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Age-specific changes in regeneration potential in CNS: molecular analysis on animal model of traumatic spinal cord injury

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To my family

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1. INTRODUCTION

1.1 THE AGING CNS

Aging of brain and spinal cord is not well understood. It remains mystery, how neurons can stay functional for more than 100 years. Aging affects most prominently the speed of information processing, which gradually declines throughout the adult life span (Craik et al., 1994). Age-related memory changes are attributed to reduced activation of the prefrontal cortex as well as to reduction in white matter and in synapse density which is to some extent compensated by activating larger cortical areas and even contralateral hemisphere (Hedden et al., 2004; Bartzokis et al., 2003; Liu et al., 1996; Bourgeois and Rakic, 1996). Other cognitive aspects are age-stable such as attention span or some get even better such as emotional components of memory (Cartensen et al., 2011).

There is no significant loss of neurons in most regions of the aging neocortex determined by the up-to-date stereological methods of neuronal quantification, in contrast to earlier reports suffering from technical limitations (Burke and Barnes, 2006). Similarly, there is no significant loss of dendritic branching in the aging hippocampus, moreover it can be even increased in some hippocampal regions in aged individuals (Buell and Coleman, 1979). Counting of spinal motoneuronsn of aged individuals and animals has revealed small losses (10-20%), even when clinical symptoms of hindlimb motor incapacities were apparent (Johnson et al., 1995; Kawamura et al., 1977; Tomlinson et al., 1977; Hashizume et al., 1988; Xie et al., 2000). Off note, motoneurons of the 30-months-old rats had lost half of their bouton coverage, surrounded by glial fibrillary acidic protein (GFAP)-positive processes and showed increased alpha-calcitonin gene related peptide (CGRP) and detectable growth-associated protein-43 (GAP-43) immunoreactivity (Johnson et al., 1995). This might indicate that normal aging is accompanied with damage to neuron integrity eliciting responses similar to axon severance in adults. In contrast, a recent study found increased numbers of neurons in the cervical spinal cord of aged female rats indicating that neurogenesis persists into old age (Portiansky et al., 2011). Both the total area occupied by neurons and the number of neurons increased significantly with age, the latter increase ranging from 16% (cervical segment C6) to 34% (cervical segment C2). Taking the total number of cervical neurons the age-related increase ranged from 19% (C6) to 51% (C3), C3 being the segment that grew most in length in the aged animals. The ratio gray matter area to whole area did not change significantly between the 30-month-old and 5-month-old rats. Such increase indicates that pre-existing neuroblasts and/or possible neurogenesis might occur during the entire life span as proliferating neuronal cells were identified.

Gene expression microarrays provide a powerful technology for investigating brain aging as expression of thousands of genes can be monitored in parallel (Schena et al., 1996; Lockhart and Barlow, 2001; Lee et al., 2000; Jiang et al., 2001). It appears that aging changes the expression of ca 4% of genes across different species (Yankner et al., 2008; Lu et al., 2004). There is a robust age-associated induction of stress response genes such as antioxidant defense, DNA repair, and immune function. It is possible that the wide-spread neuroinflammatory response in brain aging is triggered by mitochondrial dysfunction (Gemma et al., 2002). Aging mitochondria produce reactive oxygen species (ROS) which may mediate oxidative damage of DNA. In young adult brain, DNA damage is repaired efficiently, whereas it persists in the aged brain. It is proposed that during normal aging, neurons likely survive in the presence of unrepaired DNA damage by silencing damaged areas via transcriptional repression (Yankner et al., 2008). In fact, there are indications that oxidative DNA damage accumulates in the promotors of a subset of age-downregulated genes associated with synaptic function, protein transport, and mitochondrial function (Lu et al., 2004; Ohno et al., 2006). However, some uncertainty remains as to whether the damage can be random (Yankner et al., 2008). Indeed, aging downregulates genes involved in mitochondrial function, vesicle-mediated protein transport, synaptic plasticity, including glutamate receptor subunits, synaptic vesicle proteins, signal transduction systems that mediate long term potentiation (LTP), several calcium binding proteins such as calbindin 1, 2 and calmodulin (Geula et al., 2003; Lu et al., 2004; Fraser et al., 2005). Altered calcium homeostasis in the aged brain might contribute to altered synaptic plasticity and render neurons more vulnerable to a variety of toxic insults mediated by calcium, such as excitotoxicity.

In addition to mitochondrial/oxidative stress theory, another model of brain aging is proposed based on microarray results. Alterations in neuronal activity (Demerens et al., 1996) or metabolism (Kalman et al., 1997) might trigger chronic demyelination process, which then activates myelin and cholesterol synthesis and reults subsequently in neuroinflammation (Blalock et al., 2003). Interestingly, this study with the 4-, 14- and 24-month-old rats showed, that nearly all genomic alterations began before midlife (75% of the maximal change occurred between the young and mid-aged groups and the young and aged groups) (Blalock et al., 2003). Majority of the aging- and cognition-related genes which were upregulated had the maximal change between the young and aged groups – meaning these changes continued to advance between midlife and late life. Some of the aging-associated gene profiles can be reversed by caloric restriction, which also increases lifespan (Hyun et al., 2006; Lee et al., 2000).

*C. elegans and Drosphila**	*Rattus norvegicus*	*Mus musculus*	*Homo sapiens*
Whole organism (1)	Hippocampus (CA1) (7)	Neocortex and cerebellum (5)	Prefrontal cortex (9)
DNA repair proteins Peptidase Hydrolase Carbon-carbon lyase Mitochondrial membrane Molecular function: carriers Primary active transporters Ion transporters	Oxidative stress response Protein processing Inflammatory response Glial/structure Myelin-related proteins Metal ion homeostasis Growth/maintenance Signal transduction (Ca^{2+} related) Negative transcriptional regulation Mitochondrial and metabolism Synaptic/neurite plasticty Biosynthesis Signaling (extracellularly regulated) Extracellular matrix/structure Protein trafficking	Stress response genes Oxidative stress response Heat shock genes Proteases Inflammatory response Cathepsins Neural plasticity CNS development	Stress response genes DNA repair genes Inflammatory response Metal ion homeostasis Myelin-related proteins Synaptic function Vesicular transport Neuronal survival Mitochondrial function Amino acid modification Ca^{2+} homeostasis
*cross-species study		Cortex (6)	Prefrontal cortex (8)
C. elegans		Proteases Mitochondrial genes Stress response genes Neural plasticity Synaptic transmission ATPases	**Glial enriched** Immune response/cellular defense Growth factors Microtubule structure & function
Whole organism (2)			**Neuronal enriched** Synaptic transmission Ca^{2+} homeostasis Voltage-gated ion channels G protein–coupled receptors Microtubule structure and function Kinase-phosphatase
Stress response genes Insulin signaling pathway Tc3 transposons Heat shock genes (up then down)			
D. melanogaster			
Thorax and abdomen			
Stress response genes (3,4) Oxidative stress genes (3) Proteases (4) Mitochondrial genes (3,4) Metabolism (3,4) Reproduction (3)			

Table 1. Processes affected most by aging across species include upregulation of responses associated with stress (red) and downregulation of mitochondrial genes (blue), from Yankner et al., 2008: (1) McCarroll et al., 2004; (2) Lund et al., 2002; (3) Zou et al., 2000; (4) Pletcher et al., 2002; (5) Lee et al., 2000; (6) Jiang et al., 2001; (7) Blalock et al., 2003; (8) Erraji-Benchekroun et al., 2005; (9) Lu et al., 2004.

1.2. THE AGING SPINAL CORD

Spinal cord is a part of CNS which controls voluntary movements and posture and receives sensory information from limbs and trunk. It also controls viscera and circulation in thorax, abdomen, pelvis (The Spinal cord, 2009). Spinal cord lays protected inside the canal of vertebras surrounded with cerebrospinal fluid similarly to brain (Trepel, 2006). It is divided into segments according to the places in which spinal nerves leave the spinal cord: 8 cervical segments (C1-C8), thoracal (T1-T12), lumbar (L1-L5) and sacral (S1-S5), coccygeal cord (in humans rudimental, in rat Co1-Co6). The spinal cord of rat is 9 cm long, whereas individual segments of human spinal cord can be 1.5-1.6 cm in length. The longest is T6 with 2.2 cm (The Spinal Cord, 2009). Spinal canal gets smaller and vertebra bigger with age (Ishikawa et al., 2003; Tanaka, 1984). Transverse area of spinal cord increases from teenagers to adults and then decreases at older age in humans determined by MRI (Ishikawa et al., 2003), although in rats such decrease at older age is not observed (Portiansky et al., 2011).

Spinal cord is composed of gray matter and white matter. White matter consist of millions of descending and ascending axons, which bundle together to form axon tracts with similar function, and also of glial cells. Ascending fibers project to brain transducing sensory information such as touch and pain from periphery, whereas descending motor fibers project from brain to spinal cord to initiate movements and regulate posture. Propriospinal (PS) fibers connect spinal cord segments. The long PS fibers located to cervical spinal cord (C3-C5) descend in ventral and lateral funiculi to the dorsal horn in lumbosacral spinal cord (Alstermark et al., 1987a; Alstermark et al., 1987b) and coordinate limb movements (Jankowska et al., 1974; Miller et al., 1973). The short PS neurons connect higher cervical (C3-C4) segments with the lower cervical segments (C6-T1) and are involved in visual guided hand movements (grasping) (Alstermark et al., 1987c; Alstermark et al., 1990). Spinal cord gray matter contains mostly cell bodies of motoneurons and glial cells. Functions of the body including locomotion is coordinated by interaction of different axon tracts, most of important which are introduced below (Fig 1).

1.2.1. Corticospinal tract

The corticospinal tract (CST) is the biggest descending tract which innervates α-motorneurons in the gray matter. This tract originates from the pyramidal corticospinal neurons in layer V of the cerebral cortex. In rat the main component of the CST (70-90%) decussates at the spinomedullary junction to form the dorsal CST (dCST) which runs in the ventral-most part of the contralateral dorsal funiculus (Tracey, 1995). There are also decussated fibers that run in the contralateral dorsolateral white matter and form the dorsolateral CST (Brosamle and Schwab, 1997). The remaining fibers which do not decussate run through the ipsilateral ventromedial funiculus and form the ventral CST (Steward et al., 2004). dCST terminates mostly in the medial area of dorsal horn, in the intermediate gray matter (Rexed Laminae 3, 4, 5 and 6), and to lesser extent on the interneuron pools of the ventral horn (Elbert et al., 1999). In contrast to rodents, in primates the dCST is allmost absent and thr dorsolateral CST makes up the major fraction of fibers, a part of which is able to make direct synaptic contacts with motoneurons in humans. The primary function of CST is controlling of learned motor skills, such as taking of things (Whishaw et al., 1998), but also some aspects of locomotion and posture (Metz et al., 1998).

The rubrospinal tract (RST) originates from the nucleus ruber in the mesencephalon. Its decussated projections run in the far lateral regions of the dorsal spinal cord. The RST projects to gray matter interneurons which innervate α-motoneurons.

Aging does not decrease the numbers of CST neurons, and the proportion of myelinated fibers is higher in the older animals (80374.2 ± 6829.9) than in the younger intact animals (62949.7 ± 8419.7; $p < 0.001$) (Nielson et al., 2010; Leenen et al., 1989). Only a very small

fraction of total axons (11.50 ± 1.91; 0.014 ± 0.002% of total) showed some signs of degeneration in intact aged rats.

1.2.2. Noradrenergic and dopaminergic (catecholamineric) fiber tracts

The descending spinal catecholaminergic projections originate predominantly from the A11 dopamine-containing cell group (Skagerberg and Lindvall, 1985; Qu et al., 2006) and the A4-A7 noradrenergic cell groups in particular Locus coeruleus (Hynes and Rosenthal, 1999; Clark et al., 1993; Westlund et al., 1983) and from the C1-C3 groups of adrenaline-synthesizing neurons in the medulla (Minson et al., 1990). Dopaminergic fibers descend in the dorsolateral funiculus and near the central canal, the projections of coeruleospinal tract (CoST) run in the ventrolateral funiculus of white matter (Sluka and Westlund, 1992). Dopaminergic neurons synthesize from tyrosine dopamine, whereas the noradrenergic neurons are able to convert it further to noradrenaline.

It is well known that dopamine plays an important role in controlling locomotion. Data concerning the dopamine levels in the aged brain however, is quite conflicting (Dorce and Palermo-Nietto, 1994; Reimann et al., 1993; Emerich et al., 1993). Goicoechea et al. (1997) associated the reduced striatal dopamine levels with diminuation of general locomotion in the 25-months-old rats. Friedemann and Gerhardt (1992) found no age-related differences in the striatal dopamine levels, dopamine metabolite and turnover in the 6- and 30-months-old rats. Ponzio et al. (1982) measured decreased levels of dopamine and noradrenaline, but later it was shown that only the dorsal regions of the lumbar and sacral spinal cord have changed levels and not the ventral regions (Lovell et al., 2000; Ko et al., 1997). Ranson et al. (2003) reported of reduced levels of TH-like immunoreactivity in regions containing preganglionic neurons, which play an important role in controlling mictruration behaviours and sexual reflexes. A decrease of more than half of neurons in the Locus coeruleus in man between the age 10 and 100 has been reported, though this was most pronounced in the rostral part of the nucleus which does not project to the spinal cord (Manaye et al., 1995). No significant decline in the neuron numbers was observed between the ages of 12 and 32 months in rats (Goldman and Coleman, 1981).

1.2.3. Serotonergic fibers

Serotonergic axons in the spinal cord mainly arise form Raphe nuclei (Tork 1990; Skagerberg and Björklund, 1985). Neurons of *Raphe magnus* project in the dorsolateral funiculus and terminate mostly in the ventral horn (Mason, 1999), whereas neurons of *Raphe obscurus* and *Raphe pallidus* project in the ventrolateral part of white matter and terminate in the intermediate gray matter and motorneurons in the dorsal horn (Tracey et al., 2004). The serotonergic axons form synaptic connections with α-and γ-motorneurons as well as with the

preganglionic sympaththetic neurons in *Substantia gelatinosa* (Inman et al., 2003). Some few local serotonergic neurons are located around the central canal and dorsal horn (Yoshimura et al., 2006). The neurotransmitter serotonin (5-HT; 5-Hydroxytryptamin) plays a role in important motor circuits by facilitating the exitation of motoneurons; the raphespinal tract (RaST) executes neuromuscular reactions on motoric functions such as rhytmic movements (Gerin et al., 1995), but also regulates autonomic, reproductive and excretory functions (Ono and Fukuda, 1995; Mason, 1999; Deumens et al., 2005).

Aging may affect serotonergic system. Reports about the serotonin levels in the aged spinal cord are controversial, whereas consistently it has been reported that the turnover of serotonin is significantly higher in the spinal cord and brain of aged rats (Goicoechea et al., 1997; Rodriguez-Gomez et al., 1995; Venero et al., 1993; Johnson et al., 1993). In several studies, no age-related changes in 5-HT levels in the spinal cord of rats were found (Lovell et al., 2000; Johnson et al., 1993; Algeri et al., 1983; Ponzio et al., 1982). In contrast, Ko et al. (1997) as well as Goicoechea et al. (1997) reported that 5-HT levels in the spinal cord are reduced. In the latter study, this correlated well with impaired exploratory behaviour in the aged rats. Indeed, reduced serotonergic transmission is implicated in behavioural analysis using an agonist for 5-HT_2 receptors, expression of which is lower in aged animals (Morgan et al., 1987). In such a way reduced 5-HT transmission led to impaired motor performance in both young and aged animals, although for aged animals it took twice as long to recover (Freo et al., 1991). Johnson et al. (1993) reported that in the 30-month-old rats there were less 5-HT axons with a normal morphology in the ventral horn and in the dorsal horn of the lumbosacral spinal cord, whereas signs of degeneration were clearly less evident in the thoracic and cervical spinal cord segments. These changes in axon morphology varied between aged litter-mates and were apparent as disturbances of hindlimb function in about 40% of the aged rats. The serotonin transporter mRNA increases in aged rats suggesting that compensatory mechanism exist, as to capture more serotonin from the synaptic cleft (Meister et al., 1995). The attempts to quantify the 5-HT-containing neurons in Raphe nuclei at young and old age have led to different results. In humans no difference was found (Kloppel et al., 2001),whereas in rat the decline was 15%, however, it was not clear whether the spinally projecting cathecholaminergic neurons were affected (Agnati et al., 1985).

1.2.4. Calcitonin gene-related peptide (CGRP) fibers

CGRP-labeled sensory axons are local sensory axons with synapses in the spinal cord. These axons ascend for 1-2 segments. The CGRP-containing sensory fibers project to the lamina I, outer layer of lamina II and lamina V of the dorsal horn are probably transmitting pain, temperature and damaging and undamaging mechanical stimuli (Ondarza et al. 2003). CGRP is involved in trophic effects of muscle innervation in the peripheral nervous system

(PNS) e.g. increases muscle contraction, elevation of cyclic AMP (cAMP) level, supporting the synthesis of nicotine acetylcholine receptor, regulation of phosphorylation of these receptors and formation of neuromuscular connections (Zheng et al., 2008). In development, this neuropeptide is involved in sprouting of motor endplates and there is evidence that its expression is influenced by neuronal activity, which suggests its putative role in regenerative responses following injury (Wang et al., 1993).

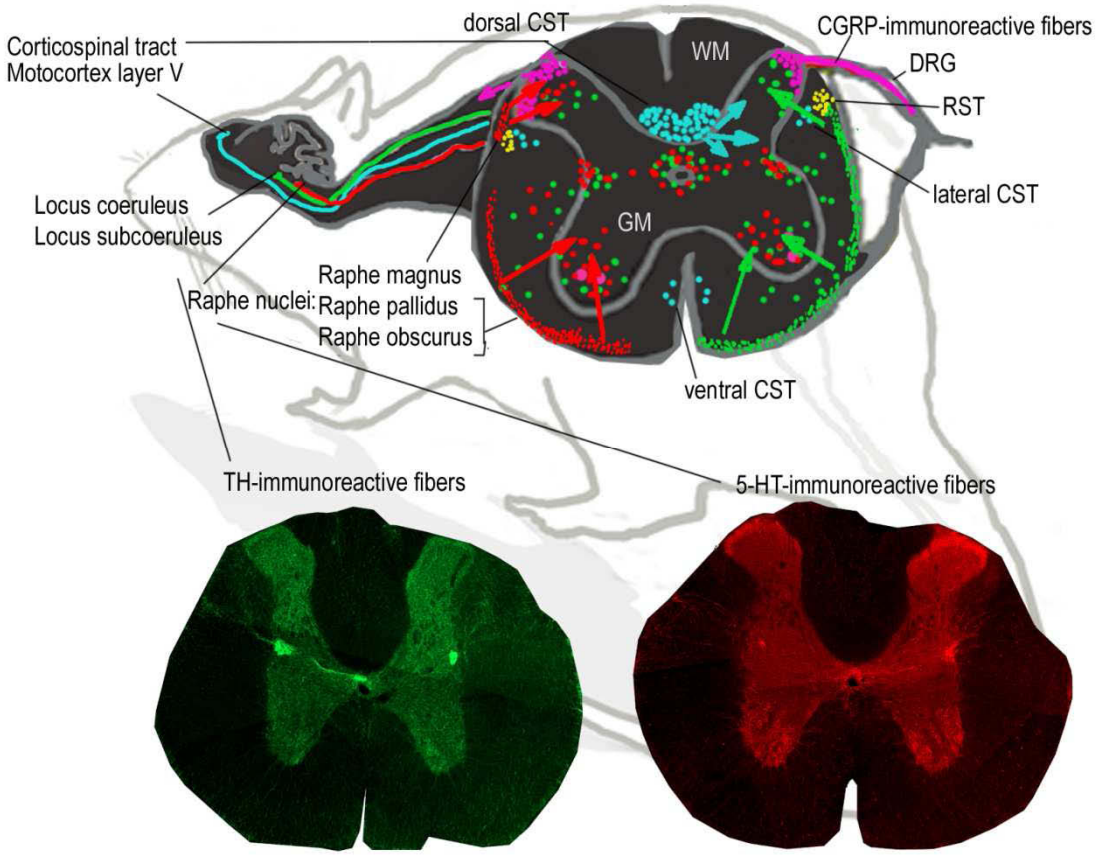

Fig 1. Schematic presentation of fiber tracts important for locomotion: corticospinal tract (CST, blue), rubrospinal tract (RST, yellow), serotonergic (5-HT, red), cathecholaminergic (TH, green) and CGRP axon projections (arrows). Schema and immunostaining images by A. J. based on Holstege and Kuypers, 1987 and The Spinal Cord, 2009). GM, gray matter, WM, white matter.

1.3. TRAUMA IN AGING CNS

1.3.1. SCI demographics

Spinal cord injury (SCI) disables motor, sensory and autonomic functions being devastating to both patients and people close to them. World-wide 2 million people are suffering from SCI (Afshari et al., 2009) and 10.4 to 59 new incidences per million inhabitants of countries across the world occur annually (Wyndaele and Wyndaele, 2006). The most notable tendency is growing incidence of SCI among older individuals, predominate etiology of which

is falls (Jackson et al., 2004; Furlan et al., 2008). The percentage of patients older than 60 years at the time of injury has increased from 4% to 11% since 2000 (NSCISC, 2011), especially in elderly females (Furlan et al., 2005; Krassioukov et al., 2003; Sekhon and Fehlings, 2001). The average age has increased from 28.7 years in the 1970's to the current age of 40.7 years (NSCISC, 2011) reflecting the reduced mortality rates (DeVivo, 2007; Jakob et al., 2009), increasing median age of the general population as well as the life-long activity of older generation.

Aging-associated physiological changes have been accepted as a major reason for increased susceptibility to acute injury and for slow recovery (Gershkoff et al., 1993). Analysis of large clinical database of SCI patients in Canada (n=485) revealed that mortality was higher among patients older than 65 years, but importantly, among survivors motor and sensory outcomes did not depend on age (Furlan et al., 2008). In the same study, analysis of post-mortem spinal cord tissue revealed no significant age-related differences for extent of myelin degeneration or number of intact axons within sensory, motor and autonomic spinal cord tracts post-SCI. The analysis of data gathered by European network of 17 SCI rehabilitation centers (237 traumatic SCI subjects) surprisingly showed that elderly patients had a better recovery of motor deficit. However, elderly patients had difficulties in translating an improvement in neurological outcome into functional changes (Jakob et al., 2009). The authors suggested that the unexpectedly good findings in elderly patients might be explained by the "ischemic preconditioning"-like mechanisms. Such transient ischemic attacks induce neuroprotective mechanisms on a cellular level resulting in smaller overall damage which is common in stroke patients (Moncayo et al., 2000). Since post-traumatic ischemia plays an important role as a secondary lesion mechanism after SCI (Tator and Fehlings, 1991) and is more prevalent among the older than younger SCI subjects, it might contribute to neuroprotection in older SCI patients.

1.3.2. SCI pathology

Immediately after injury, damage to blood vessels leads to bleeding and ischemia. Microglial cells get activated within minutes to hours. Immune cells start to infiltrate, first neutrophils followed by monocytes (Hawthorne and Popovich, 2011). Within weeks cystic cavitations and the lesion scar forms involving much larger damaged area than by the initial injury (Fitch et al., 1999). The secondary tissue damage comprises cell death and also demyelination of axons (Lin et al., 2007).

The distal axon stump will be degraded in the Wallerian degeneration process (von Meyenburg, 1998; Oudega et al., 1999), whereas the rostral part of the axon builds retraction bulb and retracts (die back). CST axons have reported to retract up to 2.5 mm from the site of injury (Bernstein and Stelzer, 1983; Oudega et la., 1999). The reactions to axotomy as

well as to a lesion scar are not homogenous in different axon tracts (Brazda and Müller, 2009). Compared to callosal neurons which become dystrophic and rapidly die back into white matter (similarly to CST axons), axotomized serotonergic fibers persist the penumbra of the lesion and sprout within regions containing many inflammatory cells, laminin and high levels of chondroitin sulfate proteoglycans (CSPGs). By *in vitro* imaging it was recently demonstrated that the growth cones of serotonergic neurons remain more active similarly to the growth cones of the embryonic dorsal root ganglion neurons (DRG) when interacting with inhibitory CSPG-rich substrates (Hawthorne et al., 2011). Despite of that, all attempts of injured axons of various tracts to regrow cease, because of the growth inhibitory surrounding and insufficient intrinsic growth capacities.

Nonetheless, axon death after transection is not so common as previously thought. Recent design-based counts of myelinated axons revealed no decrease in axon number in the medullary pyramid after SCI, regardless of injury level, severity, or time after injury – meaning that no obvious retrograde cell death takes place in dCST (Nielson et al., 2010). Injury, however, may reduce an age-dependent increase in the number of myelinated axons that would otherwise occur (Leenen et al., 1989) and affect ongoing myelination of axons during aging as the spinal cord-injured rats had fewer myelinated axons in the medullary pyramid at one year after injury than the aged matched controls (Nielson et al., 2010).

1.3.3. Lesion scar

At CNS injury site forms a lesion scar, which seals the damaged area. The lesion scar consits of a fibrotic scar inside the lesion site, which is rich of collagen type IV (Col IV), produced by different cells that infiltrate the lesion site: meningeal fibroblasts, astrocytes, endothelial cells (Klapka and Müller, 2006; Hermanns et al., 2000). The fibrotic scar is surrounded by the glial scar containing reactive astrocytes expressing GFAP (Grimpe and Silver 2002; Silver and Miller 2004; Fawcett and Asher, 1999). The major impediment for axon regrowth is dense fibrotic scar because it facilitates the accumulation of various axon growth inhibitory molecules such as the myelin-associated glycoprotein (Filbin, 2003), semaphorins (Pasterkamp et al., 1999; Pasterkamp and Verhaagen, 2001; Goldberg et al., 2004), ephrins (Miranda et al., 1999; Benson et al., 2005) and different CSPGs (Bartus et al., 2011; Morgenstern et al., 2002).

1.3.4. Effect of age on regeneration potential in peripheral nervous system (PNS)

In contrast to the limited regeneration attempts of injured axons in the CNS, peripheral axons show spontaneous regeneration after axotomy and therefore, much more information about age-specific changes in regeneration capacities in the PNS is available compared to the CNS.

The potential for regeneration in the PNS remains throughout life, although delay and decline in efficacy is often reported (Kerezoudi and Thomas, 1999). The age-related decline in nerve regeneration after injury may be attributed to changes in neuronal, axonal, Schwann cell and macrophage responses. Delay in initiation of regeneration is associated with reduced axonal transport as well as with slower clearance of myelin debris (Gutmann, 1942; Hofteig et al., 1981) and Wallerian degeneration (Choi et al., 1995) in older animals. Several myelin abnormalities in elderly subjects may be due to a decrease in the expression of the major myelin proteins (P0, PMP22, MBP). Axonal atrophy may be explained by a reduction in the expression and axonal transport of cytoskeletal proteins, decline in nerve conduction velocity, muscle strength, sensory discrimination, autonomic responses, and endoneurial blood flow. Interestingly, biophysical studies of cultured sensory neurons revealed that the speed of regeneration was reduced by 40% in adult compared to neonatal neurons (11.5 μm/hour vs 8.2 μm/hour). The slower regeneration speed was assigned to 3 times higher axonal stiffness (viscosity) of adult neurons determined by the force calibrated towing needles (Lamoureux et al., 2010).

Nonetheless, no difference was detected in the elongation rate of the regenerating sensory axons (Gutmann et al., 1942; Campbell and Pomeranz, 1993) or completedness of reinneravation (Navarro and Kennedy, 1988; Vaughan, 1992). Pestronk et al. (1980) found despite of reduced sprouting capacity in aged rats, that there is a population of faster-growing fibers which is the same at all ages.

Myelination by Schwann cells is not affected by age (Vaughan, 1992), but the formation of a 1:1 Schwann cell to axon relationship which precedes remyelination is delayed (Tanaka and Webster, 1991) and the amount of trophic and tropic factors secreted by reactive Schwann cells and target organs are lower in older subjects than they are in younger subjects. Interestingly, reduced regeneration capacity of aged nerves has been associated with reduced numbers of endoneurial macrophages in aging nerves, as suppression of macrophages led to significantly smaller numbers of the regeneration fibers in mice (Tanaka et al., 1992). Willcox and Scott (2004) demonstrated that the expression of growth-associated molecules [alpha(1)-tubulin, neurofilament NF-L] is reduced with age. At motor nerve terminals, the capacity to produce ultraterminal sprouting secondary to partial denervation is more pronounced in young animals (Pestronk et al., 1980), but not the capacity to eliminate terminal sprouts or reinnervate (Robbins and Nakashiro, 1993). Frequency and accuracy of reoccupation of the sites of motor nerve terminals are impaired – more often nerve terminals greatly overshoot the original border in old animals (Robbins and Nakashiro, 1993). Nerve transection is more likely to result in loss of the parent neurons following nerve transection in younger than in older animals (Romanes, 1946; La Velle and La Velle, 1984; Schmalbruch, 1984). Axon reaction (chromatolysis) is more intense and does

not return to normal as rapidly in old animals, although the degree of retrograde axonal atrophy is less, which suggests a diminished dependence on growth factor support.

1.3.5. Spinal cord injury in aged animals

There are limited numbers of experimental studies that have investigated how the age at the time of SCI affects the pathology and outcome (Table 1). Only few of these works have used rodents which can be qualified as aged, as an average life span of rats is around 24 months (Deerberg et al., 1980). Despite of unavailability of very aged animals for experimental research, particularly the studies with rats older than 18 months are of interest if parallels between elderly patients are to be drawn.

	Publication	**SCI model**	**Age**
1	Kumamaru et al. (2011) Age-related differences in cellular and molecular profiles of inflammatory responses after spinal cord injury. *J Cell Physiol.*	Contusion	1 and 2.5 months Mice
2	Siegenthaler et al. (2008) Myelin pathogenesis and functional deficits following SCI are age-associated. *Exp Neurol. 213:363-71.*	Contusion T10	2, 12 and 24 months female SD
3	Siegenthaler et al. (2008) Voluntary running attenuates age-related deficits following SCI. *Exp Neurol. 210:207-16.*	Contusion T10	2 and 12 months male SD
4	Genovese et al. (2006) Increased oxidative-related mechanisms in the spinal cord injury in old rats. *Neurosci Lett. 393:141-6.*	Clip compression T5–T8	3 and 18 months male SD
5	Chaovipoch et al. (2006) 17beta-estradiol is protective in spinal cord injury in post- and pre-menopausal rats. *J Neurotrauma. 23:830-52.*	Complete crush T8–9	2 and 12 months female SD
6	Gwak et al. (2004). Effect of age at time of spinal cord injury on behavioral outcomes in rat. *J Neurotrauma. 21:983-93.*	Unilateral hemisection T13	40 days 2 and 12 months male SD

Table 2. Studies of experimental SCI in aged animals. SD, Sprague-Dawley rats.

Experimental studies of SCI in aged rats observed an age-dependent decline in locomotor recovery (Gwak et al., 2004; Genovese et al., 2006; Siegenthaler et al., 2008) and increased inflammatory reactions (Genovese et al., 2006) as well as increased myelin pathology and reduced remyelination (Siegenthaler et al., 2008).

1.3.6. Genome-wide expression studies in SCI

Microarray experiments combined with advances in bioinformatic approaches to analyze the resulting datasets allow studying SCI pathology as well as the effects of therapeutic interventions in animal models in unbiased way. High-density oligonucleotide microarray platforms make it possible to measure transcript levels of virtually all genes on the rat genome simultaneously, compared to only some selected groups at the early days of array studies. It is thus possible to go further from hypothesis-driven research.

In the last decade several array works have been conducted to investigate the gene expression response in the CNS after brain injury (Salin and Chesselet, 1992; Abankwa et al., 2002; Bareyre et al., 2002; Raghavendra Rao et al., 2003; Küry et al., 2004; Israelsson et al., 2006). Only little is known about the transcriptional response in affected brain regions following SCI, because most of the studies have concentrated on the lesion site in the spinal cord and nearby tissue (De Biase et al., 2005). A study from our laboratory could show that SCI elicits massive changes in gene expression in the cortex, starting as early as 1 day post-operation (dpo); these responses increased over time peaking at 3 weeks post injury and decreasing slightly thereafter at 60 dpo (Kruse et al., 2008). Moreover, upon treatment with AST, the dCST transected young animals showed significant changes in the expression pattern of regulated genes which are growth-associated and neuroprotective (Kruse et al., 2008; Kruse, in press). The differences in transcriptomes of intact, axotomixed and regenerating neuronal systems represent the corresponding state of neurons.

Transcriptomic profiles of SCI in aged animals are unknown. In stroke, distinct gene expression profiles in aged and young animals have been reported with growth-inhibitory molecules induced earlier and growth-promoting factors with delay in aged peri-infarct cortex (Carmichael et al., 2003). Moreover, genome-wide expression analysis of aged and young animals revealed that different transcriptomes were responsible for stroke-induced sprouting of cortical neurons (Li et al., 2010). Nonetheless, selected genes representing the regenerative response were similarly induced in both 3-and 20-month-old rats after stroke indicating that the potential for regenerative response of brain still remained at older age (Petcu et al., 2008).

1.4. LESION MODEL AND TREATMENT OF SPINAL CORD INJURY

1.4.1. Experimental strategies to suppress collagenous lesion scar

Only in recent years, treatments have been developed which are able to induce regeneration in mature CNS and, therefore, may provide a basis to investigate the regeneration potential in the aging CNS. The fibrotic lesion scar forming after CNS injury is a major impediment for axonal regeneration (Stichel and Müller, 1998; Hermanns and Müller, 2001; Klapka and Müller, 2006; Brazda and Müller, 2009). The attempts to suppress the Col IV-containing lesion scar by enzymatic digestion led to extensive bleeding as the basal membrane surrounding blood vessels was also degradated (Feringa et al., 1979; Guth et al., 1980). For this reason, an innovative pharmacological approach was established in the Molecular Neurobiology Laboratory of the University of Düsseldorf which suppresses the formation of this regeneration barrier via targeted inhibition of *de novo* biosynthesis of collagen by an iron chelator (2,2'-bipyridine-5,5'-dicarboxylic acid; BPY-DCA) (Stichel et al., 1999).

The iron chelator is required for scavenging Fe^{2+}, a co-factor for the prolyl 4-hydroxylase, which is a key enzyme in collagen synthesis. Resulting iron-deprivation transiently inhibits the formation of the stable triple collagen helices. This scar-suppressing strategy (anti-scarring treatment; AST) was at first successfully implemented by a postcommissural fornix transection as a brain lesion model in rat (Stichel et al., 1999) and has been independently reproduced by Kawano et al. (2005), who showed that loss of spontaneous regeneration capacity of nigrostriatal dopaminergic axons in early postnatal development can be effectively counteracted by prevention of collagen biosynthesis and scar formation.

AST was successfully applied also by the lesion model in the spinal cord in a modified form (Hermanns et al., 2001; Klapka et al., 2005). In addition to local injections of the iron chelator, AST in these studies included an application of 8-bromo-cyclic AMP (cAMP), further suppressing the scar formation by inhibiting the prolifearation of fibroblasts (Berry et al., 1983) and astrocytes (Liesi and Kauppila, 2002) which are the major producers of Col IV. Both cell types express connective tissue growth factor (CTGF) which is involved in proliferation and production of extracellular matrix (ECM). Via increasing the intracellular levels of cyclic AMP (cAMP), CTGF-mediated effect would be decreased resulting in reduced proliferation and production of ECM (Duncan et al., 1999).

The dorsal hemisection model at T8 includes transection of dorsal CST, lateral RST and dorsal columns, as well as parts of the RaST and CoST with a Scouten wire knife. The CST-transection leads to transient functional deficits in rats, whereas permanent functional deficits are observed when the RST is transected simultaneously together with CST, indicating the compensatory ability of RST (Kennedy, 1990).

Suppression of scar formation by AST has been shown to promote long-distance regeneration of cut CST axons, neuroprotection of projecting cortical neurons and functional

improvement of treated young adult rats after SCI (Klapka et al., 2005). In addition, significantly increased density of descending CST, serotonergic raphespinal, catecholaminergic coerulospinal tracts and increased denstity of sensory CGRP-immunoreactive fibers into the lesion zone at 5 weeks, and further enhanced the number of the descending axons into and beyond the lesion zone at 12 weeks post-injury upon AST has been demonstrated (Schiwy et al., 2009).

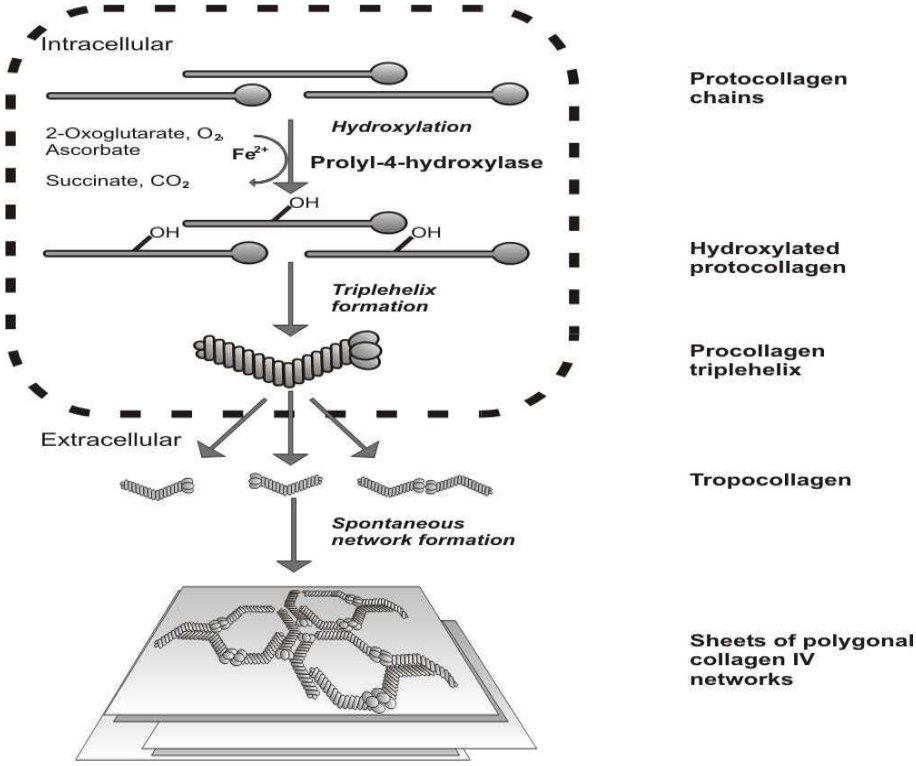

Fig 2. Schematic presentation of collagen biosynthesis.

1.4.2. Experimental strategies to increase axonal sprouting

In recent years axonal sprouting has been recognized as an important contributor to rapid spontaneous locomotor recovery after acute SCI via reorganization of connections (Bradbury and McMahon, 2006; Maier and Schwab, 2006; Fouad and Tse, 2008). New intraspinal circuits include contacts between sprouting axons and the long PS neurons that bridge the lesion and arborize on lumbar motoneurons (Bareyre et al., 2004). Several approaches have been shown to increase axonal sprouting following SCI such as neutralizing Nogo-A (Raineteau et al., 2002) and enzymatic degradation of CSPGs in lesion area by Chondroitinase ABC (Hafidi et al., 2004). Previously a study from our laboratory could show that injury-induced sprouting of CST (Opatz et al., 2009) can be further enhanced by local intrathecal infusion of a chemokine stromal cell derived factor (SDF-1)/CXCL12 into the lesion area (Opatz et al., 2009).

2. AIM OF THE THESIS

The influence of aging on the regenerative growth potential of central nervous system (CNS) axons following spinal cord injury (SCI) is not known. Only in recent years, treatments have been developed which are able to induce regeneration in mature CNS and, therefore, may provide a basis to investigate the regeneration potential in aging CNS. The aim of this thesis is to analyse age-dependent changes in the regeneration capacity of injured axons in the model of SCI in rat. Experimental studies of SCI in rats show a decline in locomotor recovery as well as an increase in myelin pathology and in oxidative stress with age, suggesting that aging may have negative impact on axon plasticity in CNS similarly to peripheral nervous system (PNS). In that case, it should be attempted to reactivate the regeneration capacity in the injured spinal cord of aged animals by following therapeutic strategies - suppression of collagenous lesion scarring (anti-scarring treatment, AST) which has been shown to enhance regeneration of various axon tracts, and by infusion of chemokine stromal cell-derived factor 1 (SDF-1) which increases sprouting of corticospinal tract (CST) in young animals. We also would determine whether the treatment-promoted regeneration capacity is reduced at older age and whether the decline in regeneration potential in different axon tracts is similar with increasing age or whether there are neuron-specfic differences.

1. The effect of old age on axonal regeneration and sprouting would be studied in geriatric (older than 22 months) and young (2-3 months) rats in the dorsal hemisection model of SCI at thoracic level T8. (i) Rostral sprouting of serotonergic (5-HT), catecholaminergic (TH) and anterogradely traced CST following intrathecal SDF-1 infusion, and (ii) regenerative growth of these descending tracts and sensory CGRP axons into the lesion area following AST would be quantified using immunohistological methods at five weeks after spinal cord hemisection.
2. Using genome-scale transcriptional profiling, the age-dependent changes on transcriptomic level will be studied. These might reflect the regenerative growth potential in aging CNS. We want to investigate whether the AST-induced regeneration program, identified in young animals, can be activated in aged animals. Both SCI and aging produce profound changes in gene expression; therefore, aging might interfere with the success of such therapeutic interventions. We would investigate the extent and nature of the difference between the dynamic cortical (layer V/VI of sensorimotor cortex) gene expression profiles of aged and young rats following thoracic corticospinal tract (CST) transection (the dorsal hemisection SCI) with and without AST at 1, 7 and 35 dpo, which represent the acute, subacute and chronic stages of SCI, respectively.

3. OUTLINE

The studies were intiated by establishing hemisection SCI model in very old rats together with the triple-staining immunostaining protocol in order to investigate the effect of old age on two types of axon growth after SCI: rostral sprouting and regenerative growth into lesion site, along with the infusion of the stromal cell-derived growth factor-1 (SDF-1/CXCL12) and anti-scarring treatment (AST), respectively (**Chapter 4.1**). As we could determine that SDF-1 infusion enhances sprouting of CST, 5-HT and TH fibers in young and aged animals, we wanted to know whether these effects could have been elicited by direct mechanisms, therefore the expression of SDF-1 receptors on these axon tracts was analysed (CST in young animals, **chapter 4.2**; CST, 5-HT, TH in young and aged animals, **chapter 4.1**). Dynamic cortical gene expression profiles at 1, 7, 35 days following SCI in young and old rats were analysed with the aim to determine how aging affects the responses to SCI and whether the AST-induced regeneration program can be activated in aged animals (**chapter 4.3**). In **Chapters 4.4 and 4.5,** the role of SDF-1 in SCI and that of chemokines in CNS injury such as SCI, traumatic brain injury and stroke, respectively, were analysed and discussed.

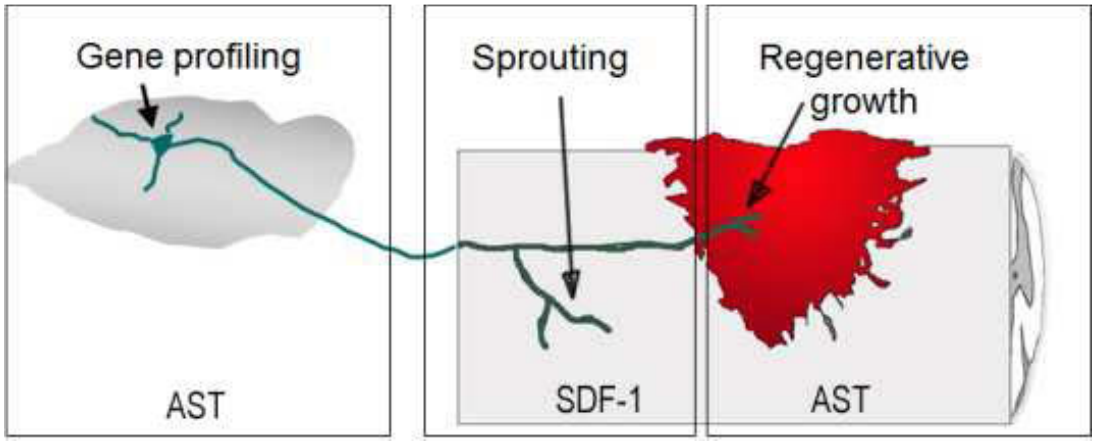

Fig 3. Schematic overview of the three main topics of this thesis.

4. PUBLICATIONS

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Regular Article

Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury

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ABSTRACT

The demographics of acute spinal cord injury (SCI) are changing with an increased incidence in older age. However, the influence of aging on the regenerative growth potential of central nervous system (CNS) axons following SCI is not known. We investigated axonal sprouting along with the efficiency of the infusion of the stromal cell-derived growth factor-1 (SDF-1/CXCL12) and regenerative growth along with the anti-scarring treatment (AST) in young (2–3 months) and geriatric (22–28 months) female rats following SCI. AST included local injection of iron chelator (2,2′-dipyridine-5,5′-dicarboxylic acid) and 8-bromo-cyclic adenosine monophosphate solution into the lesion core. Axon outgrowth was investigated by immunohistological methods at 5 weeks after a partial dorsal hemisection at thoracic level T8. We found that aging significantly reduces spontaneous axon sprouting of corticospinal (CST), serotonergic (5-HT) raphespinal and catecholaminergic (TH) coerulospinal tracts in distinct regions of the spinal cord rostral to the lesion. However, impairment of axon sprouting could be markedly attenuated in geriatric animals by local infusion of SDF-1. Unexpectedly and in contrast to rostral sprouting, aging does not diminish the regenerative growth capacity of 5-HT-, TH- and calcitonin gene-related peptide (CGRP)-immunoreactive axons at 5 weeks after SCI. Moreover, 5-HT and TH axons maintain the ability to react upon AST with significantly enhanced regeneration in aged animals. These data are the first to demonstrate, that old age compromises axonal plasticity, but not regenerative growth, after SCI in a fiber tract-specific manner. Furthermore, AST and SDF-1 infusions remain efficient, which implicates that therapy in elderly patients is still feasible.

Introduction

The demographics of acute SCI are changing. Although mostly young people are affected, the percentage of patients older than 60 years at the time of injury has increased from 4% to 11% since 2000 along with the average age from 27 years in 1970s to 40 years since 2005 (NSCI, 2010). Thus, there is considerable clinical interest in determining the consequence of aging on the regenerative capacity of injured CNS axons in order to develop SCI therapies that remain effective at older ages.

Regenerative capacity of injured axons in the spinal cord is age-dependent. While neonatal animals regenerate very well, spontaneous regeneration of the mature CNS is limited (Schwab and Bartholdi, 1996). However, there are no studies describing the growth capacity of spinal axons at older ages. In the aging CNS biological systems involved in synaptic function, mitochondrial energy metabolism, and stress resistance change (Boveris and Navarro, 2008; Kyrkanides et al., 2001; Yankner et al., 2008), possibly turning the CNS more susceptible to trauma. Indeed, experimental studies of SCI in aged rats observed an age-dependent decline in locomotor recovery (Genovese et al., 2006; Gwak et al., 2004; Siegenthaler et al., 2008) and increased inflammatory reactions (Genovese et al., 2006) as well as increased myelin pathology and reduced remyelination (Siegenthaler et al., 2008). None of these SCI studies, however, investigated age-dependent axonal growth responses, which is considered a key predictor of neurological outcome in SCI (Fehlings and Tator, 1995; Medana and Esiri, 2003). In brain, it has been demonstrated that catecholaminergic axons sprout less in the senescent rat in response to denervation of the septum and hippocampus (Scheff et al., 1978). On the other hand, it has been demonstrated that olfactory sensory neurons and retinal ganglion cells regenerate well at old age (24 months) following axotomy (Luo et al., 2010; Morrison and Costanzo, 1995), suggesting that aging might severely impair the regenerative capacity of CNS axons in a neuron-specific manner.

Only in recent years, treatments have been developed which are able to induce regeneration in mature CNS and, therefore, may provide a basis to investigate the regeneration potential in aging CNS. The fibrotic lesion scar forming after CNS injury is a major impediment for axonal regeneration (Brazda and Muller, 2009; Klapka and Muller, 2006; Silver and Miller, 2004). A scar-suppressing treatment (anti-scarring treatment; AST) that has been established in our laboratory and which includes local injections of an iron chelator

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(2,2′-bipyridine-5,5′-dicarboxylic acid; BPY-DCA) and 8-bromo-cyclic adenosine monophosphate (8-Br-cyclicAMP) into the lesion core has been shown to promote long-distance regeneration of cut corticospinal tract (CST) axons, neuroprotection of projecting cortical neurons and functional improvement of treated young adult rats after SCI (Klapka et al., 2005). In addition, we recently showed significantly increased density of descending CST, serotonergic raphespinal (5-HT, RaST), catecholaminergic coerulospinal [tyrosine hydroxylase (TH), CoST] tracts and increased density of sensory calcitonin gene-related peptide (CGRP)-immunoreactive fibers in the lesion zone at 5 weeks, and further enhanced numbers of descending axons in and beyond the lesion zone at 12 weeks post-injury upon AST (Schiwy et al., 2009). In recent years axonal sprouting has been recognized as an important contributor to rapid spontaneous locomotor recovery after acute SCI via reorganization of connections (Bradbury and McMahon, 2006; Fouad and Tse, 2008; Maier and Schwab, 2006). We have shown previously that injury-induced sprouting of CST (Opatz et al., 2009) can be further enhanced by local intrathecal infusion of the chemokine SDF-1 into the lesion area.

Having established these AST and SDF-1 treatment strategies in young adult animals, we have now investigated their effectiveness to promote axon growth in aged animals and explored whether the responses observed depend on the nature of the descending motor tracts mentioned above. At 5 weeks after partial dorsal hemisection and treatment we have quantified the effect of aging on two types of axon growth: (i) sprouting rostral to the site of injury in serotonergic, dopaminergic and anterogradely traced corticospinal tract (CST) following intrathecal SDF-1 infusion, and (ii) regenerative growth of these descending tracts and ascending CGRP axons into the lesion area following AST using immunohistological methods.

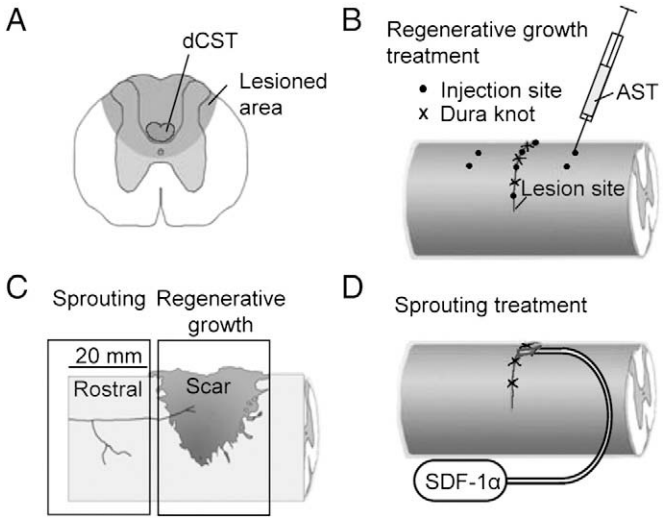

Fig. 1. Schematic illustrations of the partial dorsal hemisection, application of treatments and quantification of regenerative axon growth. (A) Dorsal columns, including dorsal corticospinal tract (dCST) and gray matter, were transected with a Scouten wire knife at thoracic level 8 as shown in a cross section of the spinal cord. Two treatments were applied in this study. (B) In one experiment, aged and young animals either received anti-scarring treatment (AST) to promote regenerative axon growth or buffer injection into the spinal cord after dura suture. In these groups, axon profiles were quantified in the glial fibrillary acidic protein (GFAP)-immunonegative scar area in every other sagittal section (C, right box). (D) In the other experiment, the aged and young rats received an intrathecal catheter connected to an Alzet osmotic minipump either to infuse stromal cell-derived growth factor-1α (SDF-1) to promote axon sprouting, or vehicle into the lesion site for 1 week. In the latter animal groups, axonal sprouting was quantified in 12 cross sections per animal within a region up to 20 mm rostral to the lesion site (C, left box).

Materials and methods

SCI and treatments

Young adult (178–242 g; 9–14 weeks) and geriatric (258–485 g; 22–28 months) female Wistar rats (HanTac:WH; Taconic, Denmark) received a partial dorsal hemisection at thoracic level T8 under isoflurane anesthesia (Forene, Abbott, Germany; 2–3% in O_2 and NO_2 at a ratio of 1:2). The dura was opened at T8 and the dorsal CST (dCST), lateral rubrospinal tracts and dorsal columns, as well as parts of the RaST (Heise and Kayalioglu, 2009) and CoST (Rajaofetra et al., 1992), were cut with a Scouten wire knife (Bilaney, Germany) to the depth of the central canal (Fig. 1A) (Klapka et al., 2005). After dura suture the animals either received AST, SDF-1α or corresponding buffer solutions. AST was applied as described in Klapka et al., 2005 with minor changes, not affecting the treatment efficacy. Similarly to the previous protocol, 8 × 0.2 µl injections of iron chelator BPY-DCA (2,2′-bipyridine-5,5′-dicarboxylic acid, 40 mM in Tris buffer), 4 injections into the lesion site and 2 injections each at a distance of 1 mm rostral and caudal to the lesion using a glass capillary penetrating the spinal cord for 1 mm were made. Instead of 4 mg solid 8-Br-cyclicAMP, 4 × 0.25 µl of 8-Br-cyclicAMP (100 µg in Tris buffer) was injected into the lesion site (Fig. 1B). The lesioned control animals received Tris buffer following the same injection protocol. The lesion site was covered with a piece of Elvax (ethylene vinyl acetate; a gift from Erbslöh GmbH, Velbert, Germany) copolymer sheet loaded with BPY-DCA for AST-treated animals or Tris buffer for control animals. The overlying tissue was sutured, and the animals (n = 8–10/group) were allowed to recover.

SDF-1α infusion protocol is described in Opatz et al., 2009. Briefly, after dura suture and filling the catheter, a small hole was made into the dura at a distance of 5 mm caudal to the lesion (Fig. 1D). The intrathecal catheter (#0007740, Alzet; 0.36 mm outer diameter; 0.18 mm inner diameter) was inserted into the subarachnoid space and directed towards the side of injury. The catheter was connected to an osmotic minipump (Alzet, model 2001) loaded with 200 µl of either SDF-1α (10 µM in 0.4% BSA/PBS buffer; R&D Systems) or vehicle alone (0.4% BSA/PBS buffer). Following fixation of catheter and minipump, the lesion site was covered with a piece of Elvax sheet loaded with Tris buffer. The overlying tissue was sutured, and the animals (n = 5–6 animals/group) were allowed to recover. Application of either SDF-1α or PBS/BSA buffer was carried out at a flow rate of 1 µl/h over a time period of 7 days. One week after lesion, the pump was removed in a second surgery.

Survival time for all the animals in these studies was 5 weeks. Three weeks prior to sacrifice CST was traced with biotinylated dextrane amine (BDA) (Klapka et al., 2005). Post-operative care included treatment with antibiotics (Baytril; Bayer Health Care, Germany) for 1 week and manual bladder emptying. Institutional guidelines for animal safety and comfort were adhered to, and all surgical interventions and pre- and post-surgical animal care were provided in compliance with the German Animal Protection Law (State Office, Environmental and Consumer Protection of Northrhine Westfalia, LANUV NRW).

Immunohistology

Isoflurane-anesthetized animals (Hypnorm, Janssen-Cilag, Germany) were perfused transcardially with ice-cold 1 M phosphate-buffered saline (PBS) for 2 min and 4% paraformaldehyde in 0.1 M PBS (PFA; Merck, Germany) for 15 min. Spinal cords were removed and postfixed for 24 h at 4 °C in the same fixative and cryoprotected in 30% sucrose at 4 °C before cutting into coronal or parasagittal serial 50 µm sections using a freezing microtome.

For the analysis of regenerative axon growth, every second free-floating 50 µm parasagittal spinal cord section per animal was triple-stained. The triple-staining protocol for the analysis of regenerative

axon growth is as follows: washing with PBS, blocking with 5% donkey serum for 1 h at room temperature (RT), incubation with primary antibodies against TH/CGRP/GFAP or 5-HT/BDA/GFAP (Table 1) in PBS/5% donkey serum overnight at 4 °C. Streptavidin Oregon green 488 (MoBiTec, Germany; 1:1000) was used for BDA visualization. After overnight incubation sections were washed with PBS and incubated with secondary antibodies for 1 h at RT: donkey anti-goat Alexa 594, donkey anti-rabbit Alexa 488 and donkey anti-mouse Alexa 647 (Molecular Probes) or donkey anti-rabbit Alexa 594 and donkey anti-mouse Alexa 647, respectively.

For the analysis of rostral axonal sprouting in coronal free-floating 50 μm sections prepared from the 2 cm piece of spinal cord rostral to lesion, 5-HT and TH were separately stained in adjacent sections. For the evaluation of rostral sprouting the anterogradely traced CST was stained with DAB as described in Opatz et al. (2009). Briefly, slices were incubated with an avidin–biotin-peroxidase complex (ABC, Vector Laboratories) at 4 °C overnight. The next day, tissue was washed in PBS and reacted in 0.015% diaminobenzidine (DAB; Sigma) for 1 min. A brown staining reaction was obtained by adding 0.004% H_2O_2 to the DAB solution, and the staining process was stopped by washing with PBS buffer. The sections were mounted onto slides and embedded in Fluoromount-G (Dako). Images were taken with a BZ8000 Keyence fluorescent digital microscope. Filters for GFP, Texas Red, and Cy5 were used to take images of Alexa 488, Alexa 594 and Alexa 647, respectively.

For double staining of SDF-1 receptors and axon tracts, free-floating coronal spinal cord sections were pretreated for 20 min with 3% hydrogen peroxide in PBS to quench any endogenous peroxidase activity, then blocked in 5% donkey and horse serum and 0.1% Triton-X-100 in PBS for 1 h at RT. Samples were stained with the following primary antibodies in 3% horse serum and 0.1% Triton-X-100 in PBS at 4 °C over night: goat-anti-CXCR4 and goat-anti CXCR7 simultaneously with the above mentioned antibodies for 5-HT or TH. BDA was stained with streptavidin Oregon green 488. For SDF receptor signal amplification, a TSA (Tyramide Signal Amplification) fluorescence procedure (Perkin Elmer-NEN, Boston, MA, USA) was used in the double-stainings of 5-HT and TH with SDF receptors. Therefore, after incubation, sections were rinsed extensively (3×, for 15 min each) with PBS and incubated for 1 h in a 1:150 dilution of biotin-conjugated horse anti-goat antibody (Vector, Burlingame,CA, USA) directed to the primary CXCR4 and CXCR7 antibody. Donkey anti-rabbit Alexa 488-conjugated antibody (1:250 dilution) was used as secondary antibody against 5-HT or TH. Sections were then washed with PBS (3×, for 10 min each). Briefly, sections were incubated for 30 min in a 1:100 dilution of streptavidin-conjugated horseradish peroxidase (Amersham Pharmacia Biotech, UK). Then sections were rinsed extensively with PBS and incubated in a 1:50 dilution of Fluorescein Cy5-conjugated Tyramide (Perkin Elmer-NEN, Boston, MA, USA) for 5 min. Double staining of receptors and BDA was performed without TSA fluorescence procedure and Alexa-conjugated secondary antibodies were used to detect the SDF receptors. Slices with receptor double stainings were analyzed using a Zeiss LSM 510 confocal microscope. Three laser lines emitting at 488, 543 and 633 were used to excite Alexa 488, Alexa 594 and Cy5, respectively. The channels were recorded separately, and 4 serial stack images of 1–2 μm thickness were collected and projected into a single plane. The digitalized pictures were saved in TIF format.

Table 1
Antibody specifications.

Antibody	Type	Host	Commercial source	Dilution
CGRP	Polyclonal IgG	Goat	1720-9007, Serotec, UK	1:1500
TH	Polyclonal IgG	Rabbit	ab112, Abcam, UK	1:750
GFAP	Monoclonal IgG1	Mouse	MAB3402, Chemicon, USA	1:500
5-HT	Polyclonal IgG	Rabbit	Se100, Biologo, Germany	1:30
CXCR4	Polyclonal IgG	Goat	sc-6190, Santa Cruz, USA	1:100
CXCR7	Polyclonal IgG	Goat	sc-107515, Santa Cruz, USA	1:100

Axon quantification

For assessment of regenerative axon growth of each fiber population, axon profiles were counted throughout all focal planes of every second parasagittal 50 μm tissue sections (10–20 sections/animal) in the GFAP-negative lesion area (Fig. 1C).

To quantify rostral sprouting of 5-HT- and TH-labeled axons, images of the central canal, dorsal horn, ventral horn and white matter near intermediolateral column were captured in a series of 12 coronal sections obtained at every 1.5 mm in a spinal cord segment up to 20 mm rostral to the lesion border (Fig. 1C). In ImageJ, the original tif images were converted to 8-bit before the application of the 'smallest Hessian' was performed (Grider et al., 2006). The resulting images were converted to a binary image by setting a threshold for pixel intensity at which 5-HT or TH positive axons were above threshold (Grider et al., 2006). After setting the threshold, a rectangle of 500×350 or 350×500 pixels in size, respectively, for the above mentioned spinal cord areas was placed over the image and the percent area occupied by 5-HT or TH positive axons was determined.

Quantification of CST sprouting was performed by counting the BDA-labeled axon profiles in gray matter using a light microscope. For normalization of tracing efficiency, the number of counted fiber profiles was divided by the pixel density of the dorsal CST measured by ImageJ for each animal.

Statistics

For all data the two-sided Mann–Whitney *U*-test for paired comparison and Holm–Bonferroni correction for multiple testing with the type I error rate $\alpha = 0.05$ was applied using WinStat software (Version 2005.1 for Microsoft Excel). The paired comparisons were made to test for significant elevation in axon profiles of treated compared to control animals as well as young compared to aged animals; four comparisons per fiber population in total. The experimental groups were considered significantly different at $p \leq 0.05$.

Results

Aging does not decrease the density of axon profiles growing into the lesion core in response to scar inhibition

The majority of injured axons in both aged and young control animals stopped at the border of the lesion (Fig. 2). Nonetheless, all axon populations, except CST, showed some in-growth into the primary fibrous scar area in young and, interestingly, also in aged animals lacking treatment (gray bars in Figs. 2M–O). According to these values, aging did not impair the spontaneous capacity of CGRP, TH and 5-HT axons to grow into the fibrous scar area at 5 weeks post-injury.

When AST was applied immediately after hemisection to transiently suppress fibrotic scarring, enhanced 5-HT, TH and CGRP axon densities could be observed in the region of the secondary or delayed scar at 5 weeks post-injury and treatment. The results showed a remarkably enhanced regenerative axon growth in aged animals which was significant for TH and 5-HT fibers in treated vs. control animals (Mann–Whitney *U-test*, Figs. 2N,O). In young animals the significance level of all three fiber systems was marginally missed after Holm–Bonferroni correction. The CGRP axons showed a clear trend towards a more prominent in-growth into the scar area under AST in both young and aged animals. None of the tracts was found to regenerate beyond the site of injury into the intact distal spinal cord tissue within 5 weeks post-injury. In contrast to the other tracts studied, CST axons were not found beyond the border of the fibrous scar in both age and treatment groups (data not shown). We also

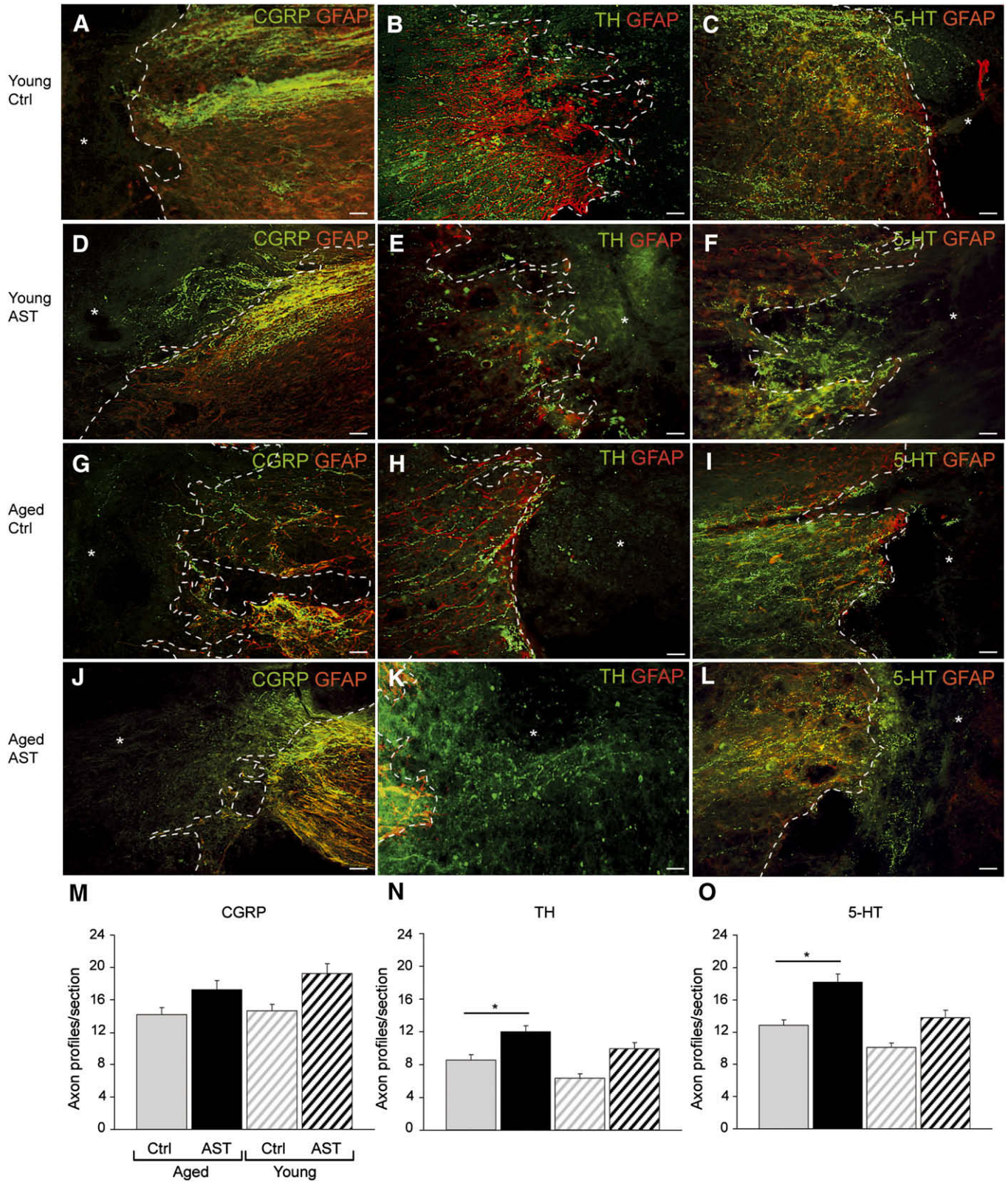

Fig. 2. Regenerative growth of 5-HT, TH and CGRP axons in young and aged rats. Representative immunohistologically stained 50-μm-thick sagittal spinal cord sections of young (A–C) and aged (G–I) control animals (Ctrl) and anti-scarring treatment (AST)-treated young (D–F) and aged animals (J–L). Corresponding quantification of axon profiles within the lesion core (M–O). Orientation dorsal = up, caudal = right. In the aged and young control animals, majority of the calcitonin gene-related peptide (CGRP)-, tyrosine hydroxylase (TH)- and serotonin (5-HT)-immunoreactive axons (green) did not cross the border of the glial scar (dotted line) in 5 weeks post-injury, whereas in AST-treated animals, numerous axons of these tracts did grow into the GFAP-immunonegative lesion area (asterisk). Note that ascending CGRP tract axons grow into the lesion caudally. Scale bar = 50 μm. (M–O) Axon profiles in the GFAP-negative fibrous scar region of every second sagittal section of each animal were counted and presented as axon profiles per section in each experimental group. There were significantly more TH- and 5-HT-immunoreactive axon profiles in the lesion core of AST-treated aged animals compared to buffer-treated controls. *$p<0.05$, Mann–Whitney *U*-Test, Holm–Bonferroni correction. Error bars: SEM.

quantified the GFAP-negative area of damaged tissue in which the axon profiles were counted and found that the lesion area did not differ significantly between the aged and young animal groups ruling out that alterations in lesion size determined the differences in the number of regenerating axons (Suppl. Fig. 1). Together these results indicate that aging does not impair the regenerative capacity of, at least, 5-HT, TH and CGRP axons and these axon tracts remain responsive to AST in old age.

Rostral sprouting of CST after SCI and SDF-1 infusion is impaired by aging

CST was visualized by anterograde labeling, whereby the tracing efficacy was not worse in the aged animals (data not shown). Nonetheless, we calculated the sprouting index (labeled CST axon profiles in the entire gray matter of the cross section divided by the pixel number of labeled fibers in the dorsal CST) to count for tracing variations in individual animals. Even if the absolute number of CST axons (higher than the number of traced axons) would decrease with aging, not supported by the literature (Leenen et al., 1989 and Nielsen et al., 2010), our analysis of the sprouting capacity would be independent of that. In coronal sections of intact spinal cord of young and aged animals we did not observe CST axons in the gray matter suggesting that axons which we detected in gray matter of lesioned animals must have sprouted after SCI (data not shown). Similarly to our recent study (Opatz et al., 2009), SDF-1 significantly increased rostral CST sprouting in young animals (Mann–Whitney *U*-test; Fig. 3A, striped bars). In the aged control rats, only a very low number of axon arbors were extending from the dorsal CST into gray matter. However, significantly more collateral axon sprouts were seen in the aged rats that had received SDF-1. Although the SDF-1-mediated elevation of axonal sprouting was significant and about twofold in aged animals it never reached the level of CST sprouting in young animals. We further observed an interesting difference in the distribution of axon sprouts in the gray matter in aged and young animals. Whereas in young animals sprouting axons were observed both in dorsal and ventral gray matter (Opatz et al., 2009), in aged rats sprouting CST axons were restricted to dorsal gray regions (Figs. 3B–E).

Aging impairs rostral sprouting of 5-HT and TH axons after SCI and SDF-1 infusion in distinct regions of spinal cord rostral to the lesion site

Here we studied whether hemisection SCI induces rostral sprouting of 5-HT and TH axons in young and aged animals and whether sprouting can be further enhanced by SDF-1 treatment.

Fiber distribution was similar in intact and injured aged and young animals for both serotonergic raphespinal (5-HT, RaST) and catecholaminergic coerulospinal [tyrosine hydroxylase (TH), CoST] tracts. A dense plexus of 5-HT axons and TH axons was present in the intermediolateral column (IML), around the central canal (CC), but 5-HT and TH fibers were more diffusely distributed throughout the ventral horn (VH) and the dorsal horn (DH). Fig. 4 gives an example of axonal sprouting of TH fibers around CC, before and after setting a threshold in order to select and count axonal structures.

Serotonergic innervation in the intact aged and young animals did not differ in the spinal cord regions investigated and axon densities were even somewhat higher in aged animals, except for the white matter region (Figs. 5E–H). Johnson et al. (1993) also found no differences in 5-HT axon densities in the thoracic and cervical regions of 2–3-months-old and aged 30-months-old rat spinal cords. The area occupied by 5-HT positive axon structures around CC was significantly smaller in the aged control group than in the young control group, whereas no significant age-dependent differences in lesion-induced 5-HT axon sprouting were observed in the areas VH, DH and WM (Fig. 5). Age-dependent differences in rostral axon densities between lesioned aged and young control groups are more pronounced for TH-immunoreactive axons. Significantly lower TH axon densities of lesioned aged control animals were observed in the areas CC, DH and

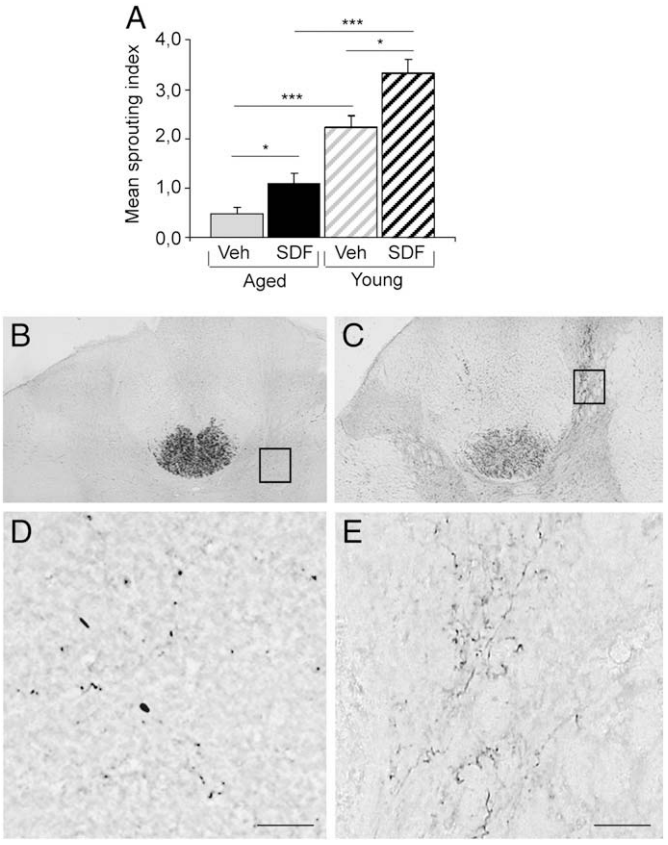

Fig. 3. Quantification of CST sprouting rostral to the lesion following local intrathecal SDF-1 or vehicle infusion. (A) Sprouting index presents the number of biotinylated dextran amine (BDA)-labeled CST axon profiles in the entire gray matter of the cross section divided by the pixel number of labeled fibers in the dorsal CST multiplied by 1000 for each animal to account for differences in BDA labeling between individual rats. Twelve 50 μm thick cross sections taken at every 1.5 mm in a region rostral to the lesion border were analyzed per animal. Aging significantly reduced CST sprouting in vehicle- and SDF-1-treated animals. SDF-1 infusion significantly increased sprouting in both young and aged animals. (B, C) Cross sections rostral to the lesion site with labeled CST in aged vehicle-treated animal (B) and in aged SDF-1-treated animal (C). Gray matter (GM). (D, E) Higher magnification of BDA-traced fibers of the regions which had the highest density of fibers boxed in (B, C), respectively. Scale bar = 50 μm. *$p<0.05$, ***$p<0.0005$ Mann–Whitney *U*-Test, Holm–Bonferroni correction. Error bars: SEM.

WM but not in the region of the VH. However, the significantly lower TH axon density in the dorsal horn and white matter regions of the lesioned aged animals compared to young is due to the significant age-specific decline in the TH innervation of these regions in the intact aged animals (Figs. 5I–L). Similarly to 5-HT, lesion-induced sprouting of TH is significantly reduced in the central canal region as innervation in this region was not changed with aging.

SDF-1 increased 5-HT axon densities only around CC in aged animals, whereas SDF-1 in young animals significantly enhanced 5-HT sprouting densities in the areas CC, DH and WM. In contrast, rostral sprouting of TH axons was enhanced by SDF-1 in the areas of CC, DH and WM of young rats and in the areas CC and DH of aged animals, respectively. Interestingly, the ventral horn seems to be the only area, thus far, in which rostral sprouting of both 5-HT and TH fibers following partial dorsal hemisection is not affected by aging regardless whether SDF-1 treatment is applied or not (Fig. 5B).

Age-dependent and fiber tract-specific expression of SDF-1 receptors CXCR4 and CXCR7

We investigated whether the two known SDF-1 receptors, CXCR4 and CXCR7, are expressed on CST, 5-HT and TH axons in the spinal cord of aged animals. Recently, we have shown that both receptors are localized on CST axons in young rats (Opatz et al., 2009) and thus SDF-

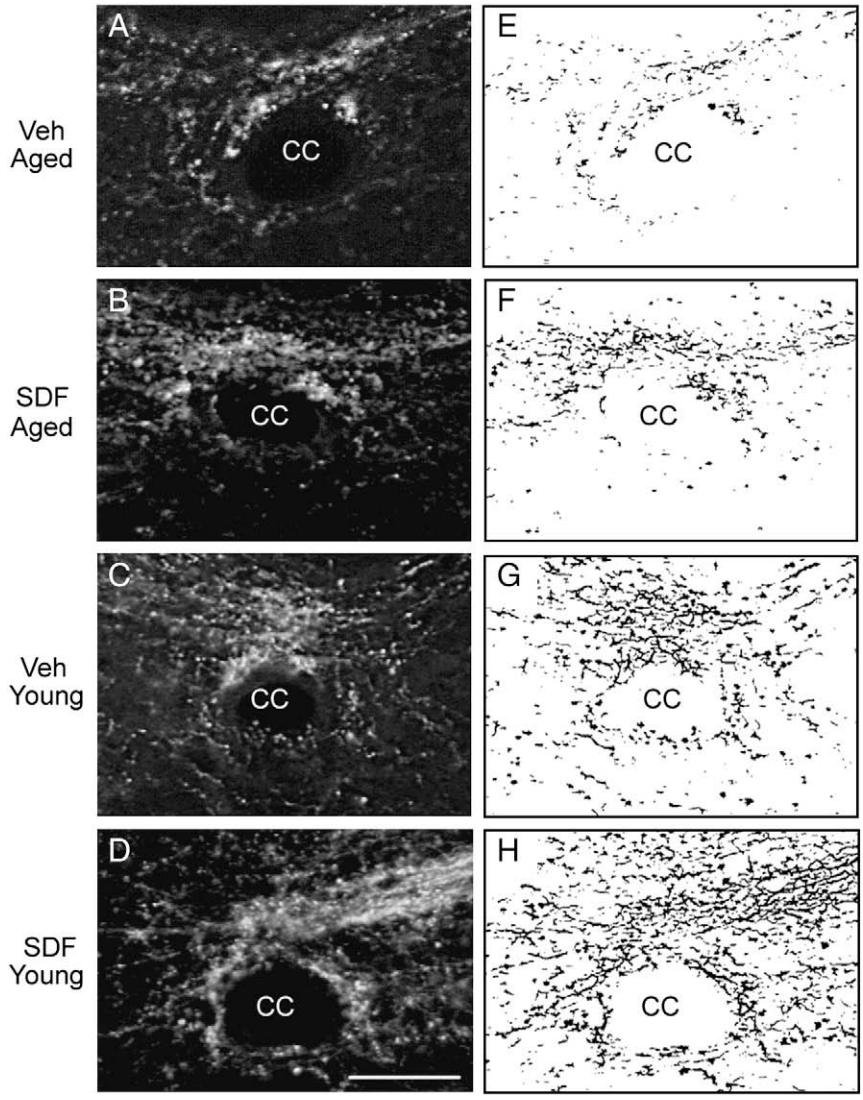

Fig. 4. Rostral sprouting of tyrosine hydroxylase (TH)-immunoreactive axons around the central canal region and corresponding images after processing with ImageJ. Cross sections of the vehicle-treated aged (A, E) and young rats (C, G), and SDF-1-treated aged (B, F) and young (D, H) animals. Image analysis with ImageJ included conversion to a binary picture by setting a threshold for pixel intensity such that TH-immunoreactive axon structures were above the threshold and the percent area occupied by the labeled axons could be determined as in Grider et al. (2006). CC, central canal. Scale bar = 100 μm.

1 might directly exert its effect on CST. Confocal laser scanning microscopy of double-stained BDA-labeled CST and CXCR4 in aged animals revealed localization of CXCR4 on CST axons (Figs. 6A–C, see also Supplementary Movie 1, boxed area from Fig. 6A). The patchy distribution of CXCR4 on CST axons in old animals resembles the staining seen in young rats. However, CXCR7 could not be detected on CST fibers in aged animals (Figs. 7A–C), which is in contrast to young rats. Further, none of the SDF-1 receptors could be detected on 5-HT or TH axons in spinal cord of both young (Figs. 6 and 7D–I), respectively, and aged animals (data not shown). We found that both SDF receptors are expressed on astrocytes (Suppl. Fig. 2), in the ventral horn cell population, probably motor neurons (Figs. 7D–F and data not shown, respectively) and CXCR4 very strongly in the ependymal cell layer around central canal (Figs. 6G–I).

Discussion

Here we demonstrate that rostral axon sprouting in traumatic SCI is markedly impaired at old age, but axon re-growth into the lesion zone is not. Moreover, AST and SDF-1 treatment led to significant improvement in both regenerative axon growth and rostral sprouting in geriatric rats indicating that therapy from spinal cord trauma in elderly patients is still feasible.

Rostral sprouting

Our results are the first to indicate that aging impairs the sprouting capacity of CST, RaST and CoST axon tracts in the injured spinal cord. These observations fit the overall notion of the decline of CNS plasticity with increasing age. This is commonly explained with systemic changes in CNS such as increased inflammation (Jurgens and Johnson, 2010; Kyrkanides et al., 2001; Yankner et al., 2008) and/or reduction of axon growth support (Hayashi et al., 1997; Hoffman et al., 1992). Reduced axon densities observed here reflect impaired lesion-induced sprouting capacity and not impaired innervation in the intact aged animals, as (i) for CST we calculated a sprouting index which was independent of tracing efficacy and absolute numbers of CST fibers, and as (ii) serotonergic innervation and TH-fiber density in the central canal region of intact aged animals was comparable to young. However, the decreased densities of TH-fibers in the dorsal horn and white matter regions in the lesioned aged animals could be explained by impaired innervation in intact aged animals. On the

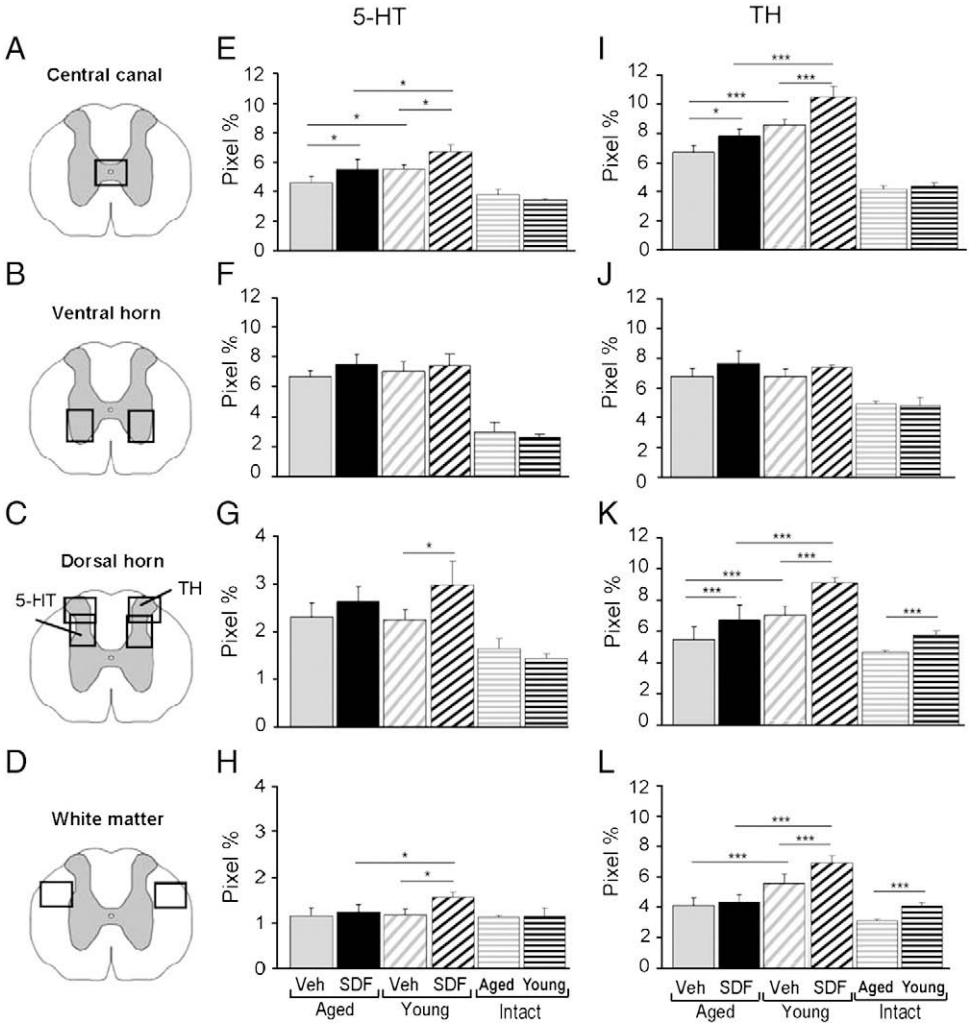

Fig. 5. Rostral sprouting of 5-HT- and TH-immunoreactive axons is impaired by aging in region-specific manner. Quantitative assessment of 5-HT- (E–H) and TH-immunoreactive (I–L) axon densities rostral to the injury around the central canal CC (A, E, I), in the ventral horn VH (B, F, J), dorsal horn DH (C, G, K) and white matter WM (D, H, L) regions upon SDF-1 or vehicle infusion, respectively, in sections of the lesioned animals and in corresponding areas of the intact animals. Axon density in E–L is presented as percent of area occupied by 5-HT- or TH-immunofluorescent structures in the regions of interest boxed in A–D in the respective cross sections. Aging impaired lesion/vehicle-induced sprouting around the CC for 5-HT- and TH-immunoreactive axons (E) and (I), respectively. The significant differences in the axon densities in the DH and WM regions for TH-immunoreactive axons (K, L) were due to significantly decreased innervation of these regions in aged intact animals. SDF-1 increased 5-HT axon densities around the CC in aged and around the CC and in the DH, WM in young animals (E and E, G, H, respectively), whereas TH axon sprouting was enhanced by SDF-1 around the CC and in the DH and WM in both young and aged animals (I, K and L, respectively). SDF-1 effect was significantly reduced by aging around the CC and in the WM for 5-HT axons (E, H) and around the CC and in the DH and WM for TH axons (I, K, L). *$p<0.05$, ***$p<0.0005$ Mann–Whitney *U*-Test, Holm–Bonferroni correction. Error bars: SEM.

other hand, it could be possible (but is still unproven) that perineuronal nets (Celio et al., 1998; Rhodes and Fawcett, 2004) around axons and synapses may restrict plasticity predominantly in the aged rather than in the young CNS.

As shown here, aging impaired rostral sprouting capacity in a tract- and region-specific manner. Among the fiber tracts investigated, the CST was most severely affected. In 22-months-old rats the rostral sprouting response of CST was only about 25% of the sprouting activity found in young adult (2–3-months-old) animals. Compared to CST, age-dependent differences in the magnitude of sprouting in RaST (5-HT) and CoST (TH) were smaller. Among the latter fiber tracts TH axons were more susceptible to aging than serotonergic fibers, as age-dependent reduction in sprouting capacity of TH axons reached significance in at least 3 out of 4 spinal cord cross sectional regions (CC, DH, WM) as compared to only one region (CC) for 5-HT axons. Importantly, aging did not affect sprouting in the VH, in which serotonergic and noradrenergic innervation was found to modulate locomotion (Rajaofetra et al., 1992; Rekling et al., 2000). Increased density of these tracts in the VH might contribute to recovery from SCI by elevating the muscle tone (Muir and Steeves, 1997). Innervation of the DH contributes to the complex circuitry that modulates sensory information, among which dopaminergic innervation is established in anti-nociception (Millan, 2002). We observed no signs of neuropathic pain in aged and young animals during our experiments, although TH density was less increased in aged animals after SCI than in young animals. Using the plantar test no evidence of increased neuropathic pain in SDF-treated young animals could be observed (N. Brazda, personal communication). It has previously been shown by Gwak et al. (2004)that young animals develop more robust central neuropathic behaviors than middle-aged rats after SCI. Furthermore, aging-impaired sprouting around central canal and in the lateral white matter suggests that optimal maintenance of regulation of sympathetic outflow (Allen and Cechetto, 1994; Jacobs et al., 2002) might be affected in aged animals.

SDF-1 elicited increased rostral axonal sprouting at old age. Moreover, the magnitude of enhanced sprouting, which SDF-1 added upon the spontaneous lesion-induced sprouting response, seems to be similar in aged and young animals. However, in all fiber systems tested the levels of SDF-1 induced sprouting in aged animals are significantly lower than in young rats. In CST, which bears both SDF-1 receptors (CXCR4, CXCR7) in young rats and the CXCR4 in aged animals, intrathecal infusion of SDF-1 elicited a two-fold increase in sprouting, but compared to young animals, this increase started from

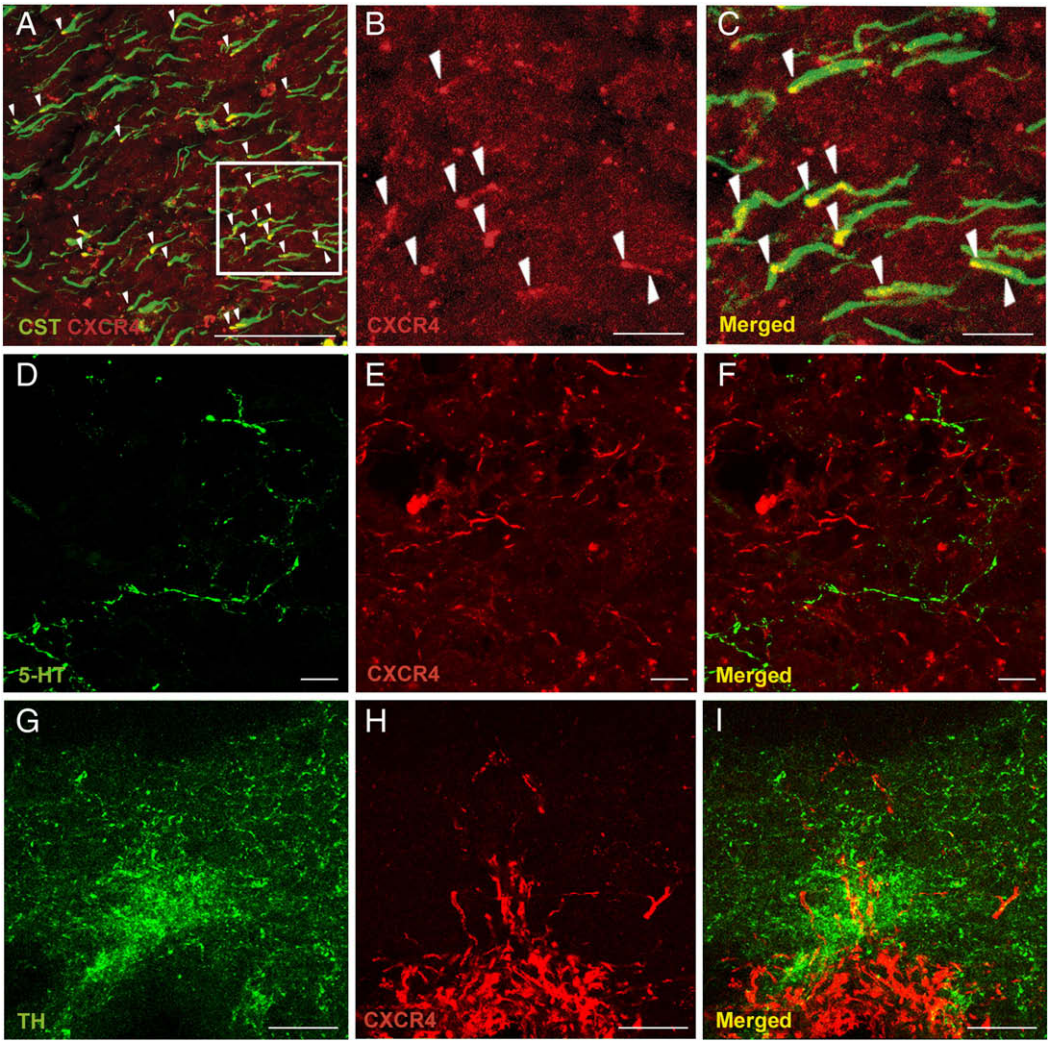

Fig. 6. Axonal localization of CXCR4 receptor. (A) Confocal analysis of CST axons double-stained for BDA (green) and CXCR4 (red) revealed that CXCR4 is present on many CST axons (arrowheads). (B, C) Boxed area in (A) is enlarged in (B, C). (D–F) No localization of CXCR4 receptors (E) on 5-HT-positive axons (D) could be detected. (G–I) Ependymal cell layer around central canal shows strong CXCR4 expression (H) which does not colocalize with TH-positive axons (G). Although there appears some yellow labeling in (I), no colocalization was observed on none of the Z-stack plane images (data not shown). The images in A–C were taken from the dorsal corticospinal tract of SDF-1-treated intact aged animals, the images in D–F from the intermediate gray regions and the images in G–I from the central canal area in spinal cord cross sections from young injured animals. Scale bar in A, G–I = 50 μm, in B–C = 10 μm, D–F = 20 μm.

a markedly lower level. As both G-protein coupled SDF-1 receptors are present on CST axons, the chemokine-induced sprouting response is probably due to direct ligand–receptor interactions. Tysseling et al. (2011) could, however, not detect CXCR4 expression on the corticospinal axons and motor neurons, probably because they used GFP-transgene mice and not immunohistology, where the immunoreactivity has to be enhanced with tyramide signal amplification (TSA) (Banisadr et al., 2002). Moreover, Dugas et al. (2008) showed that purified corticospinal motorneurons express CXCR4 receptors.

On the other hand, TH and 5-HT fibers bear no detectable levels of the SDF-1 receptors, indicating that SDF-1-stimulated sprouting enhancement observed for RaST and CoST is most likely due to indirect (paracrine) effects presumably mediated via astrocytes, a cell type which carries both SDF-1 receptors (Supplementary Fig. 2), or via neuronal progenitor cells from the ependymal cell layer around central canal and/or infiltrating macrophages expressing strongly CXCR4 (Fig. 6; Tysseling et al., 2011). Determination of the individual functional roles of the SDF-1 receptors awaits the generation of conditional knockout mutants for both receptor genes in future studies. Nonetheless, CXCR7 has recently been considered to function as a scavenger receptor regulating chemokine responsiveness (Naumann et al., 2010; Sánchez-Alcañiz et al., 2011) rather than transducing signaling (Balabanian et al., 2005; Burns et al., 2006). This is in contrast to CXCR4, which, upon ligand binding, has been described to elevate intracellular cAMP levels (Chalasani et al., 2003) and subsequent induction of regeneration-associated genes such as Arginase I, which catalyzes production of polyamines (Cai et al., 2002).

SDF-1 is the first compound identified that effectively increases plasticity after trauma in aged spinal cord. Previously, increasing axonal plasticity in subcortical regions triggered by anti-NogoA treatment was shown to improve motor task performance after stroke in geriatric rats (Markus et al., 2005). Thus improving axonal sprouting in spinal cord and brain may not only improve plasticity in young rats but also in aged animals.

Axonal regeneration

Axonal regeneration differs from rostral sprouting, as lesioned axons proximal to the lesion grow out to overcome the lesion zone and reinnervate the target area distal to the site of injury rather than remodeling local circuitry. Here we demonstrate that AST-promoted regenerative axon growth into the scar area can be initiated in several lesioned fiber tracts in spinal cord. We found that the majority of TH,

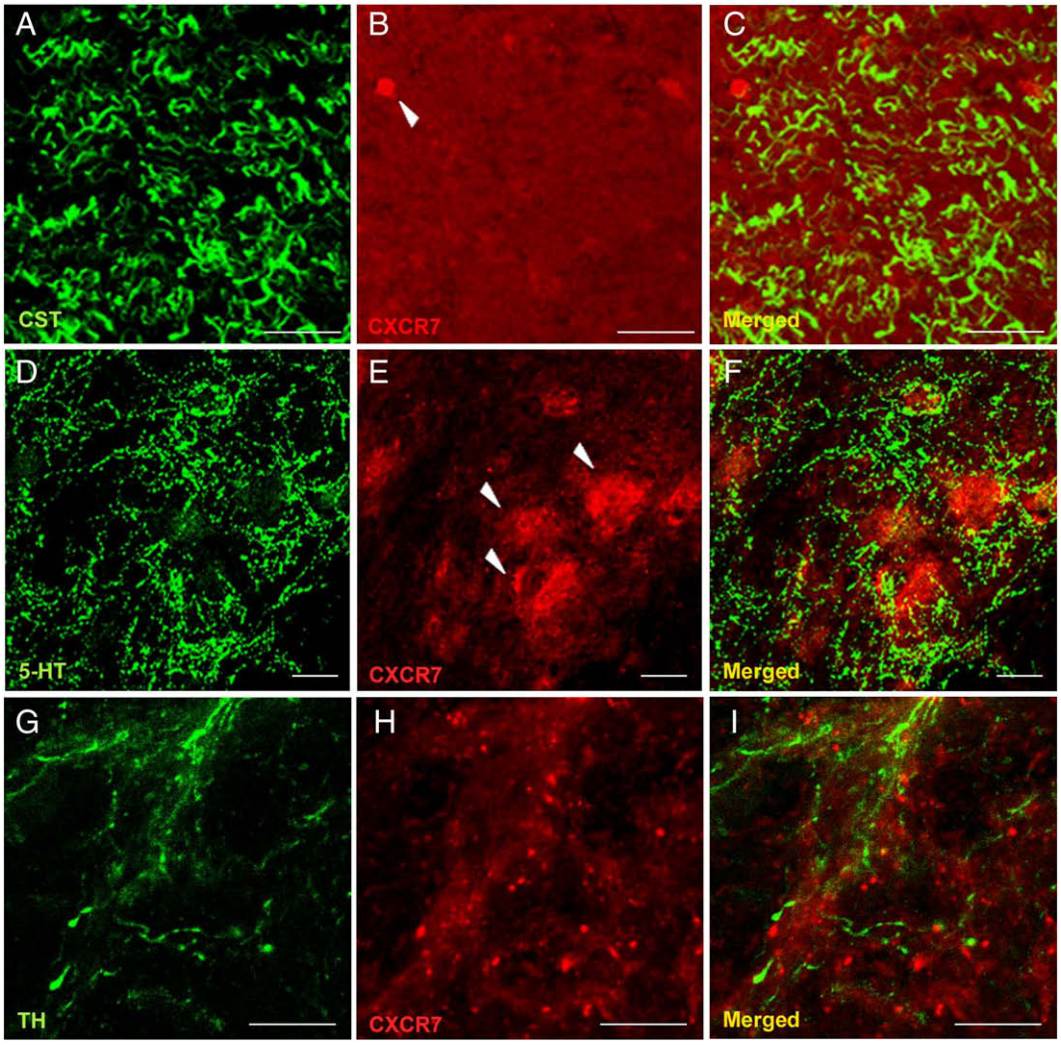

Fig. 7. Localization of CXCR7 receptor. (A–C) CST axons (A) of aged animals lack CXCR7 immunoreactivity (B). A round cell in (B, arrowhead) is most likely an infiltrating macrophage. The 5-HT-positive fibers (D) are in close proximity to but do not colocalize with CXCR7-immunoreactive structures in the ventral horn which probably represent large motor neurons (E, arrowheads). TH-positive (G) fibers do not bear CXCR7 receptors (H). Images in A–C were taken from the dorsal corticospinal tract of intact aged animals and images in D–F and G–I from the ventral horn regions and from the intermediate gray regions in spinal cord cross sections from young injured animals, respectively. Scale bar in A–I = 25 μm.

5-HT, CST and CGRP axons stopped at the border of the lesion in both young and aged injured control animals. Upon AST substantial numbers of TH, 5-HT and CGRP axons had grown into the scar. We had expected to find a somewhat reduced axon growth capacity at old age compared to young animals (Gwak et al., 2004; Siegenthaler et al., 2008). However, to our surprise, the degree of AST-promoted growth of 5-HT and TH axons in aged animals was, in fact, at least as vigorous as in young rats (see Figs. 2N, O).

The numbers of axons found in the lesion area were highest for CGRP followed by 5-HT, TH and CST in aged animals repeating the order detected at young age (Schiwy et al., 2009). This order presumably reflects the declining inherent regenerative growth capacity of the particular neuron types, not taking into account, however, the overall but unknown numbers of axons present in the individual spinal tracts. Although we could not detect CST fibers entering the scar area in both age groups within 5 weeks, CST axons at the border of the lesion indicated that the fibers may have, at least, overcome the distance of die-back as it has been reported that in the absence of any treatment, the CST degenerates up to a millimeter rostral to the lesion (von Meyenburg et al., 1998). Note, that we have previously found CST axons traversing the lesion zone in young AST-treated animals in long-term (12 weeks post-injury) experiments (Klapka et al., 2005; Schiwy et al., 2009).

The importance of the current study is that aged rats still retain the potential to initiate axon regeneration, a prerequisite for the growth beyond the scar to the targets and functional recovery. We have shown previously in long-term experiments that significantly increased numbers of CST, 5-HT and TH axons in young animals grow not only into the lesion but also beyond the site of injury upon AST (Schiwy et al., 2009). At the 5 week timepoint used in this study, we did not expect that axons would grow beyond the scar and re-establish connections, because the time period is too short. The axon growth we demonstrate is, therefore, unlikely of functional relevance. However, carrying out long-term studies for 12-weeks or more with the 24–28-month-old animals, which are already older than the average life span of Wistar rats and show a very high probability to die and/or develop tumors during the experiment (Deerberg et al., 1980) would have been unethical.

Our data clearly demonstrate that the inherent growth potential of damaged TH, 5-HT and CGRP axons remains preserved till senescence (in rats for at least up to an age of 2 years), allowing regeneration of those fibers when inhibitory growth barriers such as scarring are suppressed. Our observation is in line with the very recent report of Furlan et al. (2010) showing that axonal pathology in geriatric patients is very similar but not worse compared to young patients. Furthermore, it was previously demonstrated that age does not affect

the ability of axons of the olfactory sensory neurons and of retinal ganglion cells to regenerate after injury (Luo et al., 2010; Morrison and Costanzo, 1995). Our dissenting results in aged rats, with impaired rostral sprouting on the one hand, and the unimpaired regenerative axon growth on the other hand, are not necessarily in conflict with published data, as both axonal growth modes might be based on distinct molecular mechanisms such as organization of the microtubule cytoskeleton (Witte and Bradke, 2008), activity of specific set of genes (Smith and Skene, 1997) and signaling pathways and different requirements for growth signals (Bernstein-Goral et al., 1997). It is generally assumed that the molecular machinery which is fully active during elongating axon growth is suppressed to some extent when the arborising mode is switched on (Fawcett et al., 2001; Rossi et al., 2007). Different effectors of a signaling pathway may make the difference. For example, the Ras effector Raf promotes axon elongation, whereas another Ras effector, Akt, causes an increase in axon caliber and branching (Markus et al. 2002). As SDF-1 reduces axon elongation but promotes axon branching in hippocampal neurons (Pujol et al., 2004), it was hypothesized that SDF-1 may activate the Ras–Akt pathway as shown in several neuronal models (Floridi et al., 2003; Peng et al., 2004) but not Raf-1.

Conclusions

This study shows, for the first time, that aging restricts axonal plasticity while concomitant regenerative axon growth is not impaired in SCI. Despite tract specific differences in spontaneous axonal growth responses in the injured spinal cord of young and aged rats, we observed significant supporting effects on axon sprouting and regenerative growth upon therapeutic interventions with both SDF-1 and pharmacological suppression of scarring in both young and aged animals. This finding is of particular importance in light of our increasingly active older population.

Supplementary materials related to this article can be found online at doi:10.1016/j.expneurol.2011.07.002.

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References

Allen, G.V., Cechetto, D.F., 1994. Serotoninergic and nonserotoninergic neurons in the medullary raphe system have axon collateral projections to autonomic and somatic cell groups in the medulla and spinal cord. J. Comp. Neurol. 350, 357–366.

Balabanian, K., Lagane, B., Infantino, S., Chow, K.Y., Harriague, J., Moepps, B., Arenzana-Seisdedos, F., Thelen, M., Bachelerie, F., 2005. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J. Biol. Chem. 280, 35760–35766.

Banisadr, G., Fontanges, P., Haour, F., Kitabgi, P., Rostène, W., Mélik Parsadaniantz, S., 2002. Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. Eur. J. Neurosci. 16, 1661–1671.

Bernstein-Goral, H., Diener, P.S., Bregman, B.S., 1997. Regenerating and sprouting axons differ in their requirements for growth after injury. Exp. Neurol. 148, 51–72.

Boveris, A., Navarro, A., 2008. Brain mitochondrial dysfunction in aging. IUBMB Life 60, 308–314.

Bradbury, E.J., McMahon, S.B., 2006. Spinal cord repair strategies: why do they work? Nat. Rev. Neurosci. 7, 644–653.

Brazda, N., Muller, H.W., 2009. Pharmacological modification of the extracellular matrix to promote regeneration of the injured brain and spinal cord. Prog. Brain Res. 175, 269–281.

Burns, J.M., Summers, B.C., Wang, Y., Melikian, A., Berahovich, R., Miao, Z., Penfold, M.E., Sunshine, M.J., Littman, D.R., Kuo, C.J., Wei, K., McMaster, B.E., Wright, K., Howard, M.C., Schall, T.J., 2006. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J. Exp. Med. 203, 2201–2213.

Cai, D., Deng, K., Mellado, W., Lee, J., Ratan, R.R., Filbin, M.T., 2002. Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. Neuron 35, 711–719.

Celio, M.R., Spreafico, R., De Biasi, S., Vitellaro-Zuccarello, L., 1998. Perineuronal nets: past and present. Trends Neurosci. 21, 510–515.

Chalasani, S.H., Sabelko, K.A., Sunshine, M.J., Littman, D.R., Raper, J.A., 2003. A chemokine, SDF-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. J. Neurosci. 23, 1360–1371.

Deerberg, F., Rapp, K., Rehm, S., Pittermann, W., 1980. Genetic and environmental influences on lifespan and disease in Han:Wistar rats. Mech. Aging Dev. 14, 333–343.

Dugas, J.C., Mandemakers, W., Rogers, M., Ibrahim, A., Daneman, R., Barres, B.A., 2008. A novel purification method for CNS projection neurons leads to the identification of brain vascular cells as a source of trophic support for corticospinal motor neurons. J. Neurosci. 28, 8294–8305.

Fawcett, J.W., Rosser, E., Dunnett, S.B., 2001. Brain Damage, Brain Repair. Oxford University Press, Oxford.

Fehlings, M.G., Tator, C.H., 1995. The relationships among the severity of spinal cord injury, residual neurological function, axon counts, and counts of retrogradely labeled neurons after experimental spinal cord injury. Exp. Neurol. 132, 220–228.

Floridi, F., Trettel, F., Di Bartolomeo, S., Ciotti, M.T., Limatola, C., 2003. Stromal cell-derived factor 1-mediated CXCR4 signaling in rat and human cortical neural progenitor cells. J. Neuroimmunol. 135, 38–46.

Fouad, K., Tse, A., 2008. Adaptive changes in the injured spinal cord and their role in promoting functional recovery. Neurol. Res. 30, 17–27.

Furlan, J.C., Bracken, M.B., Fehlings, M.G., 2010. Is age a key determinant of mortality and neurological outcome after acute traumatic spinal cord injury? Neurobiol. Aging 31, 434–446.

Genovese, T., Mazzon, E., Di Paola, R., Crisafulli, C., Muia, C., Bramanti, P., Cuzzocrea, S., 2006. Increased oxidative-related mechanisms in the spinal cord injury in old rats. Neurosci. Lett. 393, 141–146.

Grider, M.H., Chen, Q., Shine, H.D., 2006. Semi-automated quantification of axonal densities in labeled CNS tissue. J. Neurosci. Methods 155, 172–179.

Gwak, Y.S., Hains, B.C., Johnson, K.M., Hulsebosch, C.E., 2004. Locomotor recovery and mechanical hyperalgesia following spinal cord injury depend on age at time of injury in rat. Neurosci. Lett. 362, 232–235.

Hayashi, M., Yamashita, A., Shimizu, K., 1997. Somatostatin and brain-derived neurotrophic factor mRNA expression in the primate brain: decreased levels of mRNAs during aging. Brain Res. 749, 283–289.

Heise, C., Kayalioglu, G., 2009. Spinal cord transmitter substances. In: Watson, C., Paxinos, G., Kayalioglu, G. (Eds.), The Spinal Cord — a Christopher and Dana Reeve Text and Atlas. Academic Press, London, pp. 191–208.

Hoffman, A.R., Lieberman, S.A., Ceda, G.P., 1992. Growth hormone therapy in the elderly: implications for the aging brain. Psychoneuroendocrinology 17, 327–333.

Jacobs, B.L., Martin-Cora, F.J., Fornal, C.A., 2002. Activity of medullary serotonergic neurons in freely moving animals. Brain Res. Brain Res. Rev. 40, 45–52.

Johnson, H., Ulfhake, B., Gerlind, E., Bennett, G.W., Fone, K.C.F., Hokfelt, T., 1993. The serotoninergic bulbospinal system and brainstem–spinal cord content of serotonin-, TRH-, and substance P-like immunoreactivity in the aged rat with special reference to the spinal cord motor nucleus. Synapse 15, 63–89.

Jurgens, H.A., Johnson, R.W., 2010. Dysregulated neuronal–microglial cross-talk during aging, stress and inflammation. Exp. Neurol. Nov 24. [Epub ahead of print].

Klapka, N., Muller, H.W., 2006. Collagen matrix in spinal cord injury. J. Neurotrauma 23, 422–435.

Klapka, N., Hermanns, S., Straten, G., Masanneck, C., Duis, S., Hamers, F.P., Muller, D., Zuschratter, W., Muller, H.W., 2005. Suppression of fibrous scarring in spinal cord injury of rat promotes long-distance regeneration of corticospinal tract axons, rescue of primary motoneurons in somatosensory cortex and significant functional recovery. Eur. J. Neurosci. 22, 3047–3058.

Kyrkanides, S., O'Banion, M.K., Whiteley, P.E., Daeschner, J.C., Olschowka, J.A., 2001. Enhanced glial activation and expression of specific CNS inflammation-related molecules in aged versus young rats following cortical stab injury. J. Neuroimmunol. 119, 269–277.

Leenen, L.P., Meek, J., Posthuma, P.R., Nieuwenuys, R., 1989. Differences in the fiber composition of the pyramidal tract in two- and 14-month-old rats. Neuroscience 28, 635–643.

Luo, J.M., Geng, Y.Q., Zhi, Y., Zhang, M.-Z., van Rooijen, N., Cui, Q., 2010. Increased intrinsic neuronal vulnerability and decreased beneficial reaction of macrophages on axonal regeneration in aged rats. Neurobiol. Aging 31, 1003–1009.

Maier, I.C., Schwab, M.E., 2006. Sprouting, regeneration and circuit formation in the injured spinal cord: factors and activity. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361, 1611–1634.

Markus, A., Zhong, J., Snider, W.D., 2002. Raf and akt mediate distinct aspects of sensory axon growth. Neuron 35, 65–76.

Markus, T.M., Tsai, S.Y., Bollnow, M.R., Farrer, R.G., O'Brien, T.E., Kindler-Baumann, D.R., Rausch, M., Rudin, M., Wiessner, C., Mir, A.K., Schwab, M.E., Kartje, G.L., 2005. Recovery and brain reorganization after stroke in adult and aged rats. Ann. Neurol. 58, 950–953.

Medana, I.M., Esiri, M.M., 2003. Axonal damage: a key predictor of outcome in human CNS diseases. Brain 126, 515–530.

Millan, M.J., 2002. Descending control of pain. Prog. Neurobiol. 66, 355–474.

Morrison, E.E., Costanzo, R.M., 1995. Regeneration of olfactory sensory neurons and reconnection in the aging hamster central-nervous-system. Neurosci. Lett. 198, 213–217.

Muir, G.D., Steeves, J.D., 1997. Sensorimotor stimulation to improve locomotor recovery after spinal cord injury. Trends Neurosci. 20, 72–77.

Naumann, U., Cameroni, E., Pruenster, M., Mahabaleshwar, H., Raz, E., Zerwes, H.G., Rot, A., Thelen, M., 2010. CXCR7 functions as a scavenger for CXCL12 and CXCL11. PLoS One 5, e9175.

Nielson, J.L., Sears-Kraxberger, I., Strong, M.K., Wong, J.K., Willenberg, R., Steward, O., 2010. Unexpected survival of neurons of origin of the pyramidal tract after spinal cord injury. J. Neurosci. 30, 11516–11528.

NSCISC (National Spinal Cord Injury Statistical Center), 2010. Facts and Figures at Glance-2010. [Online]. Available: https://www.nscisc.uab.edu/public_content/pdf/Facts%20and%20Figures%20at%20a%20Glance%202010.pdf. [accessed July 23, 2010].

Opatz, J., Kury, P., Schiwy, N., Jarve, A., Estrada, V., Brazda, N., Bosse, F., Muller, H.W., 2009. SDF-1 stimulates neurite growth on inhibitory CNS myelin. Mol. Cell. Neurosci. 40, 293–300.

Peng, H., Huang, Y., Rose, J., Erichsen, D., Herek, S., Fujii, N., Tamamura, H., Zheng, J., 2004. Signalling pathways involved in the chemotactic activity of CXCL12 in cultured rat cerebellar neurons and CHP100 neuroepithelioma cells. J. Neurosci. Res. 76, 35–50.

Pujol, F., Kitabgi, P., Boudin, H., 2004. The chemokine SDF-1 differentially regulates axonal elongation and branching in hippocampal neurons. J. Cell Sci. 118, 1071–1080.

Rajaofetra, N., Ridet, J.L., Poulat, P., Marlier, L., Sandillon, F., Geffard, M., Privat, A., 1992. Immunocytochemical mapping of noradrenergic projections to the rat spinal cord with an antiserum against noradrenaline. J. Neurocytol. 21, 481–494.

Rekling, J.C., Funk, G.D., Bayliss, D.A., Dong, X.W., Feldman, J.L., 2000. Synaptic control of motoneuronal excitability. Physiol. Rev. 80, 767–852.

Rhodes, K.E., Fawcett, J.W., 2004. Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? J. Anat. 204, 33–48.

Rossi, F., Gianola, S., Corvetti, L., 2007. Regulation of intrinsic neuronal properties for axon growth and regeneration. Prog. Neurobiol. 81, 1–28.

Sánchez-Alcañiz, J.A., Haege, S., Mueller, W., Pla, R., Mackay, F., Schulz, S., López-Bendito, G., Stumm, R., Marín, O., 2011. Cxcr7 controls neuronal migration by regulating chemokine responsiveness. Neuron 69, 77–90.

Scheff, S.W., Bernardo, L.S., Cotman, C.W., 1978. Decrease in adrenergic axon sprouting in the senescent rat. Science 202, 775–778.

Schiwy, N., Brazda, N., Muller, H.W., 2009. Enhanced regenerative axon growth of multiple fibre populations in traumatic spinal cord injury following scar-suppressing treatment. Eur. J. Neurosci. 30, 1544–1553.

Schwab, M.E., Bartholdi, D., 1996. Degeneration and regeneration of axons in the lesioned spinal cord. Physiol. Rev. 76, 319–370.

Siegenthaler, M.M., Ammon, D.L., Keirstead, H.S., 2008. Myelin pathogenesis and functional deficits following SCI are age-associated. Exp. Neurol. 213, 363–371.

Silver, J., Miller, J.H., 2004. Regeneration beyond the glial scar. Nat. Rev. Neurosci. 5, 146–156.

Smith, D.S., Skene, J.H., 1997. A transcription-dependent switch controls competence of adult neurons for distinct modes of axon growth. J. Neurosci. 17, 646–658.

Tysseling, V.M., Mithal, D., Sahni, V., Birch, D., Jung, H., Miller, R.J., Kessler, J.A., 2011. SDF1 in the dorsal corticospinal tract promotes CXCR4+ cell migration after spinal cord injury. J. Neuroinflammation 8, 16.

von Meyenburg, J., Brösamle, C., Metz, G.A., Schwab, M.E., 1998. Regeneration and sprouting of chronically injured corticospinal tract fibers in adult rats promoted by NT-3 and the mAb IN-1, which neutralizes myelin-associated neurite growth inhibitors. Exp. Neurol. 154, 583–594.

Witte, H., Bradke, F., 2008. The role of the cytoskeleton during neuronal polarization. Curr. Opin. Neurobiol. 18, 479–487.

Yankner, B.A., Lu, T., Loerch, P., 2008. The aging brain. Annu. Rev. Pathol. 3, 41–66.

SUPPLEMENTARY MATERIALS

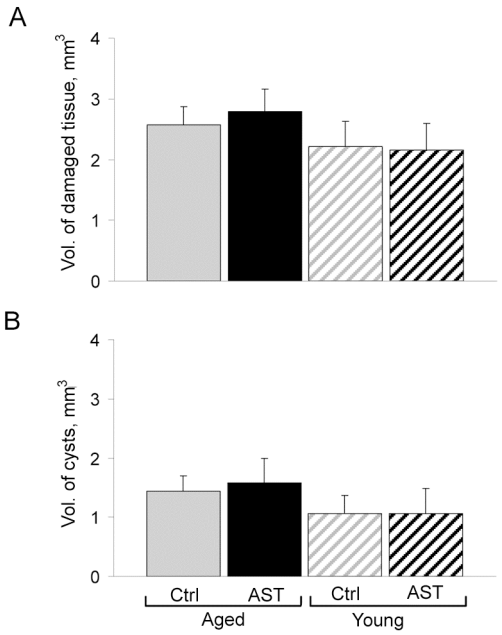
MATERIALS AND METHODS

Quantification of lesion size and cyst volume

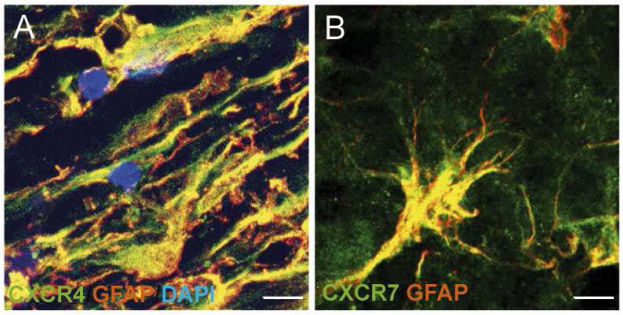
Serial GFAP-stained 50 µm parasagittal sections (spaced 200 µm apart) including the lesion area were used for quantification of damaged spinal tissue and cyst volume. The GFAP-immunonegative lesion areas were outlined and measured using ImageJ. Lesion volume was calculated using the Cavalieri's estimation method (Rosen and Harry, 1990). For cyst volume, cystic cavities were outlined and areas summed per slice.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. **No significant differences in the volume of damaged tissue and cystic cavities between buffer- and AST-treated aged and young animals.** (A) Damaged tissue was defined as GFAP-immunonegative lesion area including cavities. Such areas were outlined and measured with ImageJ in the 50-µm-thick sagittal sections spaced 200 µm apart spanning the entire lesion. Acquired area values were used for the calculation of volume with Cavalieri principle. (B) Mean volume of cystic cavitations was calculated similarly to the damaged area volume. Mann-Whitney U-Test, Holm-Bonferroni correction. Error bars: SEM.



Supplementary Figure 2. **CXCR4 and CXCR7 receptor expression on astrocytes.** Confocal analysis of astrocytes double-stained for GFAP (red) and CXCR4 (green) revealed that CXCR4 (A) as well as CXCR7 (B) is present on astrocytes. Pictures were taken in spinal cord cross sections from young animals. Scale bar = 10 µm.



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SDF-1 stimulates neurite growth on inhibitory CNS myelin

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ABSTRACT

Impaired axonal regeneration is a common observation after central nervous system (CNS) injury. The stromal cell-derived factor-1, SDF-1/CXCL12, has previously been shown to promote axonal growth in the presence of potent chemorepellent molecules known to be important in nervous system development. Here, we report that treatment with SDF-1α is sufficient to overcome neurite outgrowth inhibition mediated by CNS myelin towards cultured postnatal dorsal root ganglion neurons. While we found both cognate SDF-1 receptors, CXCR4 and CXCR7/RDC1, to be coexpressed on myelin-sensitive dorsal root ganglion neurons, the distinct expression pattern of CXCR4 on growth cones and branching points of neurites suggests a function of this receptor in chemokine-mediated growth promotion and/or arborization. These *in vitro* findings were further corroborated as local intrathecal infusion of SDF-1 into spinal cord injury following thoracic dorsal hemisection resulted in enhanced sprouting of corticospinal tract axons into white and grey matter. Our findings indicate that SDF-1 receptor activation might constitute a novel therapeutic approach to promote axonal growth in the injured CNS.

Introduction

Limited central nervous system (CNS) recovery (Ramón y Cajal, 1928) has been ascribed to multiple factors such as the absence of neurotrophins (Lu et al., 2004; Blackmore and Letourneau, 2005) or the formation of a lesion scar acting as a regeneration barrier at the site of injury (Stichel and Müller, 1998; Fawcett and Asher, 1999; Klapka and Müller, 2006). Furthermore, myelin-associated inhibitors, MAIs, were shown to contribute to regenerative failure (Schwab and Bartholdi, 1996; Fournier and Strittmatter, 2001; Filbin, 2003; Domeniconi et al., 2005). In postnatal mammalian CNS injury, Nogo-A (Chen et al., 2000; GrandPrè et al., 2000), myelin-associated glycoprotein (MAG, McKerracher et al., 1994; Tang et al., 2001), and oligodendrocyte-myelin glycoprotein (OMgp, Wang et al., 2002; Vourc'h and Andres, 2004), are efficient axonal outgrowth inhibitors. Interestingly, these molecules were described to abrogate axonal outgrowth upon interaction with a common tripartite receptor complex (Fournier et al., 2001; Filbin, 2003; Domeniconi et al., 2005) in a manner which largely depends on neuronal age and origin (McKerracher et al., 1994; Mukhopadhyay et al., 1994; DeBellard et al., 1996; Ng and Lozano, 1999). Recent studies documented that this impaired tolerance of inhibitory cues is related to the intrinsic state of the affected cells (Song et al., 1998; Ng and Lozano, 1999; Blackmore and Letourneau, 2005). In dorsal root ganglion (DRG) neurons, an increased sensitivity towards the myelin-associated axon growth inhibitor MAG coincides with a molecular switch at postnatal day three/four (P3/P4) which is characterized by decreased intracellular cAMP levels (DeBellard et al., 1996; Cai et al., 2001).

Cyclic AMP regulates numerous cellular processes through different signalling pathways (Antoni, 2000), and elevation of intracellular cAMP levels turned out to be a tool for increasing the regenerative capacity of neuronal cells (Cai et al., 1999; Neumann et al., 2002; Qiu et al., 2002).

One of the extrinsic factors which can promote cAMP-dependent alteration of axonal outgrowth behaviour was shown to be the chemokine SDF-1/CXCL12. Recent studies on guidance cues in embryonic neural development revealed an improvement in neurite outgrowth in the presence of potent neurorepellent molecules such as semaphorin 3A and slit after application of this chemokine (Chalasani et al., 2003, 2007). The α-chemokine SDF-1 is the only known ligand for CXCR4, a coreceptor of T-tropic HIV-1 strains (Oberlin et al., 1996; Nagasawa et al., 1998). While CXCR4- and SDF-1-deficient mice show similar developmental defects and die perinatally (Nagasawa et al., 1996; Ma et al., 1998; Lu et al., 2002), SDF-1 was recently shown to signal through an additional receptor, CXCR7/RDC1 (Balabanian et al., 2005).

In this study, we analyzed the ability of SDF-1α to overcome CNS myelin-associated outgrowth inhibition of myelin-sensitive postnatal

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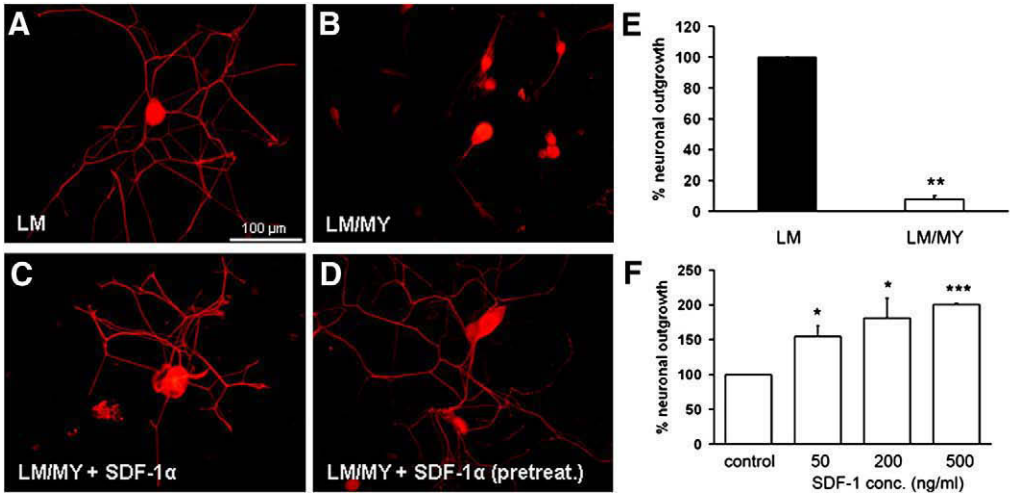

Fig. 1. SDF-1α mediates neurite outgrowth on adult CNS myelin substrate. On a laminin substrate cultured postnatal (P6) DRG neurons show extensive neurite outgrowth (A, E), whereas neurite outgrowth was significantly impaired on a culture substrate containing CNS myelin (B, E). The addition of SDF-1α to DRG cultures plated on a CNS myelin substrate results in improved neurite outgrowth (C). Further preincubation of the neurons with SDF-1α prior to plating of the cells on myelin leads to a significant and dose-dependent increase in the proportion of outgrowing neurons (D, F) when compared to myelin controls lacking the chemokine supplement (B, F). LM, laminin; LM/MY, laminin/myelin. Results in (E, F) are shown as mean ± SEM and are derived from three independent experiments ($n = 3$) each in which the percentage of neurons displaying three or more neurites with a length of at least the cell body diameter was determined. Values are normalized for numbers of untreated cells growing out on laminin (E) or on myelin (F), respectively. *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$ (student's t-test).

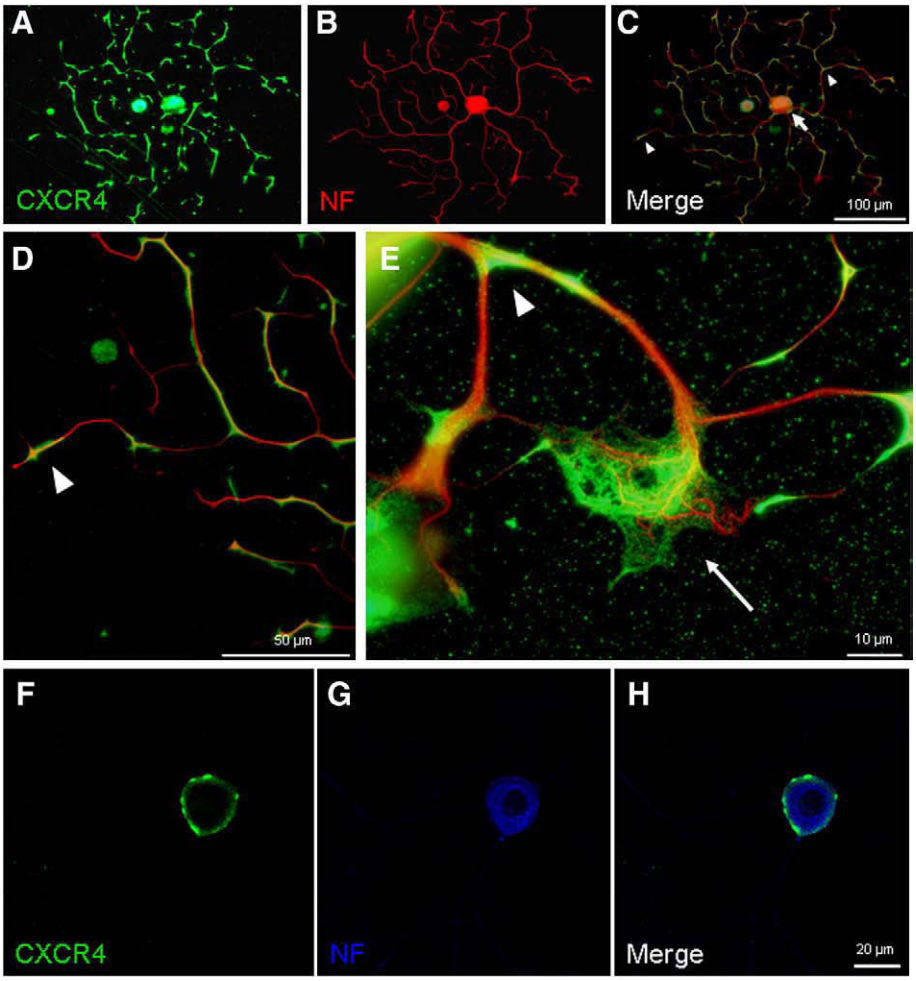

Fig. 2. CXCR4 is expressed on DRG neurons. The cognate receptor to SDF-1, CXCR4, is expressed on cultured DRG neurons, where it is located in a characteristic pattern on neurofilament (NF)-positive cell bodies and neurites (A–C, in C arrow and arrowheads, respectively). CXCR4-immunoreactivity on neurites is prominent near branching points (D, E, arrowheads) and at growth cones (E, arrow). Cell surface localization of CXCR4 is confirmed by confocal laser scan microscopy (F–H). Pictures chosen for presentation are representative of at least five independent experiments ($n \geq 5$).

DRG neurons following preconditioning with this chemokine and investigated the distinct localization and distribution of both CXCR4 and CXCR7/RDC1 receptor molecules in these neurons. Furthermore, we identified both SDF-1 receptors on injured CST axons in spinal cord and observed significantly enhanced axonal sprouting upon continuous local infusion of SDF-1α into the spinal cord lesion.

Results

SDF-1α abrogates impaired axon outgrowth on CNS myelin

To test the neurite growth inhibitory effects of a CNS myelin preparation applied in this study, P6 DRG neurons were plated on substrates composed of either laminin or laminin plus myelin. After 24 h, neurons were immunostained for neurofilaments to visualize cell bodies and neurites and were screened for outgrowth performance. Quantitative analysis of neurite growth on culture substrates with or without myelin revealed a severe impairment of outgrowth in the presence of myelin. Neurite growth was significantly decreased by approximately 80% in comparison to DRG neurons on a myelin-free laminin substrate (Figs. 1A, B, E). The outgrowth impairment of DRG neurons on myelin was not due to a reduction in cell adhesion, as the number of neurons attached to myelin-coated surfaces was even slightly increased as compared to the laminin control (data not shown).

To assess whether SDF-1α is able to reverse myelin-induced neurite outgrowth inhibition, this chemokine was applied to P6 DRG neurons at a concentration range of 50–500 ng/ml for 24 h. Quantitative analysis revealed that in the presence of SDF-1α up to 35% more P6 DRG neurons developed three or more neurites on myelin-coated slides (Figs. 1B, C). Outgrowth improvement was most prominent, although statistically not significant, at a concentration of 500 ng/ml of SDF-1α (data not shown). Conversely, DRG neurons grown on laminin alone displayed no marked effects following application of this chemokine.

Preincubation with SDF-1 significantly enhances neurite outgrowth activity on myelin

To test whether the growth-promoting effect of SDF-1α can be increased by a preincubation period prior to cell seeding, dissociated DRGs were incubated with SDF-1α at a final concentration of 50–500 ng/ml for 1 h and were then plated on a laminin or laminin plus myelin substrate. Following preincubation and plating, cells were cultivated in the presence of SDF-1α for additional 23 h, then fixed and stained for neurofilaments. Quantitative analysis demonstrated that cells pretreated with SDF-1α showed significantly enhanced outgrowth on myelin with a doubling of the number of neurons displaying three or more neurites (Figs. 1D, F), compared to control cultures lacking SDF-1α preincubation (Fig. 1F). Neither preincubation alone nor cultivation of neurons in the presence of SDF-1α without pretreatment led to a significant increase in the number of cells showing neurite outgrowth (data not shown) demonstrating that pretreatment was necessary, but not sufficient, to induce significantly enhanced neurite growth on a CNS myelin substrate.

SDF-1 receptors CXCR4 and CXCR7/RDC1 are coexpressed on cultured DRG neurons

We have characterized the spatial and temporal distribution of the two known G protein-coupled receptors for SDF-1, CXCR4 and CXCR7/RDC1, on myelin-sensitive P6 DRG neurons by double-immunofluorescence. Neurons were counterstained for neurofilaments to visualize cell bodies and neurites. Staining for CXCR4

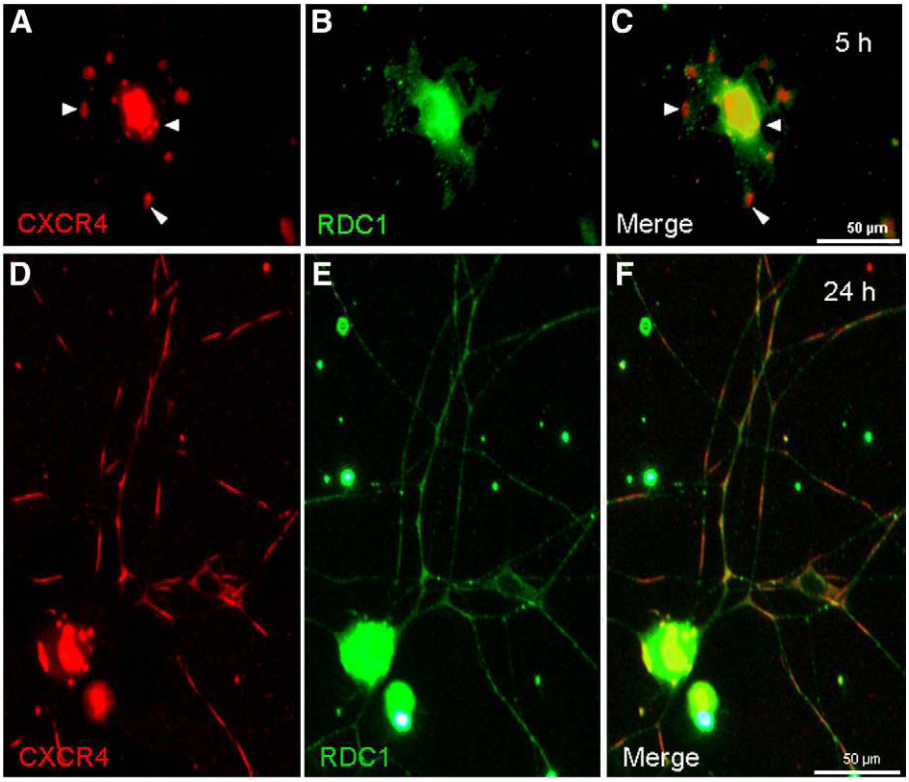

Fig. 3. CXCR4 and CXCR7/RDC1 are coexpressed on cultured DRG neurons. CXCR4 and CXCR7/RDC1-immunoreactivity show distinct localization patterns in early cultures of P6 DRG neurons (A–C). While both receptors to SDF-1 are coexpressed in P6 DRG neurons, a region-specific distribution of CXCR4 (A), but not CXCR7/RDC1 (B), is observed within 5 h after cell plating. Arrowheads indicate CXCR4-immunoreactivity at the tips and bases of outgrowing neurite stumps. After 24 h *in vitro*, CXCR7/RDC1-immunoreactivity is evenly distributed on CXCR4-positive cell bodies and neurites in a granular pattern (D–F). Pictures chosen for presentation are representative of at least three independent experiments ($n \geq 3$).

showed a strong expression of this receptor in nearly all of the DRG neurons after 24 h *in vitro* (Figs. 2A–C). Immunocytochemical analysis at higher magnifications revealed that CXCR4-immunoreactivity was distributed in a characteristic patchy pattern (Figs. 2C–E) along neurites, where it was predominantly localized at branching points and at the growing tips of neurites. A patchy distribution of CXCR4 could also be observed on the outer surface of neuronal cell bodies (Fig. 2C, arrow), as demonstrated by confocal laser scanning microscopy (Figs. 2F–H, see also Supplementary movie 1 and 2). CXCR4-immunoreactivity gradually decreased during prolonged culture periods and was barely detectable on neurofilament-positive neurites after 72 h while, at the same time, staining for CXCR4 remained strong on cell bodies, where it became more evenly distributed (data not shown). A very similar staining pattern could be obtained using a second anti-CXCR4 antibody from a different provider (data not shown).

The second known receptor for SDF-1, CXCR7/RDC1, was expressed and evenly distributed on the cell surface of nearly all DRG neurons, both on neurites and cell bodies colocalizing, in part, with CXCR4 (Figs. 3A–F). A region-specific distribution of CXCR4-immunoreactivity on neuronal cell bodies and on neurites could already be observed within 5 h *in vitro*, where it was preferentially localized to the tips of outgrowing neurite stumps as well as to sites where processes emerged (Figs. 3A, C arrowheads). Conversely, CXCR7/RDC1 was

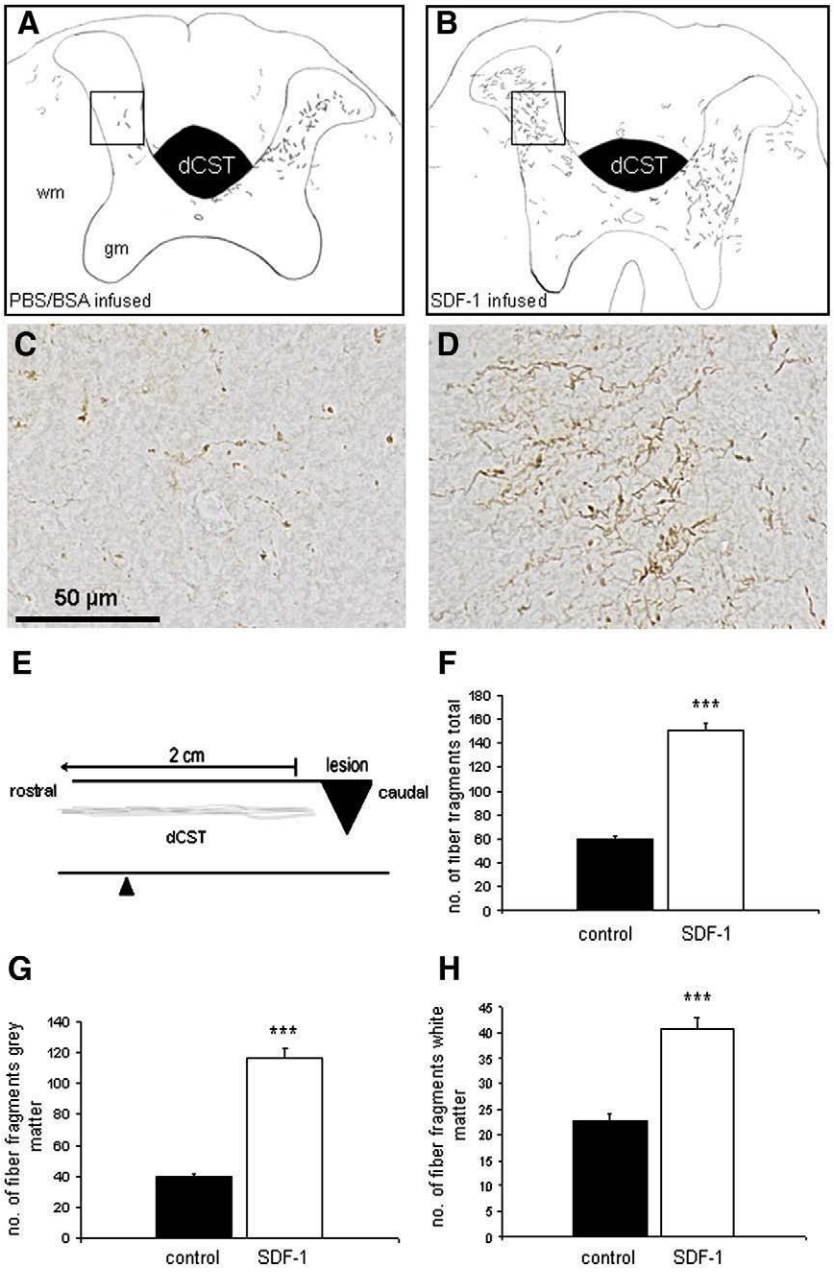

Fig. 4. Quantification of SDF-1α-induced axon sprouting of the lesioned dorsal corticospinal tract (dCST) following local intrathecal chemokine infusion. (A, B) Camera lucida reconstructions of coronal spinal cord sections rostral to the lesion site (indicated by arrowhead in E) with labelled dCST in (A) PBS/BSA infused control rat ($n=6$; gm, grey matter; wm, white matter) and in (B) SDF-treated animals ($n=5$). (C, D) Higher magnification of BDA labelled fibers of the regions boxed in (A, B), respectively. Scale bar in C, 50 µm. (E) Schematic parasagittal section of the spinal cord including the lesion and the counting area (large horizontal bar: 1–20 mm rostral to the lesion). Arrowhead indicates the position where the sections shown in A and B were taken. (F–H) Diagrams show the mean of total CST fibers (F), CST fibers in grey matter (G) and CST fibers in white matter (H) per animal counted in twelve 50 µm sections taken at every 1.5 mm in a region rostral to the lesion as indicated by the large horizontal bar in E. In all areas counted, SDF-1 treated animals show higher numbers of fiber fragments than controls. ***$p<0.001$ (Student's t test). Data are represented as mean ± SEM.

evenly distributed in these cells both at early (Figs. 3B, C) and at late (Figs. 3E, F) stages of cultivation. Our findings demonstrate that both cognate receptors to SDF-1, CXCR4 and CXCR7/RDC1, are present on cultured P6 DRG neurons but appear in different distribution patterns.

Intrathecal infusion of SDF-1α into lesioned rat spinal cord promotes sprouting of corticospinal tract axons

To confirm the neurite outgrowth promoting effects of SDF-1α *in vivo*, we applied this chemokine in a rat model of spinal cord injury. Animals underwent a dorsal hemisection of the spinal cord including the major part of the dorsal corticospinal tract (dCST) and subsequently received an intrathecal infusion of either SDF-1α or PBS/BSA buffer over a period of 1 week. After 5 weeks, animals were sacrificed, BDA-labelled CST axons were immunohistochemically visualized (Fig. 4,A–D) and in each animal axon sprouting was analyzed by counting the fiber fragments in the white and grey matter of 12 coronal sections taken from a region between 1 and 20 mm rostral to the lesion site (Fig. 4E, large horizontal bar). The total number of counted axons in the control group was 59.9±2.8. This was significantly increased in rats which received the SDF-1α chemokine, reaching 150.4±5.6 fibers/animal (Fig. 4F).

In the buffer-infused control animals, BDA-labelling revealed only little sprouting of the dorsal CST in the grey (39.4±2.3 fibers/animal, Fig. 4G) and white matter (22.7±1.4 fibers/animal, Fig. 4H), respectively. The density of CST fibers was significantly higher in the SDF-1α-treated rats, reaching 116.8±6.1 fibers/animal in grey (Fig. 4G) and 40.7±2.1 fibers/animal in white matter (Fig. 4H) regions, respectively.

We further investigated whether the SDF-1α receptors were expressed in intact and lesioned spinal cord of rat. In both intact and lesioned spinal cord numerous BDA-traced CST axons showed immunoreactivity for CXCR4 (Figs. 5A–D) and CXCR7/RDC1 (data not shown). Confocal images revealed that both receptors were present along the axons but tended to be enriched in branching points or endbulb-like structures (Figs. 5E–L). Triple-staining for BDA, CXCR4 and CXCR7/RDC1 revealed that occasionally both receptors colocalized along the same axon fragment (Figs. 5M–P). Immunostainings were confirmed by using a second set of anti-CXCR4 or anti-CXCR7/RDC1 antibodies from a different provider (data not shown).

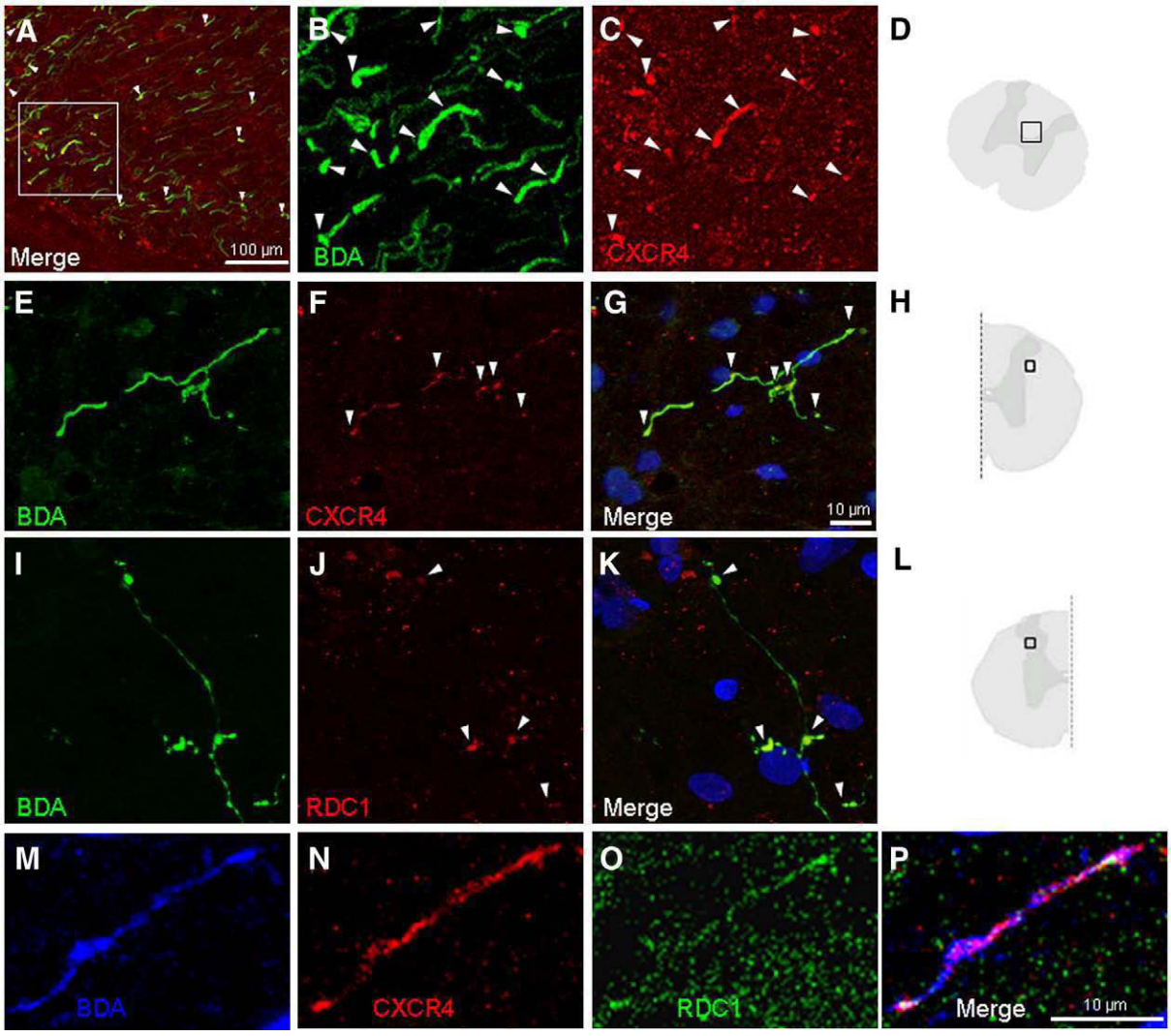

Fig. 5. Both CXCR4 and RDC1 are expressed on numerous CST axons *in vivo*. (A–C) Confocal analysis of BDA-traced CST axons double stained for BDA (B) and CXCR4 (C) reveals that CXCR4 is present on many CST axons (arrowheads in A). Boxed area in (A) is enlarged in (B, C). CXCR4 is enriched in putative branching points and endbulbs of axons (F, G arrowheads). (I–K) Similarly, immunoreactivity for CXCR7/RDC1 is observed in endbulb-like structures (J,K arrowheads). (D, H, L) Images were taken from regions at the grey/white matter border in coronal spinal cord sections taken at 5–10 mm rostral to the lesion from an SDF-1α-treated animal (boxed areas). (M–P) Triple-staining of BDA (M), CXCR4 (N) and CXCR7/RDC1 (O) reveals that both chemokine receptors occasionally overlap along axon fragments (P, merged). (G,) Nuclei are visualized by DAPI labelling.

Discussion

Increasing the regenerative capacity of adult neuronal cells is a challenging aim of modern neurobiological research. Myelin proteins were reported to be among the major impediments to neuronal repair in the injured adult mammalian CNS (Filbin, 2003; Schwab et al., 2005) contributing to neurological deficits which accompany spinal cord injury, presumably by acting through activation of a Rho-dependent pathway (Domeniconi et al., 2005; Alabed et al., 2006; McKerracher and Higuchi, 2006). In this study, we show that neurite outgrowth of postnatal DRG neurons is significantly impaired on rat CNS myelin compared to laminin-only control substrate, and we demonstrate that the α-chemokine SDF-1 is able to promote neurite outgrowth of myelin-sensitive neurons. Application of SDF-1α to postnatal DRG neurons resulted in an efficient abrogation of growth inhibition and led to improved neurite outgrowth in a dose-dependent manner.

Our observations are in accordance with recent findings by Chalasani et al. (2003, 2007) showing that SDF-1 abolishes inhibitory effects of potent repulsive guidance molecules such as semaphorin 3A and C or slit-2 on embryonic retinal ganglion cells. In our study, neurite growth-promoting effects of SDF-1α on inhibitory myelin were significantly increased if cells underwent a preincubation phase in the presence of this chemokine prior to plating on myelin substrate. However, SDF-1α pretreatment of neurons revealed to be necessary, but not sufficient, for neurite outgrowth on a CNS myelin substrate, as withdrawal of SDF-1α after pretreatment largely led to an abrogation of the growth supporting effects. Thus, pretreatment with SDF-1α may act as a conditioning process which facilitates subsequent neurite outgrowth on myelin-coated surfaces. Similar priming effects have been reported for neurotrophins, cAMP or conditioning lesions (Neumann and Woolf, 1999; Cai et al., 1999; Neumann et al., 2002) to improve regenerative capacities. Recent evidence suggests that SDF-1 elevates intracellular cAMP levels through a CXCR4-dependent pathway (Chalasani et al., 2003).

We have also characterized the expression and distribution of the G protein-coupled SDF-1 receptors CXCR4 and CXCR7/RDC1 in myelin-sensitive DRG neurons. CXCR4 is prominently expressed on cultured P6 DRG neurons at early stages of cultivation and is localized to both cell bodies and neurites in a characteristic pattern with preferential appearance at branching points and growth cones. As these structures represent regions of directional growth, CXCR4 might affect neurite branching and/or neurite extension in these cells. Interestingly, a similar region-specific axonal distribution of CXCR4-immunoreactivity was described for hippocampal neurons (Pujol et al., 2004).

Until recently, CXCR4 was regarded as the only SDF-1 receptor (Lazarini et al., 2003; Kucia et al., 2004). However, it could be shown that the former vasoactive intestinal peptide–receptor CXCR7/RDC1 is activated by SDF-1 (Balabanian et al., 2005). As neuronal localization of the CXCR7/RDC1 receptor has not been described thus far, we characterized its spatial and temporal expression patterns on DRG neurons. CXCR7/RDC1 is coexpressed with CXCR4 on P6 DRG neurons, where it could be mapped to cell bodies and neurites. In contrast to the distinct localization of CXCR4, CXCR7/RDC1 is more evenly distributed and its expression remains prominent during the entire period (72 h) of cultivation. SDF-1 may thus activate two different receptors on DRG neurons and presumably affect multiple signalling pathways.

Our *in vitro* observations on the neurite growth supporting function of SDF-1α on inhibitory CNS myelin substrate were further corroborated by *in vivo* studies in a model of traumatic spinal cord injury in rat. Following local intrathecal infusion of SDF-1α into the lesion area, we observed extensive sprouting of transected CST axons into grey and white matter of the rostral stump. Conversely, in injured control animals lacking SDF-1α infusion axon sprouting was very limited.

Both cognate receptors to SDF-1α, CXCR4 and CXCR7/RDC1, are expressed by numerous CST axons in intact and lesioned spinal cord. This is in line with the observations that these receptors are present at low constitutive levels in intact mature cortex neurons (Schönemeier et al., 2008). The presence of CXCR4 and CXCR7/RDC1 receptors in the injured spinal cord, as shown here, suggests direct interactions of SDF-1α with its neuronal receptors to promote axon sprouting. However, it should be noted that, besides direct interactions with neuronal SDF-1 receptors, indirect axon growth-promoting mechanisms could be activated through binding of SDF-1α to receptor-bearing nonneuronal cells. Furthermore, at the current stage it cannot be excluded that in order to react with a regenerative sprouting response, axonal tips or branching points need to express both receptors, CXCR4 and CXCR7/RDC1. Determination of their individual functional roles awaits the generation of conditional knockout mutants for both receptor genes as well as viable receptor-deficient double mutants.

Taken together, we have characterized SDF-1α as a potent activator of *in vitro* as well as *in vivo* neurite regeneration and demonstrated its capacity to overcome CNS myelin-induced neurite outgrowth inhibition of both postnatal PNS and adult CNS neurons. We were able to demonstrate coexpression of both receptor proteins in cultured rat DRG neurons as well as on sprouting CNS axons. Further experiments will be required in order to elucidate the complete pathway employed by SDF-1α to abrogate myelin-induced inhibition of axon growth.

Experimental methods

Animals

Postnatal day 6 (P6) Wistar rat pups were used for preparation of primary cells and adult Wistar rats (200–250 g) were used for spinal cord injury. Animals were bred within the institutional animal facility under pathogen-free conditions and in temperature- and humidity-controlled housing. All experiments were conducted in compliance with the German Animal Protection Law.

Preparation of DRG neurons

DRG neurons were isolated from P6 Wistar rats according to DeBellard et al. (1996). Briefly, rats were anaesthetized and decapitated. DRGs (Th8-L6) were harvested, mechanically dissociated and incubated in 0.25% trypsin/EDTA and 0.3% collagenase type I in DMEM at 37 °C and 10% CO_2 for 30 min. For each experiment, the DRGs of four animals were combined. Following dissociation, the cells were resuspended in DMEM medium (Gibco) containing 10% fetal bovine serum (PAA), nerve growth factor-2.5S (10 ng/ml, Sigma), glutamine (20 mM, Gibco) penicillin/streptomycin (500 U/ml; Gibco) and 5′-fluoro-2′-desoxyuridine, 5′dFUrd (100 nM, Sigma).

Preparation of myelin from adult rat brain

Myelin was prepared from brains of adult female Wistar rats as previously described (Cai et al., 2002). Briefly, whole brains were collected and frozen at −20 °C. The brain stem was harvested and myelin was prepared by ultracentrifugation in a sucrose step gradient. Following determination of total protein amount (Biorad) using bovine serum albumin (fraction IV; Sigma) as a standard, the myelin preparation was diluted to a final protein concentration of 10 μg/ml in 10 mM HEPES buffer (pH 7.15).

Immunocytochemistry

Cultured P6 DRG neurons were fixed in 4% paraformaldehyde (PFA) at room temperature for 10 min and preincubated in normal goat serum at a final concentration of 3% in phosphate buffered saline (PBS). Samples were stained with the following primary antibodies at 4 °C over night: rabbit polyclonal antibody NA 1297 directed against the NF-L, NF-M, and NF-H forms of neurofilament (1:2000, Biotrend),

mouse monoclonal antibody MAB170 to CXCR4 (12G5 clone; 1:100, R&D Systems), rabbit polyclonal antibody BP2211 to CXCR4 (1:100; Acris), rabbit polyclonal antibody IMG-71142 to CXCR7/RDC1 (1:100, Imgenex), and isotype control antibody 550339 to mouse monoclonal IgG_{2A} (1:50, BD Biosciences). For detection of primary antibodies, samples were stained with Biotin-, Alexa 594- or Alexa 488- (1:500, MoBiTec) conjugated anti-mouse or anti-rabbit secondary antibodies at room temperature for 2 h and finally mounted in Fluoromount-G (SouthernBiotech). For staining of neurofilaments, Triton was applied at a final concentration of 0.05% in PBS. Picture acquisition on a Nikon Eclipse TE 200 microscope and data analysis were performed using the Lucia software (Nikon).

Neurite outgrowth assay

For the neurite outgrowth assay, 8-well Permanox labtek chamber slides (Nalge Nunc) were coated with poly-D-lysine (1 mg/ml, Sigma) at room temperature for 1 h and subsequently with laminin (1 μg/ml, Sigma) at 37 °C for 1 h. For myelin coating, myelin preparation from adult rat brain was left to dry on poly-D-lysine/laminin coated slides at room temperature over night at a final concentration of 2 μg of total protein/well. After washing the coated slides once with PBS, the cells were seeded at a final density of 5×10^4 cells/well and incubated in DMEM medium containing 10% fetal bovine serum, nerve growth factor-2.5S (10 ng/ml, Sigma), penicillin/streptomycin (500 U/ml, Gibco), and 5′-fluoro-2′-desoxyuridine (100 nM, Sigma). Cells were left to grow under different conditions at 37 °C and 10% CO_2 for up to 24 h, fixed with 4% PFA and then stained for neurofilaments. For quantitative analysis, nine pictures were taken randomly per well at 4× magnification and the number of neurons showing neurite outgrowth as well as the total number of neurons were determined. We defined neurite growing neurons as neurofilament-positive cells showing three or more neurites each with a length of at least the cell body diameter (Ng and Lozano, 1999; Qiu et al., 2005). In stimulation experiments, cells were incubated with SDF-1α (350-NS, R&D Systems) at a final concentration of 50–500 ng/ml. SDF-1α was applied in a preincubation step at 37 °C and 10% CO_2 for 1 h prior to cell plating.

Spinal cord surgery and infusion of SDF-1α

Spinal cord surgery in adult female Wistar rats (200–250 g), which included transection of the dorsal corticospinal tract (CST), was performed as described previously (Hermanns et al., 2001). The dura was opened at the thoracic level 8 (Th8), and the dorsal CST and dorsal columns were transected using a Scouten wire knife (Bilaney, Germany). Following surgery, the dura was sutured. After filling the catheter, a small hole was made into the dura at a distance of 5 mm caudal to the lesion. The silicon tube catheter was inserted into the subarachnoid space and directed towards the side of injury. The catheter was connected to an osmotic minipump (Alzet) loaded with 200 μl of either SDF-1α (10 μM in PBS/BSA; R&D Systems) or PBS/BSA buffer. Following fixation of catheter and minipump, the overlying tissue was sutured, and the animals were allowed to recover. Application of either SDF-1α or PBS buffer was carried out at a flow rate of 1 μl/h over a time period of 7 days. One week after lesion, the pump was removed in a second surgery.

Anterograde CST axon tracing and immunohistochemistry

Three weeks before sacrifice, all rats received CST axon tracing as described previously (Klapka et al., 2005). A total volume of 3.2 μl biotinylated dextrane amine (BDA, 10%, Molecular Probes) was stereotactically injected into both hemispheres of the sensorimotor cortex. After five weeks, the rodents underwent injection of a lethal dose of hypnorm/dormicum and were then transcardially perfused with 4% PFA (Merck). The spinal cord was removed, postfixed in 4% PFA, and cryoprotected in sucrose (30%, Sigma) for 3–5 days. Spinal cord samples were serially sectioned in a coronal plane in 50 μm thick slices using a freezing microtome. Immunostaining of spinal cord slices for BDA was performed as described previously (Klapka et al., 2005). Briefly, free floating sections were washed in PBS followed by incubation with an avidin–biotin-peroxidase complex (ABC, Vector Laboratories) at 4 °C over night. The next day, tissue was washed in PBS and reacted in 0.015% DAB for 1 min. A brown staining reaction was obtained by adding 0.004% H_2O_2 to the DAB solution, and the staining process was stopped by washing with PBS buffer. The sections were mounted onto slides and embedded in DPX (Fluka). Images were captured with a Keyence BZ-8100E microscope.

For detection of receptor expression *in vivo*, 50 μm free-floating cryostat coronal spinal cord sections were preincubated in 5% donkey serum and 0.1% Triton-X-100 in PBS for 1 h at RT. Samples were stained with the following primary antibodies in 3% donkey serum and 0.1% Triton-X-100 in PBS at 4 °C over night: rabbit polyclonal antibody sc-6190 directed against CXCR4, (1:100, Santa Cruz), mouse monoclonal antibody MAB170 to CXCR4 (12G5 clone; 1:100, R&D Systems), rabbit polyclonal antibody ab12870 to CXCR7/RDC1 (1:100; Abcam) and mouse monoclonal antibody MAB4227 to CXCR7/RDC1 (1:100; R&D Systems). For detection of primary antibodies, samples were stained with Alexa 594- or Alexa 488- (1:300, MoBiTec) conjugated anti-mouse or anti-rabbit secondary antibodies, respectively, and for triple staining BDA was stained with streptavidin-conjugated Alexa 350 (Invitrogen) at room temperature for 2 h and finally mounted in Fluoromount-G (SouthernBiotech).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mcn.2008.11.002.

References

Alabed, Y.Z., Grados-Munro, E., Ferraro, G.B., Hsieh, S.H.K., Fournier, A.E., 2006. Neuronal responses to myelin are mediated by rho kinase. J. Neurochem. 96, 1616–1625.

Antoni, F.A., 2000. Molecular diversity of cyclic AMP signalling. Front. Neuroendocrinol. 21, 103–132.

Balabanian, K., Lagane, B., Infantino, S., Chow, K.Y.C., Harriague, J., Moepps, B., Arenzana-Seisdedos, F., Thelen, M., Bachelerie, F., 2005. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J. Biol. Chem. 280, 35760–35766.

Blackmore, M., Letourneau, P.C., 2005. Changes within maturing neurons limit axonal regeneration in the developing spinal cord. J. Neurobiol. 66, 348–360.

Cai, D., Shen, Y., DeBellard, M.E., Tang, S., Filbin, M.T., 1999. Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. Neuron 22, 89–101.

Cai, D., Qiu, J., Cao, Z., McAtee, M., Bregman, B.S., Filbin, M.T., 2001. Neuronal cyclic cAMP controls the developmental loss in ability of axons to regenerate. J. Neurosci. 21, 4731–4739.

Cai, D., Deng, K., Mellado, W., Lee, J., Ratan, R.R., Filbin, M.T., 2002. Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin *in vitro*. Neuron 35, 711–719.

Chalasani, S.H., Kabelko, K.A., Sunshine, M.J., Littman, D.A., Raper, J.A., 2003. A chemokine, SDF-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. J. Neurosci. 23, 1360–1371.

Chalasani, S.H., Sabol, A., Xu, H., Gyda, M.A., Rasband, K., Granato, M., Chien, C.B., Raper, J.A., 2007. Stromal cell-derived factor-1 antagonizes slit/robo signaling *in vivo*. J. Neurosi. 27, 973–980.

Chen, M.S., Huber, A.B., van der Haar, M.E., Frank, M., Schnell, S., Spillmann, A.A., Christ, F., Schwab, M.E., 2000. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403, 434–439.

DeBellard, M.E., Tang, S., Mukhopadhyay, G., Shen, Y.J., Filbin, M.T., 1996. Myelin-associated glycoprotein inhibits axonal regeneration from a variety of neurons via interaction with a sialoglycoprotein. Mol. Cell. Neurosci. 7, 89–101.

Domeniconi, M., Zampieri, N., Spencer, T., Hilaire, M., Mellado, W., Chao, M.V., Filbin, M.T., 2005. MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. Neuron 46, 849–855.

Fawcett, J.W., Asher, R.A., 1999. The glial scar and central nervous system repair. Brain Res. Bull. 49, 377–391.

Filbin, M.T., 2003. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat. Rev., Neurosci. 4, 1–11.

Fournier, A.E., Strittmatter, S.M., 2001. Repulsive factors and axon regeneration in the CNS. Curr. Opin. Neurobiol. 11, 89–94.

Fournier, A.E., GrandPrè, T., Strittmatter, S.M., 2001. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409, 341–346.

GrandPrè, T., Nakamura, F., Vartanian, T., Strittmatter, S.M., 2000. Identification of the Nogo inhibitor of axon regeneration as a reticulon protein. Nature 403, 439–444.

Hermanns, S., Klapka, N., Müller, H.W., 2001. The collagenous lesion scar — an obstacle for axonal regeneration in brain and spinal cord injury. Restor. Neurol. Neurosci. 19, 130–148.

Klapka, N., Müller, H.W., 2006. Collagen matrix in spinal cord injury. J. Neurotrauma 23, 422–435.

Klapka, N., Hermanns, S., Straten, G., Masanneck, C., Duis, S., Hamers, F.P.T., Müller, D., Zuschratter, W., Müller, H.W., 2005. Suppression of fibrous scarring in spinal cord injury of rat promotes long distance regeneration of corticospinal tract axons, rescue of primary motoneurons in somatosensory cortex and significant functional recovery. Eur. J. Neurosci. 22, 3047–3058.

Kucia, M., Jankowski, K., Reca, R., Wysoczynski, M., Bandura, L., Allensdorf, D.J., Zhang, J., Ratajczak, J., Ratajczak, M.Z., 2004. CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. J. Mol. Histol. 35, 233–245.

Lazarini, F., Tham, T.M., Casanova, P., Arenzana-Seisdedos, F., Dubois-Dalcq, M., 2003. Role of the α-chemokine Stromal cell-derived factor-1 (SDF-1) in the developing and mature central nervous system. Glia 42, 139–148.

Lu, M., Grove, E.A., Miller, R.J., 2002. Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. Proc. Natl. Acad. Sci. U. S. A. 99, 7090–7095.

Lu, P., Yang, H., Jones, L.L., Filbin, M.T., Tuszynski, M.H., 2004. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. J. Neurosci. 24, 6402–6409.

Ma, Q., Jones, D., Borghesani, D.R., Segal, R.A., Nagasawa, T., Kishimoto, T., Bronson, R.T., Springer, T.A., 1998. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc. Natl. Acad. Sci. 95, 9448–9453.

McKerracher, L., Higuchi, H., 2006. Targeting Rho to stimulate repair after spinal cord injury. J. Neurotrauma 23, 309–317.

McKerracher, L., David, S., Jackson, D.L., Kottis, V., Dunn, R.J., Braun, P.E., 1994. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite outgrowth. Neuron 1, 805–811.

Mukhopadhyay, G., Doherty, P., Walsh, F.S., Crocker, P.R., Filbin, M.T., 1994. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 13, 757–767.

Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., Kishimoto, T., 1996. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 382, 635–638.

Nagasawa, T., Tachibana, K., Kishimoto, T., 1998. A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: their functions in development, hematopoiesis and HIV infection. Semin. Immunol. 10, 179–185.

Ng, W.P., Lozano, A.M., 1999. Neuronal age influences the response to neurite outgrowth inhibitory activity in the central and peripheral nervous system. Brain Res. 836, 49–61.

Neumann, S., Woolf, C.J., 1999. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. Neuron 23, 83–91.

Neumann, S., Bradke, F., Tessier-Lavigne, M., Basbaum, A.I., 2002. Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. Neuron 34, 885–893.

Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J.L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J.M., Clark-Lewis, I., Legler, D.F., Loetscher, P., Baggiolini, M., Moser, B., 1996. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 382, 833–835.

Pujol, F., Kitabgi, P., Boudin, H., 2004. The chemokine SDF-1 differentially regulates axonal elongation and branching in hippocampal neurons. J. Cell. Sci. 118, 1071–1080.

Qiu, J., Cai, D., Dai, H., McAtee, M., Hoffman, P.N., Bregman, B.S., Filbin, M.T., 2002. Spinal axon regeneration induced by elevation of cyclic AMP. Neuron 34, 895–903.

Qiu, J., Cafferty, W.B.J., McMahon, S.B., Thompson, S.W.N., 2005. Conditioning injury-induced spinal axon regeneration requires signal transducer and activator of transcription 3 activation. J. Neurosci. 25, 1645–1653.

Ramón y Cajal, S., 1928. Degeneration and Regeneration of the Nervous System. Oxford Univ. Press, London.

Schönemeier, B., Schulz, S., Hoellt, V., Stumm, R., 2008. Enhanced expression of the CXCl12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain. J. Neuroimmunol. 198, 39–45.

Schwab, M.E., Bartholdi, D., 1996. Degeneration and regeneration of axons in the lesioned spinal cord. Physiol. Rev. 76, 319–369.

Schwab, J.M., Bernard, F., Moreau-Fauvarque, C., Chèdotal, A., 2005. Injury reactive myelin/oligodendrocyte-derived axon growth inhibition in the adult mammalian central nervous system. Brain Res. Rev. 49, 295–299.

Song, H., Ming, G., He, Z., Lehmann, G., Tessier-Lavigne, M., Poo, M.M., 1998. Conversion of neuronal growth cone responses form repulsion to attraction by cyclic nucleotides. Science 281, 1515–1518.

Stichel, C.C., Müller, H.W., 1998. The CNS lesion scar: new vistas on an old regeneration barrier. Cell Tissue Res. 294, 1–9.

Tang, S., Qiu, J., Nikulina, E., Filbin, M.T., 2001. Soluble myelin-associated glycoprotein released from damaged white matter inhibits axonal regeneration. Mol. Cell. Neurosci. 18, 259–269.

Vourc'h, P., Andres, C., 2004. Oligodendrocyte myelin glycoprotein (OMgp): evolution, structure and function. Brain Res. Rev. 45, 115–124.

Wang, K.C., Koprivica, V., Kim, J.A., Sivasankaran, R., Guo, Y., Neve, R.L., He, Z., 2002. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417, 941–944.

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4.3. AGE-ASSOCIATED CORTICAL TRANSCRIPTOME DURING SPINAL CORD REPAIR AFTER TRAUMA

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Abstract

Both spinal cord injury (SCI) and aging produce profound changes in gene expression; therefore, aging likely interferes with the success of such therapeutic interventions which were tailored for young patients. Using genome-scale transcriptional profiling, we found that the age-dominated profiles of rat sensorimotor cortex during the acute and subacute phases of thoracic corticospinal tract transection could be shaped to resemble regeneration-associated profiles by local pharmacological treatment to suppress fibrotic scarring. The anti-scarring treatment (AST) consisted of an iron chelator and cAMP that were applied to both aged (22 months) and young adult (2 months) rats suffering from SCI. In combination with previous studies demonstrating regenerative axon growth upon AST in aged animals, these findings support the feasibility of successful AST therapy in elderly patients.

Introduction

There is a growing incidence of spinal cord injury (SCI) among older individuals. The percentage of patients older than 60 years at the time of injury has increased from 4% to 11% since 2000, and the average age has increased from 28.7 years in the 1970's to the current age of 40.7 years [27]. Considering the recent and future dramatic increases in the aging population, there is substantial clinical interest in developing SCI therapies that are effective, regardless of age.

Aging has a profound effect on gene expression [40], whereby down-regulation of mitochondrial genes and up-regulation of the genes involved in inflammation mediate the conserved hallmarks of aging [40, 41]. Dysfunction of energy metabolism and increased inflammation are only two of the important factors that may render an aged nervous system more vulnerable to injury and/or diminish the efficacy of therapies originally established for the young. Transcriptomic profiles of SCI in aged animals have, thus far, not been defined. Following stroke, distinct gene expression profiles in aged and young animals have been reported, which include growth-inhibitory molecules that are induced acutely and growth-promoting factors that have a delayed expression profile in the aged peri-infarcted cortex [5]. Moreover, genome-wide expression analysis of aged and young animals has revealed that different transcriptomes are responsible for stroke-induced sprouting of cortical neurons [23]. Nonetheless, selected genes relating to the regenerative response were similarly induced in both 3- and 20-months-old rats after stroke, indicating that the potential for regenerative responses in the brain remains intact at an older age [30].

SCI elicits massive changes in gene expression in the spinal cord [8] and, as we have previously reported [20, 19], in sensorimotor cortex, starting as early as 1 day post-operation (dpo). These responses increase over time. Moreover, we previously identified a regeneration-associated transcriptomic program underlying long distance axon regeneration [20, 19] along with functional recovery in young adult rats following local injections at the lesion site of an anti-scarring treatment (AST) comprised of an iron chelator (2,2'-dipyridine-5,5'-dicarboxylic acid) and 8-bromo-cyclic adenosine monophosphate (8Br-cAMP) [18, 35].

In this study, we investigated the extent and nature of the difference between the dynamic cortical gene expression profiles of aged (22-months-old) and young (2-months-old) rats following thoracic corticospinal tract (CST) transection and whether the AST-induced regeneration program can be activated in aged animals. GeneChip analyses were performed on layers V/VI of the rat sensorimotor cortex at 1, 7 and 35 dpo, which represented the acute, subacute and chronic stages of SCI, respectively.

Materials and methods

Animal groups and experimental SCI

Dorsal spinal cord hemisection, which included transection of the corticospinal tract at thoracic level eight [18] (lesion group), lesions plus the anti-scarring treatment (AST group) and laminectomy alone (sham group) were investigated in 36 young (150-220 g; 2-months-old) and 36 geriatric (252-449 g; 22-months-old) female Wistar rats (HanTac:WH; Taconic, Ry, Denmark) at 1, 7 and 35 days post-operation (dpo) (Fig. 1A). In total, 72 animals in 18 groups (4 animals/group) were individually analyzed. Anti-scarring treatment (AST) consisted of 8 x 0.2 µl injections of the iron chelator 2,2'-bipyridine-5,5'-dicarboxylic acid (40 mM in Tris buffer) and 4 x 0.25 µl of 8-Br-cAMP (100 µg in Tris buffer) into the lesion site [16]. Control lesion animals received Tris buffer injections. Post-operative care included treatment with the pain killer Rimadyl (Carprofen, 5 mg/kg s.c.), which was delivered 2 days post injury at one injection per day, and with antibiotics (Baytril; Bayer HealthCare, Leverkusen, Germany) for 1 week. The bladder was emptied manually. Institutional guidelines for animal safety and comfort were followed, and all of the surgical interventions and pre- and post-surgical animal care were provided in compliance with the German Animal Protection law (State Off ce, Environmental and Consumer Protection of Nordrhein Westfalia, LANUV NRW).

RNA isolation, processing and hybridization to Affymetrix GeneChips

At 1, 7 and 35 dpo, animals were deeply anesthetized (Narcoren, 100 mg/kg i.p.) and decapitated. Snap frozen left hemispheres were cut (Bregma 0.96 to -1.96) into forty 50-µm-thick coronal cryosections, which were mounted on Superfrost microscopy slides in the cryostat cabinet. The cortical layer V/VI was rapidly dissected out and immediately frozen on dry ice (Fig. 1A). RNA was extracted using a Qiagen RNeasy Microarray tissue kit according to the manufacturer's protocol (version November 2009) in conjunction with on-column DNase digestion. From 150 ng of total RNA, the sense-strand DNA targets were amplified and biotinylated using the two-cycle target labeling protocol (Affymetrix, Santa Clara, CA, whole transcript sense target labeling manual 701880 Rev. 5). Samples from single animals were hybridized individually to the Rat Gene 1.0 ST array (Affymetrix) by BMFZ Core Laboratories at the University of Düsseldorf, Germany. Confirmation of the intactness of the RNA (RNA Integrity Numbers ranged from 8.8 to 10) and the completeness of fragmentation of the biotinylated sense strand DNA was performed with an Agilent Bioanalyzer 2100. The efficiency of hybridization and cRNA amplification were excellent and confirmed using the manufacturer's controls.

Microarray analysis

Quantile normalization, summarization with the RMA16 algorithm and baseline transformation of the data to medians of all samples, among other analysis, were performed with GeneSpring GX software (Agilent Technologies, Santa Clara CA, USA). No arrays failed the quality-control analysis, and the correlation coefficients between biological replicate arrays were high; the lowest value was 0.95. Clustering analysis included building a global cluster of all 27,342 genes using self-organizing maps (SOM) and condition trees using average-linkage hierarchical clustering. All microarray data have been deposited in the ArrayExpress at EMBL-EBI.

The total dataset was analyzed with a 3-way ANOVA (p-value cut-off 0.05, Benjamini-Hochberg correction for the false discovery rate). Comparisons between differentially expressed lesion vs. sham and between AST vs. lesion genes in young and aged animals were made by filtering for the $\log_2$-transformed fold-change of +/-0.25 and by performing t-tests (alpha< 0.12) with the Benjamini-Hochberg correction. Classification according to gene ontology was performed using the online tool DAVID [14], pathway analysis using Ingenuity Pathway Analysis program (Ingenuity Systems, Redwood City, CA, USA) and gene set enrichment analysis using ErmineJ software [12].

Four different gene expression profiles shown in Fig. 2 were validated with RT-qPCR, one gene per each profile-group.

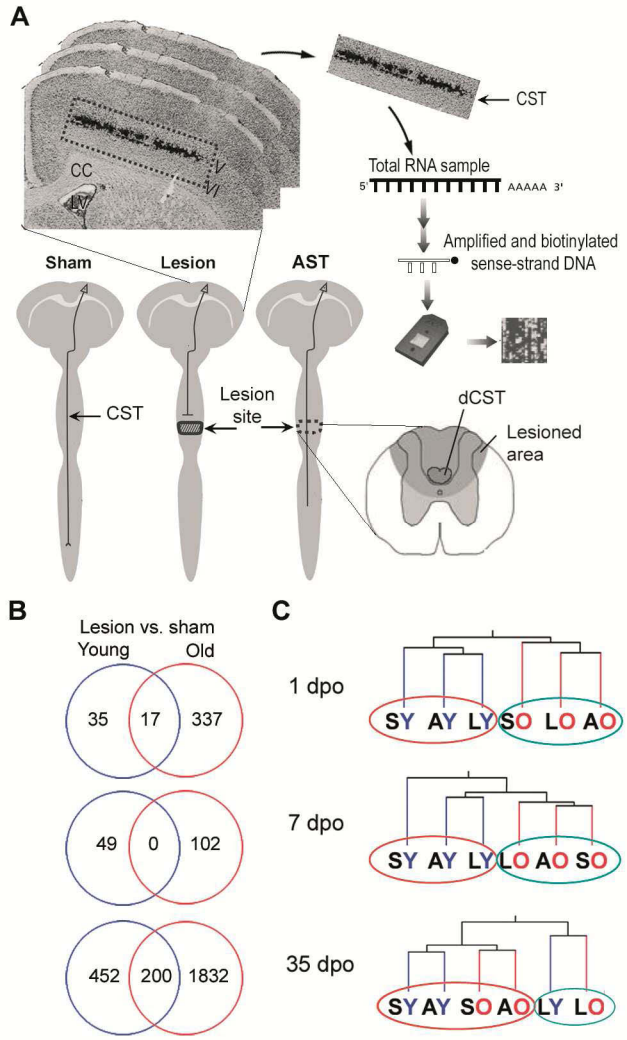

Fig. 1 (A) Sensorimotor cortex layer V containing the corticospinal tract (CST) motor neurons and the subjacent part of layer VI were dissected out of coronal brain cryosections of sham-operated, spinal injured and AST-treated spinal injured 2- and 22-months-old rats at 1, 7 and 35 dpo (n=4 per group). Samples from individual animals were prepared and hybridized each to an individual GeneChip. LV, lateral ventricle, CC, corpus callosum, dCST, dorsal CST. **(B)** There is little overlap between significantly lesion- vs. sham-regulated genes in old and young animals. **(C)** Condition trees at acute, subacute and chronic stages demonstrate the role of age and treatment following SCI. At 1 and 7 dpo, young and aged groups segregated. At 35 dpo, regardless of age, sham and AST-treated groups were more related to each other than to the lesion-only groups. SY = sham young, AY = AST young, LY = lesioned young, SO = sham old, LO = lesioned old, AO = AST old.

Results

Genome-scale transcriptional profiling revealed that the responses to spinal cord injury (SCI) in aged and young animals were distinct. We observed only a small overlap among the genes that were significantly regulated in SCI (Fig. 1B). It should be noted that the gene expression changes in sham-operated animals were very dynamic; this finding has also been reported by others [8]. Therefore, these injury responses were best correlated with their respective sham profiles for age- and time-matched animals (Fig. 2). Over-representation and gene set enrichment analysis of these genes demonstrated that, in addition to common biological processes (such as lipid metabolism), other pathways (such as activation of the complement cascade) were specific to aged animals (Table 1). Different pathways were over-represented in young and aged animals. For example, ubiquitination pathways were up-regulated in the young animals, whereas the

complement system was increased in aged animals at 1 dpo. In addition, cAMP and chemokine signaling were up-regulated in the young animals, and complement system and Notch signaling were up-regulated in the aged animals at 7 dpo. RhoA, Erk5 and PI3K/Akt were up-regulated in the young animals, whereas CNTF and mTOR were up-regulated in aged animals at 35 dpo (Fig. 3).

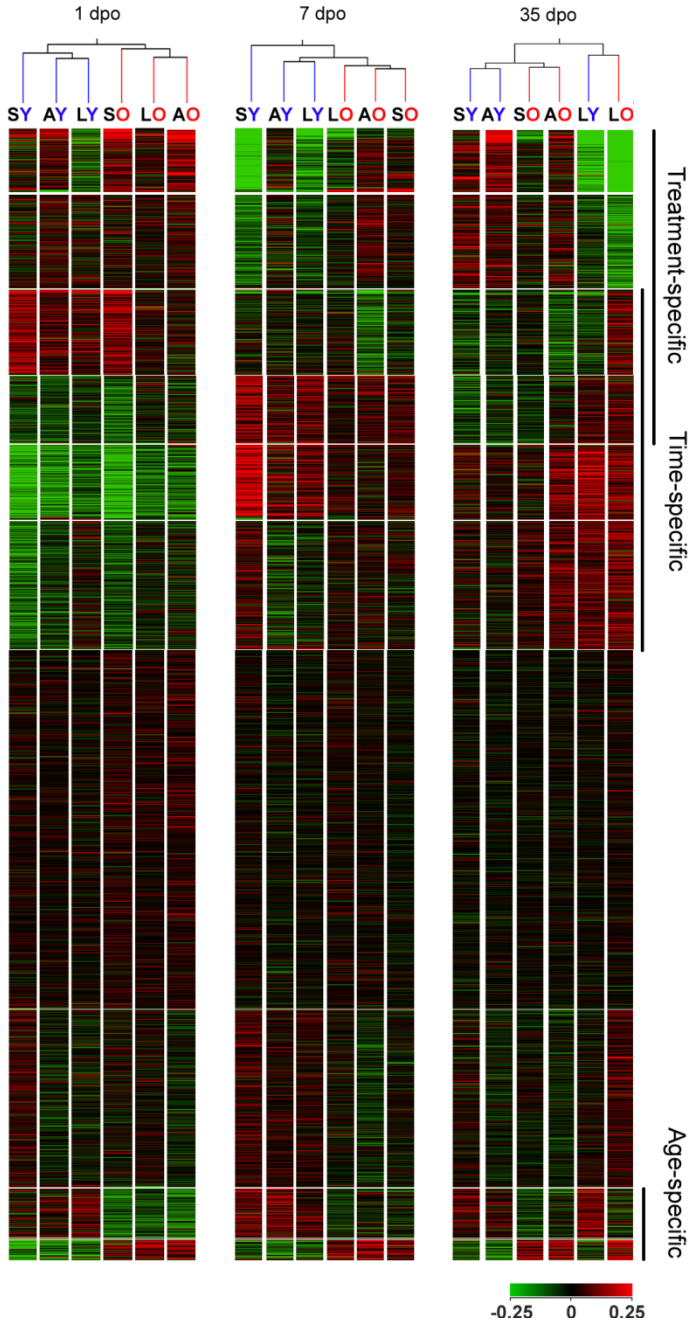

Fig. 2. Global cluster of all 27,342 genes (across all samples), built using the self-organizing maps and average linkage hierarchical clustering of conditions at different time points. SY = sham young, AY = AST young, LY = lesioned young, SO = sham old, LO = lesioned old, AO = AST old.

An increased number of genes (2,617 from a total of 27,342 probe sets) were differentially expressed in an age-specific manner when the treatment conditions and time points were pooled together (3-way ANOVA, $p<0.05$) (Fig. 4). Biological processes, such as axonogenesis/axon guidance, myelination, synaptic transmission, cell adhesion

and activation of immune responses, were enriched. Importantly, there was no interaction between the age and treatment effects, thereby demonstrating the absence of converse regulation in aged and young animals during different treatment conditions.

Group	1 dpo	7 dpo	35 dpo
Young/ aged	Lipid metabolism (Y:D,Cor; A:D)	Ras pathway (Y:Roc, Cor; A:Cor)	Transcription (D)
	Glycolysis (Y:Roc; A:Cor)	RNA splicing (Cor)	Translation (Y:Roc, D; A:D)
	Ras pathway (Cor)	Proteolysis (Y:Roc, Cor; A:Cor)	Ribosome biogenesis (D)
	Microtubule cytoskeleton organization (Cor)	Chromatin modification (Cor)	RNA processing (D)
	Regulation of neurotransmitter levels (Cor)	Glycolysis (Cor)	mRNA splice site selection
Young	Mitochondrial respiratory chain complex assembly (Cor)		
	Activity of caspases and peptidases (Roc)	Cholsesterol metabolic process (Roc, Cor)	
	Ubiquitin-dependent protein degradation (Roc, Cor)	Negative regulation of synaptogenesis	
	Dephosphorylation (Roc)	Dopamine transport (Roc, Cor),	
	GABA signaling pathway (Cor)	Regulation of translation (Cor)	
	Regulation of transcription initiation (Cor)	Nuclear import (Cor)	
Aged	Potein kinase cascade (D)	Complement activation (D)	Transport (D)
	Bone development (D)	Acute inflammatory response (D)	Neuritogenesis (D)
	Apoptosis (D)	Response to wounding	Neurogenesis (Roc,D)
	Chromatin modification (Cor)	Microtubule polymerization (Roc, Cor, D)	Cell cycle (D)
	Regulation of cell adhesion (Cor)	RNA localization (Cor)	Chromatin modification (D)
	Protein processing (Cor)		MAPKKK cascade
			I-kappaB kinase/NF-kappaB cascade
			Small GTPase mediated signal transduction (D)
			Protein modification by small protein removal (D)
			Regulation of cell-cell adhesion (Roc)

Table 1. Enriched biological processes of significantly lesion-regulated genes in young and aged animals during the acute, subacute and chronic stages of SCI determined by DAVID (D), ErmineJ (Roc, receiver-operator curves, Cor, correlation resampling).

Anti-scarring treatments elicited similar gene expression profiles regardless of age at the chronic stage of SCI (Fig. 1C and Fig. 2). In contrast to the age-specific clustering of experimental groups at the acute and subacute stages of SCI, gene expression profiles for AST and sham groups of aged and young animals were more similar to each other at 35 dpo compared to the lesion-only groups. In the most prominent cluster of co-regulated genes (Fig. 5, cluster 1) the AST uniformly counteracted the lesion-induced down-regulation in both aged and young animal groups back towards or even above sham-like expression levels. This gene cluster included growth promoting factors, such as insulin-like growth factor, ciliary neurotrophic factor and brain-derived neurotrophic factor, and signaling molecules, such as CREB1 and JAK2. The remaining three profiles (clusters 2-4) included lesion-induced up-regulation in aged, in young or in both young and aged animals. Importantly, AST-specific regulation did not further boost the lesion-elicited

responses, and AST-specific gene regulation was not reciprocal between young and aged animals.

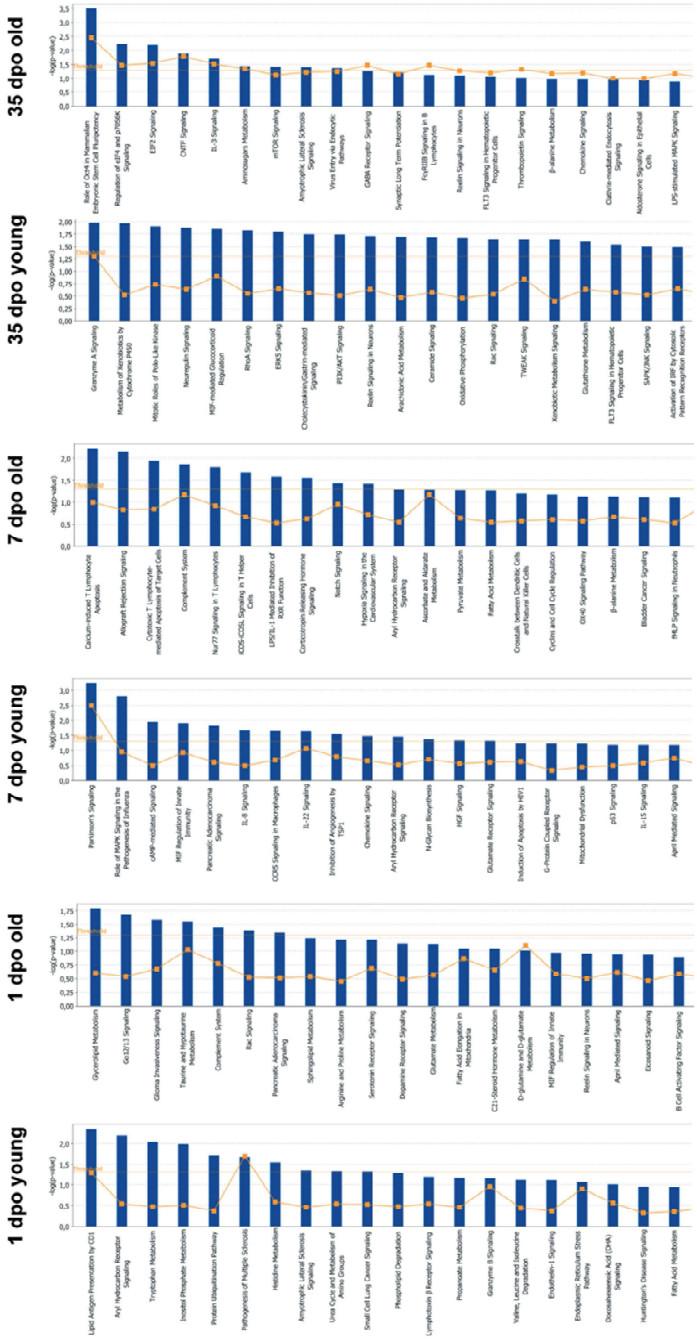

Fig. 3. Pathway analysis of the significantly lesion- vs. sham-regulated genes in aged and young animals at different time points using the Ingenuity curated database of gene interactions of over 23,900 human, rat, and mouse genes. This analysis identifies several key signaling cascades in SCI in young and aged adult by testing the number of genes that were regulated in SCI in a specific pathway versus total number of genes for that pathway in this (right-tailed Fisher's exact test), displayed as the red line with square points. The significance is a measurement for the likelihood that the pathway is associated with the dataset by random chance, the straight red line in the graphs indicates the threshold for a significant association, the –log (0.05).

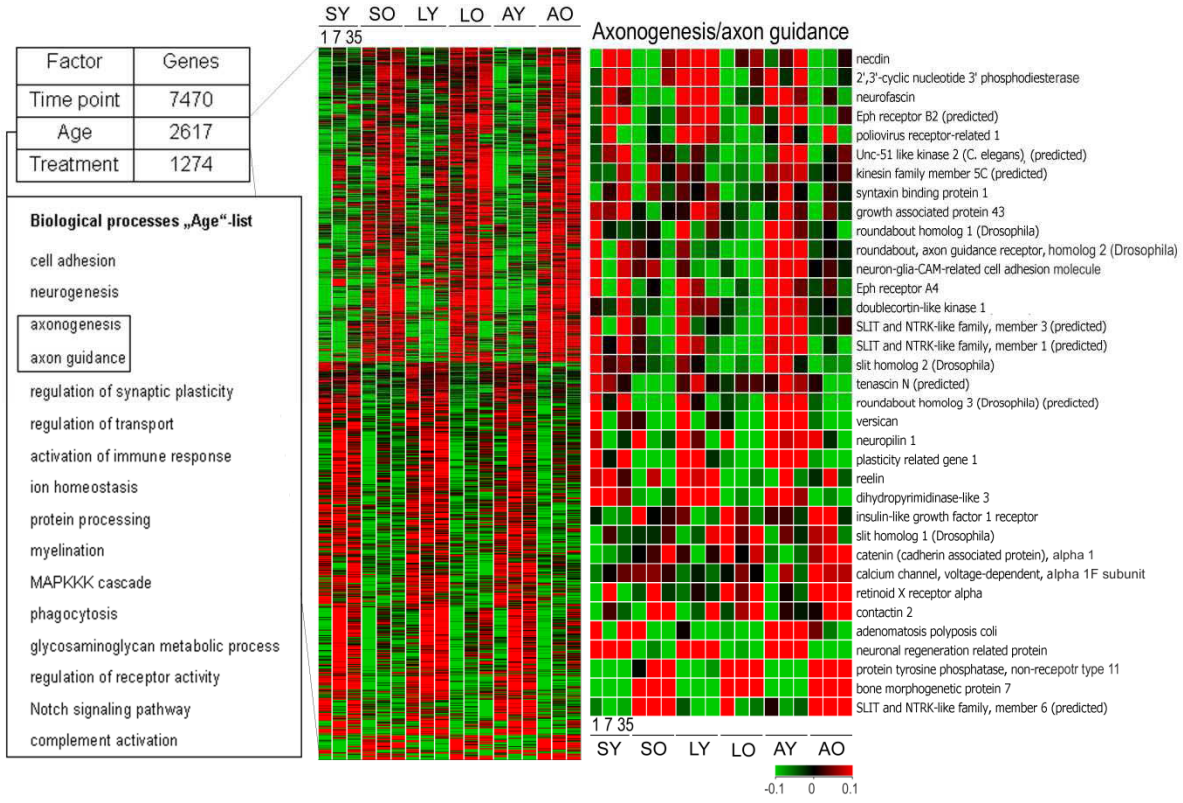

Fig. 4. Aging changes the cortical expression of genes that are relevant to SCI. **(A)** Variance analysis of the total data set indicated that in total 11,361 out of 21,798 gene sets were significantly regulated. **(B)** There were 2,617 genes regulated by age if the data are pooled over time points and treatment conditions. **(C)** Over-representation of biological processes of the age-specific genes and **(D)** axonogenesis/axon guidance group gene expression.

Discussion

Using a systematic approach, we revealed that the initially divergent patterns of lesion-induced gene expression in aged and young animals converge to a similar profile at the chronic stage of SCI upon the therapeutic intervention of an anti-scarring treatment.

The cortical transcriptomes following SCI were age-specific because there was little overlap of significantly SCI-regulated genes between aged and young animals and because several of the biological processes exerted by these genes were unique, including complement activation in aged animals. This particular pathway might be especially unfavorable to regeneration, as it has been shown that inhibition of complement pathways promotes functional recovery and reduces tissue damage [31, 39, 11]. Complement component 3, which is generated upon activation of the complement pathways and leads to production of C3b and iC3b, two opsonins that target apoptotic cells

and promote their clearance by macrophages and microglia [32], and their levels were higher in aged compared to young lesioned animals in all three time points. On the other hand C3a has recently been shown to inhibit proinflammatory cytokines and to contribute to stem cell chemotaxis into areas of inflammation, potentially enhancing tissue repair [36]. Complement component 1 q subcomponent, beta polypeptide (C1qB) was upregulated at 1 dpo and 35 dpo, the levels were significantly higher in aged animals at 1 dpo. In fact, this recognition component of the complement system may be a response to injury as it is upregulated in brain ischemia/reperfusion [15]. Cortical deafferentation has been shown to cause elevations in striatal C1qB mRNA that coincided temporally and overlapped anatomically with the course of degeneration of corticostriatal afferent fibers [29]. In the absence of other complement proteins such as C3a or C5a (this is, at least, the case in young animals at 1 dpo) C1q,improves neuronal viability and neurite outgrowth [1]. It is proposed by analogy with the known role of complement factors in fat tissue, that it is likely that local expression of these factors plays a role in the regulation of fatty acid homeostasis and in energy metabolism cross-talk between different compartments of the peripheral nerve [6].

Lesion-regulated genes were enriched for lipid metabolism, which in the context of SCI has only received little attention. Fatty acids play an important role in neurite outgrowth as structural building blocks for extensive membranes. Polyunsaturated fatty acids (PUFA) such as arachidonic acid (AA) and docosahexaenoic acid (DHA), which are taken up by neurons, provide the needed flexibility and fluidity for membranes [22]. Acyl-CoA synthetases (ACSs) are rate-limiting for fatty acid internalization and, in line with that, ACS2 enhances neurite outgrowth by promoting PUFA internalization [24]. Acyl-CoA synthetase long-chain family member 1 is upregulated at 1 dpo in young animals.

Mitochondria-associated genes which were involved in energy production were enriched in lesioned animals indicating the importance of energy supply for cortical motor neurons (motor cortex layer V) after axotomy. These cells have a higher energy demand than the rest of the cortex [34]. Most genes involved in glycolysis were upregulated already at 1 dpo.

Age-specific expression profiles have also been reported in stroke [5] and stroke-induced sprouting [23]. Paradoxically, in these studies the sprouting neurons in aged animals showed up-regulation of myelin and ephrin receptors. Therefore, the whole transciptome matters, although it might contain genes which are unfavourable to regenerative growth. In our study there was no significant reciprocal gene regulation in either age group. Lesion-dependent gene regulation ranked behind aging-dependent regulation at the acute and subacute stages of SCI. This is likely because of the high proportion of age-specific regulated genes (9.5% of total probe sets), which is in agreement with the literature [40, 41]. Moreover, the age-specific regulated genes were preferentially involved in axonal function, which is highly relevant to SCI.

Anti-scarring treatment, which was locally applied immediately after the lesion, elicited massive changes in gene expression in the cortex at the chronic stage of SCI. As early as 1 and 7 dpo, the variation in gene expression was much less in AST-treated animals relative to lesion-only animals. This difference culminated in highly homogeneous profiles for aged and young animals at 35 dpo, which included the up-regulation of many lesion-dependent down-regulated genes (e.g., growth factors) to the sham-level or higher. This finding is consistent with previous reports demonstrating that an activated regeneration program underlies the increased axonal regrowth of CST in young animals upon AST [18, 35]. Down-regulated by lesion and upregulated by AST were ciliary neurotrophic factor (CNTF), insulin-like growth factor 1 (IGF-1), brain-derived growth factor (BDNF), Kruppel-like growth factor 7 (KLF7), doublecortin (DCX) and activating transcription factor 2 (ATF2). CNTF is able to prevent motoneuron degeneration after axotomy [37, 7] and to support corticospinal motor neuron growth via direct mechanisms [17]. Recently, it was shown that cortical neurons up-regulate a CNTF-mediated neuroprotective signalling pathway in response to the chronic insults or stress in the pathogenesis of multiple sclerosis [10]. Similarly, IGF-1 together with BDNF was shown to enhance specifically axon outgrowth of corticospinal motor neurons [28]. DCX, an endogenous marker of immature neurons, was recently hypothesized to play a role in cortical plasticity and brain repair [2]. DCX-positive cells were present in the whole primate cerebral cortex and expressed glial and/or neuronal markers. However, only the DCX/GFAP positive cells were able to proliferate and reacquire progenitor characteristics [2]. KLF7 is required for neuronal morphogenesis and axon guidance in selected regions of the nervous system including the cortex [21], and this transcription factor might be involved in neurite outgrowth following axotomy. In fact, KLF7 silencing leads to impairment of neurite outgrowth in the neuronal cell line PC12 [4], and Moore et al. showed that KLF7 is the most effective among KLFs to promote neurite outgrowth in cortical and retinal ganglion primary cultures [26]. Interestingly, following axotomy ATF2 is reduced in the dorsal motor nucleus of the vagus nerve and the hypoglossal nucleus in rats [38] and upregulated in sprouting cortical neurons following stroke [23]. Rapid and persistent down-regulation of ATF-2 is a constituent of the long-term neuronal stress response and the reappearance of ATF-2 after several weeks indicates the normalization of neuronal gene transcription following brain injury [25].

Besides changes in neuronally expressed genes we detected changes in non-neuronal (glial) genes. For example, GFAP was increasingly up-regulated following SCI. Interestingly, the cell adhesion molecule ALCAM was found upregulated in our

investigation and also in the study by Li et al. [23]. ALCAM is involved in neurite extension via heterophilic and homophilic interactions [3, 9] and it may further play a role in the binding of T- and B-cells to activated leukocytes and/or mediate interactions between cells of the nervous system. Hemoglobin is usually not associated with neuronal cells. However, hemoglobin protein chains were recently shown to be expressed by neurons and astrocytes and might play a novel role in lesion responses [33]. Hemin-induced HbA and HbB expression in cultured neurons was reduced by deferoxamine treatment [13]. As our AST treatment also includes an iron chelator, the finding that lesion-induced HbB in young rats was reduced by AST is in line with previous observations.

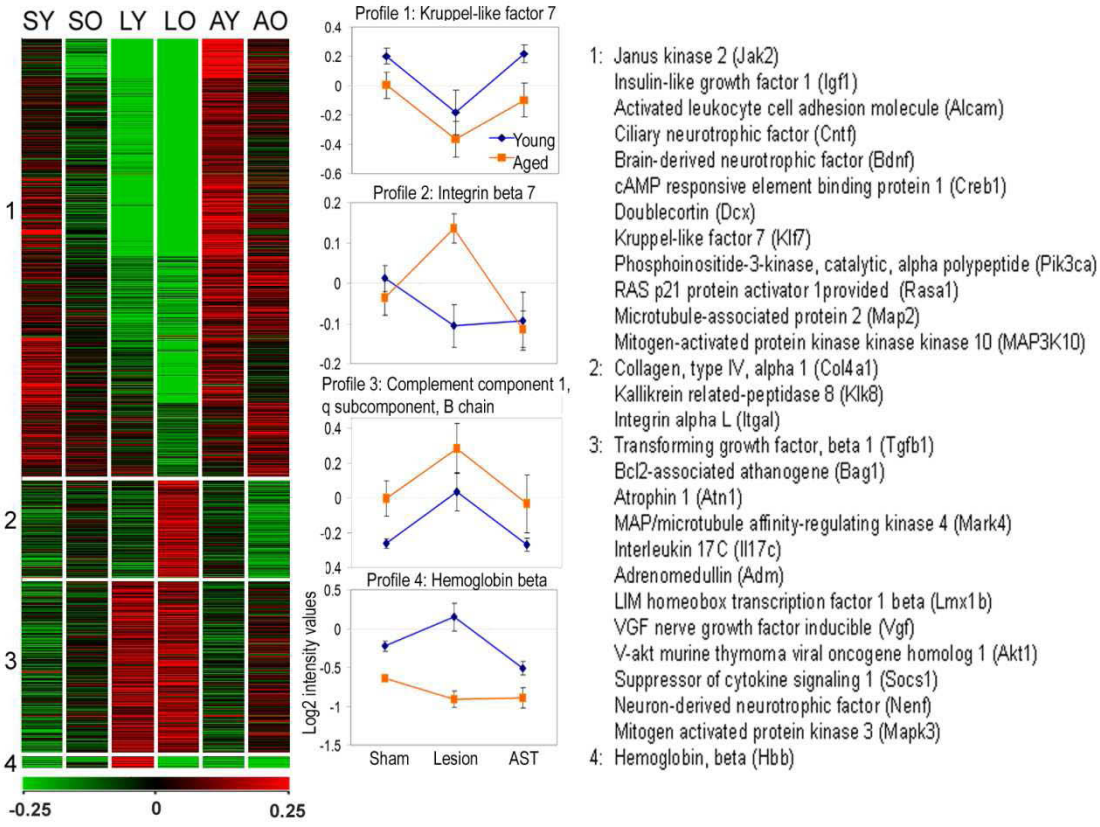

Fig. 5. A total of 2,747 genes that were significantly affected by lesion (sham vs. lesion) and AST (lesion vs. AST) at 35 dpo, grouped into 4 clusters with self-organizing maps according to the expression of the representative genes. SY = sham young, AY = AST young, LY = lesioned young, SO = sham old, LO = lesioned old, AO = AST old.

Age cannot be neglected as a factor in therapeutic responses. As was shown here for SCI and as others have reported for stroke, age-related changes produce distinct transcriptomes. However, despite the initial age-specific differences in gene expression, the local therapeutic intervention in the injured spinal cord by AST activated similar molecular programs in both aged and young animals. Thus AST overruns the age-

specific differences in transcriptomic lesion responses. Together with previous observations regarding axonal regrowth upon AST in aged animals [16], the present results further imply that anti-scarring treatment may offer a feasible therapeutic option in elderly SCI patients.

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References

1. Benoit ME, Tenner AJ Complement protein C1q-mediated neuroprotection is correlated with regulation of neuronal gene and microRNA expression. J Neurosci 31:3459-3469
2. Bloch J, Kaeser M, Sadeghi Y, Rouiller EM, Redmond DE, Jr., Brunet JF (2011) Doublecortin-positive cells in the adult primate cerebral cortex and possible role in brain plasticity and development. J Comp Neurol 519:775-789
3. Burns FR, von Kannen S, Guy L, Raper JA, Kamholz J, et al. (1991) DM-GRASP, a novel immunoglobulin superfamily axonal surface protein that supports neurite extension. Neuron 7:209-220
4. Caiazzo M, Colucci-D'Amato L, Esposito MT et al (2010) Transcription factor KLF7 regulates differentiation of neuroectodermal and mesodermal cell lineages. Exp Cell Res 316:2365-2376
5. Carmichael ST (2003) Gene expression changes after focal stroke, traumatic brain and spinal cord injuries. Curr Opin Neurol 16:699-704
6. Chrast R, Verheijen MH, Lemke G (2004) Complement factors in adult peripheral nerve: a potential role in energy metabolism. Neurochem Int 45:353-359
7. Dale SM, Kuang RZ, Wei X, Varon S (1995) Corticospinal motor neurons in the adult rat: degeneration after intracortical axotomy and protection by ciliary neurotrophic factor (CNTF). Exp Neurol 135:67-73

8. De Biase A., Knoblach SM, Di Giovanni S et al (2005) Gene expression profiling of experimental traumatic spinal cord injury as a function of distance from impact site and injury severity. Physiol Genomics 22:368-381
9. DeBernardo AP, Chang S (1996) Heterophilic interactions of DM-GRASP: GRASP-NgCAM interactions involved in neurite extension. J Cell Biol 133:657-666
10. Dutta R, McDonough J, Chang A et al (2007) Activation of the ciliary neurotrophic factor (CNTF) signalling pathway in cortical neurons of multiple sclerosis patients. Brain 130:2566-2576
11. Galvan MD, Luchetti S, Burgos AM et al (2008) Deficiency in complement C1q improves histological and functional locomotor outcome after spinal cord injury. J Neurosci 28:13876-88
12. Gillis J, Mistry M, Pavlidis P (2010) Gene function analysis in complex data sets using ErmineJ. Nat Protoc 5:1148-59
13. He Y, Hua Y, Keep RF, Liu W, Wang MM, et al. Hemoglobin expression in neurons and glia after intracerebral hemorrhage. Acta Neurochir Suppl 111:133-137
14. Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44-57
15. Huang J, Kim LJ, Mealey R et al (1999) Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. Science 285:595-599
16. Järve A, Schiwy N, Schmitz C, Mueller HW (2011) Effect of aging on axon sprouting and regenerative growth in spinal cord injury. Exp Neurol 231:284-294
17. Junger H, Junger WG (1998) CNTF and GDNF, but not NT-4, support corticospinal motor neuron growth via direct mechanisms. Neuroreport 9:3749-3754

18. Klapka N, Hermanns S, Straten S et al (2005) Suppression of fibrous scarring in spinal cord injury of rat promotes long-distance regeneration of corticospinal tract axons, rescue of primary motoneurons in somatosensory cortex and significant functional recovery. Eur J Neurosci 22:3047-3058
19. Kruse F, Bosse F, Vogelaar CF et al Cortical gene expression in spinal cord repair: insight into the complexity of the neural regeneration program. Front Mol Neurosci. 4:26; doi:10.3389/fnmol.2011.00026
20. Kruse F, Brazda N., Kuery P, Bosse F, Mueller HW (2008) Analyzing complex gene expression profiles in sensorimotor cortex following spinal cord injury and regeneration promoting treatment. In: Müller HW, editor. Neural Degeneration and Repair. Gene Expression Profiling, Proteomics and Systems Biology. Weinheim: WILEY-VCH. pp. 61-89
21. Laub F, Lei L, Sumiyoshi H et al (2005) Transcription factor KLF7 is important for neuronal morphogenesis in selected regions of the nervous system. Mol Cell Biol 25:5699-5711
22. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40:1-94
23. Li S, Overman JJ, Katsman D et al (2010) An age-related sprouting transcriptome provides molecular control of axonal sprouting after stroke. Nat Neurosci 13:1496-1504
24. Marszalek JR, Kitidis C, Dararutana A, Lodish HF (2004) Acyl-CoA synthetase 2 overexpression enhances fatty acid internalization and neurite outgrowth. J Biol Chem 280:23882-23891
25. Martin-Villalba A, Winter C, Brecht S, Buschmann T, Zimmermann M, et al. (1998) Rapid and long-lasting suppression of the ATF-2 transcription factor is a common response to neuronal injury. Brain Res Mol Brain Res 62:158-166

26. Moore DL, Blackmore MG, Hu Y, Kaestner KH, Bixby JL, et al. (2009) KLF family members regulate intrinsic axon regeneration ability. Science 326:298-301
27. NSCISC (National Spinal Cord Injury Statistical Center). Facts and Figures at Glance-2011.[Online]. https://www.nscisc.uab.edu/public_content/pdf/Facts%202011%20Feb%20Final.pdf, Accessed 19.06.2011
28. Ozdinler PH, Macklis JD (2006) IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. Nat Neurosci 9:1371-1381
29. Pasinetti GM, Johnson SA, Rozovsky I et al (1992) Complement C1qB and C4 mRNAs responses to lesioning in rat brain. Exp Neurol 118:117-125
30. Petcu EB, Sfredel V, Platt D, Herndon JG, Kessler C, Popa-Wagner A (2008) Cellular and molecular events underlying the dysregulated response of the aged brain to stroke: a mini-review. Gerontology 54:6-17
31. Qiao F, Atkinson C, Song H, Pannu R, Singh I, Tomlinson S (2006) Complement plays an important role in spinal cord injury and represents a therapeutic target for improving recovery following trauma. Am J Pathol 169:1039-1047
32. Rahpeymai Y, Hietala MA, Wilhelmsson U, et al (2006) Complement: a novel factor in basal and ischemia-induced neurogenesis. The EMBO Journal 25:1364–1374
33. Richter F, Meurers BH, Zhu C, Medvedeva VP, Chesselet MF (2009) Neurons express hemoglobin alpha- and beta-chains in rat and human brains. J Comp Neurol 515:538-547
34. Rossner MJ, Hirrlinger J, Wichert SP et al (2006) Global transcriptome analysis of genetically identified neurons in the adult cortex. J Neurosci 26:9956-9966
35. Schiwy N, Brazda N, Muller HW (2009) Enhanced regenerative axon growth of multiple fibre populations in traumatic spinal cord injury following scar-suppressing treatment. Eur J Neurosci 30:1544-1553

36. Schraufstatter IU, DiScipio RG, Zhao M, Khaldoyanidi SK (2009) C3a and C5a are chemotactic factors for human mesenchymal stem cells, which cause prolonged ERK1/2 phosphorylation. J Immunol 182:3827–3836.
37. Sendtner M, Kreutzberg GW, Thoenen H (1990) Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. Nature 345:440-441
38. Shimokawara T, Yamada E, Masui K et al (2002) Changes in expression of p38 mitogen-activated protein kinase in the dorsal motor nucleus of the vagus nerve and hypoglossal nucleus after axotomy in adult rats. Neuropathology 22: 261-268.
39. Singhrao SK, Neal JW, Rushmere NK, Morgan BP, Gasque P (2000) Spontaneous classical pathway activation and deficiency of membrane regulators render human neurons susceptible to complement lysis. Am J Pathol 157:905–918
40. Yankner BA, Lu T and Loerch P (2008) The aging brain. Annu Rev Pathol 3:41-66
41. Zahn JM and Kim SK (2007) Systems biology of aging in four species. Curr Opin Biotechnol 18:355-359

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Molecules in focus

SDF-1/CXCL12: Its role in spinal cord injury

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ABSTRACT

The chemokine stromal cell-derived factor 1 (SDF-1/CXCL12), is not only the most ancient, but also one of the most potent chemotactic factors. Orchestrating the migration of cells as well as promoting axon outgrowth in the presence of myelin inhibitors, SDF-1 is fundamental to central nervous system development, homeostasis and traumatic injury. SDF-1 attracts endogenous stem/precursor cells and immune cells to the injury site and, upon local infusion, enhances axonal sprouting following spinal cord injury. Together these features make SDF-1 a very exciting molecule for spinal cord repair.

1. Introduction

SDF-1, also known as CXC-family chemokine CXCL12, was originally cloned from a mouse bone marrow stromal cell line by signal sequence trap cloning strategy (Tashiro et al., 1993) and first described as a pre-B cell stimulating factor (Nagasawa et al., 1994). Two decades of intensive research have revealed the central role of this chemokine, conserved from zebra fish to human, in development and physiology (Zlotnik et al., 2006). Deletion of the genes encoding SDF-1, or its G-protein coupled receptors CXCR4 (Oberlin et al., 1996) and CXCR7 in mouse (Balabanian et al., 2005) is perinatally lethal (Nagasawa et al., 1996; Zou et al., 1998; Sierro et al., 2007). Interestingly, apart from predominant heart defects in the CXCR7 knockout mice, the CXCR4 or SDF-1 deficient mice show, in addition, disturbed hematopoiesis, lymphopoiesis and developmental abnormalities in the hippocampus, cerebellum, retina and spinal cord. The latter mutants presumably suffer from perturbed proliferation, survival and migration of neuronal progenitors and deficits in axonal pathfinding. Here, we focus on biological functions of SDF-1 in spinal cord injury (SCI).

2. Structure

All six human (α, β, γ, δ, ε, φ) and three known rat and murine SDF-1 isoforms (α, β, γ) are alternative splicing products of one common mRNA precursor molecule (Shirozu et al., 1995; Gleichmann et al., 2000; Yu et al., 2006) and differ only in their use or non-use of an alternative fourth exon (Fig. 1).

SDF-1 has been reported to shift between a monomeric and dimeric structure depending on solution conditions. While SDF-1 dimers, which preferentially form at higher concentrations halt cell migration, monomers promote chemotaxis *in vivo* (Drury et al., 2011). Numerous basic AAs in SDF-1 give rise to a notable positive charge which further increases with the length of the splicing variants. It is assumed that the positive charges at the C-terminus stabilize receptor binding by interaction with glycosaminoglycans (GAGs) on the cell surface (Laguri et al., 2007).

3. Expression, activation and turnover

SDF-1 and its receptors are ubiquitously expressed in many tissues including the CNS. In the spinal cord, the main sources of SDF-1 are the dorsal corticospinal tract (dCST) and the meninges, as observed in the SDF-GFP mouse (Tysseling et al., 2011). CXCR4 is strongly expressed in the ependymal cell layer around the central canal (Jaerve et al., 2011; Tysseling et al., 2011). Notable expression of CXCR7 is also associated with the ependymal cell layer and, in addition, with blood vessels in the spinal cord (A.J., unpublished data). A similar distribution of CXCR7 was recently described for brain (Schönemeier et al., 2008). Immunohistochemistry combined with the tyramide signal amplification allowed the detection of low levels of both CXCR4 and CXCR7 on dCST, astrocytes and on structures resembling motor neurons in the ventral horn, whereby CXCR7 staining in dCST was fainter than that of CXCR4 which was restricted to branching points of axons (Opatz et al., 2009; Jaerve et al., 2011).

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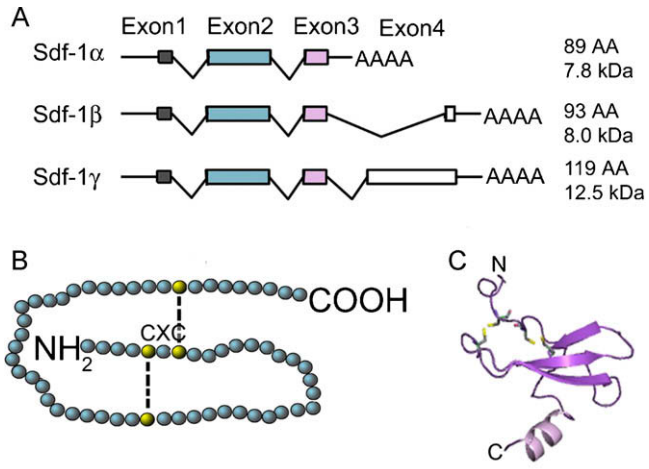

Fig. 1. Structure of SDF-1. (A) Alternative splicing gives rise to several isoforms which differ in their carboxyterminal length. SDF-1α is the smallest but predominant isoform with 89 amino acids (AA) including a signaling peptide of 19 AA, which is cleaved off in the mature protein. SDF-1β isoform carries four additional AA at the C-terminus (93 AA), whereas SDF-1γ is 30 carboxyterminal AA residues longer than SDF-1α (119 AA) because of an in-frame insertion of 2572 nucleotides downstream of codon 89. All splice variants encode functional 8–14 kDa secretory proteins (Yu et al., 2006). (B) SDF-1 carries four conserved cysteine residues comprising two disulphide bonds (indicated as yellow perls), the two amino-terminal cysteins are separated by a single residue that is characteristic to the CXC family of chemokines. (C) 3-D structure according to Crump et al. (1997); N and C indicate the N- and C-termini, respectively. SDF-1 has the typical chemokine fold: a flexible N-terminal region, a loop following the CXC motif, three antiparallel β-strands in a Greek key-like arrangement, and a C-terminal α-helix (Crump et al., 1997). According to the two-site model of receptor activation, the core region (12–17 AA) is docking first to the receptor and then the N-terminal signaling domain can bind and activate the receptor.

SDF-1 binding to CXCR4, but not to CXCR7, is known to elevate intracellular Ca^{2+} levels (Levoye et al., 2009). Chemotaxis and survival have been reported to be controlled by CXCR4 signal transduction via PI3K, PLC/PKC, and MAPKp24/44 (ERK1/2), while CXCR7 seems to regulate primarily the availability of membrane-bound CXCR4 by sequestering SDF-1 from the cell surface (Sánchez-Alcañiz et al., 2011). Astrocytic proliferation is dependent on ERK and PI3K pathways (Bajetto et al., 2001), and chemotaxis of immature neurons depends on ERK signaling (Lazarini et al., 2000). Antirepellent activity was shown to be mediated by the CXCR4/$G\alpha_i$ pathway in which calcium activated calmodulin stimulates an adenylate cyclase to elevate cAMP levels and subsequently activates PKA (Chalasani et al., 2003). However, activation of G-proteins of the $G\alpha_{i/o}$ class typically inhibits adenylate cyclase, an enzyme generating cAMP, whereas the $G\alpha_s$ type directly stimulates it. Therefore, the rather paradoxical finding of the latter study was explained by Twery and Raper (2011) in a model in which multiple G protein components cooperate to produce the cAMP levels required for SDF1 antirepellent activity. Control of axon elongation via an SDF-1alpha/Rho/mDia pathway in cultured cerebellar granule neurons has been described (Arakawa et al., 2003). Moreover, intracellular interaction of CXCR4 and CXCR7 has recently been shown to change SDF-1 signaling (Levoye et al., 2009).

Steady-state levels of SDF-1 are maintained through regulation of the degradation process, which is rapid in blood and tissues (Davis et al., 2005). Splice variant-specific persistence in the blood may be the cause for different inflammatory reactions following stroke – heavy in rodents, milder in humans – as only the α-isoform is found in the human brain, whereas in the mouse and rat brains SDF-1β and SDF-1γ are most abundant, respectively (Stumm et al., 2002; Gleichmann et al., 2000; Yu et al., 2006).

4. Biological function

4.1. SDF-1 orchestrates cell migration in SCI

The rise in SDF-1 levels following CNS injury is mostly associated with astrocytes (Hill et al., 2004; Miller et al., 2005) and forms a gradient to guide immune and stem cells towards the injury site (Fig. 2). Through interaction with CXCR7 secreted SDF-1 could promote astrocytic proliferation, which is characteristic to CNS trauma (Odemis et al., 2010). SDF-1/CXCR4 signaling, on the other hand, induces fast elevation of intracellular Ca^{2+} and glutamate release from astrocytes and thus might modulate neuronal excitability. Unfortunately, in the presence of activated microglia, which is activated within minutes to hours after SCI, the release of glutamate along with TNFα and prostaglandins is dramatically amplified and probably cytotoxic to neurons (Bezzi et al., 2001). Because prolonged exposure to lipopolysaccharide or TNFα leads to down-regulation of CXCR4 in primary astrocytes (Han et al., 2001), down-regulation of CXCR4 could be a mechanism to diminish glutamate excitotoxicity *in vivo* as well. Microglial upregulation of CXCR4 (Tysseling et al., 2011) and migration towards SDF-1 could be beneficial, as these cells produce also neuroprotective molecules such as TGFβ, macrophage colony stimulating factor receptor (MCSFR) and IL-10 (Wang et al., 2008; David and Kroner, 2011).

In comparison to posttraumatic astroglial production of SDF-1, meningeal cells express high levels of SDF-1 in both intact and injured spinal cord (Tysseling et al., 2011). The latter cells have been shown to ssignificantly improve remyelination if cotransplanted with olfactory ensheating cells (OECs) (Lakatos et al., 2003) and to establish a permissive environment for axon regeneration after SCI in newts (Zukor et al., 2011). Therefore, the role of meningeal cells in SDF-1 expression and function following SCI deserves further investigation.

In SCI, a peak in SDF-1 mRNA was measured at 7 days post injury (dpi) at the lesion site and correlated well with the recruitment of stem cells which were injected into the blood stream (Takeuchi et al., 2007). Ependymal cells, an endogenous stem cell pool in the adult spinal cord (Barnabé-Heider and Frisén, 2008), continuously express high levels of CXCR4 (Tysseling et al., 2011; Jaerve et al., 2011). It has been reported that at 4 dpi ependymal cells start to migrate towards the injury site in the dorsal funiculus, accumulate in the glial scar over time and differentiate to scar-forming glial cells and, to a lesser extent, to oligodendrocytes (Meletis et al., 2008). It has been shown recently that oligodendrocyte precursor cells (OPC) maturate and remyelinate upon SDF-1 interaction with CXCR7 (Göttle et al., 2010), whereas signaling via CXCR4 influences OPC migration and survival (Dziembowska et al., 2005).

SDF-1 is known to attract immune cells into CNS lesions. A massive infiltration of $CXCR4^+$ macrophages was first observed at 2 weeks following SCI (Tysseling et al., 2011). Importantly, alternatively activated (AA/M2) macrophages which can be growth promoting show increased expression levels of CXCR4, while classically activated (CA/M1) macrophages, which can be cytotoxic, predominantly express CCR7 (Martinez et al., 2006). Furthermore, AA macrophages show a higher motility than CA macrophages and are preferentially attracted by neuronal conditioned medium (Vereyken et al., 2011).

4.2. SDF-1 stimulates axon outgrowth in SCI

Besides stimulation of cell migration, SDF-1 is able to promote neurite outgrowth of cultured neurons in the presence of inhibitory CNS myelin as we and others have previously shown (Chalasani et al., 2003; Opatz et al., 2009). The lack of limb innervation in

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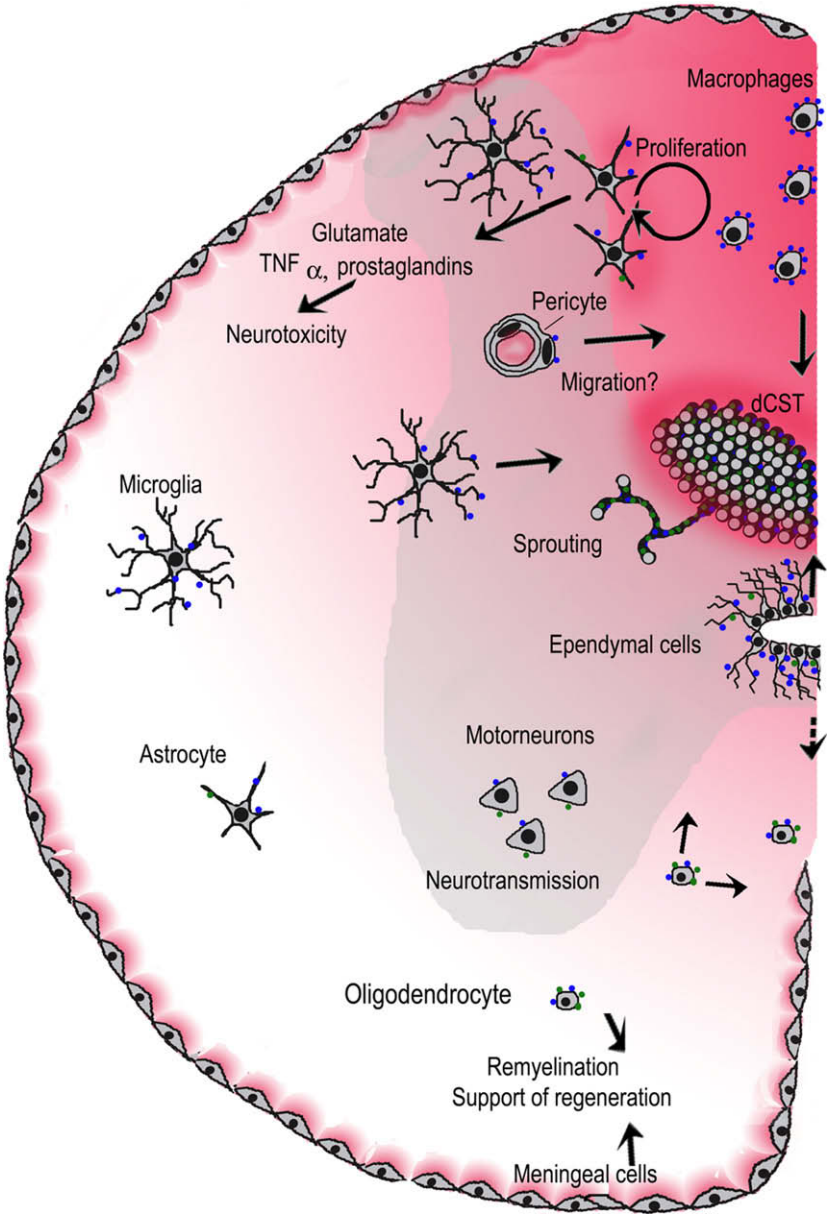

Fig. 2. Biological role of SDF-1 in SCI. Following SCI, SDF-1 is produced by astrocytes, endothelial cells and infiltrating macrophages as well as by the dorsal corticospinal tract (dCST) and meningeal cells, which express SDF-1 also in the intact spinal cord. Consequently, a gradient of SDF-1 (red) forms in the spinal cord (one half of spinal cord is depicted), which attracts CXCR4 expressing cells such as microglial cells/macrophages, ependymal stem cells, vascular precursors, oligodendrocytes, macrophages, bone marrow-derived stem cells and presumably pericytes to the injury site (here, the injury site is the dorsal part of the spinal cord; the migration is indicated by arrows; note that SDF-1 expressing meninges also attracts cells). SDF-1 increases axonal sprouting and recruits stem cells which contribute to neuroprotection. Vascular progenitor cells are involved in neovascularization which supports neuronal regeneration. Ependymal spinal cord stem cells give rise to astrocytes and oligodendrocytes. Oligodendrocyte precursors proliferate, differentiate and remyelinate axons upon SDF-1. Infiltrating macrophages and activated microglia can either be supportive or detrimental to regeneration. For example, activated microglia together with astrocytes release high amounts of glutamate, TNFα and prostaglandins causing further damage to neurons. However, it is possible that especially CXCR4$^+$ anti-inflammatory macrophages are recruited by SDF-1, facilitating regenerative processes. GM, grey matter; WM, white matter.

CXCR4-deficient mice supports the idea that SDF-1 may counteract neurite repellent cues such as semaphorin 3A (Odemis et al., 2005). As myelin inhibition is considered to be a major cause of the very limited axon regeneration in adult CNS, the ability of SDF-1 to neutralize myelin inhibition is highly desirable for SCI. We could demonstrate that local infusion of SDF-1 into the injured spinal cord resulted in enhanced sprouting of the dCST axons as well as serotonergic and tyrosine hydroxylase-positive fibers into white and grey matter rostral to the injury site (Opatz et al., 2009; Jaerve et al., 2011), possibly through counteracting myelin inhibitors. In the latter two fiber systems expression of SDF-1 receptors could not be detected by immunohistochemistry. Thus sprouting of 5-HT and TH-positive axon tracts is probably indirectly mediated by SDF-1, whereas in the dCST, which bears both receptors, one or both of the SDF-1 receptors could be directly involved. SDF-1/CXCR4 signaling has been described to elevate intracellular cAMP levels (Chalasani et al., 2003) and subsequently induce regeneration-associated genes such as Arginase I, which catalyzes the production of polyamines (Cai et al., 2002). Our preliminary cell culture data support a functional role of CXCR4, as blocking of CXCR7 further increased neurite outgrowth (F.B., unpublished data). However, proof of principle regarding the role of CXCR4 and CXCR7 in axon growth should come from future studies with receptor conditional knockout mice.

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5. Potential role of SDF-1 in promoting regenerative responses in SCI

SDF-1α is an interesting compound for regenerative medicine for its ability to attract different kinds of stem/progenitor cells and promote axon growth. Several studies have demonstrated that the CXCL12:CXCR4 pathway regulates homing of engrafted stem cells to sites of tissue damage within the CNS (Takeuchi et al., 2007; Shyu et al., 2008a; Carbajal et al., 2010) which improved recovery processes in SCI, stroke and multiple sclerosis, respectively. Supporting this, treatment with anti-SDF-1α blocking serum resulted in a marked impairment in migration and proliferation of engrafted neural stem cells (Carbajal et al., 2010). It has not been demonstrated whether endogenous stem cells/progenitor cells are also mobilized by SDF-1 and if application of SDF-1 would further enhance their mobilization in order to activate/enhance body's own repair processes, largely because of technical challenges in tracking the small number of endogenous stem cells in static studies (Tysseling et al., 2011). However, evidence from skeletal regeneration and wound healing studies supports this hypothesis, as locally administered SDF-1 stimulates recruitment of endogenous stem/progenitor cells which promote skeletal regeneration (Fujio et al., 2011), and hydrogel-delivery of SDF-1 significantly promotes wound healing by enhancing endothelial cell invasion into the wound (Henderson et al., 2011). For example, effective recruitment of pericytes may serve as a therapeutic option to improve microcirculation, or promotion of oligodendroglia recruitment and differentiation could support remyelination in the injured CNS. SDF-1 infused into the lesion site promotes axonal sprouting in SCI (Opatz et al., 2009; Jaerve et al., 2011). Axon sprouting is known to contribute to plasticity-associated recovery. In addition, Shyu et al. (2008b) has shown that transplanted OECs promote axonal regeneration after stroke likely via SDF/CXCR4-mediated signaling.

Before SDF-1 can be successfully translated into clinical medicine, concerns about its tumorigenic potential (Zlotnik, 2008) as wells as neuropathic pain (Oh et al., 2001) should be addressed. Although, we never have observed any of these effects during a 4-months follow-up period in spinal injured rats which had initially received intrathecal SDF-1 infusion for one week, longer studies might be necessary. Further characterization of the immune responses and of the individual biological functions of the two receptors will, hopefully, reveal the full potential of SDF-1 for the treatment of CNS injury.

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References

Arakawa Y, Bito H, Furuyashiki T, Tsuji T, Takemoto-Kimura S, Kimura K, et al. Control of axon elongation via an SDF-1alpha/Rho/mDia pathway in cultured cerebellar granule neurons. J Cell Biol 2003;61:381–91.

Bajetto A, Barbero S, Bonavia R, Piccioli P, Pirani P, Florio T, et al. Stromal cell-derived factor-1alpha induces astrocyte proliferation through the activation of extracellular signal-regulated kinases 1/2 pathway. J Neurochem 2001;77:1226–36.

Balabanian K, Lagane B, Infantino S, Chow KY, Harriague J, Moepps B, et al. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J Biol Chem 2005;280:35760–6.

Barnabé-Heider F, Frisén J. Stem cells for spinal cord repair. Cell Stem Cell 2008;3:16–24.

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, et al. CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. Nat Neurosci 2001;4:702–10.

Cai D, Deng K, Mellado W, Lee J, Ratan RR, Filbin MT, et al. and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin *in vitro*. Neuron 2002;35:711–9.

Carbajal KS, Schaumburg C, Strieter R, Kane J, Lane TE. Migration of engrafted neural stem cells is mediated by CXCL12 signaling through CXCR4 in a viral model of multiple sclerosis. Proc Natl Acad Sci USA 2010;107:11068–73.

Chalasani SH, Sabelko KA, Sunshine MJ, Littman DR, Raper JA. A chemokine, SDF-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. J Neurosci 2003;23:1360–71.

Crump MP, Gong JH, Loetscher P, Rajarathnam K, Amara A, Arenzana-Seisdedos F, et al. Solution structure and basis for functional activity of stromal cell-derived factor-1; dissociation of CXCR4 activation from binding and inhibition of HIV-1. EMBO J 1997;16:6996–7007.

David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci 2011;12:388–99.

Davis DA, Singer KE, De La Luz Sierra M, Narazaki M, Yang F, Fales HM, et al. Identification of carboxypeptidase N as an enzyme responsible for C-terminal cleavage of stromal cell-derived factor-1alpha in the circulation. Blood 2005;105:4561–8.

Drury LJ, Ziarek JJ, Gravel S, Veldkamp CT, Takekoshi T, Hwang ST, et al. Monomeric and dimeric CXCL12 inhibit metastasis through distinct CXCR4 interactions and signaling pathways. PNAS USA 2011;108:17655–60.

Dziembowska M, Tham TN, Lau P, Vitry S, Lazarini F, Dubois-Dalcq M. A role for CXCR4 signaling in survival and migration of neural and oligodendrocyte precursors. Glia 2005;50:258–69.

Fujio M, Yamamoto A, Ando Y, Shohara R, Kinoshita K, Kaneko T, et al. Stromal cell-derived factor-1 enhances distraction osteogenesis-mediated skeletal tissue regeneration through the recruitment of endothelial precursors. Bone 2011;49:693–700.

Gleichmann M, Gillen C, Czardybon M, Bosse F, Greiner-Petter R, Auer J, et al. Cloning and characterization of SDF-1gamma, a novel SDF-1 chemokine transcript with developmentally regulated expression in the nervous system. Eur J Neurosci 2000;12:1857–66.

Göttle P, Kremer D, Jander S, Odemis V, Engele J, Hartung HP, et al. Activation of CXCR7 receptor promotes oligodendroglial cell maturation. Ann Neurol 2010;68:915–24.

Han Y, Wang J, He T, Ransohoff RM. TNF-alpha down-regulates CXCR4 expression in primary murine astrocytes. Brain Res 2001;888:1–10.

Henderson PW, Singh SP, Krijgh DD, Yamamoto M, Rafii DC, Sung JJ, et al. Stromal-derived factor-1 delivered via hydrogel drug-delivery vehicle accelerates wound healing *in vivo*. Wound Repair Regen 2011;19:420–5.

Hill WD, Hess DC, Martin-Studdard A, Carothers JJ, Zheng J, Hale D, et al. SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury. J Neuropathol Exp Neurol 2004;63:84–96.

Jaerve A, Schiwy N, Schmitz C, Mueller HW. Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. Exp Neurol 2011(July) [Epub ahead of print].

Laguri C, Sadir R, Rueda P, Baleux F, Gans P, Arenzana-Seisdedos F, et al. The novel CXCL12gamma isoform encodes an unstructured cationic domain which regulates bioactivity and interaction with both glycosaminoglycans and CXCR4. PLoS One 2007;2(October (10)):e1110.

Lakatos A, Smith PM, Barnett SC, Franklin RJ. Meningeal cells enhance limited CNS remyelination by transplanted olfactory ensheathing cells. Brain 2003;126:598–609.

Lazarini F, Casanova P, Tham TN, De Clercq E, Arenzana-Seisdedos F, Baleux F, et al. Differential signalling of the chemokine receptor CXCR4 by stromal cell-derived factor 1 and the HIV glycoprotein in rat neurons and astrocytes. Eur J Neurosci 2000;12:117–25.

Levoye A, Balabanian K, Baleux F, Bachelerie F, Lagane B. CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling. Blood 2009;113:6085–93.

Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol 2006;177:7303–11.

Meletis K, Barnabe-Heider F, Carlen M, Evergren E, Tomilin N, Shupliakov O, et al. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol 2008;6:e182.

Miller JT, Bartley JH, Wimborne HJ, Walker AL, Hess DC, Hill WD, et al. The neuroblast and angioblast chemotaxic factor SDF-1 (CXCL12) expression is briefly up regulated by reactive astrocytes in brain following neonatal hypoxic–ischemic injury. BMC Neurosci 2005;6:63.

Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635–8.

Nagasawa T, Kikutani H, Kishimoto T. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc Natl Acad Sci USA 1994;91:2305–9.

Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, et al. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 1996;382:833–5.

Odemis V, Lamp E, Pezeshki G, Moepps B, Schilling K, Gierschik P, et al. Mice deficient in the chemokine receptor CXCR4 exhibit impaired limb innervation and myogenesis. Mol Cell Neurosci 2005;30:494–505.

Odemis V, Boosmann K, Heinen A, Küry P, Engele J. CXCR7 is an active component of SDF-1 signalling in astrocytes and Schwann cells. J Cell Sci 2010;123:1081–8.

Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ. Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. J Neurosci 2001;21:5027–35.

Opatz J, Kury P, Schiwy N, Järve A, Estrada V, Brazda N, et al. SDF-1 stimulates neurite growth on inhibitory CNS myelin. Mol Cell Neurosci 2009;40:293–300.

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Sánchez-Alcañiz JA, Haege S, Mueller W, Pla R, Mackay F, Schulz S, et al. Cxcr7 controls neuronal migration by regulating chemokine responsiveness. Neuron 2011;69:77–90.
Schönemeier B, Schulz S, Hoellt V, Stumm R. Enhanced expression of the CXCl12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain. J Neuroimmunol 2008;198:39–45.
Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, Shinohara T, et al. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. Genomics 1995;28:495–500.
Shyu WC, Liu DD, Lin SZ, Li WW, Su CY, Chang YC, et al. Implantation of olfactory ensheathing cells promotes neuroplasticity in murine models of stroke. J Clin Invest 2008a;118:2482–95.
Shyu WC, Lin SZ, Yen PS, Su CY, Chen DC, Wang HJ, et al. Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. J Pharmacol Exp Ther 2008b;324:834–49.
Sierro F, Biben C, Martínez-Muñoz L, Mellado M, Ransohoff RM, Li M, et al. Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. Proc Natl Acad Sci USA 2007;104:14759–64.
Stumm RK, Rummel J, Junker V, Culmsee C, Pfeiffer M, Krieglstein J, et al. A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J Neurosci 2002;22:5865–78.
Takeuchi H, Natsume A, Wakabayashi T, Aoshima C, Shimato S, Ito M, et al. Intravenously transplanted human neural stem cells migrate to the injured spinal cord in adult mice in an SDF-1- and HGF-dependent manner. Neurosci Lett 2007;426:69–74.
Tashiro K, Tada H, Heilker R, Shirozu M, Nakano T, Honjo T. Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. Science 1993;261:600–3.
Twery EN, Raper JA. SDF1-induced antagonism of axonal repulsion requires multiple G-protein coupled signaling components that work in parallel. PLoS One 2011;6:e18896.
Tysseling VM, Mithal D, Sahni V, Birch D, Jung H, Belmadani A, et al. SDF1 in the dorsal corticospinal tract promotes CXCR4$^+$ cell migration after spinal cord injury. J Neuroinflamm 2011;8:16.
Vereyken EJ, Heijnen PD, Baron W, de Vries EH, Dijkstra CD, Teunissen CE. Classically and alternatively activated bone marrow derived macrophages differ in cytoskeletal functions and migration towards specific CNS cell types. J Neuroinflamm 2011;8:58.
Wang X, Li C, Chen Y, Hao Y, Zhou W, Chen C, et al. Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1alpha activation. Biochem Biophys Res Commun 2008;371:283–8.
Yu L, Cecil J, Peng SB, Schrementi J, Kovacevic S, Paul D, et al. Identification and expression of novel isoforms of human stromal cell-derived factor 1. Gene 2006;374:174–9.
Zlotnik A, Yoshie O, Nomiyama H. The chemokine and chemokine receptor superfamilies and their molecular evolution. Genome Biol 2006;7:243.
Zlotnik A. New insights on the role of CXCR4 in cancer metastasis. J Pathol 2008;215:211–3.
Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393:595–9.
Zukor KA, Kent DT, Odelberg SJ. Meningeal cells and glia establish a permissive environment for axon regeneration after spinal cord injury in newts. Neural Dev 2011;6:1.

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4.5. CHEMOKINES IN CNS INJURY AND REPAIR

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Submitted

Abstract

Chemokines are small chemotactic factors that are known for their role in recruiting inflammatory cells into the CNS injury site. In addition, several chemokines were shown to be essential for stem/progenitor cell attraction, with CXCL12/SDF-1 as the most potent chemotactic cytokine. Chemokines thus participate in remyelination, neovascularization and neuroprotection, which are important prerequisites for CNS repair after trauma. Among other chemokines CXCL12 stands out, as it is able to promote neurite outgrowth in the presence of growth inhibitory CNS myelin and to enhance axonal sprouting after spinal cord injury (SCI). However, in contrast to CXCL12 application, blocking of action may be required for other chemokines, e.g. neutralization of CXCL10, in order to achieve axon sprouting, likely via reduction of neuroinflammation, improved tissue preservation as well as neovascularization. The aim of the review is to summarize the data about these exciting functions of chemokines in CNS trauma such as spinal cord injury, traumatic brain injury (TBI) and stroke, and discuss the potential of chemokines in CNS repair. Chemokines are promising targets not only to reduce secondary damage after injury, but also to stimulate regenerative processes by recruiting stem cells as well as promoting axonal sprouting.

Introduction

Chemokines are small (8-14 kDa) proteins, a subtype of cytokines with the ability to chemoattract cells. First they were defined by their ability to induce directional migration and activation of leukocyte subsets into inflammatory sites. Chemokines represent one of the first completely known molecular superfamilies which has expanded via gene duplication (DeVries et al., 2006) to about 50 members in mammals, chicken, zebrafish, sharks, jawless fish and C. elegance (Zlotnik et al., 2006). Chemokines are classified into four subfamilies according to the position of two conserved cystein residues in the mature protein: CXC, CC, XC and CX3C. Their structural features also convey their ability to recruit specific cells. Chemokines are produced in relatively high amounts at the traumatic injury site by resident tissue cells, activated resident and recruited leukocytes, cytokine-activated endothelial cells and by some neurons.

Functions other than those in inflammation, such as coordination of cell migration during development and homeostasis of the CNS, were discovered only recently, although it is obvious that they must have existed in lower organisms long before the rise of the immune system. In fact, the role in inflammation, homeostasis or both goes along with the evolutionary story of each chemokine and can be elucidated by looking at the chromosomal location (Table 1). More ancient and conserved chemokines, such as CXCL12, have distinct chromosomal locations and are capable of exerting homeostatic functions, whereas others, located in clusters on chromosomes, have changed after the segregation of human and mice

and mostly function in inflammation (Zlotnik et al., 2006). Arising redundancy in signalling networks and functions is further complicated by promiscuity of binding to only about 18 known receptors. Uniquely, CXCL12 binds to two different receptors CXCR4 and CXCR7.

Studies using knockout mice deficient in distinct chemokines and their receptors have often been disappointing, as defects are subtle, as, e.g., in the homozygous CXCR2 mutants (Cacalano et al., 1994), CCL3 mutants or CCR5 deficient mice/humans (Liu et al., 1996; Samson et al., 1996) with only slight differences in responses to viral infections. In contrast, the deletion of the genes encoding SDF-1, or its G-protein coupled receptors CXCR4 (Oberlin et al., 1996) and CXCR7 in mouse (Balabanian et al., 2005) is perinatally lethal (Nagasawa et al., 1996; Zou et al., 1998; Sierro et al., 2007). CXCR7 knockout mice have heart problems, whereas CXCL12 and CXCR4 deficiency further impaired hematopoiesis, lymphopoiesis and CNS development. Deficits in CNS development were accounted to disturbed proliferation, survival and migration of neuronal progenitor cells as well as axonal pathfinding.

Although the good or bad role of inflammation is still under debate (Yong and Rivest, 2009), the immunomodulatory role of chemokines is now being translated into anti-inflammatory interventions to reduce the destruction arising from infiltration of inflammatory cells and consequent secondary damage after injury (Donelly et al., 2011). Such studies have reported also enhanced neuroprotection, tissue preservation and axon sprouting (Glaser et al., 2004; Glaser et al., 2006). We and other labs have recently shown that CXCL12 directly promotes neurite outgrowth on inhibitory myelin in cultured neurons and, moreover, enhances sprouting of axons after SCI (Chalasani et al., 2003; Opatz et al., 2009; Jaerve et al., 2011). Many other groups have shown its role in recruiting stem and progenitor cells into the lesion site (Takeuchi et al., 2007). Reduction of secondary damage and stimulation of regenerative processes such as axonal sprouting make chemokines a promising target for repair of SCI, TBI and stroke.

Molecular structure

Chemokines have a typical tertiary fold due to 1-3 disulfide bonds between conserved N-terminal cysteins stabilizing the structure; a three-stranded anti-parallel β-sheet and a carboxyterminal α-helix between disordered termini, of which the N-terminus is important for signalling. CXC chemokines have one amino-acid residue separating two conserved cysteins near the amino terminus, whereas CC chemokines carry two adjacent cysteines.

Name	Acronyme	Receptor	Chrom. location	Inflammatory (I), homeostatic (H),dual (D) or unknown (U) function; type of recruited leukocyte	
CXC chemokine family					
CXCL1	GROα,KC CINC-1	CXCR2 CXCR1	4q13.3	I	Neutrophil, monocyte
CXCL2	MIP-2, GROβ	CXCR2	4q13.3	I	Neutrophil, monocyte
CXCL3	Groγ DcipI	CXCR2	4q13.3	I	Neutrophil, monocyte
CXCL4	PF4	CXCR3B	4q13.3	U	Neutrophil, monocyte, T cell
CXCL5	Lix ENA-78	CXCR2 CXCR1	4q13.3	I	Neutrophil
CXCL6*	GCP-2	CXCR2 CXCR1	4q13.3	I	Neutrophil
CXCL7	Ppbp, NAP-2	CXCR2	4q13.3	I	Neutrophil
CXCL8*	IL-8	CXCR1 CXCR2	4q13.3	I	Neutrophil
CXCL9	MIG	CXCR3 CXCR3B	4q21.1	I	Monocyte, T cell, NK
CXCL10	IP-10	CXCR3A	4q21.1	I	Monocyte, T cell, NK
CXCL11	I-TAC	CXCR3 CXCR3B CXCR7	4q21.1	I	Monocyte, T cell, NK
CXCL12	SDF-1α/β	CXCR4 CXCR7	10q11.1	H	Monocyte, T cell, B cell, dendritic cell
CXCL13	BLC BCA-1	CXCR5	4q21	H	Monocyte, B cell
CXCL14	BRAK, bolekine	U	5q31.1	I	Neutrophil, monocyte, dendritic cell
CXCL15 #	Weche, lungkine	CXCR2	U	I	Neutrophil
CXCL16		CXCR6	17p13.2	I	T cell
CXCL17		U	19q13.2	U	
CC chemokine family					
CCL1	I-309, TCA-3	CCR8	17q11.2	I	Monocyte, T cell
CCL2	MCP-1, JE	CCR2	17q11.2	I	Neutrophil, eosinophil, basophil, monocyte, T cell, dendritic cell, NK
CCL3	MIP-1α, LD78α	CCR1 CCR5	17q11.2	I	Eosinophil, monocyte,T cell, B cell, dendritic cell, NK
CCL4	MIP-1β	CCR1 CCR5	17q12	I	Monocyte, T cell, dendritic cell, NK
CCL5	Rantes	CCR1 CCR3 CCR5	17q12	I	Eosinophil, basophil, monocyte, T cell, dendritic cell, NK
CCL6#		U		I	
CCL7	MCP-3, MARC	CCR1 CCR2 CCR3 CCR5	17q11.2	I	Eosinophil, basophil, monocyte, T cell, dendritic cell, NK
CCL8	MCP-2	CCR2 CCR5 CCR1 CCR3	17q11.2	I	Eosinophil, basophil, monocyte, T cell, dendritic cell, NK
CCL9#		U		I	
CCL10#		U		I	
CCL11	Eotaxin	CCR3 CCR5	17q11.2	I	Eosinophil, basophil, monocyte, T cell, dendritic cell
CCL12#	MCP-5	CCR2		I	Neutrophil, basophil, monocyte, T cell, dendritic cell, NK

CCL13*	MCP-4	CCR1 CCR3 CCR2 CCR5	17q11.2	I	Eosinophil, basophil, monocyte, T cell, dendritic cell, NK
CCL14*	HCC-1	CCR1 CCR3 CCR5	17q12	H	T cell, monocyte
CCL15*	MIP-1γ, HCC-2 MMRP2, CCF18	CCR1 CCR3	17q12	H	Eosinophil, basophil, monocyte, T cell
CCL16*	HCC-4, LEC	CCR1 CCR2 CCR5 CCR8 HRH4	17q12	H	Monocyte, T cell
CCL17	TARC	CCR4 CCR8	16q13	D	Monocyte, T cell, dendritic cell
CCL18*	PARC	U	17q12	H	Monocyte, T cell
CCL19	MIP-3β, ELC	CCR7	9p13.3	H	Monocyte, T cell, dendritic cell
CCL20	MIP-3α, LARC	CCR6	2q36.3	D	Monocyte, dendritic cell
CCL21	SLC	CCR7	9p13.3	D	T cell, dendritic cell
CCL22	MDC, ABCD-1	CCR4	16q13	D	Monocyte, T cell, dendritic cell
CCL23*	MPIF-1, C10	CCR1 FPRL-1	17q12	I	Monocyte, T cell
CCL24	Eotaxin-2	CCR3	7q11.23	I	Eosinophil, basophil, monocyte, T cell, dendritic cell
CCL25	TECK	CCR9	19p13.2	H	Monocyte, T cell, dendritic cell
CCL26	Eotaxin-3	CCR3	7q11.23	I	Eosinophil, basophil, monocyte, T cell
CCL27	CTACK, ILC	CCR10	9p13.3	H	T cell
CCL28	MEC	CCR10 CCR3	5p12	U	T cell
C chemokine family					
XCL1	Lympho- tactin α	XCR1	1q24.2	D	T cell
XCL2*	Lympho- tactin β	XCR1	1q24.2	D	T cell
CX3C chemokine family					
CX3CL1	Fractal- Kine	CX3CR1	16q13	I	Monocyte, T cell, NK

Table 1. Chemokines, receptors, inflammatory function. Based on Zlotnik et al., 2006; David and Kroner, 2011, Keeley et al., 2010. ** human or # mouse only; I, inflammatory; H, homeostatic; D, dual; U, unknown

Structural features are decisive for specificity of receptor binding and, consequently, of chemoattraction. For example, CXC family chemokines are generally chemotactic for neutrophils due to the glutamic acid–leucine–arginine (ELR) tripeptide motif in their N-terminal domain with which they bind CXCR2. CC chemokines or others (such as CXCL12) lacking this motif, attract primarily lymphocytes (Table 1) (Zlotnik and Yoshie, 2000).

Chemokines are secreted from cells with the exception of CXCL16 and CX3CL1, which are first expressed as transmembrane proteins, but upon proteolytic cleavage the extracellular

domain is released as a soluble fragment (Ludwig and Weber, 2007). The transmembrane form of CXCL16 functions as an adhesion molecule and a scavenger receptor for oxidized low density lipoprotein and bacteria, whereas the soluble form is chemoattractive to immune cells. CXCL16, containing 254 amino acids, is larger than other chemokines and has the chemokine domain linked to the membrane anchor by a long mucin-like stalk in addition to a transmembrane domain and a cytoplasmic tail. The intracellular domain is required for efficient membrane expression and constitutive internalization (Andrzejewski et al., 2010; Huang et al., 2009). CX3CL1 has also a mucin stalk which is heavily glycosylated (Bazan et al., 1997; Matloubian et al., 2000) and is required for adhesion (Imai et al., 1997). CX3CL1 is also constitutively internalized to protect it from protease-dependent release (Huang et al., 2006). Upon inflammation and apoptosis, disintegrin and metalloproteases ADAM-10 and ADAM-17 increase CX3CL1 shedding from neuronal membranes (Chapman et al., 2000; Hatori et al., 2002).

Many chemokines oligomerize (Salanga and Handel, 2011). Oligomerization is only at the beginning to unravel its importance to the signalling responses of certain chemokines and their interaction with glycosaminoglycans. The latter compounds are negatively charged and thus stabilize structures of highly positively charged chemokines that would not form in solution. CXC chemokine dimers, such as CXCL8, are densely packed, whereas many CC chemokines, like CCL2, dimerize into elongated structures. Moreover, XCL1 exists in two three-dimensional structures under physiological conditions, a monomeric and a unique dimeric β-sheet conformation (Camillioni and Sutto, 2009), which exert different biological functions, one binding to glycosaminoglycans and the other activating the XCR1 receptor, respectively. In addition to oligomerization, stable tetramers of CXCL4 and CX3CL1 have been reported (Zhang et al., 1994). CCL3, CCL4 and CCL5 can form very stable polymers, whereas CCL2 and CXCL12 are in dynamic equilibrium between monomer and dimer depending on solution conditions (Veldkamp et al., 2005; Paavola et al., 1998). CCL27 forms tetramers at high millimolar concentrations and CCL7 does not oligomerize (Kim et al., 1996). Recent findings suggest that oligomeric forms seem to be required for migration *in vivo*, while binding of monomeric CCL2, CCL4, CCL5 and CCL10 to receptors induces cell migration *in vitro*,. In contrast, dimeric CXCL12 was unable to induce cell migration, but elevated intarcellular Ca^{2+} levels and may stop cell migration at high concentrations. Moreover, receptors of chemokines have been reported to dimerize or heterodimerize further complicating the understanding of chemokine biology (Wang and Norcross, 2008). Recently, new structural features of several G-protein coupled receptors were determined, including CXCR4 (Wu et al., 2010), as the ligand binding pocket was found much larger as previously anticipated. Based on this structural phenomenon different ligand : receptor binding stoichiometries, such as 1:2 and 2:2, became possible.

Receptors and signalling

Chemokine signalling is mediated by members of a family of 7-transmembrane–spanning, G-protein–coupled receptors. Upon ligand binding these receptors (CXCR1-6, CCR1-10, XCR1 and CX3CR1) are internalized from the cell surface and a wide range of intracellular pathways such as phosphatidylinositol-3 kinase, mitogen-activated protein kinases, and protein kinase C are activated (Stamatovic et al, 2005; Wain et al, 2002).

G-protein-mediated phosphoinositide hydrolysis generates diacylglycerol and inositol 1,4,5-trisphosphate, which then activate protein kinase C allowing the mobilization of calcium to initiate cellular responses (Wu et al, 1993). It appears that the newly discovered receptor for CXCL12 and CXCL11, CXCR7, is unable to induce Ca^{2+} elevation and, rather than signalling, primarily functions as scavenger receptor regulating the availability of chemokines. This is similar to the Duffy Antigen Receptor for Chemokines (DARC), D6 and CCX-CKR (Mantovani et al, 2006) that removes chemokines from inflammatory sites. Sánchez-Alcañiz et al. (2011) showed recently that, upon interaction with CXCR4, CXCR7 on cortical interneurons prevented the normally very rapid internalization of CXCR4 and the subsequent time- and energy-consuming biosynthesis of new molecules of the latter receptor. Thus CXCR4 responsiveness to CXCL12 was retained and desensitization was prevented even at high levels of this chemokine ligand in the developing cortex.

Chemokine–receptor complexes are phosphorylated and endocytosed after signaling through clathrin-dependent pathways, and once internalized, may either be degraded or transported back to the cell membrane for reexpression (Rose et al, 2004).

Enzymatically, chemokines are processed by aminoterminal truncation into moderately active (CXCL5) or more active (CXCL7, CXCL8, CCL4, CCL14, CCL15, CCL23) molecules or receptor specific isoforms (CCL3L1, CCL5) (Mortier et al., 2011). Further processing of chemokines can produce natural GPCR signalling antagonists or lead to total inactivation or degradation of chemokines (Mortier et al., 2011). Modification of an arginine residue into citrulline which was detected on natural CXCL8 and CXCL10 reduces the inflammatory activity *in vivo*. Glycosylation (CCL2, CCL11, CCL14, XCL1, CX3CL1) has probably a modulatory rather than a decisive role, and is important, e.g., for CCL2 functional stability, receptor interaction and surface expression of CX3CL1. The NH2-terminal glutamine residue is converted in pyroglutamic acid in CCL2, CCL7, CCL8 and CCL13 protecting these chemokines from N-terminal cleavage by aminopeptidases and DPP IV. Proteolytic cleavage and inactivation of CXCL12 in the bone marrow microenvironment has been proposed to be the mechanism underlying mobilization of hematopoietic stem and progenitor cells by G-CSF (Jin et al., 2008). NH2-terminal truncation by either DPP IV, leukocyte elastase and cathepsin G, and COOH-terminal truncation by cathepsin X reduce the chemotactic activity of CXCL12 for human leukocytes, whereas the proteolytic cleavage by the MMP-2 results in

a highly neurotoxic molecule, SDF(5–67), that is not able to bind its receptor CXCR4 but recognises CXCR3 which is known for its deleterious effects in HIV-associated neurodegeneration (Vergote et al., 2006). Insulin-degrading enzyme rapidly degrades monomeric CCL3 and CCL4. However, polymerization of CCL3 or CCL4 inhibits the insulin-degrading enzyme-dependent degradation (Ren et al., 2010).

Chemokine expression

In an oversimplified view, chemokines are either inflammatory and expressed by cells of the immune system (leukocytes) and glial, epithelial, endothelial cells as well as fibroblasts upon activation, or they are homeostatic and expressed in specific locations in the absence of apparent activating stimuli.

Constitutive expression in the brain has been reported for CCL3 (Xia and Hyman, 1999) and CCL2. The latter chemokine was found in astrocytes, microglia, endothelial cells (Glabinski et al, 1996; 2002; Harkness et al, 2003) and in neurons including cortical motor neurons (Banisadr et al., 2005).. CXCL12 is expressed in neurons of, e.g., the subventricular zone, white matter and cerebellum as well as in blood vessels of the brain, and, furthermore, along the dorsal corticospinal tract (dCST) and in meningeal cells of the spinal cord (Tysseling et al., 2011). CX3CL1 is expressed in healthy neurons (Harrison et al., 1998). CCL21 was found to be transported in presynaptic processes to nerve terminals (de Jong et al., 2005). CCL19 and CCL21, on the other hand, are constitutively expressed in cerebrovascular endothelium and the choroid plexus (Lalor and Segal, 2010).

The corresponding receptors are also constitutively expressed, that is: CX3CR1 on microglia, CCR2 in neurons (brain, spinal cord) (Gosselin et al., 2005), astrocytes, microglia, neural progenitor cells and microvascular endothelial cells (Banisadr et al., 2002, 2005; Coughlan et al, 2000; Gourmala et al, 1997; Stamatovic et al, 2005; Horuk et al., 1997). CXCR4 is constitutively expressed on cortical and hippocampal neurons, astrocytes, microglia and ependymal cells (Stumm et al., 2002; Banisadr et al., 2002) in the brain and on ependymal cells, as well as at low levels on dCST axons (Tysseling et al., 2011; Opatz et al., 2009; Jaerve et al., 2011). CXCR7 expression was observed in neurons of various brain regions (including cortical layer IV-V), astrocytes and endothelial cells (Schonemeier et al., 2008). Recently we have also detected low levels of CXCR7 on dCST axons in the spinal cord (Opatz et al., 2009; Jaerve et al., 2011).

Chemokine expression following CNS injury

In CNS injuries, chemokine and chemokine receptor expression is upregulated within minutes to hours depending on severity of the injury, and expression is maintained for at least several days after trauma (Table 2).

	Injury	Peak	Source	Reference
CXCL1	SCI	3 -12 h	Astrocytes (mRNA)	Pineau et al., 2010
		6 h	mRNA (30x up)	McTigue et al., 1998
	TBI	1 dpi	CSF	Helmy et al., 2011
		6 h	choroid plexus (mRNA, protein)	Szmydynger-Chodobska et al., 2009
	Stroke	12 h, 3-6 h	Protein, serum	Yamasaki et al., 1995
		24, 72 h	monocyte, neutrophils (mRNA, >10x up)	Brait et al., 2011
		within 24 h to 7 dpi	CSF, not serum Serum	Losy et al., 2005 Ormstad et al., 2011
CXCL2	SCI	12 h	Astrocytes (mRNA)	Pineau et al., 2010
		24 h	mRNA	Ma et al., 2002
	TBI	6 h	choroid plexus (mRNA)	Szmydynger-Chodobska et al., 2009
	Stroke	24, 72 h	monocyte, neutrophils (mRNA >10x up)	Brait et al., 2011
CXCL3	SCI	15 min to 24 h	mRNA	Rice et al., 2007
	TBI	6 h	choroid plexus (mRNA)	Szmydynger-Chodobska et al., 2009
CXCL5	SCI	6-12 h	mRNA	McTigue et al., 1998
	Stroke	Within 24 h	CSF, not serum	Zaremba et al., 2006
CXCL8	SCI	1 to 4 h 12 h	mRNA protein	Spitzbarth et al., 2011 Hirose et al., 2000
		within 3 dpi	CSF	Kwon et al., 2010
	TBI	24 h 1 dpi	mRNA CSF	Stefini et al., 2008 Helmy et al., 2011
	Stroke	24 h to 7 dpi 1-7 dpi	Serum; CSF blood mononuclear cells (mRNA)	Grau et al., 2001; Tarkowski et al., 1997; Kostulas et al., 1998
CXCL10	SCI	30 min to 24 h 6 h	mRNA mRNA	Rice et al., 2007 Gonzalez et al., 2003
		6h	mRNA	McTigue et al., 1998
		6 h, till 7 dpi	mRNA, cells near injury site	Lee et al., 2000
	TBI	24 h to 7 dpi 2 dpi	mRNA (very low levels) CSF, higher than plasma	Stefini et al., 2008 Helmy et al., 2011
	Stroke	3h, 6 h and 10-15 dpi	mRNA	Wang et al., 1998
CXCL12	SCI	7 dpi	mRNA	Takeuchi et al., 2007
		In intact and injured; 14 dpi	dCST, meninges; infiltratingmonocyte (protein)	Tysseling et al., 2011
	Stroke	7 dpi	Astrocytes, endothelial cells, neurons (protein)	Hill et al., 2004
CCL2	SCI	Starting <5 min; 4 dpi	Mainly astrocytes, also microglia, oligodendrocytes, neurons, blood vessels (mRNA);monocyte/microglia	Pineau et al., 2010
		7 dpi (reduced)	mRNA	Jones et al., 2005
		24 h	mRNA	Ma et al., 2002
		15 min to 24 h	mRNA	Rice et al., 2007
		within 72 h	CSF	Kwon et al., 2010
		1 h	cells near injury site (mRNA)	Lee et al., 2000
	TBI	14 h and 8 dpi; 1 dpi	mRNA; CSF higher than plasma	Stefini et al., 2008; Helmy et al., 2011
		6-12 h	mRNA; endothelial cells,monocyte, some astrocytes (protein)	Berman et al., 1996
	Stroke	2-3 dpi 6h-2 dpi; 4 dpi 2 dpi	Neurons, astrocytes (protein) Astrocytes;monocyte/microglia (mRNA) protein	Che et al, 2001 Gourmala et al, 1997 Yamagami et al., 1999
		24 h	CSF, but not serum	Losy et al., 2001; Zaremba et al., 2006
CCL3	SCI	15 min to 24 h	mRNA	Rice et al., 2007
		within 1h	gray matter of spinal cord (microglia), at 4 dpi cellular infiltrate (mRNA)	Bartholdy and Schwab, 1997; Lee et al., 2000

	TBI	3.5 h and 8 dpi; 1 dpi	mRNA; CSF, higher than serum	Stefini et al., 2008; Helmy et al., 2011
	Stroke	6h	mRNA, astrocytes (protein)	Kim et al., 1995
		4-6 h	Microglia, astrocytes (mRNA)	Takami et al., 1997
		8 to 72 h	Monocyte/microglia (protein)	Cowell et al., 2002
CCL4	SCI	within 1 h 4 dpi	gray matter of spinal cord (microglia) cellular infiltrate (mRNA)	Bartholdy and Schwab, 1997
	TBI	3.5 h 1 dpi	mRNA CSF higher than plasma	Stefini et al., 2008 Helmy et al., 2011
CCL5	SCI	90 min to 3 h	mRNA	Rice et al., 2007
		7dpi to 21 dpi	mRNA	Jones et al., 2005
		24 h	mRNA, microvascular endothelial cells	Benton et al., 2008
	TBI	6 dpi 1 dpi	mRNA (very low levels) CSF	Stefini et al., 2008 Helmy et al., 2011
	Stroke	24 h	blood-derived cells, resident cells (protein)	Terao et al., 2008
CCL7	SCI	24 h	mRNA	Ma et al., 2002
	TBI	Within 5 dpi	CSF higher than plasma	Helmy et al., 2011
	Stroke	12 h	mRNA	Wang et al., 1999
CCL8	SCI	12 to 24 h	mRNA	McTigue et al., 1998
CCL12	SCI	12, 24 h	mRNA	McTigue et al., 1998
CCL20	SCI	6-12 h	mRNA	McTigue et al., 1998
	Stroke	24 h	mRNA, protein	Terao et al., 2009
CCL21	SCI	4 weeks	dorsal horn neurons, thalamus (protein)	Zhao et al., 2007
	Stroke	6h to 4 dpi	cortical neurons and not glia (mRNA)	Biber et al., 2001
CX3CL1	SCI	7 dpi (downregulated)	mRNA, protein	Donelly et al., 2011
	TBI	24 h	CSF, not serum, no changes in mouse model (mRNA, protein)	Rancan et al., 2004

Table 2. Chemokine expression in CNS injury.

It is argued that chemokine gradients persist indefinitely or reoccur periodically, which would explain why infiltrated cells are observed for longer periods - about 6 weeks for neutrophils and over 6 months for monocytes - in the lesioned spinal cord, despite of their short lifespan of roughly 5 days and 2 months, respectively (Pillay et al., 2010; Hawthorn and Popovich, 2011).

The chemokine profiles following brain and spinal cord trauma are different, however, common is the high upregulation of CCL2. In head trauma and in cortical stab wound injury, CCL2, was the most strongly expressed chemokine (Stefini et al, 2008; Helmy et al., 2011; Berman et al., 1996; Glabinski et al., 1996). In SCI, CCL2 was also highly upregulated by 18-fold at 12 h. However, CXCL1 was elevated even 30-fold at 6 h and CCL8 35-fold at 12 h (McTigue et al., 1998). CXCL10 was upregulated approximately 7-fold at 6 h following SCI (McTigue et al., 1998) but was almost absent in TBI (Stefini et al., 2008). CCL20 was elevated 11-fold at 6-12 h and CCL3 5-fold at 24 h (McTigue et al., 1998). In TBI, CCL3 and CCL4 followed in their levels CCL2 and CXCL8 (Helmy et al., 2011). CCL4 as well as CCL5

were elevated in cortical stab wound injury (Berman et al., 1996; Glabinski et al., 1996), whereas the latter was expressed at very low levels in both SCI and TBI.

Monitoring chemokine levels could enhance the precision by the assessment of injury severity, which is one of the major challenges in management of SCI. Levels of CXCL8 and CCL2 in cerebrospinal fluid (CSF) at 24 h post-SCI accurately predicted the ASIA grade for acutely injured patients, and prognosis of segmental motor recovery at 6 months was more precisely predicted than with the patients' baseline ASIA grade (Kwon et al., 2010). Interestingly, elevated CCL2 levels in blood samples of ischemic stroke patients correlated well with the clinical outcome already a few hours after onset of symptoms (Worthmann et al., 2010).

The expression of several chemokines can be regulated by levels of oxygen and cytokines. For example, CCL2 production is induced by changes in oxygen levels in rat peripheral blood mononuclear cells (Reale et al., 2003). Treatment with lipopolysaccharide, interferon, interleukin-1 beta (IL-1β), colony-stimulating factor-1, transforming growth factor-β, and tumor necrosis factor-α (TNFα) can induce the expression of several chemokines as has been shown for CCL2 in different cell types *in vivo* and *in vitro* (Harkness et al, 2003; Hurwitz et al, 1995; Thibeault et al, 2001).

Chemokines and their receptors as therapeutic targets in CNS injury

CXCL1-CXCL8: CXCR2 ligands

Chemokines CXCL1 to CXCL8 recruit neutrophils, which express the CXCR2 receptor, to the site of CNS injury. As source for CXCL1 and CXCL2 hyperthrophic astrocytes have been identified which promote the entry of neutrophils and inflammatory monocytes in an IL-1 receptor/MyD88-dependent fashion (Pineau et al., 2010). SCI-induced expression of CXCL1 was reduced in myeloid cell-specific IkB kinase conditional knockout mice. IkB kinase deficiency may be responsible for the reduced neutrophil infiltration in these mice and improved recovery after SCI (Kang et al., 2011).

On the other hand, CXCL1 has been shown to be beneficial. Stimulation of the CXCL1/CXCR2 pathway in the presence of thrombin and a thrombin receptor agonist peptide has been shown to protect astrocytes from ceramide-induced apoptosis (Wang et al., 2006). CXCR2 signaling plays a role in remyelination and oligodendrocyte precursor cell (OPC) biology. However, the underlying mechanisms remain controversial. By conditional overexpression of CXCL1 in a mice model of multiple sclerosis, neuroprotection and remyelination were observed (Omari et al., 2009). This is further supported by the finding that neutralization of CXCL1 receptor CXCR2 delayed clinical recovery of the mouse model of

viral-induced demyelination, inducing apoptosis of OPCs (Hosking et al., 2010). In contrast, Kerstetter et al. (2009) showed that inhibition of CXCR2 signalling promotes functional recovery in the EAE model as differentiation of myelin producing cells was increased. Omari et al. (2006) proposed that OPCs which constitutively express CXCR2 are recruited to demyelination/damage areas by CXCL1. The latter chemokine is released by reactive astrocytes and is known to halt OPCs differentiation *in vivo* (Tsai et al., 2002). However, this concept could not be proven by cuprizone-induced demyelination in CXCR2 deficient mice, as no functional impairment in OPC recruitment was observed (Lindner et al., 2008).

CXCL4 as a strong chemoattractant for neutrophils and fibroblasts has been proposed to play a role in inflammation and wound repair (Eisman et al., 1990). It efficiently neutralizes heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting local antithrombin III activity and promoting coagulation. Under neurodegenerative conditions, the brain microglial cells have been shown to express CXCL4 and migrate towards CXCL4 via mechanisms involving the CXCR3b receptor. Furthermore, CXCL4 attenuates lipopolysaccharide-induced microglial phagocytosis and nitric oxide production in microglia and BV-2 cells (de Jong et al., 2008).

CXCL8/IL-8 is the most intensely investigated pro-inflammatory chemokine recruiting neutrophils. Blocking of its action was shown to be beneficial in CNS injury. A CXCL8-neutralising antibody was able to reduce brain oedema and cerebral infarct size (Matsumoto et al., 1997). Inhibition of CXCL8 receptor by reparixin caused the same beneficial effect in rats that underwent permanent and transient cerebral ischaemia resulting in the attenuation of neurological deficits (Villa et al., 2007). In the clamp SCI, a steroid analog therapeutic lazaroid U-74389G, which inhibits lipid peroxidation, but does not have the side effects of steroid therapy (Kavanagh and Kam, 2001), was shown to reduce the production of systemic and spinal CXCL8 (Kunihara et al. 2000). CXCL8 was shown to increase the expression of matrix metalloproteinases, cell cycle and pro-apoptotic proteins, as well as cell death in the cultured neurons. Moreover, CXCL8 levels were found to be elevated in the Alzheimer's disease brain (Thirumangalakudi et al., 2007).

On the other hand, a neuroprotecitve effect of CXCL8 cannot be excluded. This chemokine was shown to recruit human neural precursor cells across brain endothelial cells (Rainey-Barger et al., 2010). Furthermore, CXCL8 as well as CXCL2 and CXCL1 could protect hippocampal neurons from beta-amyloid (1-42) induced cell death (Watson and Fan, 2005).

Taken together, neutrophil infiltration is generally associated with secondary tissue damage. In line with this, in CXCR2-deficient mice suffering from closed head injury recruitment of neutrophils to the injury site as well as tissue damage and neuronal apoptosis were decreased but no functional effects were observed (Semple et al., 2010). In contrast, wound healing in CXCR2 knockout mice was delayed (Devalaraja et al., 2000).

CXCL9, CXCL10 and CXCL11: CXCR3 ligands

CXCR3 ligands are potent chemoattractants for T lymphocytes and natural killer cells. Their expression can be induced by interferon-gamma (Shurin et al., 2007). The location of CXCR3 on the surface of endothelial cells suggests its role in maintaining the cytokine gradient in the region of CNS inflammation (Ghersa et al., 2002). Blocking CXCR3 might thus be beneficial in neuroinflammation.

CXCL10, if over-expressed, has been shown to cause neuronal apoptosis (Sui et al., 2004). Administration of a neutralizing antibody against CXCL10 in spinal cord-injured mice reduced neuroinflammation (Gonzalez et al., 2003) and tissue damage (Glaser et al., 2004). Decreased infiltration of monocytes (lacking CXCR3) was likely due to decreased numbers of infiltrating pathogenic Th1 T cells, which predominate in SCI as well as in MS and EAE (Balashov et al., 1999; Sorensen et al., 1999; Jones et al., 2005), and attract monocytes by secreting CCL5 (Fransen et al., 2000). Anti-CXCL10 treatment significantly enhanced sprouting of the corticospinal axons and increased their number caudal to the injury site (Glaser et al., 2006). Locomotory recovery was found significantly improved (Gonzalez et al., 2003).

CXCL10 has also been shown to inhibit angiogenesis *in vitro* and *in vivo* (Strieter et al., 1995; Angiolillo et al., 1995; Luster et al., 1995). Bodnar et al. (2009) showed that CXCL10 induced dissociation of newly formed blood vessels. Neutralization of CXCL10 almost doubled the number of newly formed blood vessels following SCI, which might, together with supportive changes in gene expression, be the cause for the increased axon growth (Glaser et al., 2004). Interestingly, in cultured cortical neurons, the expression of CXCL10 was not regulated by stress or injury, instead, it was found to be constantly released at low levels by microglia. CXCL10 is expressed in developing brain and might, therefore, play a role in the recruitment and homing of glial cells during embryogenesis (Vinet et al., 2010).

CXCL11 mRNA increased in lymph nodes and spinal cord tissue in EAE rats, similarly to CXCR3 and CXCL10, but not as intensely and rapidly. Therefore, CXCL11 may not be essential for the initial migration of CXCR3-bearing cells (McColl et al., 2004). The main source of CXCL11 were astrocytes (McColl et al., 2004). CXCL9 levels, on the other hand, were very low.

In contrast to the obvious detrimental role of CXCR3 ligands, in the CXCR3-deficient mice, the EAE chronic disease was more severe, demyelination and axonal damage were increased (Müller et al., 2007). In CXCR3 knock-out mice following entorhinal cortex lesion, microglial migration into the area of axonal degeneration was impaired. However, CXCR3 seems not to be important for microglia proliferation as no differences were observed in the proliferation specific model of facial nerve axotomy (Rappert et al., 2004). Clearly, microglia

has also supportive functions in CNS injury by releasing also factors that are neuroprotective (David and Korner, 2011).

CXCL12 – ligand for CXCR4 and CXCR7

CXCL12/SDF-1 has extraordinary features among the chemokine family of proteins. CXCL12 plays a very important role in CNS development, but also in CNS homeostasis (neurogenesis, neuromodulation) and CNS injury. We have shown that infusion of this ligand to the lesion site after dorsal hemisection in rat spinal cord promotes sprouting of the dorsal corticospinal tract (dCST) sprouting rostrally to the lesion site. Fibers grow into the gray and white matter of the spinal cord (Opatz et al., 2009). In addition to dCST, rostral sprouting of serotonergic and tyrosine hydroxylase positive fibers was increased in both young and aged rats (Jaerve et al., 2011). The mechanism of action for dCST sprouting likely involves signalling via CXCR4 and/or CXCR7 as both receptors could be detected on CST axons. However, direct evidence for this hypothesis remains to be proven. Sprouting of 5-HT and TH-fibers, on the other hand, could be due to indirect mechanisms, as they do not express detectable levels of CXCL12 receptors.

CXCL12 appears to be one of the most potent coordinators of stem and progenitor cell migration. CXCL12 recruits mesenchymal stem cells (Li et al., 2010), intracerebrally transplanted bone marrow-derived mesenchymal stem cells (Wang et al., 2008), monocytes (Hill et al., 2004), endothelial cell progenitors which are important participants of neovascularization (Bogoslovsky et al., 2011) and neuroblasts (Cui et al., 2007; Imitola et al., 2004; Robin et al., 2006; Fan et al., 2010) through signalling via CXCR4. In addition, upregulation of CXCL12 and CXCR4 in murine olfactory ensheathing cells has been shown to promote axonal regeneration *in vitro* and *in vivo* (Shyu et al., 2008a). In stroke, CXCL12 has been demonstrated to moblize bone marrow-derived cells stem cells, promote angiogenesis and protect neurons (Shyu et al., 2008b). In SCI elevated levels of CXCL12 correlated well with the accumulation of neuronal progenitor cells, which were injected at the injury site one week after SCI in mice (Takeuchi et al., 2007). Ependymal cells in the spinal cord, which constitute the endogenous stem cell pool, express high levels of CXCR4 (Tysseling et al., 2011; Jaerve et al., 2011) and are, therefore, likely guided to the lesion site by its ligand. CXCL12 is also involved in neurotransmission and modulates neuronal activity, as GABAergic inputs of immature neurons at the infarct boundary were enhanced by this chemokine (Bhattacharyya et al., 2008). Also survival supporting effects for neural progenitor cells mediated via the second CXCL12 receptor, CXCR7, have been reported (Bakondi et al., 2011). Signalling by binding to CXCR7 has been implicated in OPC differentiation and remyelination (Patel et al., 2010; Göttle et al., 2010). Likely, signalling via both receptors can activate parallel and protective pathways in ischaemic stroke. Developmental regulation of

neuronal migration and axonal pathfinding by CXCL12/CXCR4 also suggests a role of this ligand-receptor interaction in regeneration in the adult brain (Stumm and Höllt, 2007). Very surprising is the finding that CXCL12 reduces senescence of an endothelial progenitor subpopulation through telomerase activation and telomere elongation (Zheng et al., 2010). From the findings described here it is obvious that numerous cell biological aspects of this interesting chemokine remain to be discovered in the future.

CXCL13, CXCL14 and CXCL16

CXCL13 levels are often elevated in the inflamed CNS and shown to recruit B cells in animal models of infectious and inflammatory demyelinating disease (Rainey-Barger et al., 2011). However, B cell depletion can lead to progressive multifocal leukoencephalopathy highlighting the importance of B cell surveillance of the CNS. As reported above for CXCL8, enhanced levels of CXCL13 have been found in active demyelination areas and presumably assist systemically injected neural precursor cells (NPCs) across brain endothelium and favour functional recovery in animal models of multiple sclerosis (MS) (Weiss et al., 2010; Bagaeva et al., 2006). CXCL14 is also involved in myelination and is up-regulated in the sciatic nerve of a mouse model of Charcot-Marie-Tooth disease type 1A and alters myelin gene expression in cultured Schwann cells (Barbaria et al., 2009).
Chemokine CXCL16 levels were reported to be dramatically elevated in EAE, up to 10-fold higher in the spinal cord as compared to the brain (Kim et al., 2010). This rise could be due to the invasion of inflammatory cells into the spinal cord (Matloubian et al., 2000; Fukumoto et al., 2004; Ludwig et al., 2005) which, in addition to the astrocytes and endothelial cells, produce CXCL16. Interestingly, when attracted by CXCL16 infiltration of neutrophil and monocytes into injured muscle promoted muscle regeneration and suppressed fibrosis (Zhang et al., 2009). The role of this chemokine in CNS injury might be worth of more detailed investigation.

MCP family: CCL2, CCL7, CCL8, CCL12 and CCL13

Each member of the monocyte chemotactic protein (MCP) family (designated as MCP-1-5, respectively) attracts a different subset of leukocytes after binding with different affinities to several receptors (Gouwy et al, 2004). Although CCL2, CCL7, and CCL8 signal through the CCR2 receptor, CCL2 is the most potent in activating signal transduction pathways which lead to monocyte transmigration (Sozzani et al, 1994) and is probably the most studied chemokine.
CCL2 and its receptor CCR2 are considered pro-inflammatory. There is strong evidence for its detrimental effect. Over-expression of CCL2 increases infarct volume (Chen et al., 2003),

exacerbates responses to brain injury (Muessel et al., 2002), and mouse mutants deficient in CCL2 or CCR2 genes showed decreased inflammatory infiltration and infarct size (Hughes et al., 2002; Dimitrijevic et al., 2007). Similar results were obtained with the chemokine receptor-antagonist treatment (Minami et al., 2003) or with anti-CCL2 antibody which decreased the permeability of the BBB after ischaemia/reperfusion (Xia and Sui, 2009). In CCR2 deficient mice, the number of infiltrating monocytes in the lesion epicentre, myelin degradation and expression of CCR1 and CCR5 were decreased at 1 week after SCI (Ma et al., 2002). In line with this is the observation that CCL2 mainly attracts the monocyte "inflammatory population" and not $Gr1^{-}/Ly6C^{low}CCR2^{-}CX3CR1^{high}$ cells which were shown to be neuroprotective (Woollard et al., 2010; Weber et al., 2008).

Nonetheless, microglia appears not to be responsible for neuron death accompanied with elevated CCL2 levels, although the latter chemokine can induce migration and proliferation of microglial cells (Hinojosa et al., 2011). CCL2 might recruit bone marrow stromal cells (MSCs) to the ischaemic brain (Wang et al., 2002). As these cells have neural phenotypes, they could be beneficial to recovery after stroke. Also recruitment of newly formed neuroblasts from neurogenic regions (the subventricular zone and the posterior peri-ventricular region) to damaged cerebral areas after focal ischaemia is likely (Yan et al., 2007). CCL2 and CCR2 are involved in CNS cytoarchitectural organization (Meng et al, 1999; Rezaie et al., 2002). CCL2 and CCL7 promote a dopaminergic phenotype in cultured rat embryonic cells (Edman et al, 2008). *In vivo*, CCL2 induce excitability of dopaminergic neurons, dopamine release, and locomotor activity in rats (Guyon et al., 2009). Conclusively, while CCL2 first worsens the stroke, it later promotes neurogenesis.

CCL3, CCL4, CCL5

CCL3 recruits monocytes and microglial cells in the injured brain (Cowell et al., 2002; Deshmane et al., 2009) via CCR1 and CCR5 (Cheng et al., 2001). CCL3 is also a potent neutrophil chemoattractant in mice and humans (Reichel et al., 2009) in the presence of other pro-inflammatory molecules, such as TNF-α or insulin (Montecucco et al., 2008a). Intra-cerebro-ventricular injection of mouse CCL3 increases the infarct volume (Takami et al., 1997).

On the other hand, CCL3 levels showed an inverse correlation with functional deficits in stroke (Zaremba et al., 2006). CCL3 has been proposed as a potential anti-apoptotic agent in stroke (Hau et al., 2008). In fact, CCL3 was shown to promote human umbilical cord blood (HUCB) cell migration in ischaemic regions (Jiang et al., 2008). it is likely that CCL3 together with its receptor CCR1 plays an important role in the subpial white matter neural stem- and progenitor-cell niche after SCI as increased immunoreactivity for CCL3 (and CCL2, CXCL12)

colocalized with astroglial, oligodendroglial markers, nestin, 3CB2 and BLBP in cells morphologically resembling radial glia (Knerlich-Lukoschus et al., 2010).

CCL4 is involved in monocyte migration (Montecucco et al., 2008b). It increased reactive oxygen species (ROS) production and adhesion of THP-1 cells to human umbilical vein endothelial cells *in vitro* (Tatara et al., 2009). Elevated serum levels have been shown to independently predict stroke and cardiovascular events in hypertensive patients (Tatara et al., 2009).

CCL5 binds CCR1, CCR3 and CCR5 (Braunersreuther et al., 2007). In the CCL5 knockout mice or in the wild-type mice receiving CCL5-deficient bone marrow the infarct volumes and BBB permeability were smaller (Terao et al., 2008). CCL5 could also increase cerebral damage through the secondary induction of other potent pro-inflammatory cytokines such as interleukin 6 (Shahrara et al., 2006). In contrast, the mutant mice for receptor of CCL5, CCR5, showed larger cerebral infarct size, with increased neuronal death and neutrophil infiltration as compared to wild-type mice (Sorce et al., 2010). CCL5, but also CCR5 are upregulated in astrocytes, microglia, endothelial cells and neurons following CNS injury (Spleiss et al., 1998; Tripathy et al., 2010), but have been proposed to mediate neuronal protection and survival (Rostene et al., 2007). Indeed, in the cell culture CCL5 increases neuronal survival and protects against the toxicity of thrombin and sodium nitroprusside (Tripathy et al., 2010).

CCL19 and CCL21 – ligands of CCR7, and CCL20 – ligand of CCR6

CCL19/CCR7 has been implicated in immune surveillance of the CNS by lymphocytes (Lalor and Segal, 2010; Shannon et al., 2010). CCL21 is rapidly upregulated in the cortical neurons upon ischemic injury and glutamate mediated excitotoxicity, transported in secretory granules along axons to presynaptic structures and, in such a way, activates remote microglia to express CXCR3 (Biber et al., 2001; Biber et al., 2011). Consequently, a chloride current and chemotaxis is triggered in microglia (Kettenmann et al., 2011; Rappert et al., 2002).

CCL20–CCR6 interactions attract inflammatory monocytes and activate microglia and are thus likely to contribute to neuroinflammation following brain injury (Terao et al., 2009). Indeed, hypothermia was shown to be effective in suppressing these effects as was neutralization with MAB540 antibody to reduce the ischaemic area (Terao et al., 2009).

CX3CL1 - ligand of CX3CR1

CX3CL1 is expressed on neurons, while CX3CR1 is found on microglia, and the interaction between them is thought to maintain microglia in a resting state, thereby suppressing the

neurotoxic and phagocytic activity of these cells (Cardona et al., 2006). Blockade of CX3CR1 signaling leads to better anatomical and functional recovery from SCI in mice (Donelly et al., 2011). The production of inflammatory cytokines by spinal microglia and monocyte-derived monocytes is reduced as well as the number of pro-inflammatory monocytes in the injury site. Knocking out the CX3CR1 gene attenuates hyperalgesia and allodynia in a modality-dependent fashion (Staniland et al., 2010). In focal cerebral ischaemia (Denes et al., 2008) or cerebral ischaemia-reperfusion models of stroke (Soriano et al., 2002), mice lacking the fractalkine receptor are less susceptible to damage. However, in Parkinson's disease and amyotrophic lateral sclerosis models the lack of CX3CR1 exacerbates neuronal loss (Cardona et al., 2006), indicating that the result depends on the neurological condition.

Clinical situation

Only a limited number of therapeutics targeting the chemokine system has been approved for clinical use. Among which is the CXCR4 antagonist mozobil for stimulating mobilization of hematopoietic stem cells from the bone marrow to the bloodstream, and the inhibitor of CCR5 maraviroc for HIV prevention. Many potential therapeutics are in trials for HIV, MS and rheumatoid arthritis (but none for CNS injuries or stroke) and no anti-inflammatory agents have been tested in TBI (Proudfoot et al., 2010; Mirabelli-Badenier et al., 2011).

Therapeutic appoaches include small molecule inhibitors or neutralizing antibodies, but increasing attention gain the capabilities of modified chemokines (Chevigné et al., 2011). Those that block receptor activation are especially effective to inhibit leukocyte recruitment. For example, the CCL2 (9–76) fragment acts as receptor antagonist and inhibits mononuclear cell infiltration in a mouse model of arthritis (Gong et al., 1997). A broad spectrum inhibitor of CC and CXC chemokines, NR58–3.14.3, a retroinverso analogue of a 12-mer peptide, reduced the inflammatory response, the lesion size and consequently improved neurologic function in a rat model of cerebral ischaemia (Beech et al., 2001).

Pathogens and viruses or even ticks have intelligent ways to escape the immune system, e.g., via production of homologues of chemokines (receptor antagonist) or of receptors (chemokine scavengers), or scavengers of secreted chemokines (Murphy et al., 2000; Mantovani et al., 2006), or 'evasins' (chemokine antagonists) (Frauenschuh et al., 2007; Déruaz et al., 2008; Vieira et al., 2009; Russo et al., 2010; Tarkowski et al., 1997). These molecules have, indeed, a great potential. For example, a chemokine analogue peptide encoded by Kaposi sarcoma-associated herpes virus, viralmonocyte inflammatory protein-II (vMIP-II) is a broad chemokine antagonist that protects the brain against focal cerebral ischemia in mice (Takami et al., 2001). Ghirnikar et al. (2000) showed that infusion of vMIPII suppressed gliotic reactions, reduced neuronal damage and dramatically halted inflammatory

cellular infiltration following SCI. Endogenous apoptosis inhibitor was also increased and neuronal apoptosis reduced (Ghirnikar et al., 2001). Subsequently, it could be demonstrated that this chemokine antagonist also reduced inflammatory infiltrates as well as axonal degeneration, but it increased neuronal survival (Ghirnikar et al., 2001).

Function	Chemokine	Intervention	Injury	Reference
Axon sprouting	CXCL12	Infusion	SCI	Opatz et al., 2009; Jaerve et al., 2011
	CXCL10	Neutralization	SCI	Glaser et al., 2006
Remyelination	CXCL12	Stimulation	EAE	Göttle et al., 2010
	CXCL1	Cond. over-expression	EAE	Omari et al., 2009
Demyelination	CXCR3	Deletion	EAE	Müller et al., 2007
Stem/progenitor cell recruitment	CXCL12	Stimulation	stroke	Cui et al., 2007; Imitola et al., 2004; Robin et al., 2006; Fan et al., 2010; Wang et al., 2008; Li et al., 2010
	CCL2	Stimulation	stroke	Yan et al., 2007
	CCL3	Stimulation	stroke	Hughes et al., 2002 Jiang et al., 2008
	CXCL8 CXCL13	Stimulation	EAE	Weiss et al., 2010
Neovascularization	CXCL10	Neutralization	SCI	Glaser et al., 2004
Reduced tissue damage	CXCL10	Neutralization	SCI	Glaser et al., 2004
	CX3CL1	Deletion	SCI	Donelly et al., 2011
	CXCL8	Neutralization	stroke	Matsumoto et al., 1997
	CXCR2	Deletion	TBI	Semple et la., 2010
	CXCR2	Inhibiton	stroke	Villa et al., 2007
	CCL2	Deletion Neutralization	stroke	Hughes et al., 2002 Xia and Sui, 2009
	CCR2	Deletion Inhibition	stroke	Dimitrijevic et al., 2007 Minami et al., 2003
	CCL20	Neutralization	stroke	Terao et al., 2009
	CX3CR1	Deletion	stroke	Soriano et al., 2002 Denes et al., 2008

Table 3. Chemokines or their receptors as targets in CNS injury.

Other substances such as cannabinoids were protective against ischaemic stroke not corroborating responsiveness to bacterial infections (Klehmet et al., 2009). Estrogen has been shown to reduce CXCL2 mRNA in the brain, suppress inflammation, elevate CCR7 expression and the number of cerebroprotective regulatory T cells (Zhang et al., 2010). Antiplatelet drugs have been shown to inhibit post-stroke upregulation of inflammatory chemokines (such as CXCL8) in circulating leukocytes (Al-Bahrani et al., 2007).

A special case among the CNS injuries considered here is stroke, as chemokines might be involved in increasing the risk of the disease. For example, CCL2, CCL4, CXCL8 and CXCL12 have been implicated in recruiting microglia and monocytes into atherosclerotic

plaques, rupture of which can also cause cerebral ischemia (Ikeda et al., 2002; Sokolov et al., 2009; Montecucco et al., 2010; Patel et al., 2010).

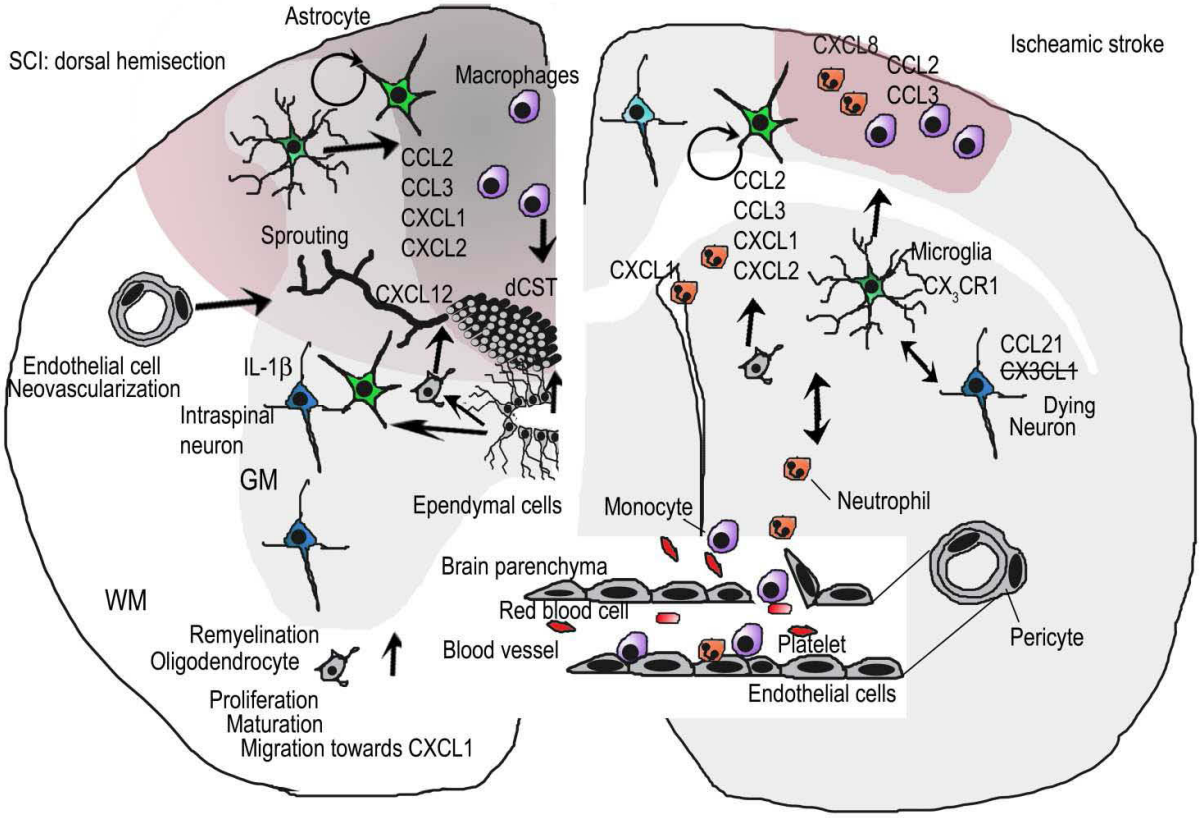

Figure 1. Role of chemokines in SCI, TBI and stroke. The blood-brain barrier (BBB) which separates the brain or spinal cord parenchyma from vascular system and consists of endothelial cells (depicted as a blood vessel) is disrupted following SCI and TBI and dysfunctional after stroke. Red and white blood cells infiltrate (in stroke after reperfusion) and interact with resident immune cells via cytokines (IL-1β, TNFα) which activate glial cells and promote their proliferation as well as production of chemokines in order to start the inflammation. Microglia migrate towards CCL2 source and produce both neurotoxic and neuro-supportive factors. Astrocytes and lesser extent endothelial cells produce CCL2, CCL3, CXCL1, CXCL2 as well as other chemokines to elicit migration of immune cells. Neutrophils are recruited by CXC chemokines CXCL1, CXCL2, CXCL8 (dependent on IL-1 receptor), also by CC chemokines CCL2 and CCL3, which, however, mostly recruit monocytes bearing CCR2. CXCL10 recruits T lymphocytes, but also monocytes from bone marrow, as does CXCL12 (which is upregulated at later time points). In TBI, neutrophils can enter via choroid plexus too, which secretes CXCL1. Recruited leukocytes themselves produce chemokines further amplifying the recruitment process. Chemokines promote regenerative processes as well. Neovascularization by infiltrating endothelial progenitor cells or tissue resident precursors is dependent on chemokines (CCL2, CCL16, CXCL12, CXCL1-8 except CXCL4). Neuroprotection by recruitment of stem cells is mediated mostly by CXCL12. In addition, CXCL12 promotes axonal sprouting. Some chemokines promote oligodendrocyte proliferation and remyelination (CXCL12, CXCL1).

Effect of age on chemokine effects

There is evidence that age may influence chemokine levels following CNS trauma. If so, it might be important to be considered for the development of therapeutic strategies. For example, after visual cortex ablation CCL2 and CCL5levels were higher in the thalamus of old mice at all time points suggesting a negative effect of age on outcome of brain injury (Sandhir et al., 2004). In very old spinal cord-injured rats, down-modulation of inflammatory responses was reported to be disturbed resulting in persistent neutrophil infiltrates. This finding was assessed by measurement of levels of the specific granulocyte enzyme MPO at 4h post injury (Genovese et al., 2006). In aged animals, microglia is activated more rapidly at 3 dpi in comparison to young animals at 14 dpi after mild cerebral ischemia (Badan et al., 2003). Nonetheless, following SCI, microglia in aged mice produced lower levels of pro-inflammatory cytokine CXCL1 at 3 h which correlated well with attenuation of infiltration of neutrophils (Kumamaru et al., 2010).

The immature nervous system might also have a different susceptibility to chemokine effects (Anthony et al., 1998). In TBI, exclusive induction of CXCL1 upon IL-1β in young but not adult rat brain was attributed to dramatic BBB breakdown and neutrophil response in young compared to adult rats. Also CXCL3 induction was higher in juvenile than in adult brain (Campbell et al., 2002). Treatment with anti-CXCL1 antibody attenuated these effects in young rats.

Circulating levels of cytokines are influenced by aging. With increased age, reduced levels of CCL2 (Kim et al., 2011; Tripathy et al., 2010; Reale et al., 2003) and CX3CL1 (Wynne et al., 2010) were reported, whereas the levels of most other chemokines were shown to be increased. CCL2 (Seidler et al., 2010; Inadera et al., 1999; Antonelli et al., 2006), CCL3, CCL4, CCL5 (Felzien et al., 2001; Chen et al., 2003), CXCL1 (Fimmel et al., 2007), CXCL8 (Baune et al., 2008), CXCL9 (Njemini et al., 2007; Shurin et al., 2007), CXCL10 (Antonelli et al., 2006; Shurin et al., 2007) and CXCL11 (Shurin et al., 2007) were elevated at higher age. Reduced CX3CL1 levels in aged brain were associated with microglial activation during aging and suppression of neurogenesis (Bachstetter et al., 2009). Administration of CX3CL1 reversed the decline in age-associated neurogenesis. Reduced CXCL12/CXCR4 in a mouse model of Alzheimer disease also correlated with impaired learning (Parachikova and Cotman, 2007). In contrast, increased serum levels of CXCL8 were likely causing poor memory and motor performance (Baune et al., 2008). Very interesting, reduced levels of CXCL12 among other hypoxia-responsive cytokines is responsible for impaired wound healing in aged animals as neovascularization is less efficient (Loh et al., 2009). Moreover, we have shown that, despite of reduced innervation in aged rats, infusion of CXCL12 is still able to increase axonal sprouting following SCI (Jaerve et al., 2011).

Perspective

Recent findings suggest a dual role of chemokines in CNS injury. Their role in leukocyte recruitment and activation is well known. It is, however, a matter of considerable debate, whether the inflammatory response is beneficial or harmful for tissue protection and repair. Mostly, it is considered to have detrimental effects, and several studies with anti-inflammatory strategies blocking the action of chemokines (e.g anti-CXCL10 antibody) or their receptors (deficiency of CX3CR1) have been shown to improve recovery (Table 3). In several cases (e.g. CCL3, CCL5) controversial results were obtained. A possible explanation could be due to the fact, that monocytes are a very heterogenous group of cells, with pro-inflammatory or regeneration-promoting characteristics, and that monocytes change their functional states (David and Kroner, 2011). In the study of Donelly et al. (2011), blockade of CX3CR1 achieved a better anatomical and functional recovery from SCI in mice, because recruitment or maturation of monocyte-derived macrophages of neurotoxic phenotype ($Ly6C^{lo}/iNOS^{+}/MHCII^{+}/CD11c^{-}$) was reduced as well as their ability to produce inflammatory cytokines and oxidative metabolites; instead, there were more $CCR2^{+}/Ly6C^{hi}/MHCII^{-}/CD11c^{+}$ monocytes recruited.

Different types of monocytes produce and release also different chemokines. For example, M1 macrophages, which can induce neuron death *in vitro*, produce CXCL9, CXCL10, CXCL11, CXCL13, CCL8, CCL15, CCL19 and CCL20. M2 subpopulations, M2a and M2c, with anti-inflammatory and reparative properties, produce CCL13, CCL14, CCL17, CCL18, CCL22, CCL23, CCL24, CCL26 and CCL16, CCL18, CXCL13, respectively. The M2b type, which produces beneficial IL-10, but also pro-inflammatory molecules IL-12, TNFα, IL-1β and IL-6, synthesizes CCL1, CCL20, CXCL1, CXCL2, CXCL3 (David and Kroner et al., 2011). Information about the expression of the corresponding chemokine receptors by different macrophage subtypes would be valuable in order to block their action. For example, by means of gene expression profiling Martinez et al. (2006) have shown that the CXCL12 receptor CXCR4 was expressed in M2 monocytes and CCR7 in the M1 type. Interestingly, the M2 type is more motile and predominantly attracted by neuronal cultures and neuronally-conditioned medium than the M1 type (Vereyken et al., 2011).

Blocking of the action of some chemokines or their receptors by neutralizing antibodies has been efficient in reducing the infiltrating immune cells and inflammatory process or has regulated the subset of recruited cells. With reduction of inflammatory cells, tissue sparing is increased. Formation of cavitations in the lesion site is characteristic for traumatic spinal cord injury in rat and human and can be suppressed by, e.g., neutralization of the chemokine CXCL10 or blocking of CX3CR1 signalling (Glaser 2004; Donelly et al., 2011).

Chemokines can contribute to repair processes by improving neuronal survival, enhancing stem and progenitor cell homing, and increasing axonal sprouting. Reducing inflammatory signalling in microglia and monocyte-derived macrophages promotes neuroprotection, e.g., by blocking of CX3CR1 signaling (Donelly et al., 2011). Neutralization of the chemokine CXCL10 reduces apoptosis after SCI (Glaser 2006). Reducing the expression of CCL21 in stressed neurons could hamper subsequent activation of microglia. Blocking of the action of CCL2 could be beneficial. Neuroprotective abilities of CCL4 and CCL5 are under discussion.

CNS repair processes are further promoted by increased proliferation and migration of neural and other progenitor cells through chemokines such as CXCL12/CXCR4, CCL3/CCR1, CXCL8 and CXCL13. Neovascularization could be supported by recruitment of endothelial progenitor cells attracted by CXCL12. Angiogenetic properties of CCL2, CCL11, CCL16 and CXCL1-8, except CXCL4, and angiostatic properties of CXCL9-CXCL11 have been reported (Keeley et al., 2010). Neutralization of CXCL10 significantly enhanced formation of new blood vessels at the injury site after SCI (Glaser 2004).

From peripheral nerve injury, it is known that neovascularization precedes nerve regeneration. Reportedly neutralization of the chemokine CXCL10 increased axon sprouting after spinal cord injury (Glaser et al., 2006). We have shown recently that infusion of CXCL12 enhanced axonal sprouting following SCI (Opatz et al., 2009; Jaerve et al., 2011). CXCL12 is able to induce neurite arborisation by elevating cAMP levels (Chalasani et al., 2003; Pujol et al., 2005) which activates expression of regeneration supporting genes such as Arginase I which is involved in the synthesis of polyamines (Cai et al., 2002).

Remyelination by oligodendrocytes is also regulated by some chemokines. CXCL12 has been shown to induce oligodendrocyte proliferation and maturation as well as remyelination (Göttle et al., 2010). Also CXCL1/CXCR2 signalling may play a role in oligodendrocyte proliferation and recruitment to demyelinated areas. As CXCL14 alters myelin gene expression in cultured Schwann cells (Barbaria et al., 2009), it might also be worth to investigate its role in the CNS.

Some chemokines have also been implicated as potential candidates for neuropathic pain following peripheral nerve injury (Kiguchi et al., 2010) and thus might play a role in neuropathic pain following SCI. Co-expression of CCL2, CCL3 and CXCL12 have been shown together with transmitters and receptors that are involved in nociceptive processing in dorsal horns as well as in brain (Knerlich-Lukoschus et al., 2011; Van Steenwinckel et al., 2011). Antagonists of the CCL2 receptor are promising agents for treating neuropathic pain, as pain-transmission effects were prevented by the administration of INCB3344 or ERK inhibitor (PD98059). As one mechanism for neuropathic pain upregulation of P2X4 expression in microglia following stimulation through neuronally-derived CCL21 has been demonstrated (Biber et al., 2011). CX3CL1 obviously also plays a role, as the deficiency of

its receptor attenuates hyperalgesia and allodynia in a modality-dependent fashion (Staniland et al., 2010).

Conlusions

There is a huge amount of information available now about the expression, regulation, signalling and action of chemokines. Several manipulations with chemokines in experimental CNS trauma models of SCI, TBI and stroke have gained very significant improvements in regenerative processes and blocking of tissue destruction. In many cases, chemokines act very similar in these pathologies, which could facilitate the development of therapies. On the other hand, the success depends on harnessing all the details in the specific injury conditions, e.g., time-course of expression, producing cell subtypes as well as their specific actions. The chemokine system with the chemoattraction function is a very interesting therapeutic target, because the whole neuroinflammation scenario can be manipulated, starting with the lack or timely changed manner of infiltration of inflammatory cells or the subtypes of invading cells. In addition to that, the cells that are able to promote regenerative processes can be called to the scene. Importantly, at least one of the chemokines (CXCL12) can directly stimulate injured axons to grow again.

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References

Al-Bahrani A, Taha S, Shaath H, Bakhiet M (2007) TNF-alpha and IL-8 in acute stroke and the modulation of these cytokines by antiplatelet agents. Curr Neurovasc Res 4:31-37

Andrzejewski MG, Koelsch A, Kogel T, Dreymueller D, Schwarz N, Ludwig A (2010) Distinct role of the intracellular C-terminus for subcellular expression, shedding and function of the murine transmembrane chemokine CX3CL1. Biochem Biophys Res Commun 395:178-184

Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, Kleinman HK, Reaman GH, Tosato G (1995) Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. J Exp Med 182:155-162

Anthony D, Dempster R, Fearn S, Clements J, Wells G, Perry VH, Walker K (1998) CXC chemokines generate age-related increases in neutrophil-mediated brain inflammation and blood-brain barrier breakdown. Curr Biol 8:923-926

Antonelli A, Rotondi M, Fallahi P, Ferrari SM, Paolicchi A, Romagnani P, Serio M, Ferrannini E (2006) Increase of CXC chemokine CXCL10 and CC chemokine CCL2 serum levels in normal ageing. Cytokine 34:32-38

Antony VB, Hott JW, Kunkel SL, Godbey SW, Burdick MD, Strieter RM (1995) Pleural mesothelial cell expression of C-C (monocyte chemotactic peptide) and C-X-C (interleukin 8) chemokines. Am J Respir Cell Mol Biol 12:581-588

Bachstetter AD, Morganti JM, Jernberg J, Schlunk A, Mitchell SH, Brewster KW, Hudson CE, Cole MJ, Harrison JK, Bickford PC, Gemma C (2009) Fractalkine and CX(3)CR1 regulate hippocampal neurogenesis in adult and aged rats. Neurobiol Aging 32:2030-2044

Badan I, Buchhold B, Hamm A, Gratz M, Walker LC, Platt D, Kessler C, Popa-Wagner A (2003) Accelerated glial reactivity to stroke in aged rats correlates with reduced functional recovery. JCereb Blood Flow Metab 23:845-854

Bagaeva LV, Rao P, Powers JM, Segal BM (2006) CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis. J Immunol 176:7676-7685

Bakondi B, Shimada IS, Peterson BM, Spees JL (2011) SDF-1α secreted by human CD133-derived multipotent stromal cells promotes neural progenitor cell survival through CXCR7. Stem Cells Dev 20:1021-1029

Balabanian K, Lagane B, Infantino S, Chow KY, Harriague J, Moepps B, Arenzana-Seisdedos F, Thelen M, Bachelerie F (2005) The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J Biol Chem 280:35760-35766

Banisadr G, Fontanges P, Haour F, Kitabgi P, Rostene W, Melik Parsadaniantz S (2002) Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. Eur J Neurosci 16:1661-1671

Banisadr G, Gosselin RD, Mechighel P, Kitabgi P, Rostene W, Parsadaniantz SM (2005) Highly regionalized neuronal expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) in rat brain: evidence for its colocalization with neurotransmitters and neuropeptides. J Comp Neurol 489:275-292

Barbaria EM, Kohl B, Buhren BA, Hasenpusch-Theil K, Kruse F, Kury P, Martini R, Muller HW (2009) The alpha-chemokine CXCL14 is up-regulated in the sciatic nerve of a mouse model of Charcot-Marie-Tooth disease type 1A and alters myelin gene expression in cultured Schwann cells. Neurobiol Dis 33:448-458

Bartholdi D, Schwab ME (1997) Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: an in situ hybridization study. Eur J Neurosci 9:1422-1438

Baune BT, Ponath G, Golledge J, Varga G, Arolt V, Rothermundt M, Berger K (2008) Association between IL-8 cytokine and cognitive performance in an elderly general population--the MEMO-Study. Neurobiol Aging 29: 937-944

Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, Greaves DR, Zlotnik A, Schall TJ (1997) A new class of membrane-bound chemokine with a CX3C motif. Nature 385:640-644

Beech JS, Reckless J, Mosedale DE, Grainger DJ, Williams SC, Menon DK (2001) Neuroprotection in ischemia-reperfusion injury: an antiinflammatory approach using a novel broad-spectrum chemokine inhibitor. J Cereb Blood Flow Metab 21:683-689

Benton RL, Maddie MA, Worth CA, Mahoney ET, Hagg T, Whittemore SR (2008) Transcriptomic screening of microvascular endothelial cells implicates novel molecular regulators of vascular dysfunction after spinal cord injury. J Cereb Blood Flow Metab 28:1771-1785

Berman JW, Guida MP, Warren J, Amat J, Brosnan CF (1996) Localization of monocyte chemoattractant peptide-1 expression in the central nervous system in experimental autoimmune encephalomyelitis and trauma in the rat. J Immunol 156:3017-3023

Bhattacharyya BJ, Banisadr G, Jung H, Ren D, Cronshaw DG, Zou Y, Miller RJ (2008) The chemokine stromal cell-derived factor-1 regulates GABAergic inputs to neural progenitors in the postnatal dentate gyrus. J Neurosci 28:6720-6730

Biber K, Sauter A, Brouwer N, Copray SC, Boddeke HW (2001) Ischemia-induced neuronal expression of the microglia attracting chemokine Secondary Lymphoid-tissue Chemokine (SLC). Glia 34:121-133

Biber K, Tsuda M, Tozaki-Saitoh H, Tsukamoto K, Toyomitsu E, Masuda T, Boddeke H, Inoue K (2011) Neuronal CCL21 up-regulates microglia P2X4 expression and initiates neuropathic pain development. EMBO J 30:1864-1873

Bodnar RJ, Yates CC, Rodgers ME, Du X, Wells A (2009) IP-10 induces dissociation of newly formed blood vessels. J Cell Sci 122:2064-2077

Bogoslovsky T, Spatz M, Chaudhry A, Maric D, Luby M, Frank J, Warach S (2011) Stromal-derived factor-1[alpha] correlates with circulating endothelial progenitor cells and with acute lesion volume in stroke patients. Stroke 42:618-625

Brait VH, Rivera J, Broughton BR, Lee S, Drummond GR, Sobey CG (2010) Chemokine-related gene expression in the brain following ischemic stroke: no role for CXCR2 in outcome. Brain Res 1372:169-179

Braunersreuther V, Zernecke A, Arnaud C, Liehn EA, Steffens S, Shagdarsuren E, Bidzhekov K, Burger F, Pelli G, Luckow B, Mach F, Weber C (2007) Ccr5 but not Ccr1 deficiency reduces development of diet-induced atherosclerosis in mice. Arterioscler Thromb Vasc Biol 27:373-379

Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold ME, Sunshine MJ, Littman DR, Kuo CJ, Wei K, McMaster BE, Wright K, Howard MC, Schall TJ (2006) A novel

chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med 203:2201-2213

Cacalano G, Lee J, Kikly K, Ryan AM, Pitts-Meek S, Hultgren B, Wood WI, Moore MW (1994) Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. Science 265:682-684

Cai D, Deng K, Mellado W, Lee J, Ratan RR, Filbin MT (2002) Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. Neuron 35:711-719

Camilloni C, Sutto L (2009) Lymphotactin: how a protein can adopt two folds. J Chem Phys 131:245105

Campbell SJ, Wilcockson DC, Butchart AG, Perry VH, Anthony DC (2002) Altered chemokine expression in the spinal cord and brain contributes to differential interleukin-1beta-induced neutrophil recruitment. J Neurochem 83:432-441

Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nature Neurosci 9:917–924.

Chalasani SH, Sabelko KA, Sunshine MJ, Littman DR, Raper JA (2003) A chemokine, SDF-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. J Neurosci 23:1360-1371

Chapman GA, Moores K, Harrison D, Campbell CA, Stewart BR, Strijbos PJ (2000) Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. J Neurosci 20:RC87

Che X, Ye W, Panga L, Wu DC, Yang GY (2001) Monocyte chemoattractant protein-1 expressed in neurons and astrocytes during focal ischemia in mice. Brain Res 902:171-177

Chen J, Mo R, Lescure PA, Misek DE, Hanash S, Rochford R, Hobbs M, Yung RL (2003) Aging is associated with increased T-cell chemokine expression in C57BL/6 mice. J Gerontol A Biol Sci Med Sci 58:975-983

Chen Y, Hallenbeck JM, Ruetzler C, Bol D, Thomas K, Berman NE, Vogel SN (2003) Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. J Cereb Blood Flow Metab 23:748-755

Cheng SS, Lai JJ, Lukacs NW, Kunkel SL (2001) Granulocyte-macrophage colony stimulating factor up-regulates CCR1 in human neutrophils. J Immunol 166:1178-1184

Chevigne A, Fievez V, Schmit JC, Deroo S (2011) Engineering and screening the N-terminus of chemokines for drug discovery. Biochem Pharmacol 82:1438-56

Coughlan CM, McManus CM, Sharron M, Gao Z, Murphy D, Jaffer S, Choe W, Chen W, Hesselgesser J, Gaylord H, Kalyuzhny A, Lee VM, Wolf B, Doms RW, Kolson DL (2000) Expression of multiple functional chemokine receptors and monocyte chemoattractant protein-1 in human neurons. Neurosci 97:591-600

Cowell RM, Xu H, Galasso JM, Silverstein FS (2002) Hypoxic-ischemic injury inducesmonocyte inflammatory protein-1alpha expression in immature rat brain. Stroke 33:795-801

Cui X, Chen J, Zacharek A, Li Y, Roberts C, Kapke A, Savant-Bhonsale S, Chopp M (2007) Nitric oxide donor upregulation of stromal cell-derived factor-1/chemokine (CXC motif) receptor 4 enhances bone marrow stromal cell migration into ischemic brain after stroke. Stem Cells 25: 2777-2785

Danforth JM, Strieter RM, Kunkel SL, Arenberg DA, VanOtteren GM, Standiford TJ (1995) Monocyte inflammatory protein-1 alpha expression in vivo and in vitro: the role of lipoteichoic acid. Clin Immunol Immunopathol 74:77-83

David S, Kroner A (2011) Repertoire of microglial andmonocyte responses after spinal cord injury. Nat Rev Neurosci 12:388-399

de Jong EK, Dijkstra IM, Hensens M, Brouwer N, van Amerongen M, Liem RS, Boddeke HW, Biber K (2005) Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation for microglia activation remote from a primary lesion. J Neurosci 25:7548-7557

de Jong EK, Vinet J, Stanulovic VS, Meijer M, Wesseling E, Sjollema K, Boddeke HW, Biber K (2008) Expression, transport, and axonal sorting of neuronal CCL21 in large dense-core vesicles. FASEB J 22:4136-4145

Denes A, Ferenczi S, Halasz J, Kornyei Z, Kovacs KJ (2008) Role of CX3CR1 (fractalkine receptor) in brain damage and inflammation induced by focal cerebral ischemia in mouse. J Cereb Blood Flow Metab 28:1707–1721

Deruaz M, Frauenschuh A, Alessandri AL, Dias JM, Coelho FM, Russo RC, Ferreira BR, Graham GJ, Shaw JP, Wells TN, Teixeira MM, Power CA, Proudfoot AE (2008) Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. J Exp Med 205:2019-2031

Deshmane SL, Kremlev S, Amini S, Sawaya BE (2009) Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 29:313-326Devalaraja RM, Nanney LB, Du J, Qian Q, Yu Y, Devalaraja MN, Richmond A (2000) Delayed wound healing in CXCR2 knockout mice. J Invest Dermatol 115:234-244

DeVries M, Kelvin A, Xu L, Ran L, Robinson J, Kelvin D (2006) Defining the origins and evolution of the chemokine/chemokine receptor system. J Immunol 176:401-415

Dimitrijevic OB, Stamatovic SM, Keep RF, Andjelkovic AV (2007) Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. Stroke 38:1345-1353

Donnelly D, Longbrake E, Shawler T, Kigerl K, Lai W, Tovar A, Ransohoff R, Popovich P (2011) Deficient CX3CR1 signaling promotes recovery after mouse spinal cord injury by limiting the recruitment and activation of Ly6Clo/iNOS+monocytes. J Neurosci 31:9910-9922

Dugas JC, Mandemakers W, Rogers M, Ibrahim A, Daneman R, Barres BA (2008) A novel purification method for CNS projection neurons leads to the identification of brain vascular cells as a source of trophic support for corticospinal motor neurons. J Neurosci 28:8294-8305

Edman LC, Mira H, Arenas E (2008) The beta-chemokines CCL2 and CCL7 are two novel differentiation factors for midbrain dopaminergic precursors and neurons. Exp Cell Res 314:2123-2130

Eisman R, Surrey S, Ramachandran B, Schwartz E, Poncz M (1990) Structural and functional comparison of the genes for human platelet factor 4 and PF4alt. Blood 76:336-344

Fan Y, Shen F, Frenzel T, Zhu W, Ye J, Liu J, Chen Y, Su H, Young WL, Yang GY (2010) Endothelial progenitor cell transplantation improves long-term stroke outcome in mice. Ann Neurol 67:488-497

Felzien LK, McDonald JT, Gleason SM, Berman NE, Klein RM (2001) Increased chemokine gene expression during aging in the murine brain. Brain Res 890:137-146

Fimmel S, Devermann L, Herrmann A, Zouboulis C (2007) GRO-alpha: a potential marker for cancer and aging silenced by RNA interference. Ann N Y Acad Sci 1119:176-189

Floridi F, Trettel F, Di Bartolomeo S, Ciotti MT, Limatola C (2003) Signalling pathways involved in the chemotactic activity of CXCL12 in cultured rat cerebellar neurons and CHP100 neuroepithelioma cells. J Neuroimmunol 135:38-46

Frauenschuh A, Power CA, Deruaz M, Ferreira BR, Silva JS, Teixeira MM, Dias JM, Martin T, Wells TN, Proudfoot AE (2007) Molecular cloning and characterization of a highly selective chemokine-binding protein from the tick Rhipicephalus sanguineus. J Biol Chem 282:27250-27258

Fukumoto N, Shimaoka T, Fujimura H, Sakoda S, Tanaka M, Kita T, Yonehara S (2004) Critical roles of CXC chemokine ligand 16/scavenger receptor that binds phosphatidylserine and oxidized lipoprotein in the pathogenesis of both acute and adoptive transfer experimental autoimmune encephalomyelitis. J Immunol173:1620-1627

Ghersa P, Gelati M, Colinge J, Feger G, Power C, Ghersa P, Gelati M, Colinge J, Feger G, Power C, Papoian R, Salmaggi A (2002) MIG--differential gene expression in mouse brain endothelial cells. Neuroreport 13:9-14

Ghirnikar RS, Lee YL, Eng LF (2000) Chemokine antagonist infusion attenuates cellular infiltration following spinal cord contusion injury in rat. J Neurosci Res 59:63-73

Ghirnikar RS, Lee YL, Eng LF (2001) Chemokine antagonist infusion promotes axonal sparing after spinal cord contusion injury in rat. J Neurosci Res 64:582-589

Glabinski AR, Balasingam V, Tani M, Kunkel SL, Strieter RM, Yong VW, Ransohoff RM (1996) Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain. J Immunol156:4363-4368

Glaser J, Gonzalez R, Perreau V, Cotman C, Keirstead H (2004) Neutralization of the chemokine CXCL10 enhances tissue sparing and angiogenesis following spinal cord injury. J Neurosci Res 77:701-708

Glaser J, Gonzalez R, Sadr E, Keirstead H (2006) Neutralization of the chemokine CXCL10 reduces apoptosis and increases axon sprouting after spinal cord injury. J Neurosci Res 84:724-734

Gong JH, Ratkay LG, Waterfield JD, Clark-Lewis I (1997) An antagonist of monocyte chemoattractant protein 1 (MCP-1) inhibits arthritis in the MRL-lpr mouse model. J Exp Med 186:131-137

Gonzalez R, Glaser J, Liu MT, Lane TE, Keirstead HS (2003) Reducing inflammation decreases secondary degeneration and functional deficit after spinal cord injury. Exp Neurol 184:456-463

Gosselin RD, Varela C, Banisadr G, Mechighel P, Rostene W, Kitabgi P, Melik-Parsadaniantz S (2005) Constitutive expression of CCR2 chemokine receptor and inhibition by MCP-1/CCL2 of GABA-induced currents in spinal cord neurones. J Neurochem 95:1023-1034

Gottle P, Kremer D, Jander S, Odemis V, Engele J, Hartung HP, Kury P (2010) Activation of CXCR7 receptor promotes oligodendroglial cell maturation. Ann Neurol 68:915-924

Gourmala NG, Buttini M, Limonta S, Sauter A, Boddeke HW (1997) Differential and time-dependent expression of monocyte chemoattractant protein-1 mRNA by astrocytes andmonocytes in rat brain: effects of ischemia and peripheral lipopolysaccharide administration. J Neuroimmunol 74:35-44

Gouwy M, Struyf S, Catusse J, Proost P, Van Damme J (2004) Synergy between proinflammatory ligands of G protein-coupled receptors in neutrophil activation and migration. J Leukoc Biol 76:185-194

Grau AJ, Reis A, Buggle F, Al-Khalaf A, Werle E, Valois N, Bertram M, Becher H, Grond-Ginsbach C (2001) Monocyte function and plasma levels of interleukin-8 in acute ischemic stroke. J Neurol Sci 192:41-47

Greenberger MJ, Strieter RM, Kunkel SL, Danforth JM, Goodman RE, Standiford TJ (1995) Neutralization of IL-10 increases survival in a murine model of Klebsiella pneumonia. J Immunol 155:722-729

Guyon A, Skrzydelski D, De Giry I, Rovere C, Conductier G, Trocello JM, Dauge V, Kitabgi P, Rostene W, Nahon JL, Melik Parsadaniantz S (2009) Long term exposure to the chemokine CCL2 activates the nigrostriatal dopamine system: a novel mechanism for the control of dopamine release. Neurosci 162:1072-1080

Harkness KA, Sussman JD, Davies-Jones GA, Greenwood J, Woodroofe MN (2003) Cytokine regulation of MCP-1 expression in brain and retinal microvascular endothelial cells. J Neuroimmunol 142:1-9

Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhikari S, Thompson DA, Botti P, Bacon KB, Feng L (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci USA 95:10896-10901

Hatori K, Nagai A, Heisel R, Ryu JK, Kim SU (2002) Fractalkine and fractalkine receptors in human neurons and glial cells. J Neurosci Res 69:418-426

Hau S, Reich DM, Scholz M, Naumann W, Emmrich F, Kamprad M, Boltze J (2008) Evidence for neuroprotective properties of human umbilical cord blood cells after neuronal hypoxia in vitro. BMC Neurosci 9:30

Hausler KG, Prinz M, Nolte C, Weber JR, Schumann RR, Kettenmann H, Hanisch UK (2002) Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia andmonocytes. Eur J Neurosci 16:2113-2122

Hawthorne AL, Popovich PG (2011) Emerging concepts in myeloid cell biology after spinal cord injury. Neurotherapeutics 8:252-261

Hayashi M, Luo Y, Laning J, Strieter RM, Dorf ME (1995) Production and function of monocyte chemoattractant protein-1 and other beta-chemokines in murine glial cells. J Neuroimmunol60:143-150

Helmy A, Carpenter KL, Menon DK, Pickard JD, Hutchinson PJ (2011) The cytokine response to human traumatic brain injury: temporal profiles and evidence for cerebral parenchymal production. J Cereb Blood Flow Metab 31:658-670

Hill WD, Hess DC, Martin-Studdard A, Carothers JJ, Zheng J, Hale D, Maeda M, Fagan SC, Carroll JE, Conway SJ (2004) SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury. J Neuropathol Exp Neurol 63:84-96

Hinojosa AE, Garcia-Bueno B, Leza JC, Madrigal JL (2011) CCL2/MCP-1 modulation of microglial activation and proliferation. J Neuroinflammation 8:77

Hirose K, Okajima K, Taoka Y, Uchiba M, Tagami H, Nakano K, Utoh J, Okabe H, Kitamura N (2000) Activated protein C reduces the ischemia/reperfusion-induced spinal cord injury in rats by inhibiting neutrophil activation. Ann Surg 232:272-280

Horuk R, Martin AW, Wang Z, Schweitzer L, Gerassimides A, Guo H, Lu Z, Hesselgesser J, Perez HD, Kim J, Parker J, Hadley TJ, Peiper SC (1997) Expression of chemokine receptors by subsets of neurons in the central nervous system. J Immunol 158:2882-2890

Hosking MP, Tirotta E, Ransohoff RM, Lane TE (2010) CXCR2 signaling protects oligodendrocytes and restricts demyelination in a mouse model of viral-induced demyelination. PloS one 5:e11340

Huang Y, Zhu XY, Du MR, Wu X, Wang MY, Li DJ (2006) Chemokine CXCL16, a scavenger receptor, induces proliferation and invasion of first-trimester human trophoblast cells in an autocrine manner. Hum Reprod 21:1083-1091

Huffnagle GB, Strieter RM, Standiford TJ, McDonald RA, Burdick MD, Kunkel SL, Toews GB (1995) The role of monocyte chemotactic protein-1 (MCP-1) in the recruitment of monocytes and CD4+ T cells during a pulmonary Cryptococcus neoformans infection. J Immunol 155:4790-4797

Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C (2002) Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. J Cereb Blood Flow Metab 22:308-317

Hurwitz AA, Lyman WD, Berman JW (1995) Tumor necrosis factor alpha and transforming growth factor beta upregulate astrocyte expression of monocyte chemoattractant protein-1. J Neuroimmunol 57:193-198

Ikeda U, Matsui K, Murakami Y, et al. (2002) Monocyte chemoattractant protein-1 and coronary artery disease. Clin Cardiol 25:143–147

Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, Kakizaki M, Takagi S, Nomiyama H, Schall TJ, Yoshie O (1997) Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 91: 521-530

Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, Frenkel D, Li J, Sidman RL, Walsh CA, Snyder EY, Khoury SJ (2004) Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A 101: 18117-18122

Inadera H, Egashira K, Takemoto M, Ouchi Y, Matsushima K (1999) Increase in circulating levels of monocyte chemoattractant protein-1 with aging. J Inteferon Cytokine Res 19:1179-1182

Jaerve A, Schiwy N, Schmitz C, Mueller HW (2011) Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. Exp Neurol 231: 284–294

Jiang L, Newman M, Saporta S, Chen N, Sanberg C, Sanberg PR, Willing AE (2008) MIP-1alpha and MCP-1 Induce Migration of Human Umbilical Cord Blood Cells in Models of Stroke. Curr Neurovasc Res 5:118-124

Jin F, Zhai Q, Qiu L, Meng H, Zou D, Wang Y, Li Q, Yu Z, Han J, Li Q, Zhou B (2008) Degradation of BM SDF-1 by MMP-9: the role in G-CSF-induced hematopoietic stem/progenitor cell mobilization. Bone Marrow Transplant 42: 581-588

Kang J, Jiang MH, Min HJ, Jo EK, Lee S, Karin M, Yune TY, Lee SJ (2011) IKK-beta-mediated myeloid cell activation exacerbates inflammation and inhibits recovery after spinal cord injury. European J Immunol 41:1266-1277

Karpus WJ, Lukacs NW, McRae BL, Strieter RM, Kunkel SL, Miller SD (1995) An important role for the chemokinemonocyte inflammatory protein-1 alpha in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. J Immunol 155:5003-5010

Kasama T, Strieter RM, Lukacs NW, Lincoln PM, Burdick MD, Kunkel SL (1995) Interferon gamma modulates the expression of neutrophil-derived chemokines. J Investig Med 43:58-67

Kavanagh RJ, Kam PC (2001) Lazaroids: efficacy and mechanism of action of the 21-aminosteroids in neuroprotection. Br J Anaesth 86:110-119

Keeley EC, Mehrad B, Strieter RM (2010) Chemokines as mediators of tumor angiogenesis and neovascularization. Exp Cell Res, vol 317. Elsevier Inc, United States, pp 685-690

Kerstetter AE, Padovani-Claudio DA, Bai L, Miller RH (2009) Inhibition of CXCR2 signaling promotes recovery in models of multiple sclerosis. Exp Neurol 220:44-56

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91:461-553

Kiguchi N, Kobayashi Y, Maeda T, Saika F, Kishioka S (2010) CC-chemokine MIP-1alpha in the spinal cord contributes to nerve injury-induced neuropathic pain. Neurosci Lett 484:17-21

Kim HO, Kim HS, Youn JC, Shin EC, Park S (2011) Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. J Transl Med 9:113

Kim JS, Gautam SC, Chopp M, Zaloga C, Jones ML, Ward PA, Welch KM. (1995) Expression of monocyte chemoattractant protein-1 andmonocyte inflammatory protein-1 after focal cerebral ischemia in the rat. J Neuroimmunol 56:127-34

Kim JV, Jiang N, Tadokoro CE, Liu L, Ransohoff RM, Lafaille JJ, Dustin ML (2009) Two-photon laser scanning microscopy imaging of intact spinal cord and cerebral cortex reveals requirement for CXCR6 and neuroinflammation in immune cell infiltration of cortical injury sites. J Immunol Methods 352:89-100

Kim KS, Rajarathnam K, Clark-Lewis I, Sykes BD (1996) Structural characterization of a monomeric chemokine: monocyte chemoattractant protein-3. FEBS Lett 395:277-282

Klehmet J, Harms H, Richter M, Prass K, Volk HD, Dirnagl U, Meisel A, Meisel C (2009) Stroke-induced immunodepression and post-stroke infections: lessons from the preventive antibacterial therapy in stroke trial. Neurosci 158:1184-1193

Knerlich-Lukoschus F, von der Ropp-Brenner B, Lucius R, Mehdorn HM, Held-Feindt J (2010) Chemokine expression in the white matter spinal cord precursor niche after force-defined spinal cord contusion injuries in adult rats. Glia 58:916-931

Kostulas N, Kivisakk P, Huang Y, Matusevicius D, Kostulas V, Link H (1998) Ischemic stroke is associated with a systemic increase of blood mononuclear cells expressing interleukin-8 mRNA. Stroke 29:462-466

Kumamaru H, Saiwai H, Ohkawa Y, Yamada H, Iwamoto Y, Okada S (2011) Age-related differences in cellular and molecular profiles of inflammatory responses after spinal cord injury. J Cell Physiol doi:10.1002

Kunihara T, Sasaki S, Shiiya N, Ishikura H, Kawarada Y, Matsukawa A, Yasuda K (2000) Lazaroid reduces production of IL-8 and IL-1 receptor antagonist in ischemic spinal cord injury. Ann Thorac Surg 69:792-798

Kwon BK, Stammers AM, Belanger LM, Bernardo A, Chan D, Bishop CM, Slobogean GP, Zhang H, Umedaly H, Giffin M, Street J, Boyd MC, Paquette SJ, Fisher CG, Dvorak MF (2010) Cerebrospinal fluid inflammatory cytokines and biomarkers of injury severity in acute human spinal cord injury. J Neurotrauma 27:669-682

Lalor SJ, Segal BM (2010) Lymphoid chemokines in the CNS. J Neuroimmunol 224:56-61

Lee YL, Shih K, Bao P, Ghirnikar RS, Eng LF (2000) Cytokine chemokine expression in contused rat spinal cord. Neurochem Int 36:417-425

Li M, Yu J, Li Y, Li D, Yan D, Qu Z, Ruan Q (2009) CXCR4 positive bone mesenchymal stem cells migrate to human endothelial cell stimulated by ox-LDL via SDF-1alpha/CXCR4 signaling axis. Exp Mol Pathol 88:250-255

Lindner M, Trebst C, Heine S, Skripuletz T, Koutsoudaki PN, Stangel M (2008) The chemokine receptor CXCR2 is differentially regulated on glial cells in vivo but is not required for successful remyelination after cuprizone-induced demyelination. Glia 56:1104-1113

Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86:367-377

Loh SA, Chang EI, Galvez MG, Thangarajah H, El-ftesi S, Vial IN, Lin DA, Gurtner GC (2009) SDF-1 alpha expression during wound healing in the aged is HIF dependent. Plast Reconstr Surg 123:65S-75S

Losy J, Zaremba J (2001) Monocyte chemoattractant protein-1 is increased in the cerebrospinal fluid of patients with ischemic stroke. Stroke 32:2695-2696

Losy J, Zaremba J, Skrobanski P (2005) CXCL1 (GRO-alpha) chemokine in acute ischaemic stroke patients. Folia Neuropathol 43:97-102

Ludwig A, Schulte A, Schnack C, Hundhausen C, Reiss K, Brodway N, Held-Feindt J, Mentlein R (2005) Enhanced expression and shedding of the transmembrane chemokine CXCL16 by reactive astrocytes and glioma cells. J Neurochem 93:1293-1303

Ludwig A, Weber C (2007) Transmembrane chemokines: versatile 'special agents' in vascular inflammation. Thromb Haemost 97:694-703

Luster AD, Greenberg SM, Leder P (1995) The IP-10 chemokine binds to a specific cell surface heparan sulfate site shared with platelet factor 4 and inhibits endothelial cell proliferation. J Exp Med 182:219-231

Ma M, Wei T, Boring L, Charo IF, Ransohoff RM, Jakeman LB (2002) Monocyte recruitment and myelin removal are delayed following spinal cord injury in mice with CCR2 chemokine receptor deletion. J Neurosci Res 68:691-702

Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, Bronson RT, Springer TA (1998) Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci USA 95:9448-9453

Maier IC, Schwab ME (2006) Sprouting, regeneration and circuit formation in the injured spinal cord: factors and activity. Philos Trans R Soc Lond B Biol Sci 361:1611-1634

Mantovani A, Bonecchi R, Locati M (2006) Tuning inflammation and immunity by chemokine sequestration: decoys and more. Nat Rev Immunol 6:907-918

Martinez FO, Gordon S, Locati M, Mantovani A (2006) Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol 177:7303-7311

Massey KD, Strieter RM, Kunkel SL, Danforth JM, Standiford TJ (1995) Cardiac myocytes release leukocyte-stimulating factors. Am J Physiol 269:H980-987

Matloubian M, David A, Engel S, Ryan JE, Cyster JG (2000) A transmembrane CXC chemokine is a ligand for HIV-coreceptor Bonzo. Nat Immunol 1:298-304

Matsumoto T, Yokoi K, Mukaida N, Harada A, Yamashita J, Watanabe Y, Matsushima K (1997) Pivotal role of interleukin-8 in the acute respiratory distress syndrome and cerebral reperfusion injury. J Leukoc Biol 62:581-587

McColl SR, Mahalingam S, Staykova M, Tylaska LA, Fisher KE, Strick CA, Gladue RP, Neote KS, Willenborg DO (2004) Expression of rat I-TAC/CXCL11/SCYA11 during central nervous system inflammation: comparison with other CXCR3 ligands. Lab Invest 84:1418-1429

McTigue DM, Tani M, Krivacic K, Chernosky A, Kelner GS, Maciejewski D, Maki R, Ransohoff RM, Stokes BT (1998) Selective chemokine mRNA accumulation in the rat spinal cord after contusion injury. J Neurosci Res 53:368-376

Meng SZ, Oka A, Takashima S (1999) Developmental expression of monocyte chemoattractant protein-1 in the human cerebellum and brainstem. Brain Dev 21:30-35

Minami M, Satoh M (2003) Chemokines and their receptors in the brain: pathophysiological roles in ischemic brain injury. Life Sci 74:321-327

Mirabelli-Badenier M, Braunersreuther V, Viviani GL, Dallegri F, Quercioli A, Veneselli E, Mach F, Montecucco F (2011) CC and CXC chemokines are pivotal mediators of cerebral injury in ischaemic stroke. Thromb Haemost 105:409-420

Montecucco F, Lenglet S, Gayet-Ageron A, et al. (2010) Systemic and intraplaque mediators of inflammation are increased in patients symptomatic for ischemic stroke. Stroke 41:1394–1404

Montecucco F, Steffens S, Burger F, Da Costa A, Bianchi G, Bertolotto M, Mach F, Dallegri F, Ottonello L (2008a) Tumor necrosis factor-alpha (TNF-alpha) induces integrin CD11b/CD18 (Mac-1) up-regulation and migration to the CC chemokine CCL3 (MIP-1alpha) on human neutrophils through defined signalling pathways. Cell Signal 20:557-568

Montecucco F, Steffens S, Burger F, Pelli G, Monaco C, Mach F (2008b) C-reactive protein (CRP) induces chemokine secretion via CD11b/ICAM-1 interaction in human adherent monocytes. J Leukoc Biol 84:1109-1119

Mortier A, Gouwy M, Van Damme J, Proost P (2011) Effect of posttranslational processing on the in vitro and in vivo activity of chemokines. Exp Cell Res 317:642-654

Muessel MJ, Klein RM, Wilson AM, Berman NE (2002) Ablation of the chemokine monocyte chemoattractant protein-1 delays retrograde neuronal degeneration, attenuates microglial activation, and alters expression of cell death molecules. Brain Res Mol Brain Res 103:12-27

Muller M, Carter SL, Hofer MJ, Manders P, Getts DR, Getts MT, Dreykluft A, Lu B, Gerard C, King NJ, Campbell IL (2007) CXCR3 signaling reduces the severity of experimental autoimmune encephalomyelitis by controlling the parenchymal distribution of effector and regulatory T cells in the central nervous system. J Immunol 179:2774-2786

Murphy PM (2000) Viral antichemokines: from pathogenesis to drug discovery. J Clin Invest 105:1515-1517

Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 382:635-638

Nagasawa T, Nakajima T, Tachibana K, Iizasa H, Bleul CC, Yoshie O, Matsushima K, Yoshida N, Springer TA, Kishimoto T (1996) Molecular cloning and characterization of a murine pre-B-cell growth-stimulating factor/stromal cell-derived factor 1 receptor, a murine homolog of the human immunodeficiency virus 1 entry coreceptor fusin. Proc Nat Acad Sci USA 93:14726-14729

Njemini R, Bautmans I, Lambert M, Demanet C, Mets T (2007) Heat shock proteins and chemokine/cytokine secretion profile in ageing and inflammation. Mech Ageing Dev 128:450-454

Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, Schwartz O, Heard JM, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B (1996) The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 382:833-835

Omari KM, John G, Lango R, Raine CS (2006) Role for CXCR2 and CXCL1 on glia in multiple sclerosis. Glia 53:24-31

Omari KM, Lutz SE, Santambrogio L, Lira SA, Raine CS (2009) Neuroprotection and remyelination after autoimmune demyelination in mice that inducibly overexpress CXCL1. Am J Pathol 174:164-176

Opatz J, Kury P, Schiwy N, Jarve A, Estrada V, Brazda N, Bosse F, Muller HW (2009) SDF-1 stimulates neurite growth on inhibitory CNS myelin. Mol Cell Neurosci 40:293-300

Ormstad H, Aass HC, Lund-Sorensen N, Amthor KF, Sandvik L (2011) Serum levels of cytokines and C-reactive protein in acute ischemic stroke patients, and their relationship to stroke lateralization, type, and infarct volume. J Neurol 258:677-685

Paavola CD, Hemmerich S, Grunberger D, Polsky I, Bloom A, Freedman R, Mulkins M, Bhakta S, McCarley D, Wiesent L, Wong B, Jarnagin K, Handel TM (1998) Monomeric monocyte chemoattractant protein-1 (MCP-1) binds and activates the MCP-1 receptor CCR2B. J Biol Chem 273:33157-33165

Parachikova A, Cotman CW (2007) Reduced CXCL12/CXCR4 results in impaired learning and is downregulated in a mouse model of Alzheimer disease. Neurobiol Dis 28:143-153

Park DH, Eve DJ, Sanberg PR, Musso J, 3rd, Bachstetter AD, Wolfson A, Schlunk A, Baradez MO, Sinden JD, Gemma C (2010) Increased neuronal proliferation in the dentate gyrus of aged rats following neural stem cell implantation. Stem Cells Dev 19:175-180

Patel JR, McCandless EE, Dorsey D, et al. (2010) CXCR4 promotes differentiation of oligodendrocyte progenitors and remyelination. Proc Natl Acad Sci USA 107:11062–11067

Peng H, Huang Y, Rose J, Erichsen D, Herek S, Fujii N, Tamamura H, Zheng J (2004) Stromal cell-derived factor 1-mediated CXCR4 signaling in rat and human cortical neural progenitor cells. J Neurosci Res 76:35-50

Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L (2010) In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood 116:625-7

Pineau I, Sun L, Bastien D, Lacroix S (2009) Astrocytes initiate inflammation in the injured mouse spinal cord by promoting the entry of neutrophils and inflammatory monocytes in an IL-1 receptor/MyD88-dependent fashion. Brain Behav Immun 24:540-553

Proudfoot AE, Power CA, Schwarz MK (2010) Anti-chemokine small molecule drugs: a promising future? Expert Opin Investig Drugs 19:345-355

Pujol F, Kitabgi P, Boudin H (2005) The chemokine SDF-1 differentially regulates axonal elongation and branching in hippocampal neurons. J Cell Sci 118:1071-1080

Rainey-Barger EK, Rumble JM, Lalor SJ, Esen N, Segal BM, Irani DN (2010) The lymphoid chemokine, CXCL13, is dispensable for the initial recruitment of B cells to the acutely inflamed central nervous system. Brain Behav Immun 25:922-931

Rancan M, Bye N, Otto VI, Trentz O, Kossmann T, Frentzel S, Morganti-Kossmann MC (2004) The chemokine fractalkine in patients with severe traumatic brain injury and a mouse model of closed head injury. J Cereb Blood Flow Metab 24:1110-1118

Rappert A, Bechmann I, Pivneva T, Mahlo J, Biber K, Nolte C, Kovac AD, Gerard C, Boddeke HW, Nitsch R, Kettenmann H (2004) CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion. J Neurosci 24:8500-8509

Rappert A, Biber K, Nolte C, Lipp M, Schubel A, Lu B, Gerard NP, Gerard C, Boddeke HW, Kettenmann H (2002) Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl- current and chemotaxis in murine microglia. J Immunol 168:3221-3226

Reale M, Di Giulio C, Cacchio M, Barbacane RC, Grilli A, Felaco M, Bianchi G, Di Gioacchino M, Conti P (2003) Oxygen supply modulates MCP-1 release in monocytes from young and aged rats: decrease of MCP-1 transcription and translation is age-related. Mol Cell Biochem 248:1-6

Reichel CA, Rehberg M, Lerchenberger M, Berberich N, Bihari P, Khandoga AG, Zahler S, Krombach F (2009) Ccl2 and Ccl3 mediate neutrophil recruitment via induction of protein synthesis and generation of lipid mediators. Arterioscler Thromb Vasc Biol 29:1787-1793

Ren M, Guo Q, Guo L, Lenz M, Qian F, Koenen RR, Xu H, Schilling AB, Weber C, Ye RD, Dinner AR, Tang WJ (2010) Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme. EMBO J 29:3952-3966

Rezaie P, Trillo-Pazos G, Everall IP, Male DK (2002) Expression of beta-chemokines and chemokine receptors in human fetal astrocyte and microglial co-cultures: potential role of chemokines in the developing CNS. Glia 37:64-75

Rice T, Larsen J, Rivest S, Yong VW (2007) Characterization of the early neuroinflammation after spinal cord injury in mice. J Neuropathol Exp Neurol 66:184-195

Robin AM, Zhang ZG, Wang L, Zhang RL, Katakowski M, Zhang L, Wang Y, Zhang C, Chopp M (2006) Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. J Cereb Blood Flow Metab 26:125-134

Rose JJ, Foley JF, Murphy PM, Venkatesan S (2004) On the mechanism and significance of ligand-induced internalization of human neutrophil chemokine receptors CXCR1 and CXCR2. J Biol Chem 279:24372-24386

Rostene W, Kitabgi P, Parsadaniantz SM (2007) Chemokines: a new class of neuromodulator? Nat Rev Neurosci 8:895-903

Russo RC, Garcia CC, Teixeira MM (2010) Anti-inflammatory drug development: Broad or specific chemokine receptor antagonists? Curr Opin Drug Discov Devel 13:414-427

Salanga CL, Handel TM (2011) Chemokine oligomerization and interactions with receptors and glycosaminoglycans: the role of structural dynamics in function. Exp Cell Res 317:590-601

Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M (1996) Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry 35:3362-3367

Sanchez-Alcaniz JA, Haege S, Mueller W, Pla R, Mackay F, Schulz S, Lopez-Bendito G, Stumm R, Marin O (2011) Cxcr7 controls neuronal migration by regulating chemokine responsiveness. Neuron 69:77-90

Sandhir R, Puri V, Klein RM, Berman NE (2004) Differential expression of cytokines and chemokines during secondary neuron death following brain injury in old and young mice. Neurosci Lett 369:28-32

Schnyder-Candrian S, Strieter RM, Kunkel SL, Walz A (1995) Interferon-alpha and interferon-gamma down-regulate the production of interleukin-8 and ENA-78 in human monocytes. J Leukoc Biol 57:929-935

Schonemeier B, Kolodziej A, Schulz S, Jacobs S, Hoellt V, et al. (2008) Regional and cellular localization of the CXCl12/SDF-1 chemokine receptor CXCR7 in the developing and adult rat brain. J Comp Neurol 510:207–220

Seebach J, Bartholdi D, Frei K, Spanaus KS, Ferrero E, Widmer U, Isenmann S, Strieter RM, Schwab M, Pfister H, Fontana A (1995) Experimental Listeria meningoencephalitis.monocyte inflammatory protein-1 alpha and -2 are produced intrathecally and mediate chemotactic activity in cerebrospinal fluid of infected mice. J Immunol 155:4367-4375

Seidler S, Zimmermann HW, Bartneck M, Trautwein C, Tacke F (2010) Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults. BMC Immunol 11:30

Shahrara S, Park CC, Temkin V, Jarvis JW, Volin MV, Pope RM (2006) RANTES modulates TLR4-induced cytokine secretion in human peripheral blood monocytes. J Immunol 177:5077-5087

Shannon LA, Calloway PA, Welch TP, Vines CM (2010) CCR7/CCL21 migration on fibronectin is mediated by phospholipase Cgamma1 and ERK1/2 in primary T lymphocytes. J Biol Chem 285:38781-38787

Shurin GV, Yurkovetsky ZR, Chatta GS, Tourkova IL, Shurin MR, Lokshin AE (2007) Dynamic alteration of soluble serum biomarkers in healthy aging. Cytokine 39:123-129

Shyu WC, Lin SZ, Yen PS, Su CY, Chen DC, Wang HJ, Li H (2008) Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. J Pharmacol Exp Ther 324:834-849

Shyu WC, Liu DD, Lin SZ, Li WW, Su CY, Chang YC, Wang HJ, Wang HW, Tsai CH, Li H (2008) Implantation of olfactory ensheathing cells promotes neuroplasticity in murine models of stroke. J Clin Invest 118:2482-2495

Sierro F, Biben C, Martínez-Muñoz L, Mellado M, Ransohoff RM, Li M, Woehl B et al. (2007) Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. Proc Natl Acad Sci USA 104:14759-64.

Smith RE, Strieter RM, Zhang K, Phan SH, Standiford TJ, Lukacs NW, Kunkel SL (1995) A role for C-C chemokines in fibrotic lung disease. J Leukoc Biol 57:782-787

Sokolov VO, Krasnikova TL, Prokofieva LV, Kukhtina NB, Arefieva TI (2009) Expression of markers of regulatory CD4+CD25+foxp3+ cells in atherosclerotic plaques of human coronary arteries. Bull Exp Biol Med 147:726–729

Sorce S, Bonnefont J, Julien S, Marq-Lin N, Rodriguez I, Dubois-Dauphin M, Krause KH (2010) Increased brain damage after ischaemic stroke in mice lacking the chemokine receptor CCR5. Br J Pharmacol 160:311-321

Soriano SG, Amaravadi LS, Wang YF, Zhou H, Yu GX, Tonra JR, Fairchild-Huntress V, Fang Q, Dunmore JH, Huszar D, Pan Y. (2002) Mice deficient in fractalkine are less susceptible to cerebral ischemia-reperfusion injury. J Neuroimmunol 125:59–65

Sozzani S, Zhou D, Locati M, Rieppi M, Proost P, Magazin M, Vita N, van Damme J, Mantovani A (1994) Receptors and transduction pathways for monocyte chemotactic protein-2 and monocyte chemotactic protein-3. Similarities and differences with MCP-1. J Immunol 152:3615-3622

Spitzbarth I, Bock P, Haist V, Stein VM, Tipold A, Wewetzer K, Baumgartner W, Beineke A (2011) Prominent microglial activation in the early proinflammatory immune response in naturally occurring canine spinal cord injury. J Neuropathol Exp Neurol 70:703-714

Spleiss O, Appel K, Boddeke HW, Berger M, Gebicke-Haerter PJ (1998) Molecular biology of microglia cytokine and chemokine receptors and microglial activation. Life Sci 62:1707-1710

Stamatovic S, Shakui P, Keep R, Moore B, Kunkel S, Van Rooijen N, Andjelkovic A (2005) Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. J Cereb Blood Flow Metab 25:593-606

Staniland AA, Clark AK, Wodarski R, Sasso O, Maione F, D'Acquisto F, Malcangio M (2010) Reduced inflammatory and neuropathic pain and decreased spinal microglial response in fractalkine receptor (CX3CR1) knockout mice. J Neurochem 114:1143-1157

Stefini R, Catenacci E, Piva S, Sozzani S, Valerio A, Bergomi R, Cenzato M, Mortini P, Latronico N (2008) Chemokine detection in the cerebral tissue of patients with posttraumatic brain contusions. J Neurosurg 108:958-962

Strieter RM, Kunkel SL, Arenberg DA, Burdick MD, Polverini PJ (1995) Interferon gamma-inducible protein 10 (IP-10), a member of the C-X-C chemokine family, is an inhibitor of angiogenesis. Biochem Biophys Res Commun 210:51-57

Strieter RM, Polverini PJ, Arenberg DA, Kunkel SL (1995) The role of CXC chemokines as regulators of angiogenesis. Shock 4:155-160

Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J, Dzuiba J, Van Damme J, Walz A, Marriott D, et al. (1995) The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 270:27348-27357

Stumm R, Hollt V (2007) CXC chemokine receptor 4 regulates neuronal migration and axonal pathfinding in the developing nervous system: implications for neuronal regeneration in the adult brain. J Mol Endocrinol 38:377-382

Stumm RK, Rummel J, Junker V, Culmsee C, Pfeiffer M, Krieglstein J, Hollt V, Schulz S (2002) A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J Neurosci 22:5865-5878

Sui Y, Potula R, Dhillon N, Pinson D, Li S, Nath A, Anderson C, Turchan J, Kolson D, Narayan O, Buch S (2004) Neuronal apoptosis is mediated by CXCL10 overexpression in simian human immunodeficiency virus encephalitis. Am J Pathol 164:1557-1566

Szmydynger-Chodobska J, Strazielle N, Zink BJ, Ghersi-Egea JF, Chodobski A (2009) The role of the choroid plexus in neutrophil invasion after traumatic brain injury. J Cereb Blood Flow Metab 29:1503-1516

Takami S, Minami M, Nagata I, Namura S, Satoh M (2001) Chemokine receptor antagonist peptide, viral MIP-II, protects the brain against focal cerebral ischemia in mice. J Cereb Blood Flow Metab 21:1430-1435

Takami S, Nishikawa H, Minami M, Nishiyori A, Sato M, Akaike A, Satoh M (1997) Induction ofmonocyte inflammatory protein MIP-1alpha mRNA on glial cells after focal cerebral ischemia in the rat. Neurosci Lett 227:173-176

Takeuchi H, Natsume A, Wakabayashi T, Aoshima C, Shimato S, Ito M, Ishii J, Maeda Y, Hara M, Kim S, Yoshida J (2007) Intravenously transplanted human neural stem cells migrate to the injured spinal cord in adult mice in an SDF-1- and HGF-dependent manner. Neurosci letters 426:69-74

Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, Tarkowski A (1997) Intrathecal release of pro- and anti-inflammatory cytokines during stroke. Clin Exp Immunol 110:492-499

Tatara Y, Ohishi M, Yamamoto K, Shiota A, Hayashi N, Iwamoto Y, Takeda M, Takagi T, Katsuya T, Ogihara T, Rakugi H (2009)monocyte inflammatory protein-1beta induced cell adhesion with increased intracellular reactive oxygen species. J Mol Cell Cardiol 47:104-111

Terao S, Yilmaz G, Stokes KY, Russell J, Ishikawa M, Kawase T, Granger DN (2008) Blood cell-derived RANTES mediates cerebral microvascular dysfunction, inflammation, and tissue injury after focal ischemia-reperfusion. Stroke 39:2560-2570

Terao Y, Ohta H, Oda A, Nakagaito Y, Kiyota Y, Shintani Y (2009)monocyte inflammatory protein-3alpha plays a key role in the inflammatory cascade in rat focal cerebral ischemia. Neurosci Res 64:75-82

Thibeault I, Laflamme N, Rivest S (2001) Regulation of the gene encoding the monocyte chemoattractant protein 1 (MCP-1) in the mouse and rat brain in response to circulating LPS and proinflammatory cytokines. J Comp Neurol 434:461-477

Thirumangalakudi L, Yin L, Rao HV, Grammas P (2007) IL-8 induces expression of matrix metalloproteinases, cell cycle and pro-apoptotic proteins, and cell death in cultured neurons. J Alzheimers Dis 11:305-311

Tripathy D, Thirumangalakudi L, Grammas P (2010) RANTES upregulation in the Alzheimer's disease brain: a possible neuroprotective role. Neurobiol Aging 31:8-16

Tripathy D, Yin X, Sanchez A, Luo J, Martinez J, Grammas P (2010) Cerebrovascular expression of proteins related to inflammation, oxidative stress and neurotoxicity is altered with aging. J Neuroinflammation 7:63

Tsai HH, Frost E, To V, Robinson S, Ffrench-Constant C, Geertman R, Ransohoff RM, Miller RH (2002) The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. Cell 110:373-383

Tysseling VM, Mithal D, Sahni V, Birch D, Jung H, Belmadani A, Miller RJ, Kessler JA (2011) SDF1 in the dorsal corticospinal tract promotes CXCR4+ cell migration after spinal cord injury. J Neuroinflammation 8:16

Van Osselaer N, Van Damme J, Rampart M, Herman AG (1991) Increased microvascular permeability in vivo in response to intradermal injection of neutrophil-activating protein (NAP-2) in rabbit skin. Am J Pathol 138:23-27

Van Steenwinckel J, Reaux-Le Goazigo A, Pommier B, Mauborgne A, Dansereau MA, Kitabgi P, Sarret P, Pohl M, Melik Parsadaniantz S (2011) CCL2 released from neuronal synaptic vesicles in the spinal cord is a major mediator of local inflammation and pain after peripheral nerve injury. J Neurosci 31:5865-5875

VanOtteren GM, Standiford TJ, Kunkel SL, Danforth JM, Strieter RM (1995) Alterations of ambient oxygen tension modulate the expression of tumor necrosis factor andmonocyte inflammatory protein-1 alpha from murine alveolarmonocytes. Am J Respir Cell Mol Biol 13:399-409

VanOtteren GM, Strieter RM, Kunkel SL, Paine R, 3rd, Greenberger MJ, Danforth JM, Burdick MD, Standiford TJ (1995) Compartmentalized expression of RANTES in a murine model of endotoxemia. J Immunol 154:1900-1908

Veldkamp CT, Peterson FC, Pelzek AJ, Volkman BF (2005) The monomer-dimer equilibrium of stromal cell-derived factor-1 (CXCL 12) is altered by pH, phosphate, sulfate, and heparin. Protein Sci 14:1071-1081

Vereyken EJ, Heijnen PD, Baron W, de Vries EH, Dijkstra CD, Teunissen CE (2011) Classically and alternatively activated bone marrow derivedmonocytes differ in cytoskeletal functions and migration towards specific CNS cell types. J Neuroinflammation 8:58

Vergote D, Butler GS, Ooms M, Cox JH, Silva C, Hollenberg MD, Jhamandas JH, Overall CM, Power C (2006) Proteolytic processing of SDF-1alpha reveals a change in receptor specificity mediating HIV-associated neurodegeneration. Proc Natl Acad Sci U S A 103:19182-19187

Vieira AT, Fagundes CT, Alessandri AL, Castor MG, Guabiraba R, Borges VO, Silveira KD, Vieira EL, Goncalves JL, Silva TA, Deruaz M, Proudfoot AE, Sousa LP, Teixeira MM (2009) Treatment with a novel chemokine-binding protein or eosinophil lineage-ablation protects mice from experimental colitis. Am J Pathol 175:2382-2391

Villa P, Triulzi S, Cavalieri B, Di Bitondo R, Bertini R, Barbera S, Bigini P, Mennini T, Gelosa P, Tremoli E, Sironi L, Ghezzi P (2007) The interleukin-8 (IL-8/CXCL8) receptor inhibitor reparixin improves neurological deficits and reduces long-term inflammation in permanent and transient cerebral ischemia in rats. Mol Med 13:125-133

Vinet J, de Jong EK, Boddeke HW, Stanulovic V, Brouwer N, Granic I, Eisel UL, Liem RS, Biber K (2010) Expression of CXCL10 in cultured cortical neurons. J Neurochem 112:703-714

Wain JH, Kirby JA, Ali S (2002) Leucocyte chemotaxis: Examination of mitogen-activated protein kinase and phosphoinositide 3-kinase activation by Monocyte Chemoattractant Proteins-1, -2, -3 and -4. Clin Exp Immunol 127:436-444

Wang J, Norcross M (2008) Dimerization of chemokine receptors in living cells: key to receptor function and novel targets for therapy. Drug Discov Today 13:625-632

Wang L, Li Y, Chen X, Chen J, Gautam SC, Xu Y, Chopp M (2002) MCP-1, MIP-1, IL-8 and ischemic cerebral tissue enhance human bone marrow stromal cell migration in interface culture. Hematology 7:113-117

Wang X, Ellison JA, Siren AL, Lysko PG, Yue TL, Barone FC, Shatzman A, Feuerstein GZ (1998) Prolonged expression of interferon-inducible protein-10 in ischemic cortex after permanent occlusion of the middle cerebral artery in rat. J Neurochem 71:1194-1204

Wang X, Li X, Yaish-Ohad S, Sarau HM, Barone FC, Feuerstein GZ (1999) Molecular cloning and expression of the rat monocyte chemotactic protein-3 gene: a possible role in stroke. Brain Res Mol Brain Res 71:304-312

Wang Y, Deng Y, Zhou GQ (2008) SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. Brain Res 1195:104-112

Wang Y, Luo W, Stricker R, Reiser G (2006) Protease-activated receptor-1 protects rat astrocytes from apoptotic cell death via JNK-mediated release of the chemokine GRO/CINC-1. J Neurochem 98:1046-1060

Watson K, Fan GH (2005)monocyte inflammatory protein 2 inhibits beta-amyloid peptide (1-42)-mediated hippocampal neuronal apoptosis through activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. Mol Pharmacol 67:757-765

Weber C, Kraemer S, Drechsler M, Lue H, Koenen RR, Kapurniotu A, Zernecke A, Bernhagen J (2008) Structural determinants of MIF functions in CXCR2-mediated inflammatory and atherogenic leukocyte recruitment. Proc Natl Acad Sci U S A 105:16278-16283

Weiss N, Deboux C, Chaverot N, Miller F, Baron-Van Evercooren A, Couraud PO, Cazaubon S (2010) IL8 and CXCL13 are potent chemokines for the recruitment of human neural precursor cells across brain endothelial cells. J Neuroimmunol 223:131-134

Woollard KJ, Geissmann F (2010) Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol 7:77-86

Worthmann H, Tryc AB, Goldbecker A, Ma YT, Tountopoulou A, Hahn A, Dengler R, Lichtinghagen R, Weissenborn K (2010) The temporal profile of inflammatory markers and mediators in blood after acute ischemic stroke differs depending on stroke outcome. Cerebrovasc Dis 30:85-92

Wu B, Chien, EY, Mol CD, Fenalti G, Liu W, Katritch V, Abagyan R, Brooun A, Wells P, Bi FC, Hamel DJ, Kuhn P, Handel TM, Cherezov V, Stevens RC (2010) Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. Science 330:1066–1071

Wu D, LaRosa GJ, Simon MI (1993) G protein-coupled signal transduction pathways for interleukin-8. Science 261:101-103

Wynne AM, Henry CJ, Huang Y, Cleland A, Godbout JP (2010) Protracted downregulation of CX3CR1 on microglia of aged mice after lipopolysaccharide challenge. Brain Behav Immun 24:1190-1201

Xia M, Sui Z (2009) Recent developments in CCR2 antagonists. Expert Opin Ther Pat 19:295-303

Xia MQ, Hyman BT (1999) Chemokines/chemokine receptors in the central nervous system and Alzheimer's disease. J Neurovirol 5:32-41

Yamagami S, Tamura M, Hayashi M, Endo N, Tanabe H, Katsuura Y, Komoriya K (1999) Differential production of MCP-1 and cytokine-induced neutrophil chemoattractant in the ischemic brain after transient focal ischemia in rats. J Leukoc Biol 65:744-749

Yamasaki Y, Matsuo Y, Matsuura N, Onodera H, Itoyama Y, Kogure K (1995) Transient increase of cytokine-induced neutrophil chemoattractant, a member of the interleukin-8 family, in ischemic brain areas after focal ischemia in rats. Stroke 26:318-322; discussion 322-313

Yan YP, Sailor KA, Lang BT, Park SW, Vemuganti R, Dempsey RJ (2007) Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. J Cereb Blood Flow Metab 27:1213-1224

Yong VW and Rivest S (2009) Taking advantage of the systemic immune system to cure brain diseases. Neuron 64:55–60.

Zaremba J, Ilkowski J, Losy J. (2006) Serial measurements of levels of the chemokines CCL2, CCL3 and CCL5 in serum of patients with acute ischaemic stroke. Folia Neuropathol 44:282-289

Zhang B, Subramanian S, Dziennis S, Jia J, Uchida M, Akiyoshi K, Migliati E, Lewis AD, Vandenbark AA, Offner H, Hurn PD (2010) Estradiol and G1 reduce infarct size and improve immunosuppression after experimental stroke. J Immunol 184:4087-4094

Zhang L, Ran L, Garcia GE, Wang XH, Han S, Du J, Mitch WE (2009) Chemokine CXCL16 regulates neutrophil andmonocyte infiltration into injured muscle, promoting muscle regeneration. Am J Pathol 175:2518-2527

Zhang X, Chen L, Bancroft DP, Lai CK, Maione TE (1994) Crystal structure of recombinant human platelet factor. Biochemistry 33:8361-8366

Zhao P, Waxman SG, Hains BC (2007) Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. J Neurosci 27:8893-890

Zheng H, Shen CJ, Qiu FY, Zhao YB, Fu GS (2010) Stromal cell-derived factor 1alpha reduces senescence of endothelial progenitor subpopulation in lectin-binding and DiLDL-uptaking cell through telomerase activation and telomere elongation. J Cell Physiol 223:757-763

Zlotnik A, Yoshie O (2000) Chemokines: a new classification system and their role in immunity. Immunity 12:121-127

Zlotnik A, Yoshie O, Nomiyama H (2006) The chemokine and chemokine receptor superfamilies and their molecular evolution. Genome biology 7:243

Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393:595-599

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5. GENERAL DISCUSSION

Do regenerative responses exist in aged CNS following trauma?

It is general view that aging influences negatively regenerative capacities and outcome of injury. Along with that, it is often assumed that treatment at older age is less likely successful. The observations from this thesis let us conclude that potential for regeneration remains in aged CNS as does the efficacy of treatment. Therefore, the hypothesis that regenerative responses might be absent in aged CNS will be reconsidered and discussed.

Do different axon tracts age differently?

We demonstrate that aged rats appear to still retain the potential to show regenerative growth of CGRP, 5-HT and TH fibers. In line with literature, we found that it is the specificity of axon tract which determines the capacity to initiate regeneration or sprouting – being most robust in the sensory and serotonergic system, followed by TH axons, whereas corticospinal tract is the most delicate and less regenerating (Hawthorne et al., 2011; Schiwy et al., 2009). The differences in the reactions and growth capacities of axon tracts might be partially explained with gene expression. Serotonergic neurons have higher levels of GAP-43 and/or β1 integrin, part of the receptor complex for laminin compared to cortical neurons (Milner and Campbell, 2002).

The order of regrowth capacities of the axon tracts studied did not change as animals aged. It might be therefore attempting to assume that aging uniformly affects these fiber tracts. The most significant effect of aging that we found was impaired sprouting capacity of CST; the age-dependent impairment of sprouting capacitiy of 5-HT and TH axons confined area around central canal (CC). This suggests that CST might also be the most vulnerable to aging. In fact, it has been shown by microarray profiling that the neurons in the layer V of the motocortex are much more dependent on energy metabolism and protein synthesis than sensory neurons (Rossner et al., 2006). In our microarray analysis of the sensorymotor cortex following SCI, which includes transection of corticospinal neurons (located to layer V), these processes were identified as the most affected following injury. Aging is accompanied with dysfunction of mitochondria and consequently energy supply, thus the CST neurons might be rendered more susceptible to trauma at older age (e.g. compared to sensory neurons).

We demonstrated that TH axons were more susceptible to aging than serotonergic fibers, as age-dependent reduction in sprouting capacity of TH axons reached significance in at least 3 out of 4 spinal cord cross sectional regions (CC, DH, WM) as compared to only one region (CC) for 5-HT axons. Indeed, there is recent evidence that on the molecular scale different neurons age differently (Moroz and Kohn, 2010).

Survival and well-being of aged animals following SCI

Our study is the first to use a hemisection SCI model in such old rats (24-28 months), which is around their biological life span. We were confronted with literature that did not convey optimism for the survival rates of older animals following SCI. In the experimental studies of Siegenthaler et al. (2008) and Genovese et al. (2006) half of the male rats (12 and 18 months-old, respectively) died of severe bladder infections after SCI. In contrast to these studies, the geriatric rats in our studies survived the hemisection injury as well as young animals and developed no complications. The survival rates were 94 % and 91 % for young and aged groups, respectively. These differences with the above-mentioned studies might be caused by the fact that we used female rats.

Aged animals in our study were more prone to small weight loss after SCI, which however bears no substantial consequence to them, as many show a tendency towards overweight at this age (Fig 4A). Similar changes have been reported in older female rats (Chavipoch et al., 2006). Bladder recovery, which is together with the change in body weight very informative measure for recovery from injury and overall well-being of injured animals, followed the same pattern in young and aged rats during five weeks survival time (Fig 4B). Slower bladder recovery in geriatric rats was reported by Siegenthaler *et al*. (2008). In our study, a relatively high number of aged animals (17%) compared to young animals (7%) did not need manual bladder expression on the first day post injury, which might have been caused by incontinence.

Overall, the hemisection model of SCI in very old rats can be used to study the regeneration potential of CNS axons. In previos studies of our laboratory one axon tract was investigated per animal group. As only limited number of aged animals was available to us (very common situation) we established a triple-staining protocol in this thesis (described in the chapter 4.1). With such approach we could investigate four axon tracts in a single animal instead of one axon tract. This protocol helped furtheron also optimize the numbers of young adult animals used in other studies of our laboratory.

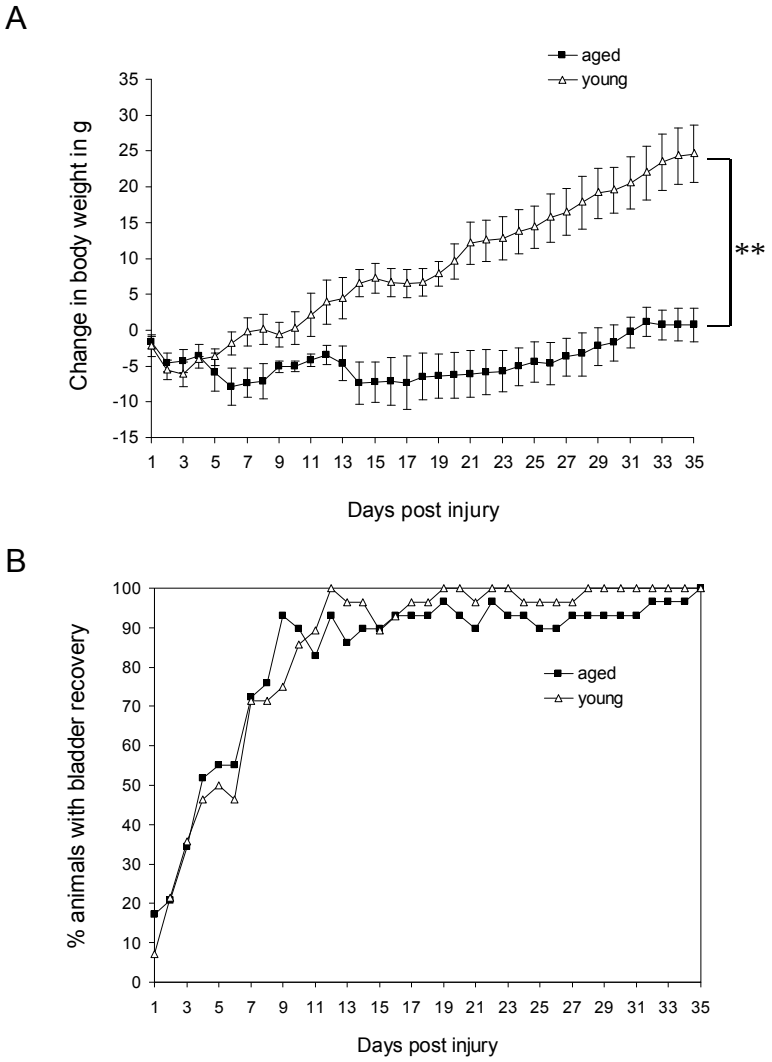

Fig 4. (A) Effect of aging on body weight and bladder function recovery after SCI. (A) Body weight was measured daily after bladder expression. Change from pre-SCI weight for young and aged animals in grams. The slopes of linear regression lines were 0.9 and 0.1445 for young and aged rats, respectively, and differed significantly (**p<0.001). Following the injury, both aged and young animals lost weight. It is not surprising that young animals, which are in a growth phase, continued to gain weight thereafter. Aged animals, on the other hand, transiently lost some weight during the first month. The maximal body weight loss in average did not exceed 10 g in aged animals, which is less than 3 % of the average body weight of these animals. A little drop in the weight of aged animals after day 14 is probably caused by BDA tracing to the motor cortex. (B) Urinary bladders were expressed once daily. No significant differences were observed in the percent of aged and young animals with bladder recovery. At day five, half of the aged and young rats had their bladder function recovered. At day 10 this had occurred in over 85% of aged and young rats and at the end of the study bladder function had recovered in all rats. Because some aged animals recovered bladder function only at five weeks, it appeared that young animals reached the 100% bladder recovery somewhat earlier than aged rats, at around day 12 post injury.

Is treatment effective at older age?

We demonstrated that the effects elicited by AST and SDF-1 infusion were comparable in young and aged animals. However, in all fiber systems tested the levels of SDF-1 induced sprouting in aged animals was significantly lower than in young rats, because this increase started from a markedly lower level in aged animals; the catecholaminergic innervation was reduced around central canal and white matter of aged rats.

AST remained efficient in enhancing the regenerative growth of TH, 5-HT and CGRP axons into lesion site in aged animals. CST fibers were not found in the scar area in both young and aged animals; indeed, the numbers of fiber fragments of CST in the lesion area at 5 week are very small (8 and 12 axon fragments in average in total per control and AST-treated animals, respectively) (Schiwy et al., 2009). Our microarray profiling showed that despite of different reactions to SCI at 1 and 7 dpo, AST elicited similar transcriptional responses in both 2- and 22-month-old rats at 5 weeks. It would be interesting to address the findings such as increased complement activation in aged animals in the future studies.

We observed that aged animals were not more disabled in their locomotion compared to young animals following SCI. This is correlates well with our findings of similar regenerative growth of TH and 5-HT fibers in aged and young animals. These tracts are very important for locomotion in rats, whereas CST is responsible for fine motorics of toes/fingers. Indeed, the differences between walking patterns of the aged and young injured animals appeared to be subtle, such as position of paw placement (more external in aged animals compared to parallel placement in aged); however, this feature was different already in some of the intact aged animals. Instead, the problem appears to be much reduced mobility and explorative locomotion in aged animals in general. Recovery following injury, however, depends highly on rehabilitation - that is physical training of limb movements (rehabilitation by rats includes special cages with enriched environment). In elderly patients, similar or even better recovery of motor deficits was evident, but they had problems in using the recovery in their everyday activities (Jakob et al., 2009). Therefore, the treatment paradigmas achieving the maximal outcome in old animals/patients likely would include both the therapeutic interventions combined with optimized rehabilitation.

SDF-1 is the first compound identified that effectively increases plasticity after trauma in aged spinal cord. Previously, increasing axonal plasticity in subcortical regions triggered by anti-NogoA treatment was shown to improve motor task performance after stroke in geriatric rats (Markus et al., 2005). Thus improving axonal sprouting in spinal cord and brain may not only improve plasticity in young rats but also in aged animals.

6. SUMMARY

Spinal cord injury (SCI) incidence is increasing at older age. However, the influence of aging on the regenerative growth potential of central nervous system axons following SCI is not known. We investigated axonal sprouting along with the efficiency of the infusion of the stromal cell-derived growth factor-1 (SDF-1/CXCL12) and regenerative growth along with the anti-scarring treatment (AST) in young (2-3 months) and geriatric (22-28 months) female rats following SCI. The AST consisted of injections of an iron chelator and cAMP. Axon outgrowth was investigated by immunohistological methods at 5 weeks after a dorsal hemisection at thoracic level T8. We found that aging significantly reduces spontaneous axon sprouting of corticospinal (CST), serotonergic (5-HT) raphespinal and catecholaminergic (TH) coerulospinal tracts in distinct regions of the spinal cord rostral to the lesion. However, impairment of axon sprouting could be markedly attenuated in geriatric animals by local infusion of SDF-1. Unexpectedly and in contrast to rostral sprouting, aging does not diminish the regenerative growth capacity of 5-HT-, TH- and calcitonin gene-related peptide (CGRP)-immunoreactive axons at 5 weeks after SCI. Moreover, 5-HT and TH axons maintain the ability to react upon AST with significantly enhanced regeneration in aged animals. These data are the first to demonstrate, that old age compromises axonal plasticity, but not regenerative growth, after SCI in a fiber tract-specific manner. Furthermore, AST and SDF-1 infusion remain efficient.

SCI causes massive changes in cortical gene expression in young animals, starting as early as 1 day post-operation (dpo). A regeneration-associated transcriptomic program has been identified underlying the long distance axon regeneration along with functional recovery in adult rat upon AST. We investigated to which extent and how the dynamic cortical gene expression profiles of aged (22-months-old) and young (2-months-old) rats following thoracic CST transection differ and whether the AST-induced regeneration program can be activated in aged animals. Therefore, total RNA isolated from layer V/VI of rat sensorimotor cortex at 1, 7, 35 dpo including the acute, subacute and chronic stages of SCI, respectively, was analysed with Affymetrix GeneChips.

With this systemic approach we could show that the cortical transcriptomes following SCI were age-specific because there was little overlap of significantly SCI-regulated genes between aged and young animals and because several of the biological processes exerted by these genes were unique, including complement activation in aged animals. We found that the age-dominated profiles of rat sensorimotor cortex during the acute and subacute phases of thoracic corticospinal tract transection could be shaped to resemble regeneration-associated profiles by AST. This result together with axonal regrowth upon AST in aged animals further implies that therapy in elderly patients might be still feasible.

7. ZUSAMMENFASSUNG

Die akuten Rückenmarkverletzungen treten mit zunehmender Häufigkeit im höheren Lebensalter auf. Allerdings liegen keine Erkenntnisse zu alterungsbedingten Änderungen im Regenerationspotential von verletzten Axonen des zentralen Nervensystems (ZNS) vor. Wir haben das axonale Aussprossen und die Infusionseffizienz des Stromal Cell-Derived Growth Factor-1 (SDF-1/CXCL12) und das regenerative Wachstum zusammen mit Anti-scarring-Behandlung (AST) in jungen (2-3 Monate) und geriatrischen (22-28 Monate) weiblichen Ratten nach Rückenmarkverletzung untersucht. AST, eine jüngst in diesem Labor entwickelte Therapie zur Unterdrückung der Narbenbildung durch lokale Applikation eines Eisen-Chelators und eines zyklischen Adenosinmonophosphats (cAMP), führt zur axonalen Regeneration über große Distanzen und zur funktionellen Erholung. Wir haben das axonale Wachstum 5 Wochen nach dorsaler Hemisektion am thorakalen Ebene T8 mit immunhistochemischen Methoden untersucht und dabei das durch die Alterung signifikant reduzierte spontane Aussprossen von dem Kortikospinaltrakt (CST), dem serotonergischen (5-HT) Raphespinaltrakt und dem catecholaminergen (TH) Coerulospinaltrakt in spezifischen Regionen von Rückenmark rostral der Läsionsstelle festgestellt.

Allerdings, konnten wir die Beeinträchtigung vom axonalen Aussprossen in den geriatrischen Tieren eine Woche nach der Verletzung ausschließlich mit lokaler Infusion von SDF-1 abmindern. Unerwartet im Gegensatz zum rostralen Aussprossen, hat die regenerative Wachstumskapazität von 5-HT-, TH- und Calcitonin-Gene-Related-Peptide(CGRP)-immunoreaktiven Axonen 5 Wochen nach Rückenmarkverletzung nicht nachgelassen.

Außerdem, behalten 5-HT und TH Axonen die Fähigkeit auf AST mit signifikant erhöhter Regeneration in alten Tieren zu reagieren. Diese Daten sind die ersten, die demonstrieren, dass das hohe Alter das Aussprossen, aber nicht das regenerative Wachstum nach Rückenmarkverletzung auf fasertrakt-spezifische Weise beeinträchtigt. Darüber hinaus, bleiben AST und SDF-1-Infusion effizient.

Schon am 1. Tag nach der Operation (days post operation, dpo) und mit wachsender Wirkung mit der Zeit führt Rückenmarkverletzung zu massiven Veränderungen in kortikaler Genexpression in jungen Tieren.

Des Weiteren konnte man ein regenerationassoziiertes transkriptionelles Programm in den AST-behandelten Tieren identifizieren, das für Regeneration und funktionelle Erholung in jungen adulten Ratten verantwortlich ist. In dieser Arbeit wurden Genexpressionsprofile der Schicht V des sensorimotorischen Kortex nach Transsektion des thorakalen Kortikospinaltrakts am 1, 7, 35 dpo, die akuten, die subakuten und die chronischen Stadien einer Rückenmarkverletzung entsprechend mit Hilfe von Microarray-Analysen (Affymetrix) untersucht. Wir haben analysiert, wie sich die dynamischen kortikalen Genexpression-Profile

der alten (22 Monate) und der jungen (2 Monate) Ratten unterscheiden und ob das AST-induzierte Regenerationsprogramm sich in alten Tieren aktivieren lässt.

Mit diesem systemischen Ansatz konnten wir demonstrieren, dass das kortikale Transkriptom nach Rückenmarkverletzung altersabhängig reguliert ist, weil wir nur kleine Überlappung in signifikant rückenmarkverletzungsregulierten Genen zwischen alten und jungen Tieren gefunden haben und weil verschiedene biologische Prozesse, die von diesen Genen reguliert werden unikal waren (wie z.B. Aktivierung des Komplements in alten Tieren). Altersdominierende Profile vom sensorimotorischen Kortex der Ratte in akuten und subakuten Phasen der thorakalen Kortikospinaltrakt-Transsektion haben sich bei AST zu regenerationsassoziierten Profilen formen lassen.

Diese Ergebnisse mit dem axonalen Wachstum auf AST in alten Tieren deuten darauf hin, dass die Therapie in älteren Patienten noch möglich sein könnte.

8. REFERENCES

Abankwa D, Kury P, Muller HW (2002) Dynamic changes in gene expression profiles following axotomy of projection fibres in the Mammalian CNS. Mol Cell Neurosci. 21:421-435.

Afshari FT, Kappagantula S, Fawcett JW (2009) Extrinsic and intrinsic factors controlling axonal regeneration after spinal cord injury. Expert Rev Mol Med. 11:e37.

Agnati LF, Fuxe K, Giardino L, Calza L, Zoli M, et al. (1985) Evidence for cholecystokinin-dopamine receptor interactions in the central nervous system of the adult and old rat. Studies on their functional meaning. Ann N Y Acad Sci 448: 315-333.

Algeri S, Calderini G, Lomuscio G, Vantini G, Toffano G, et al. (1982) Changes with age in rat central monoaminergic system responses to cold stress. Neurobiol Aging 3: 237-242.

Alstermark B, Gorska T, Lundberg A, Pettersson LG, Walkowska M (1987) Effect of different spinal cord lesions on visually guided switching of target-reaching in cats. Neurosci Res. 5: 63-67.

Alstermark B, Lundberg A, Pettersson LG, Tantisira B, Walkowska M (1987) Motor recovery after serial spinal cord lesions of defined descending pathways in cats. Neurosci Res. 5: 68-73.

Bareyre FM, Haudenschild B, Schwab ME (2002) Long-lasting sprouting and gene expression changes induced by the monoclonal antibody IN-1 in the adult spinal cord. J Neurosci. 22: 7097-7110.

Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, Schwab ME (2004) The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat Neurosci. 7: 269-77.

Bartus K, James ND, Bosch KD, Bradbury EJ (2011) Chondroitin sulphate proteoglycans: Key modulators of spinal cord and brain plasticity. Exp Neurol. In press.

Bartzokis G, Cummings JL, Sultzer D, Henderson VW, Nuechterlein KH, et al. (2003) White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. Arch Neurol. 60: 393-398.

Benson MD, Romero MI, Lush ME, Lu QR, Henkemeyer M, et al. (2005) Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. Proc Natl Acad Sci U S A. 102: 10694-10699.

Bernstein DR, Stelzner DJ (1983) Plasticity of the corticospinal tract following midthoracic spinal injury in the postnatal rat. J Comp Neurol 221: 382-400.

Berry M, Maxwell WL, Logan A, Mathewson A, McConnell P, et al. (1983) Deposition of scar tissue in the central nervous system. Acta Neurochir Suppl (Wien) 32: 31-53.

Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, et al. (2003) Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci. 23: 3807-3819.

Bourgeois JP, Rakic P (1996) Synaptogenesis in the occipital cortex of macaque monkey devoid of retinal input from early embryonic stages. Eur J Neurosci 8: 942-950.

Bradbury EJ, McMahon SB (2006) Spinal cord repair strategies: why do they work? Nat Rev Neurosci. 7: 644-653.

Brosamle C, Schwab ME (1997) Cells of origin, course, and termination patterns of the ventral, uncrossed component of the mature rat corticospinal tract. J Comp Neurol. 386: 293-303.

Buell SJ, Coleman PD (1979) Dendritic growth in the aged human brain and failure of growth in senile dementia. Science 206: 854-856.

Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. Nat Rev Neurosci. 7: 30-40.

Campbell JJ, Pomeranz B (1993) A new method to study motoneuron regeneration using electromyograms shows that regeneration slows with age in rat sciatic nerve. Brain Res. 603: 264-270.

Carmichael ST (2003) Gene expression changes after focal stroke, traumatic brain and spinal cord injuries. Curr Opin Neurol 16: 699-704.

Carstensen LL, Turan B, Scheibe S, Ram N, Ersner-Hershfield H, et al. (2011) Emotional experience improves with age: evidence based on over 10 years of experience sampling. Psychol Aging. 26: 21-33.

Chaovipoch P, Jelks KA, Gerhold LM, West EJ, Chongthammakun S, et al. (2006) 17beta-estradiol is protective in spinal cord injury in post- and pre-menopausal rats. J Neurotrauma 23: 830-852.

Choi SJ, Harii K, Lee MJ, Furuya F, Ueda K (1995) Electrophysiological, morphological, and morphometric effects of aging on nerve regeneration in rats. Scand J Plast Reconstr Surg Hand Surg 29: 133-140.

Clark FM, Proudfit HK (1993) The projections of noradrenergic neurons in the A5 catecholamine cell group to the spinal cord in the rat: anatomical evidence that A5 neurons modulate nociception. Brain Res. 616: 200-210.

Craik FI, Moscovitch M, McDowd JM (1994) Contributions of surface and conceptual information to performance on implicit and explicit memory tasks. J Exp Psychol Learn Mem Cogn 20: 864-875.

De Biase A., Knoblach SM, Di Giovanni S et al. (2005) Gene expression profiling of experimental traumatic spinal cord injury as a function of distance from impact site and injury severity. Physiol Genomics 22: 368-381.

Deerberg, F., Rapp, K., Rehm, S., Pittermann, W (1980) Genetic and environmental influences on lifespan and disease in Han:Wistar rats. Mechanisms of Aging and Development. 14: 333-343.

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, et al. (1996) Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci U S A 93: 9887-9892.

Deumens R, Koopmans GC, Joosten EA (2005) Regeneration of descending axon tracts after spinal cord injury. Prog Neurobiol. 77: 57-89.

DeVivo MJ (2007) Sir Ludwig Guttmann Lecture: trends in spinal cord injury rehabilitation outcomes from model systems in the United States: 1973-2006. Spinal Cord. England. pp. 713-721.

Dorce VA, Palermo-Neto J (1994) Behavioral and neurochemical changes induced by aging in dopaminergic systems of male and female rats. Physiol Behav. 56: 1015-1019.

Duncan MR, Frazier KS, Abramson S, Williams S, Klapper H, et al. (1999) Connective tissue growth factor mediates transforming growth factor beta-induced collagen synthesis: down-regulation by cAMP. FASEB J 13: 1774-1786.

Elbert T, Flor H (1999) Magnetoencephalographic investigations of cortical reorganization in humans. Electroencephalogr Clin Neurophysiol Suppl 49: 284-291.

Emerich DF, McDermott P, Krueger P, Banks M, Zhao J, et al. (1993) Locomotion of aged rats: relationship to neurochemical but not morphological changes in nigrostriatal dopaminergic neurons. Brain Res Bull. 32: 477-486.

Erraji-Benchekroun L, Underwood MD, Arango V, Galfalvy H, Pavlidis P, et al. (2005) Molecular aging in human prefrontal cortex is selective and continuous throughout adult life. Biol Psychiatry. 57: 549-558.

Fawcett JW, Asher RA (1999) The glial scar and central nervous system repair. Brain Res Bull. 49: 377-391.

Feringa ER, Kowalski TF, Vahlsing HL, Frye RA (1979) Enzyme treatment of spinal cord transected rats. Ann Neurol 5: 203-206.

Filbin MT (2003) Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat Rev Neurosci. 4: 703-713.

Fitch MT, Doller C, Combs CK, Landreth GE, Silver J (1999) Cellular and molecular mechanisms of glial scarring and progressive cavitation: in vivo and in vitro analysis of inflammation-induced secondary injury after CNS trauma. J Neurosci. 19: 8182-98.

Fouad K, Tse A (2008) Adaptive changes in the injured spinal cord and their role in promoting functional recovery. Neurol Res 30: 17-27.

Fraser HB, Khaitovich P, Plotkin JB, Paabo S, Eisen MB (2005) Aging and gene expression in the primate brain. PLoS Biol. 3:e274.

Freo U, Rapoport SI, Soncrant TT (1991) Age-related alterations in behavioral and cerebral metabolic responses to the serotonin agonist meta-chlorophenylpiperazine in rats. Neurobiol Aging 12: 137-144.

Friedemann MN, Gerhardt GA (1992) Regional effects of aging on dopaminergic function in the Fischer-344 rat. Neurobiol Aging. 13: 325-332.

Furlan JC, Bracken MB, Fehlings MG Is age a key determinant of mortality and neurological outcome after acute traumatic spinal cord injury? Neurobiol Aging. 31: 434-446.

Furlan JC, Krassioukov AV, Fehlings MG (2005) The effects of gender on clinical and neurological outcomes after acute cervical spinal cord injury. J Neurotrauma 22: 368-381.

Gemma C, Mesches MH, Sepesi B, Choo K, Holmes DB, et al. (2002) Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. J Neurosci. 22: 6114-6120.

Genovese T, Mazzon E, Di Paola R, Crisafulli C, Muia C, et al. (2006) Increased oxidative-related mechanisms in the spinal cord injury in old rats. Neurosci Lett. 393: 141-146.

Gerin C, Becquet D, Privat A (1995) Direct evidence for the link between monoaminergic descending pathways and motor activity. I. A study with microdialysis probes implanted in the ventral funiculus of the spinal cord. Brain Res. 704: 191-201.

Gershkoff AM, Cifu DX, Means KM (1993) Geriatric rehabilitation. 1. Social, attitudinal, and economic factors. Arch Phys Med Rehabil 74: S402-405.

Geula C, Bu J, Nagykery N, Scinto LF, Chan J, et al. (2003) Loss of calbindin-D28k from aging human cholinergic basal forebrain: relation to neuronal loss. J Comp Neurol 455: 249-259.

Glance-2011.[Online].Available https://www.nscisc.uab.edu/public_content/pdf/Facts%202011%20Feb%20Final.pdf via Internet. Accessed 19.06.2011

Goicoechea C, Ormazabal MJ, Alfaro MJ, Martin MI (1997) Age-related changes in nociception, behavior, and monoamine levels in rats. Gen Pharmacol. 28: 331-336.

Goldberg JL, Vargas ME, Wang JT, Mandemakers W, Oster SF, et al. (2004) An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J Neurosci. 24: 4989-4999.

Goldman G, Coleman PD (1981) Neuron numbers in locus coeruleus do not change with age in Fisher 344 rat. Neurobiol Aging. 2: 33-36.

Grimpe B, Silver J (2002) The extracellular matrix in axon regeneration. Prog Brain Res 137: 333-349.

Guth L, Albuquerque EX, Deshpande SS, Barrett CP, Donati EJ, et al. (1980) Ineffectiveness of enzyme therapy on regeneration in the transected spinal cord of the rat. J Neurosurg 52: 73-86.

Gutmann E (1942) Factors affecting recovery of motor function after nerve lesions. J Neurol Psychiatry 5: 81-95.

Gwak YS, Hains BC, Johnson KM, Hulsebosch CE (2004) Effect of age at time of spinal cord injury on behavioral outcomes in rat. J Neurotrauma 21: 983-993.

Hafidi A, Grumet M, Sanes DH (2004) In vitro analysis of mechanisms underlying age-dependent failure of axon regeneration. J Comp Neurol. 470: 80-92.

Hashizume K, Kanda K, Burke RE (1988) Medial gastrocnemius motor nucleus in the rat: age-related changes in the number and size of motoneurons. J Comp Neurol 269: 425-430.

Hawthorne AL, Hu H, Kundu B, Steinmetz MP, Wylie CJ, et al. The unusual response of serotonergic neurons after CNS Injury: lack of axonal dieback and enhanced sprouting within the inhibitory environment of the glial scar. J Neurosci. 31: 5605-5616.

Hawthorne AL, Popovich PG (2011) Emerging concepts in myeloid cell biology after spinal cord injury. Neurotherapeutics. 8: 252-61.

Hedden T, Gabrieli JD (2004) Insights into the ageing mind: a view from cognitive neuroscience. Nat Rev Neurosci. 5: 87-96.

Hermanns S, Klapka N, Muller HW (2001) The collagenous lesion scar--an obstacle for axonal regeneration in brain and spinal cord injury. Restor Neurol Neurosci 19: 139-148.

Hermanns S, Reiprich P, Muller HW (2001) A reliable method to reduce collagen scar formation in the lesioned rat spinal cord. J Neurosci Methods. 110: 141-146.

Hofteig JH, Vo PN, Yates AJ (1981) Wallerian degeneration of peripheral nerve. Age-dependent loss of nerve lipids. Acta Neuropathol 55: 151-156.

Holstege JC, Kuypers HG (1987) Brainstem projections to spinal motoneurons: an update. Neuroscience. 23: 809-821.

Hynes M, Rosenthal A (1999) Specification of dopaminergic and serotonergic neurons in the vertebrate CNS. Curr Opin Neurobiol. 9: 26-36.

Hyun DH, Emerson SS, Jo DG, Mattson MP, de Cabo R (2006) Calorie restriction up-regulates the plasma membrane redox system in brain cells and suppresses oxidative stress during aging. Proc Natl Acad Sci U S A. 103: 19908-19912.

Inman DM, Steward O (2003) Ascending sensory, but not other long-tract axons, regenerate into the connective tissue matrix that forms at the site of a spinal cord injury in mice. J Comp Neurol 462: 431-449.

Ishikawa M, Matsumoto M, Fujimura Y, Chiba K, Toyama Y (2003) Changes of cervical spinal cord and cervical spinal canal with age in asymptomatic subjects. Spinal Cord. 41: 159-163.

Israelsson C, Lewen A, Kylberg A, Usoskin D, Althini S, et al. (2006) Genetically modified bone morphogenetic protein signalling alters traumatic brain injury-induced gene expression responses in the adult mouse. J Neurosci Res 84: 47-57.

Jackson AB, Dijkers M, Devivo MJ, Poczatek RB (2004) A demographic profile of new traumatic spinal cord injuries: change and stability over 30 years. Arch Phys Med Rehabil. 85: 1740-1748.

Jakob W, Wirz M, van Hedel HJ, Dietz V, Group E-SS (2009) Difficulty of elderly SCI subjects to translate motor recovery--"body function"--into daily living activities. J Neurotrauma 26: 2037-2044.

Jankowska E, Lundberg A, Roberts WJ, Stuart D (1974) A long propriospinal system with direct effect on motoneurones and on interneurones in the cat lumbosacral cord. Exp Brain Res 21: 169-194.

Jiang CH, Tsien JZ, Schultz PG, Hu Y (2001) The effects of aging on gene expression in the hypothalamus and cortex of mice. Proc Natl Acad Sci U S A. 98: 1930-1934.

Jiang M, Ryu J, Kiraly M, Duke K, Reinke V, et al. (2001) Genome-wide analysis of developmental and sex-regulated gene expression profiles in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 98: 218-223.

Johnson H, Mossberg K, Arvidsson U, Piehl F, Hokfelt T, et al. (1995) Increase in alpha-CGRP and GAP-43 in aged motoneurons: a study of peptides, growth factors, and ChAT mRNA in the lumbar spinal cord of senescent rats with symptoms of hindlimb incapacities. J Comp Neurol 359: 69-89.

Johnson H, Ulfhake B, Dagerlind A, Bennett GW, Fone KC, et al. (1993) The serotoninergic bulbospinal system and brainstem-spinal cord content of serotonin-, TRH-, and substance P-like immunoreactivity in the aged rat with special reference to the spinal cord motor nucleus. Synapse 15: 63-89.

Kalman B, Lublin FD, Alder H (1997) Impairment of central and peripheral myelin in mitochondrial diseases. Mult Scler 2: 267-278.

Kawamura Y, O'Brien P, Okazaki H, Dyck PJ (1977) Lumbar motoneurons of man II: the number and diameter distribution of large- and intermediate-diameter cytons in "motoneuron columns" of spinal cord of man. J Neuropathol Exp Neurol 36: 861-870.

Kawano H, Li HP, Sango K, Kawamura K, Raisman G (2005) Inhibition of collagen synthesis overrides the age-related failure of regeneration of nigrostriatal dopaminergic axons. J Neurosci Res 80: 191-202.

Kennedy PR (1990) Corticospinal, rubrospinal and rubro-olivary projections: a unifying hypothesis. Trends Neurosci 13: 474-479.

Kerezoudi E, Thomas PK (1999) Influence of age on regeneration in the peripheral nervous system. Gerontology. 45: 301-306.

Klapka N, Hermanns S, Straten G, Masanneck C, Duis S, et al. (2005) Suppression of fibrous scarring in spinal cord injury of rat promotes long-distance regeneration of corticospinal tract axons, rescue of primary motoneurons in somatosensory cortex and significant functional recovery. Eur J Neurosci. 22: 3047-3058.

Klapka N, Muller HW (2006) Collagen matrix in spinal cord injury. J Neurotrauma 23: 422-435.

Kloppel S, Kovacs GG, Voigtlander T, Wanschitz J, Flicker H, et al. (2001) Serotonergic nuclei of the raphe are not affected in human ageing. Neuroreport 12: 669-671.

Ko ML, King MA, Gordon TL, Crisp T (1997) The effects of aging on spinal neurochemistry in the rat. Brain Res Bull. 41: 95-98.

Krassioukov AV, Furlan JC, Fehlings MG (2003) Medical co-morbidities, secondary complications, and mortality in elderly with acute spinal cord injury. J Neurotrauma 20: 391-399.

Kruse F, Bosse F, Vogelaar CF et al. Cortical gene expression in spinal cord repair: insight into the complexity of the neural regeneration program. Front Mol Neurosci. In press.

Kruse F, Brazda N., Kuery P, Bosse F, Mueller HW (2008) Analyzing complex gene expression profiles in sensorimotor cortex following spinal cord injury and regeneration promoting treatment. In: Müller HW, editor. Neural Degeneration and Repair. Gene Expression Profiling, Proteomics and Systems Biology. Weinheim: WILEY-VCH. pp. 61-89.

Kumamaru H, Saiwai H, Ohkawa Y, Yamada H, Iwamoto Y, et al. (2011) Age-related differences in cellular and molecular profiles of inflammatory responses after spinal cord injury. J Cell Physiol. doi: 10.1002/jcp.22845.

Küry P, Abankwa D, Kruse F, Greiner-Petter R, Muller HW (2004) Gene expression profiling reveals multiple novel intrinsic and extrinsic factors associated with axonal regeneration failure. Eur J Neurosci. 19: 32-42.

Lamoureux PL, O'Toole MR, Heidemann SR, Miller KE (2010) Slowing of axonal regeneration is correlated with increased axonal viscosity during aging. BMC Neurosci. 11: 140.

Lee CK, Weindruch R, Prolla TA (2000) Gene-expression profile of the ageing brain in mice. Nat Genet 25: 294-297.

Leenen LP, Meek J, Posthuma PR, Nieuwenuys R (1989) Differences in the fiber composition of the pyramidal tract in two- and 14-month-old rats. Neuroscience 28: 635-643.

Li S, Overman JJ, Katsman D, Kozlov SV, Donnelly CJ et al. (2010) An age-related sprouting transcriptome provides molecular control of axonal sprouting after stroke. Nat Neurosci 13: 1496-1504.

Liesi P, Kauppila T (2002) Induction of type IV collagen and other basement-membrane-associated proteins after spinal cord injury of the adult rat may participate in formation of the glial scar. Exp Neurol. 173: 31-45.

Lin W, Bailey SL, Ho H, Harding HP, Ron D, et al. (2007) The integrated stress response prevents demyelination by protecting oligodendrocytes against immune-mediated damage. J Clin Invest 117: 448-456.

Liu X, Erikson C, Brun A (1996) Cortical synaptic changes and gliosis in normal aging, Alzheimer's disease and frontal lobe degeneration. Dementia 7: 128-134.

Lockhart DJ, Barlow C (2001) Expressing what's on your mind: DNA arrays and the brain. Nat Rev Neurosci 2: 63-68.

Lovell JA, Novak JC, Stuesse SL, Cruce WL, Crisp T (2000) Changes in spinal serotonin turnover mediate age-related differences in the behavioral manifestations of peripheral nerve injury. Pharmacol Biochem Behav. 66: 873-878.

Lu T, Pan Y, Kao SY, Li C, Kohane I, et al. (2004) Gene regulation and DNA damage in the ageing human brain. Nature. 429: 883-891.

Lund J, Tedesco P, Duke K, Wang J, Kim SK, et al. (2002) Transcriptional profile of aging in C. elegans. Curr Biol. 12: 1566-1573.

Maier IC, Schwab ME (2006) Sprouting, regeneration and circuit formation in the injured spinal cord: factors and activity. Philos Trans R Soc Lond B Biol Sci. 361: 1611-1634.

Manaye KF, McIntire DD, Mann DM, German DC (1995) Locus coeruleus cell loss in the aging human brain: a non-random process. J Comp Neurol 358: 79-87.

Mason P (1999) Central mechanisms of pain modulation. Curr Opin Neurobiol 9: 436-441.

McCarroll SA, Murphy CT, Zou S, Pletcher SD, Chin CS, et al. (2004) Comparing genomic expression patterns across species identifies shared transcriptional profile in aging. Nat Genet. 36: 197-204.

Meister B, Johnson H, Ulfhake B (1995) Increased expression of serotonin transporter messenger RNA in raphe neurons of the aged rat. Brain Res Mol Brain Res. 33: 87-96.

Metz GA, Dietz V, Schwab ME, van de Meent H (1998) The effects of unilateral pyramidal tract section on hindlimb motor performance in the rat. Behav Brain Res 96: 37-46.

Miller S, Reitsma DJ, van der Meche FG (1973) Functional organization of long ascending propriospinal pathways linking lumbo-sacral and cervical segments in the cat. Brain Res.62: 169-188.

Milner R, Campbell IL (2002) The integrin family of cell adhesion molecules has multiple functions within the CNS. J Neurosci Res 69: 286-291.

Minson J, Llewellyn-Smith I, Neville A, Somogyi P, Chalmers J (1990) Quantitative analysis of spinally projecting adrenaline-synthesising neurons of C1, C2 and C3 groups in rat medulla oblongata. J Auton Nerv Syst 30: 209-220.

Miranda JD, White LA, Marcillo AE, Willson CA, Jagid J, et al. (1999) Induction of Eph B3 after spinal cord injury. Exp Neurol. 156: 218-222.

Moncayo J, de Freitas GR, Bogousslavsky J, Altieri M, van Melle G (2000) Do transient ischemic attacks have a neuroprotective effect? Neurology 54: 2089-2094.

Morgan DG, May PC, Finch CE (1987) Dopamine and serotonin systems in human and rodent brain: effects of age and neurodegenerative disease. J Am Geriatr Soc 35: 334-345.

Morgenstern DA, Asher RA, Fawcett JW (2002) Chondroitin sulphate proteoglycans in the CNS injury response. Prog Brain Res 137: 313-332.

Moroz LL, Kohn AB (2010) Do different neurons age differently? Direct genome-wide analysis of aging in single identified cholinergic neurons. Front Aging Neurosci 2. pii: 6.

Navarro X, Kennedy WR (1988) Effect of age on collateral reinnervation of sweat glands in the mouse. Brain Res. 463: 174-181.

Nielson JL, Sears-Kraxberger I, Strong MK, Wong JK, Willenberg R, et al. Unexpected survival of neurons of origin of the pyramidal tract after spinal cord injury. J Neurosci. 30: 11516-11528.

NSCISC (National Spinal Cord Injury Statistical Center). Facts and Figures at

Ohno M, Miura T, Furuichi M, Tominaga Y, Tsuchimoto D, et al. (2006) A genome-wide distribution of 8-oxoguanine correlates with the preferred regions for recombination and single nucleotide polymorphism in the human genome. Genome Res. 16: 567-575.

Ondarza AB, Ye Z, Hulsebosch CE (2003) Direct evidence of primary afferent sprouting in distant segments following spinal cord injury in the rat: colocalization of GAP-43 and CGRP. Exp Neurol. 184: 373-380.

Ono H, Fukuda H (1995) Pharmacology of descending noradrenergic systems in relation to motor function. Pharmacol Ther. 68: 105-112.

Opatz J, Kury P, Schiwy N, Jarve A, Estrada V, et al. (2009) SDF-1 stimulates neurite growth on inhibitory CNS myelin. Mol Cell Neurosci. 40: 293-300.

Oudega M, Vargas CG, Weber AB, Kleitman N, Bunge MB (1999) Long-term effects of methylprednisolone following transection of adult rat spinal cord. Eur J Neurosci. 11: 2453-2464.

Pasterkamp RJ, Giger RJ, Ruitenberg MJ, Holtmaat AJ, De Wit J, et al. (1999) Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural

scar tissue formed following injuries of adult but not neonatal CNS. Mol Cell Neurosci. 13: 143-166.
Pasterkamp RJ, Verhaagen J (2001) Emerging roles for semaphorins in neural regeneration. Brain Res Brain Res Rev. 35: 36-54.
Pestronk A, Drachman DB, Griffin JW (1980) Effects of aging on nerve sprouting and regeneration. Exp Neurol. 70: 65-82.
Petcu EB, Sfredel V, Platt D, Herndon JG, Kessler C, Popa-Wagner A (2008) Cellular and molecular events underlying the dysregulated response of the aged brain to stroke: a mini-review. Gerontology 54: 6-17.
Pletcher SD, Macdonald SJ, Marguerie R, Certa U, Stearns SC, et al. (2002) Genome-wide transcript profiles in aging and calorically restricted Drosophila melanogaster. Curr Biol. 12: 712-723.
Ponzio F, Calderini G, Lomuscio G, Vantini G, Toffano G, et al. (1982) Changes in monoamines and their metabolite levels in some brain regions of aged rats. Neurobiol Aging 3: 23-29.
Portiansky EL, Nishida F, Barbeito CG, Gimeno EJ, Goya RG (2011) Increased number of neurons in the cervical spinal cord of aged female rats. PLoS One. 6: e22537.
Qu S, Ondo WG, Zhang X, Xie WJ, Pan TH, et al. (2006) Projections of diencephalic dopamine neurons into the spinal cord in mice. Exp Brain Res 168: 152-156.
Raghavendra Rao VL, Dhodda VK, Song G, Bowen KK, Dempsey RJ (2003) Traumatic brain injury-induced acute gene expression changes in rat cerebral cortex identified by GeneChip analysis. J Neurosci Res 71: 208-219.
Raineteau O, Fouad K, Bareyre FM, Schwab ME (2002) Reorganization of descending motor tracts in the rat spinal cord. Eur J Neurosci. 16: 1761-71.
Ranson RN, Dodds AL, Smith MJ, Santer RM, Watson AH (2003) Age-associated changes in the monoaminergic innervation of rat lumbosacral spinal cord. Brain Res. 972: 149-158.
Reimann W, Bartoszyk GD, Kollhofer U, Schneider F, Schoenherr U (1993) Effects of ageing and long-term operant conditioning on behavior and presynaptic cholinergic and dopaminergic neuronal mechanisms in rats. Arch Int Pharmacodyn Ther 325: 5-20.
Robbins N, Nakashiro S (1993) Connections among plasticity, regeneration, and aging at the neuromuscular junction. Adv Neurol 59: 47-52.
Rodriguez-Gomez JA, de la Roza C, Machado A, Cano J (1995) The effect of age on the monoamines of the hypothalamus. Mech Ageing Dev. 77: 185-195.
Romanes GJ (1946) Motor localization and the effects of nerve injury on the ventral horn cells of the spinal cord. J Anat 80: 117-131.
Rossner MJ, Hirrlinger J, Wichert SP, Boehm C, Newrzella D, et al. (2006) Global transcriptome analysis of genetically identified neurons in the adult cortex. J Neurosci. 26: 9956-9966.
Salin P, Chesselet MF (1992) Paradoxical increase in striatal neuropeptide gene expression following ischemic lesions of the cerebral cortex. Proc Natl Acad Sci U S A 89: 9954-9958.
Schena M (1996) Genome analysis with gene expression microarrays. Bioessays 18: 427-431.
Schiwy N, Brazda N, Muller HW (2009) Enhanced regenerative axon growth of multiple fibre populations in traumatic spinal cord injury following scar-suppressing treatment. Eur J Neurosci. 30: 1544-1553.
Schmalbruch H (1984) Motoneuron death after sciatic nerve section in newborn rats. J Comp Neurol 224: 252-258.
Sekhon LH, Fehlings MG (2001) Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine (Phila Pa 1976) 26: S2-12.
Siegenthaler MM, Ammon DL, Keirstead HS (2008) Myelin pathogenesis and functional deficits following SCI are age-associated. Exp Neurol. 213: 363-371.
Siegenthaler MM, Berchtold NC, Cotman CW, Keirstead HS (2008) Voluntary running attenuates age-related deficits following SCI. Exp Neurol. 210: 207-216.
Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci. 5: 146-156.
Skagerberg G, Bjorklund A (1985) Topographic principles in the spinal projections of serotonergic and non-serotonergic brainstem neurons in the rat. Neuroscience 15: 445-480.
Skagerberg G, Lindvall O (1985) Organization of diencephalic dopamine neurones projecting to the spinal cord in the rat. Brain Res. 342: 340-351.
Sluka KA, Westlund KN (1992) Spinal projections of the locus coeruleus and the nucleus subcoeruleus in the Harlan and the Sasco Sprague-Dawley rat. Brain Res. 579: 67-73.
Steward O, Zheng B, Ho C, Anderson K, Tessier-Lavigne M (2004) The dorsolateral corticospinal tract in mice: an alternative route for corticospinal input to caudal segments following dorsal column lesions. J Comp Neurol 472: 463-477.
Stichel CC, Muller HW (1998) The CNS lesion scar: new vistas on an old regeneration barrier. Cell Tissue Res 294: 1-9.

Tanaka K, Webster HD (1991) Myelinated fiber regeneration after crush injury is retarded in sciatic nerves of aging mice. J Comp Neurol 308: 180-187.

Tanaka K, Zhang QL, Webster HD (1992) Myelinated fiber regeneration after sciatic nerve crush: morphometric observations in young adult and aging mice and the effects of macrophage suppression and conditioning lesions. Exp Neurol. 118: 53-61.

Tanaka Y (1984) [Morphological changes of the cervical spinal canal and cord due to aging]. Nihon Seikeigeka Gakkai Zasshi 58: 873-886.

Tator CH, Fehlings MG (1991) Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 75: 15-26.

The Spinal Cord. A Christopher and Dana Reeve Foundation Text and Atlas (2009).

Tomlinson BE, Irving D (1977) The numbers of limb motor neurons in the human lumbosacral cord throughout life. J Neurol Sci. 34: 213-219.

Tork I (1990) Anatomy of the serotonergic system. Ann N Y Acad Sci 600: 9-34; discussion 34-35.

Tracey DJ (1995) Ascending and descending pathways in the spinal cord. The Rat Nervous System, 2nd edn. Academic Press: San Diego, pp 67–77.

Tracey DJ (2004) Ascending and descending pathways in the spinal cord. In: "The Rat Nervous System" 3rd edn; ed. G. Paxinos. Chapter 7, pp. 149-164. Elsevier Academic Press, San Diego.

Trepel M (2006) Neuroanatomie. Struktur und Funktion.

Vaughan DW (1992) Effects of advancing age on peripheral nerve regeneration. J Comp Neurol 323: 219-237.

Venero JL, Machado A, Cano J (1993) Effect of ageing on monoamine turnover in the prefrontal cortex of rats. Mech Ageing Dev. 72: 105-118.

von Meyenburg J, Brosamle C, Metz GA, Schwab ME (1998) Regeneration and sprouting of chronically injured corticospinal tract fibers in adult rats promoted by NT-3 and the mAb IN-1, which neutralizes myelin-associated neurite growth inhibitors. Exp Neurol. 154: 583-594.

Wang XH, Iannuzzelli PG, Murphy EH (1993) Calcitonin gene-related peptide increases following axotomy of trochlear motoneurons. Exp Neurol. 123: 157-166.

Westlund KN, Bowker RM, Ziegler MG, Coulter JD (1983) Noradrenergic projections to the spinal cord of the rat. Brain Res. 263: 15-31.

Willcox BJ, Scott JN (2004) Growth-associated proteins and regeneration-induced gene expression in the aging neuron. Mech Ageing Dev. 125: 513-516.

Wyndaele M, Wyndaele JJ (2006) Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord. 44: 523-529.

Xie YY, Yao ZB, Wu WT (2000) Survival of motor neurons and expression of beta-amyloid protein in the aged rat spinal cord. Neuroreport 11: 697-700.

Yankner BA, Lu T, Loerch P (2008) The aging brain. Annu Rev Pathol 3: 41-66.

Yoshimura M, Furue H (2006) Mechanisms for the anti-nociceptive actions of the descending noradrenergic and serotonergic systems in the spinal cord. J Pharmacol Sci. 101: 107-117.

Yoshimura M, Furue H (2006) Mechanisms for the anti-nociceptive actions of the descending noradrenergic and serotonergic systems in the spinal cord. J Pharmacol Sci 101: 107-117.

Zheng LF, Wang R, Xu YZ, Yi XN, Zhang JW, et al. (2008) Calcitonin gene-related peptide dynamics in rat dorsal root ganglia and spinal cord following different sciatic nerve injuries. Brain Res. 1187: 20-32.

Zou S, Meadows S, Sharp L, Jan LY, Jan YN (2000) Genome-wide study of aging and oxidative stress response in Drosophila melanogaster. Proc Natl Acad Sci U S A. 97: 13726-13731.

9. ABBREVATIONS

5-HT	serotonin (5-hydroxytryptamine)
AST	anti-scarring treatment
BDA	biotinylated dextran amine
BPY-DCA	2,2`- bipyridine-5,5`-dicarboxylic acid (iron chelator)
C	cervical
cAMP	cyclic adenoside monophosphate
CC	central canal
CGRP	Calcitonin gene-related peptide
CSPG	chondroitin sulfate proteoglycan
CNS	central nervous system
Col4	collagen type IV
CSF	cerebrospinal fluid
CST	corticospinal tract
CTGF	connective tissue growth factor
dCST	dorsal corticospinal tract
DH	dorsal horn
dpo	days post operation
DRG	dorsal root ganglion
ECM	extracellular matrix
GAP-43	growth-associated protein-43
GFAP	glial fibrillary acidic protein, astrocyte marker
GM	gray matter
LTP	long term potentiation
MBP	myelin basic protein
MRI	magnetic resonance imaging
PNS	peripheral nervous system
P0	myelin protein P0
PMP22	peripheral myelin protein 22
PS	propriospinal
RaST	Raphe spinal tract
ROS	reactive oxygen species
RST	rubrospinal tract
SCI	spinal cord Injury
T	thoracic level
TH	tyrosine hydroxylase
VH	ventral horn
WM	white matter

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Eidesstattliche Erklärung:

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet, sowie Zitate kenntlich gemacht habe.

Dortmund, im Oktober 2011