primary cells [Fig. 4(A)], do not form tumors upon grafting as some cell lines do (Tuszynski *et al.*, 1994), migrate freely for considerable distances [Fig. 4(B)] and become integrated in neuropil or white matter, where they survived for at least 8 months after injection. The microimplanted Schwann cells did not prevent axonal degeneration of transected fornix fibers but promoted structural recovery of the projection. The regrowing fibers traversed the formerly impermeable lesion site and extended along their former pathway up to the normal target [Fig. 4(C and D)], where they reformed synaptic contacts (Stichel *et al.*, 1997). In contrast to previous studies (see above), the post-commissural fornix fibers were remyelinated by central myelin. This impressive restoration of a lesioned fiber tract in rats as well as recent reports of functional recovery of visually deprived animals in the presence of implanted Schwann cells (Fagioli *et al.*, 1997) provide an encouraging argument for pursuing this approach to an appropriate therapeutic strategy. Further exciting data in favor of this approach are studies in our lab showing that even xenogeneic Schwann cells represent a source of non-immunogenic graft material (Hermanns *et al.*, 1997), while others demonstrated that large populations of Schwann cells can be expanded from adult human PN (Morrissey *et al.*, 1995; Rutkowski *et al.*, 1995; Van den Berg *et al.*, 1995; Casella *et al.*, 1996; Hanemann *et al.*, 1997) and these human Schwann cells are also able to promote axonal regeneration (Levi *et al.*, 1994; Guest *et al.*, 1997). However, the task set to those working in that field is to analyze whether 'peripheralizing' the CNS induces changes in the physiological properties of the host tissue.

5.3.4. Astroblasts

In an attempt to avoid potential drawbacks by implanting foreign cells to the CNS and to promote axonal regrowth, several studies have explored the effects of another cellular transplant with growth-promoting capacities, the astrocytes. From studies of Smith and coworkers (Smith *et al.*, 1986, 1990; Smith and Miller, 1991; Smith and Silver, 1996) it becomes apparent that age and differentiation stage of astrocytes play an important role in their capacity to promote regeneration. Thus, in contrast to their mature counterparts immature astrocytes, called astroblasts, have been shown to exert multiple beneficial effects on the regeneration process. Astroblasts, implanted before the age of postnatal day 14, produce high concentrations of neurotrophic and neurite outgrowth promoting factors (Nieto-Sampedro *et al.*, 1982; Martin, 1992), suppress scar formation (Smith *et al.*, 1986; Wang *et al.*, 1995a; Smith and Silver, 1996), are highly plastic and motile (Goldberg and Bernstein, 1988; Bernstein

Fig. 4. Regeneration of transected postcommissural fornix after microinjection of a Schwann cell suspension into the lesion site. (A) Cultured Schwann cells exhibiting immunoreactivity for low affinity NGF receptor. (B) Distribution of prelabeled (Hoechst dye) Schwann cells microinjected into the transected postcommissural fornix. A characteristic gradient of Schwann cells is formed in a pattern radiating from the injection site. (C) At 6 weeks after transection and Schwann cell injection lesioned fornix fibers cross the former lesion and injection site and elongate within their normal pathway up to their target, the mammillary body (MB). (D) High-magnification photomicrograph shows the fibers overriding the former lesion and injection site. Arrows indicate the location of lesion/injection site. d: Distal, p: proximal. Scale bars 10 μ m (A), 100 μ m (B–D).

and Goldberg, 1989, 1991; Smith and Miller, 1991; Smith and Silver, 1996) and express a distinct form of protease (Kalderson *et al.*, 1990a). Importantly, cultured astroblasts, introduced into lesion sites either as part of embryonic tissue, coupled to prosthesis and gel matrix vehicles or as a pure cell suspension, promote the regrowth of certain axons across the corpus callosum midline (Smith *et al.*, 1986; Smith and Silver, 1996), enhance regrowth of axons across lesions in the corticospinal tract (Schreyer and Jones, 1987) and promote regeneration of dorsal root fibers (Kliot *et al.*, 1990) and of optic nerve fibers (Sievers *et al.*, 1995).

We assessed the influence of microimplanted cultured astroblasts on the growth behavior of lesioned axons in our lesion model the transected postcommissural fornix (Wunderlich *et al.*, 1994) (Fig. 5). In contrast to implanted Schwann cell suspensions [Fig. 4(B)], microinjected astroblasts formed an elliptical, well-integrated bolus of nonmigrating cells [Fig. 5(B)]. However, similar to the Schwann cell effects, they did neither prevent nor change the spatiotemporal pattern of degeneration in the proxi-

mal and distal fornix stumps, but increased the number of spontaneously sprouting axons of transected fornix. Quantitative analysis revealed a significant increase in myelinated axons of up to 40% compared to animals without implant. Regrowing axons neither entered nor bypassed the astrocytic implant, but grew along its surface and failed to elongate into the distal stump [Fig. 5(C)]. Thus, structural regeneration of the tract was prevented by the inability of regrowing fibers to cross the lesion/implantation site. While our studies confirmed the growth stimulating effects of implanted astroblasts, they argue against a scar reducing effect of astroblasts and underline the key role of the lesion scar in regeneration failure of adult mammalian CNS.

In considering using astroblasts to promote axonal regeneration in traumatic CNS lesions, the requirement for embryonic primary cells is a serious ethical and technical problem. Continuous cell lines have been proposed as an alternative to fetal tissue. Therefore, a panel of astrocyte cell lines with growth-permissive or nonpermissive properties were recently established, using retrovirus-mediated trans-

Fig. 5. Influence of microinjected astroblasts on axonal regeneration in transected postcommissural fornix. (A) Cultured immature astrocytes were prelabeled with fluorescent Dye I and (B) microinjected into the lesion site immediately after transection of the tract. (C) Anterogradely HRP-WGA labeling of the tract revealed that in animals with astroblast implant: (i) the number of spontaneously sprouting axons significantly increases; but (ii) the axons fail to enter or grow around the implant. Scale bars 50 μ m (A), 100 μ m (B and C).

fer of the gene for SV40 large T antigen (Isono *et al.*, 1992; Groves *et al.*, 1993; Fok-Seang *et al.*, 1995). These cell lines express several proteins characteristic for astrocytes like fibronectin, laminin, N-CAM, TEN and various proteoglycans and retain their *in vitro* immunocytochemical properties after transplantation. Despite the potential disadvantage of tumor formation by immortalized cells, it might be advantageous to use the astrocyte lines to stimulate axon growth.

5.3.5. Olfactory Bulb Ensheathing Cells

The olfactory bulb contains a special type of macroglia, the ensheathing glia, which appears to be responsible for the regenerative ability of olfactory axons (Doucette, 1990). Since ensheathing glia cells secrete NGF and express a variety of molecules which are known promoters of neurite initiation, attachment and growth (Ramon-Cueto and Nieto-Sampedro, 1992; Ramon-Cueto *et al.*, 1993), they are suggested to perform simultaneously the roles of

both astrocytes and Schwann cells (Doucette and Devon, 1993). While combining the favorable characteristics of the latter cells, the ensheathing cells are promising candidates for implantation into areas of CNS injury.

In fact recent implantation studies have confirmed the growth-promoting function of the ensheathing cells *in vivo*. They support the regeneration of:

1. dorsal root axons when placed inside semipermeable polymer tubes (Lustgarten *et al.*, 1991) or implanted into the dorsal root entry zone (Ramon-Cueto and Nieto-Sampedro, 1994);
2. cholinergic axons from the medial septal nucleus when implanted as ensheathing cell/collagen matrix following ablation of the fimbria fornix (Smale *et al.*, 1996); and
3. corticospinal axons when injected into the lesion site (Li *et al.*, 1997).

Another step towards clinical application is the recent establishment of a clonal olfactory bulb-ensheathing cell line (Goodman *et al.*, 1993), which has been shown to produce peripheral-type myelin sheaths around demyelinated spinal cord axons (Franklin *et al.*, 1996).

5.3.6. Microglial Cells

Microglial cells have long been known to play a key role in the regeneration process of CNS. However, the nature of their involvement in the regeneration is still a subject of controversy. Brain microglia are highly complex cells exhibiting multiple morphological forms and functional specializations [for reviews see Streit *et al.* (1988) and Thomas (1992)]. In addition to its immune function [for review see Perry *et al.* (1993)], microglia:

1. release neurotoxic substances (Giulian, 1993; Giulian *et al.*, 1993), which might propagate further destruction of neurons, but also
2. remove the cellular debris from an area of degeneration (Giulian *et al.*, 1989; Stoll *et al.*, 1989) and secrete growth factors (Nagata *et al.*, 1993), like NGF (Mallat *et al.*, 1989), thrombospondin (Rabchevsky and Streit, 1997) and laminin (Rabchevsky and Streit, 1997), functions which suggest a beneficial role for these cells in regeneration.

According to these conflicting actions, the results of experimental microglia treatments are contradictory. While suppression of the microglial metabolic activity enhances axonal regeneration (Thanos *et al.*, 1993), it has also been reported that addition of microglia or macrophages to CNS lesions (David *et al.*, 1990; Lazarov-Spiegler *et al.*, 1996; Rabchevsky and Streit, 1997; Prewitt *et al.*, 1997; Frauzeu *et al.*, 1998) overcomes CNS regeneration failure. In line with the latter data is the hypothesis; David *et al.*, 1990 that the lack of a robust microglia/macrophage response in the lesioned adult mammalian CNS may account for the lack of significant axonal regeneration (Avellino *et al.*, 1995). However, it will be of crucial relevance to perform additional experiments to define the role of microglial cells and to develop the microglia transplantation approach to a novel therapy for CNS injuries.

5.4. Neutralization of Neurite Growth Inhibitors

A tremendous effort has been made to identify neurite growth inhibiting molecules (see Section 3.2). Currently the best characterized cells mediating inhibitory signals in axonal growth are oligodendrocytes. In a series of experiments it has been shown that the growth inhibitory nature of oligodendrocytes and myelin can at least be attributed to the myelin proteins NI-35 and NI-250 (NI, neurite inhibitor) as well as the myelin-associated glycoprotein (MAG) (Berry, 1982; Schwab and Thoenen, 1985; Schwab and Caroni, 1988; Bandtlow *et al.*, 1990; Bastmeyer *et al.*, 1991; McKerracher *et al.*, 1994; Mukhopadhyay *et al.*, 1994; Li *et al.*, 1996). Based on this *in vitro* data it was hypothesized that the myelin molecules NI35/250 become exposed during the injury response and abort regeneration. Following, different attempts were started to reduce or neutralize these proteins. In one series of experiments myelin-free spinal cord has been produced in young rats by repeated X-irradiation (Savio and Schwab, 1990) or immunocytolysis (Keirstead *et al.*, 1992). These treatments resulted in substantial elongation of regrowing corticospinal and brainstem descending projections. The studies further suggest that myelin proteins represent a major nonpermissive component of CNS tissue and may serve as 'border markers' that restrict the growth of neurites to appropriate regions and layers in the CNS and may also exert an inhibitory role in the context of regenerative neurite growth. In a second series of experiments antibodies against the myelin proteins NI-35/-250 were raised to neutralize the growth inhibitory activity of these proteins. The continuous application of these antibodies by implantation of living mouse antibody-secreting hybridomas either on top of a lesion or into the lateral ventricles restores elongation of some corticospinal, optic nerve and septo-hippocampal sprouts for considerable distances distal to the lesion site (Schnell and Schwab, 1990; Cadelli and Schwab, 1991; Schnell and Schwab, 1993; Weibel *et al.*, 1994). Subsequent work has shown that coadministration of NT-3 enhances sprouting of lesioned fibers (Schnell *et al.*, 1994) which correlates with significant recovery of certain motor functions (Bregman *et al.*, 1995). In support of the growth-inhibitory role of myelin in the regeneration process are also recent observations of Cheng *et al.* (1996) and Tuszyński *et al.* (1996) in grafted spinal cord, where regenerating fibers extend only through gray matter and avoid to enter the white matter. Moreover, Eitan *et al.* (1994) showed that a single injection of the enzyme transglutaminase which triggers the apoptotic death of oligodendrocytes induced structural and partial functional recovery of transected optic nerve. Nonetheless, it has to be considered that in animals treated with antibodies against NI 35/250 the number of regenerating fibers is rather small and these fibers never succeeded in crossing through the lesion scar. Thus, the NI-glycoproteins seem to be important myelin components implicated in defining territories for axonal projections and hence are important target molecules for modulation once the fibers have passed the lesion site. However, these inhibitory molecules

do not arrest axon growth at the lesion site, one major hurdle for axonal regeneration, and therefore, neutralization of these molecules may not be the prime target for strategies initiating axon regeneration at the lesion site.

Another component of myelin that has received much attention is MAG. While studies in MAG-gene deficient mice do not support a role of MAG in regeneration failure (Bartsch, 1996), the physiological relevance of MAG remains to be determined by inhibition experiments *in vivo*.

5.5. Reduction of Scarring

Central nervous system destruction by injury leads to the formation of scars as in other organs of the body. Normal mature scarring is characterized by the deposition of a basement membrane formed by two independent networks (the collagen IV- and the laminin-network) and by astrocytosis and the permanent presence of mesodermal components like fibroblasts and macrophages (Berry *et al.*, 1983). The relationship of the lesion scar and the failure of the mammalian CNS to regenerate has a long and controversial history. Since until recently it was unclear what aspects of this glial-mesodermal cicatrix comprises the barrier, a variety of manipulations have been tried to reduce scar tissue. Attempts include excision or reduction of the lesion scar, bridging the scar by cellular or noncellular grafts (see Section 5.3) and modification of either the gliotic or the basal membrane component of the scar.

Historically, the major approach to reduce the CNS scar has been the application of pharmacological agents. The first of these, a bacterial pyrogen, piromen, was utilized to reduce or alter the nature of cicatrix formation in transected spinal cord (Windle *et al.*, 1951; Clemente, 1955). In many instances, treatment with these agents significantly altered scar formation and increased sprouting of new axons but the beneficial effects were temporary. Other classical approaches include injections of enzymes, like collagenase, trypsin or elastase to permeabilize the basal membrane of the scar (Freeman *et al.*, 1960; Puchala and Windle, 1977; Guth *et al.*, 1980) that mostly failed to significantly improve the conditions for neurite growth. One reason for this failure might be the unavoidable, additional attack of vascular basal membranes in the surrounding of the lesion, that might lead to severe changes in vascular supply to the damaged region.

More recent attempts to reduce scar formation are the use of X-irradiation and the application of glucocorticoids or antibodies to transforming growth factor β (TGF β). X-Irradiation is an elegant, while noninvasive, approach to reduce the gliotic component of the scar by inhibition of cell proliferation and to prevent tissue degeneration around the lesion site (Kalderon *et al.*, 1990b). This method has been successfully applied in transected spinal cord lesion and resulted in restitution of severed corticospinal axons (Kalderon and Fuks, 1996a) and recovery of control of hindlimb muscle (Kalderon and Fuks, 1996b). However, the regenerating axons did neither follow their previous pathway, that is important for reestablishment of topographic re-

Fig. 6. Basal membrane deposition and axonal growth arrest. (A) At *ca* 2 weeks posttransection fornix axons stop at the former lesion site. (B) Adjacent section showing that at this time the lesion site is filled by an collagen IV immunoreactive basal membrane, which is oriented perpendicular to the trajectory of the tract and forms a continuous structure. Injections of anti-Coll IV antibodies (C) or dipyrindyl (D) immediately after lesion prevent the formation of the basal membrane in the lesion center. Arrows indicate lesion/injection site. d; Distal, p; proximal. Scale bars 100 μ m.

relationships, nor did they restore topographic and coordinated synaptic reinnervation of the target. While oligodendrocytes are also reduced within this glial-deficient environment and growth-promoting Schwann cells immigrate from the PNS this experimental approach does not provide unequivocal evidence that the scar it is the sole factor limiting axonal growth after injury.

Injections of glucocorticoids (Li and David, 1996) or antibodies against TGF β (Logan *et al.*, 1994) affected the fibroblastic part of the scar but so far no analysis of axonal regeneration across the modified scar has been provided.

The overwhelming data on axonal growth stop at the lesion scar and the fact that the identity of the inhibitory scar constituent is still unclear prompted us to analyze and influence this axonal-scar relationship in a well-defined lesion model, the postcommisural fornix of the adult rat (Stichel *et al.*, 1998) (Figs 6 and 7). Transected and retracted postcommisural fornix fibers spontaneously sprout up to the lesion site, where they stop abruptly [Fig. 3(B)Fig. 6(A)]. The spatio-temporal coinci-

dence of this axonal arrest with the formation of a basal membrane in the lesion site [Fig. 6(B)] strongly suggests that the basal membrane is the inhibitory constituent of the impermeable scar. In an effort to reduce postlesion basal membrane deposition we injected locally either an antiserum to collagen IV or an iron-chelator, dipyrindyl, that inhibits the enzyme prolylhydroxylase and thereby prevents formation of collagen fibrils. And in fact, we succeeded in reducing the basal membrane formation of the scar [Fig. 6(C and D)Fig. 7(A)] and this reduction was sufficient for elongation of large numbers of fibers across the lesion site [Fig. 7(B and C)]. The regenerating fibers reinnervated their normal target [Fig. 7(E and F)], were remyelinated [Fig. 7(D)] and regained normal conduction properties. Our study provided unequivocal evidence that:

1. the lesion scar and in particular the basal membrane impedes axonal regeneration in the adult mammalian CNS; and
2. CNS axons have an innate potential for regeneration and self-organization.

Fig. 7. Reduction of basal membrane formation promotes axonal elongation of transected postcommissural fornix axons across the lesion site. Animals with immediate local injection of dipyrindyl into the transection site show reduced formation of a basal membrane in the lesion center (A) and successful regeneration across the lesion site (B). (C) High-magnification photomicrograph of fibers crossing the lesion/injection site shown in (B). Anterogradely labeling with WGA-HRP revealed that regenerating axons are remyelinated (D) and reinnervate their normal target (E and F). Filled arrows indicate the position of the lesion/injection site, open arrows mark the WGA-HRP reaction product. Scale bars 100 μm (A-C), 0.1 μm (D), 0.5 μm (E and F).

However, our experiments cannot determine the mechanism underlying this growth arrest mediated by the basal membrane. In addition to a possible mechanical barrier effect, several components with repulsive or inhibitory effects might be cross-linked to collagen and indirectly reduced by the alteration in collagen network formation. Now one of the key challenges to proceed this experimental strategy to clinical application is to analyze whether lesion scars in other brain regions or in the spinal cord can also be modified by the described treatment and whether this modification is sufficient also for other fiber tracts to overcome the major extrinsic constraint to axonal regeneration, the lesion scar.

5.6. Electric Fields

Nervous system tissue, both *in vivo* and *in vitro*, respond to an applied d.c. electric field. *In vitro*, weak electric fields stimulate neurite sprouting (Hinkle *et al.*, 1981), induce growth cone turning (Hinkle *et al.*, 1981; Patel and Poo, 1982; Erskine *et al.*, 1995), enhance nerve growth rates (McCaig, 1990a; Erskine *et al.*, 1995), enhance and direct neurite branching (McCaig, 1990b; Erskine *et al.*, 1995) and alter the distribution of growth cone filopodia (McCaig, 1986, 1987). *In vivo* responses to an electric field include increases in rate of neurite growth, orientation of neurites towards the negative pole of an electric field, increases in the amount of branching of axons and a decrease in axonal dieback (Borgens, 1982; Roederer *et al.*, 1983). However, the cellular mechanisms underlying these pronounced effects are completely unknown and only a few speculations were made, which are, a reduction of retrograde axonal degeneration by reducing the injury current-mediated dieback (Borgens *et al.*, 1979; Borgens, 1982); electrophoresis of membrane components (Poo and Robinson, 1977; Orida and Poo, 1978), that may be critical to axonal growth; electrophoresis of intra-axonal components (Borgens, 1982) and perhaps effects on the glial architecture of the scar (Borgens *et al.*, 1994).

While most studies have been performed in a variety of nonmammalian vertebrates (Marsh and Beams, 1946; Borgens *et al.*, 1979; Jaffe and Poo, 1979; Roederer *et al.*, 1983), applied electrical fields have also proved effective in promoting neuronal regeneration in adult mammalian CNS. Studies of Borgens *et al.* (1987, 1990) in the adult guinea pig and those of Fehlings and coworkers in the adult rat (Fehlings *et al.*, 1988; Fehlings and Tator, 1992) provide solid support that an electric field applied across hemisectioned spinal cord leads to some functional recovery. However, electric field-treated spinal cords contained only some axons that regenerated across the plane of transection, while most axons passed around the lesion site and elongated within the outer rim of the spinal cord which typically survives impact (Borgens and Bohnert, 1997). It seems necessary to examine the possibility of synergistic effects between fields and glial implants or pharmacological treatment, to increase the intensity of the regenerative response.

5.7. Combined Strategies

On the basis of the studies discussed in Sections 5.1, 5.2, 5.3, 5.4, 5.5 and 5.6 it is apparent that, except for some very rare cases (Stichel *et al.*, 1997), the application of a single intervention strategy is insufficient to ensure structural regeneration and recovery of function of a lesioned CNS fiber tract. Studies in the spinal cord have shown that even under conditions of little scarring and an acceptable growth environment, substantial growth of some of the long tracts does not occur (Tuszynski *et al.*, 1994; Xu *et al.*, 1995a; Nakahara *et al.*, 1996; Tuszynski *et al.*, 1996). Particular populations of neurons have specific requirements for regenerative growth. At least in the spinal cord it seems that a cocktail of strategies is required for anatomic reorganization.

In order to increase the capacity for axonal growth one type of approach is to apply NTFs in combination with growth promoting cells. Thus, administration of neurotrophic factors together with growth-promoting cells, like Schwann cells or PN graft (Varon and Conner, 1994; Xu *et al.*, 1995a,b; Cheng *et al.*, 1996; Guest *et al.*, 1997) improved the regenerative growth of long descending pathways compared to single growth-promoting approaches. Recently, Meuei and coworkers (1998) implanted Schwann cells genetically modified to express BDNF, which resulted in an enhanced regeneration of brainstem neurons. In a second type of combined approach, NTFs were applied together with antibodies to growth-inhibitory molecules to alter the environment of the lesion site (Schnell *et al.*, 1994), which enhanced the regenerative sprouting of the corticospinal tract. These studies and those of Tuszynski and coworkers (Tuszynski *et al.*, 1994; Nakahara *et al.*, 1996; Tuszynski *et al.*, 1996; Grill *et al.*, 1997) in spinal cord clearly indicate that the influence of the neurotrophins on mature injured CNS neurons is not uniform. Rather, particular populations of neurons appear to respond preferentially to particular members of the neurotrophin family (Table 2). The identification of optimal combinations of NTF(s) and growth substrates may be a useful strategy to improve axonal regeneration in adult CNS.

Another combination approach is the simultaneous application of antibodies to growth-inhibitory molecules and the implantation of a fetal graft (Schnell *et al.*, 1994) or Schwann cells (Guest *et al.*, 1997) into the lesion site. The fetal graft + IN1 combination attempted to guide the elongating axons across the lesion site, but it failed since regrowing fibers always crossed the lesion site through remaining tissue bridges. The other combination (Schwann cells + IN1) also failed to support corticospinal tract regeneration. With regard to this issue one might assume that the combination of the scar reducing approach using anti-collagen IV antibodies or dipyrindyl with the application of NTFs and/or antibodies against NI35/250 may be an exciting and promising 'cocktail approach' to restore functional deficits in the lesioned spinal cord.

6. CLINICAL PERSPECTIVES

The many basic science studies reviewed suggest that restoration of physiological function after trau-

matic CNS injury is feasible. However, as a therapeutic strategy in human disorders and injuries any approach to stimulate axonal regrowth is still in its infancy. While large clinical trials to evaluate pharmacological treatments of secondary damage following spinal cord trauma are performed since 1975 (NASCIS, national acute spinal cord studies) (Bracken *et al.*, 1984, 1990, 1997), the approaches focusing on axonal regrowth still remain on a purely experimental animal level at present. Extrapolation of the experimental data to the human situation will have to confront the issues of larger and more complex lesions, longer pathway distances and the side-effects of the various growth-stimulating procedures. On the other hand, it has been shown that as little as 5–10% of a particular pathway can often sustain recovery of function (Fehlings and Tator, 1995), implying that even modest axonal growth may have sufficient functional impact. There is much cause for optimism and it is now a matter of time and planning and designing new experiments to create the most effective 'cocktail(s)' for CNS regeneration.

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