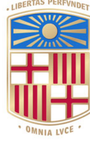
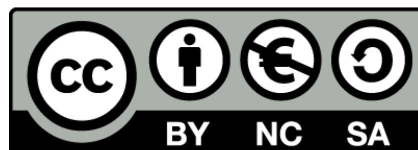


Activity-dependent mechanisms of axonal growth

Francina Mesquida Veny



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Activity-dependent mechanisms of axonal growth

Doctoral thesis

Universitat de Barcelona

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Barcelona, 2022

Institute for Bioengineering of Catalonia (IBEC)

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Programa de Doctorat en Biomedicina

Activity-dependent mechanisms of axonal growth

PhD thesis report presented by Francina Mesquida Veny to apply for a PhD degree from the Universitat de Barcelona. This doctoral thesis is inscribed in the **Programa de Doctorat en Biomedicina** of the Universitat de Barcelona.

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Dr. Arnau Hervera Abad	Dr. José Antonio del Río Fernández	Francina Mesquida Veny

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Abstract

Spinal cord injuries (SCI) are a major cause of paralysis in young adults. In this type of injuries recovery is impaired as adult central nervous system (CNS) axons fail to regenerate. This results from both a loss of intrinsic growing capacities in developmental axons when they mature, together with the presence of extrinsic factors hampering this regeneration, including a glial scar together with the production of growth-inhibitory molecules, as well as a lack of injury resolution leading to a chronic inflammation.

Unfortunately, despite research efforts, current therapies for this type of injuries only lead to mild improvements and among them, activity-based therapies seem to raise above the others. Activity-based therapies try to induce recovery by increasing neuronal activity, however, a proper physiological and molecular characterization of the rationale behind their success is still missing.

Neuronal activity has been described to regulate transcriptional and epigenetic mechanisms; moreover, it also alters neuronal secretion with an impact on cellular dialogues. These characteristics indicate neuronal activity may be modulating both of the CNS barriers for regeneration. During this doctoral thesis we aimed to explore the influence of neuronal activity on SCIs, hypothesizing specific neuronal activations were the principal responsible for success in activity-based therapies. Particularly, we studied the role of precise manipulations of neuronal activity, using optogenetic and chemogenetic tools, in axonal growth of stimulated neurons as well as the impact these activations could have on neuronal extrinsic signalling.

Our results show that optogenetic and chemogenetic stimulations of neuronal activity enhanced growth in both regenerating and refractory to regenerate neurons. However, this growth was hampered by the inhibitory molecules present in the injured CNS and did not result in functional recovery in rodent models of SCI. Our data indicated that the growth induction in specifically stimulated neurons resulted from local adjustments rather than inducing a pro-regenerative transcriptional state, as seen by our gene expression analysis of regeneration-associated genes (RAGs). Altogether, our results suggest recovery in activity-based therapies derives from the summation of various forms of plasticity, induced by their simultaneous recruitment of several circuits.

In parallel, we observed that these precise modulations of neuronal activity, while unable to alter the predominant environment after SCI, could initiate previously undescribed intricate cellular dialogues. Specifically, we found an increase in the chemokine CCL21 upon nociceptor activation which triggered the response of several cell types in the injury. In proprioceptors, this CCL21 was responsible for a growth induction after CCR7 activation, which required the MEK-ERK pathway

as well as the modulation of the actin cytoskeleton. Meanwhile, the CCL21 interaction with CXCR3 in other cells effectively aborted this regeneration.

All in all, our work reveals the existence of a complex plethora of synergic mechanisms, far from understood, contributing to the outcome of activity-based therapies and reinforces the need for further mechanistic studies which would allow the optimization of their success.

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Abbreviations

AAV: adeno-associated virus

AAV-ChR2: AAV9-hSyn-hChR2(H134R)-EYFP

AAV-hM3Dq: AAV5-hSynhM3D(Gq)-mCherry

AAV-mCherry: AAV5-hSyn-mCherry

ABP: actin-binding protein

ACKRs: atypical chemokine receptors

ADAMTS4: a disintegrin and metalloproteinase with thrombospondin motifs 4

ADF: actin-depolymerizing factor

APV: (2R)-amino-5-phosphonovaleric acid

Armcx1: armadillo repeat containing X-linked 1

Arp 2/3: actin-related protein 2/3

ATF3: activating transcription factor 3

a.u.: arbitrary units

BCI: Brain-Computer-Interfaces

BDNF: brain-derived neurotrophic factor

BMDM: Bone-marrow derived macrophages

BMS: Basso Mouse Scale

BSA: bovine serum albumin

cAMP: cyclic adenosine monophosphate

Ca^{2+}: calcium

Cbp: Creb-binding protein

CCL: C-C motif ligand

CCiTUB: Centres Científics i Tecnològics Universitat de Barcelona

cCKRs: conventional chemokine receptors

CEEA: Ethics Committee on Animal Experimentation

CGRP: calcitonin gene-related peptide

ChABC: chondroitinase ABC

ChR2: Channelrhodopsin-2

CM: conditioned media

CNO: clozapine N-oxide

CNS: central nervous system

CNTF: ciliary neurotrophic factor

CO_2: carbon dioxide

CREB: cAMP response element-binding protein

CSMNs: corticospinal motor neurons

CSPGs: chondroitin sulfate proteoglycans

CST: corticospinal tract

CTB-647: Cholera Toxin Subunit B AlexaFluor 647

CXCL: C-X-C motif ligand

CX_3CL: C-XXX-C motif ligand

C-domain: central domain

DC: dendritic cell

DCA: dorsal column axotomy

DLK: dual leucine zipper-bearing kinase

DMEM: Dulbecco's Modified Eagle Medium

DIV: days *in vitro*

DPI: days post-injury

DREADD: designer receptor exclusively activated by a designer drug

DRG: dorsal root ganglia

DSB: DNA double-strand break

ECM: extracellular matrix

EGF: epidermal growth factor

ERK: extracellular signal-regulated kinase

EV: extracellular vesicles

E15.5-16.5: embryonic stage 15.5-16.5

FACS: Fluorescence activated cell sorting

FBS: setal bovine serum

FGF2: fibroblast growth factor 2

GAG: glycosaminoglycan

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

GAP43: growth-associated protein 43

GDNF: glial-derived growth factor

GFP: green fluorescent protein

GFAP: glial fibrillary acidic protein

GM: grey matter

GPCRs: G-protein coupled receptors

GSK-3β: glycogen synthase kinase 3-β

HBSS: Hank's balanced salt solution

HDAC: histone deacetylase

ICC: immunocytochemistry

IHC: immunohistochemistry

Ig: immunoglobulin

IGF1: insulin-like growth factor 1

IL: interleukin

IL1ra: IL-1 receptor antagonist

IPSC: induced pluripotent stem cell

i.p.: intraperitoneal

i.pl. intraplantar

JAK: Janus kinase

JNK: c-Jun N-terminal kinase

KLF: Krüppel-like factors

LAR: leukocyte common antigen-related

LCCM: L929-cell conditioned media

LIF: leukemia inhibitory factor

LIMK: LIM kinase

LINGO-1: leucine-rich repeat and immunoglobulin-like domain-containing Nogo receptor-interacting protein 1

LV: lentivirus

LV-ChR2: LV-EF1α-hChR2(H134R)-EYFP-WPRE

MAG: myelin-associated glycoprotein

MAPK: mitogen-activated protein kinase

MFI: mean fluorescence intensity

MLC: myosin light chain

miR: microRNA

MSC: mesenchymal stem cells

mTOR: mammalian target of rapamycin

M1: primary motor cortex

M-CSF: macrophage-colony stimulating factor

Na^+: sodium

$NaHCO_3$: sodium bicarbonate

NGF: nerve growth factor

NgR: Nogo receptor

NHS: normal horse serum

NO: nitric oxide

NOX2: NADPH oxidase 2

NSC: neural stem cell

NT-3: neurotrophin-3

N-WASP: neural Wiskott-Aldrich syndrome protein

OEC: olfactory ensheating cell

OMgp: oligodendrocyte myelin glycoprotein

OPC: oligodendrocyte-progenitor cells

O/N: over-night

PBS: phosphate-buffered saline

PCAF: histone acetyltransferase p300/CBP-associated factor

PDMS: poly(dimethylsiloxane)

PFA: paraformaldehyde

PirB: paired immunoglobulin-like receptor B

PI3K: phosphoinositide-3-kinase

PKC: protein kinase C

PNN: perineuronal nets

PNS: peripheral nervous system

PPARα: peroxisome proliferator-activated receptor α

PSD-95: postsynaptic density protein-95

PTEN: phosphatase and tensin homolog

Ptx: pertussis toxin

PV: parvalbumin

PV-ChR2-mCherry: PV-Cre/Ai27D/CSP-Flox mice

Pyk2: proline-rich tyrosine kinase 2

P-domain: peripheral domain

P/S: penicillin/streptomycin

RAG: regeneration-associated genes

RGC: retinal ganglion cells

RGMa: repulsive guidance molecule a

RhoA: Ras homolog gene family member A

RIM: Rab3 interacting molecule

ROCK: Rho-associated kinase

ROS: reactive oxygen species

RPTPσ: receptor protein tyrosine phosphatase sigma

RT: room temperature

SCG-10: superior cervical ganglion-10 protein

SCI: spinal cord injury

SDF1: stromal cell-derived factor 1; CXCL12

sICAM: soluble intercellular adhesion molecule-1

SLC: Secondary lymphoid-tissue chemokine (SLC), CCL21

SNA: sciatic nerve axotomy

SNC: sciatic nerve crush

SOCS3: suppressor of cytokine signaling 3

SOX11: SRY-related HMG-box 11

Sprr1a: small proline-rich protein 1a

S1: primary somatosensory motor cortex

TBS: Tris-buffered saline

TET3: Ten eleven translocation 3

TGF-β: transforming growth factor β

Thy1-ChR2: B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J mice

TIMP1: tissue inhibitor of metalloproteinase 1

TNF-α: tumor necrosis factor α

Trk: tropomyosin receptor kinase

TRPV1: transient receptor potential vanilloid receptor 1

TTBS: TBS + 1% Tween

T-domain: transition zone

VGCC: voltage-gated calcium channels

VEGF: vascular endothelial growth factor

vGlut1: vesicular glutamate transporter 1

WM: white matter

WT: wild type

YFP: yellow fluorescent protein

5mC: methylated DNA cytosines

6Ckine: chemokine with 6 cysteines, CCL21

Introduction

1. General introduction

Traumatic spinal cord injuries (SCI) represent one of the leading worldwide causes of long-term disabilities. This type of injuries result mainly from traffic accidents and falls affecting principally young adults and have devastating consequences in the quality of life of the patients, which will suffer from functional deficits for the rest of their lives (Ahuja et al., 2017).

The severity degree of the injury depends on two characteristics: the level of the injury and its completeness. The area of the body affected by the injury will be determined by the spinal level of the injury as a result of the distribution of the nerves exiting the spinal cord: innervated areas below the injury level will most likely present deficits (Ahuja et al., 2017). However, that will depend on the completeness of the injury as the majority of SCI are incomplete which means some functions may be preserved as a result of spared neural tissue (O'Shea et al., 2017).

Despite years of research, current therapies for SCI have only achieved minor improvements in patients and are far to offer complete recovery. The different etiologies and thus characteristics of the lesions add extra challenges in the errand to find a cure for SCI. Together with that, one should not forget the complex wiring system that represents the spinal cord, fashioned with great precision during development, with posterior fine tuning (Hawryluk et al., 2012). Different strategies might also be of interest depending on the completeness of the injury (O'Shea et al., 2017). In addition, the spinal cord presents a handful of diverse neuronal types, each of them with unique properties, including their regenerating capacities (Deumens et al., 2005). Hence, it is now clear that the availability of proper treatment will rely on tailored therapies for each patient, further reinforcing the need to expand our knowledge about the pathophysiology of these injuries, as well as the effects each therapy might exert on each of them at a cellular and molecular level.

Patients suffering from SCI do not recover as a consequence of the incapability of the adult central nervous system (CNS) to regenerate after injury. Research has now repeatedly shown that both extrinsic and intrinsic factors prevent this regeneration. Extrinsically, the production of inhibitory molecules, the formation of a glial scar and a persistent inflammation after injury result in an adverse atmosphere for axon regrowth (Bradbury and Burnside, 2019; Mesquida-Veny et al., 2021). On the other hand, while peripheral nervous system (PNS) neurons can activate a regenerating program after a lesion, adult CNS neurons remain impassible (Mahar and Cavalli, 2018; Mar et al., 2014). Moreover, axons in the CNS present great growth and regenerating capacities during development, when they have to cover long distances to reach their final target, however, after forming connections these capacities drastically drop with age (Geoffroy et al., 2016; Hilton and Bradke, 2017). Since PNS neurons as well as developing neurons in the CNS are able to react to axonal injury with pro-regenerative responses, absent in CNS adult neurons,

the comparison of these different systems has allowed to uncover some of the mechanisms involved (Hilton and Bradke, 2017; Mahar and Cavalli, 2018). Hence, to this day, these models have helped us to understand some of the reasons behind regenerating failure after SCI as well as to identify putative targets for its treatment.

All in all, both environmental and inherent barriers underlie the lack of regeneration in the adult CNS, which translates into poor prognosis after SCI. In that direction, in spite of astonishing advances in the study of the molecular and physiological basis of both barriers, together with strategies to overcome them, it is now clear that the simultaneous fine tuning of both of them is essential to promote recovery, as approaching only one of these barriers has systematically resulted insufficient to promote functional recovery (Griffin and Bradke, 2020).

2. Brief anatomy of the spinal cord

In order to understand the pathophysiology of spinal injuries, it is important to consider the anatomy and physiology of this complex organ (Figure 1). Located inside the vertebral column, the spinal cord collects sensorial inputs from the peripheral nerves and sends them to the brain while transferring motor information from the brain to the rest of the body (Purves et al., 2004). The different peripheral nerves arrive at each of the spinal cord segments and merge via the dorsal root (sensory information) and the ventral root (motor information).

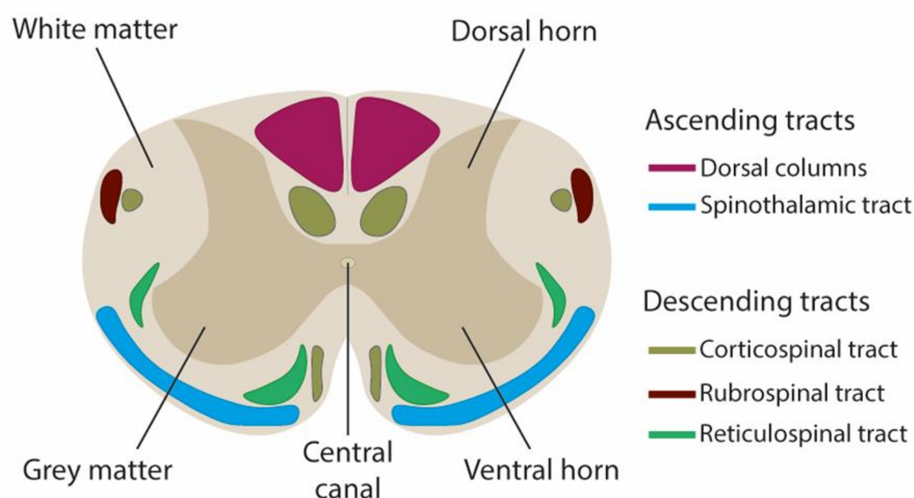

Figure 1. Anatomy of the rodent spinal cord. The location of the main ascending and descending tracts is represented in a section of the spinal cord. Adapted from (Saliani et al., 2017).

Hence, in the spinal cord long, ascending and descending tracts can be found, together with intraspinal circuits. The ascending tracts transport sensory information and are composed mainly

by the dorsal columns (responsible for mechanoception and proprioception) and the spinothalamic tract (responsible for nociception and temperature) (Purves et al., 2004). The descending motor tracts include the corticospinal tract (CST) (originating from the motor cortex), responsible for intended and brain-integrated movements, the rubrospinal tract (originating in the red nucleus) and the reticulospinal tract (originating in the brain stem), these last two with a major role in unconscious movement. Motor information is then transduced to motor neurons in the ventral horn of the spinal cord, whose axons leave the spinal cord via the ventral root and contact with the target skeletal muscles (Purves et al., 2004).

Importantly, besides merely transmitting the information between the brain and the periphery, a first integration and modulation of all the signals occurs in the spinal cord, while some responses can also be generated there, such as reflexes (Falgairolle and O'Donovan, 2019). This highlights the existence of intraspinal circuits that are able to respond to both input and output information (Koch et al., 2018).

Several models allow to study the pathophysiology of the SCI, as well as the molecular mechanisms promoting regeneration (Table 1). These models range from simplified ones like neuronal cultures, useful to study basic molecular mechanisms, to realistic injury models, allowing for a comprehensive examination of all the factors and processes involved in a SCI. In terms of specific cellular models, two particular systems have raised importance above the others due to their inherent characteristics: the dorsal root ganglia (DRG) neurons, as a result of their particular morphology as well as their location between the PNS and CNS, and the corticospinal motor neurons (CSMN), whose axons form the CST, due to the central role of this tract in the motor function and to its refractoriness to regenerate.

System	**Model**	**Specific assay/cell type**	**Useful in/for**	**Limitations**
***In vitro* neuronal cultures**	Low density cultures	Neurite outgrowth	High-content screening Preliminary studies	Lack of injury Absence of extrinsic factors
	Microfluidic devices	Axotomy	Reproducible injuries Axonal isolation	Absence of extrinsic factors
PNS	Sciatic nerve injury	Motor neurons or DRG neurons	Identifying new growth promoting pathways and favourable extrinsic factors	Different injury environment (and cell type)
PNS/CNS	Conditioning injury	Injury to the peripheral branch prior to injury to the central branch of a DRG neuron	Identifying dormant regenerative pathways	Lack of translationality
CNS	Developing neurons	Embrionary and postnatal neurons	Identifying dormant regenerative pathways	Different environment and capacities
	Optic nerve injury	Retinal ganglion cells (RGC)	First approach to CNS injury	Cell-specific unusual characteristics
	Spinal cord injury		Studying different aspects of the pathophysiology	Complexity and heterogeneity
	Severity	**Injury model**	**Useful in/for**	**Limitations**
	Complete	Transection	Strict regeneration assessment	Lack of clinical relevance Severe impairments
	Incomplete	Contusion	Modelling real injuries Clinical relevance	Spared tissue Difficulties to discern between regeneration or plasticity
		Lateral transection	Regeneration assessment with less severe impairments	Heterogeneity
		Dorsal hemisection	CST regeneration studies	Heterogeneity
		Dorsal columns axotomy	Comparison with peripheral branch injury	Lack of clinical relevance
		Pyramidotomy	CST sprouting studies	Lack of clinical relevance

Table 1. Principal models in the study of SCI and axon regeneration. Information from (Al-Ali et al., 2017; Curcio and Bradke, 2018; He and Jin, 2016; Hilton and Bradke, 2017; Lee and Lee, 2013; Steward and Willenberg, 2017). Pyramidotomy: injury of the pyramidal tract (containing the CST) at the ventral brainstem.

2.1. A special case: the DRGs

Sensory information arrives to the spinal cord through peripheral nerves along the sensory neurons. The cell body of these neurons is located in the DRG, small prominences of the peripheral nerves that can be found near the dorsal root of the spinal cord (Nascimento et al., 2018). Sensory neurons present a pseudo-unipolar morphology, meaning that their axon forms two branches: the peripheral branch in the peripheral nerve, and the central branch, which enters the spinal cord (Figure 2).

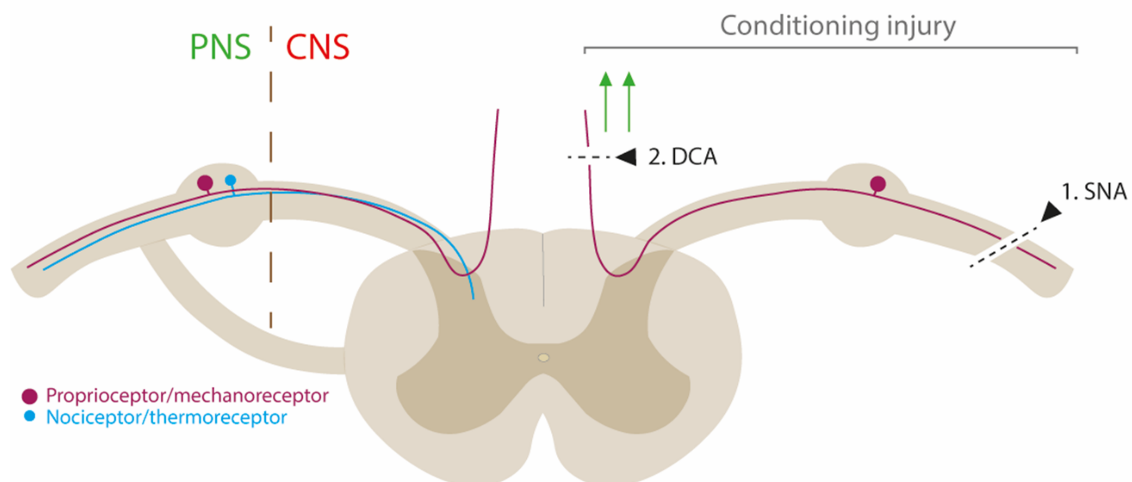

Figure 2. DRG neurons as a model for axon regeneration. Large-diameter DRG neurons (proprioceptors and mechanoreceptors) are located at the boundary between the PNS and the CNS and present fascinating growth capacities: their central branch is primed to regenerate after an injury to the peripheral branch (conditioning injury). DCA: dorsal column axotomy. SNA: sciatic nerve axotomy.

Different types of sensory neurons are present in the DRGs: proprioceptors, mechanoreceptors, nociceptors and thermoreceptors. Large-diameter sensory neurons, which include proprioceptors and some mechanoreceptors (Aβ) (Le Pichon and Chesler, 2014) have become an important model for axon regeneration studies, as their peripheral branch is able to regenerate (for instance after sciatic nerve injury), while the central branch, forming the dorsal columns, fails to do so (Ferreira et al., 2012). This discrepancy results not only from the different environment (glial scar, absence of Schwann cells, chronic inflammatory response…) but also from the incapacity of the neuron itself to mount a proper regenerative response after an injury in the central branch, including in the dorsal root (Attwell et al., 2018). Injury in the peripheral branch triggers regeneration-associated gene (RAG) expression, such as growth-associated protein 43 (GAP43), small proline-rich protein 1a (Sprr1a) and brain-derived neurotrophic factor (BDNF) (Chong et al., 1994; Starkey et al., 2009; Tonra et al., 1998), but a central injury does not (Giovanni, 2009).

The rationale behind this difference remains still unclear. In fact, when a peripheral injury precedes the central injury, the central branch presents robust regenerative capacities, a phenomenon termed “conditioning injury” (Figure 2) (Hoffman, 2010; Richardson and Issa, 1984). This means that a central injury itself lacks the capacity to reactivate the growth program in neurons. One of the factors might relate to the impossibility of central injuries to generate “injury signals”, while in peripheral injuries calcium waves, cyclic adenosine monophosphate (cAMP) or extracellular signal-regulated kinase (ERK) signaling among others allow to modify the transcriptional landscape (Mar et al., 2014). Thus, DRG neurons offer the possibility to characterize both injuries within the same neuron type, which might be key to determine the molecular mechanisms that would allow regeneration in the CNS.

2.2. The corticospinal tract

The CST is one of the major descending pathways in the spinal cord. It is mainly composed of axonal projections coming from pyramidal neurons in the primary motor cortex (M1), as well as in the primary somatosensory cortex (S1) (Welniarz et al., 2017). As a result, the CST presents an essential role in motor control, especially in movements that require fine control (Lemon, 2008). Costicospinal neuronal projections exit the layer V in the cortex, descend through the internal capsule and continue descending in the brainstem until the spinal cord, where most of the axons deviate to the other half of the spinal cord, forming the decussation (Figure 3A) (Welniarz et al., 2017). These axons then continue down the spinal cord course until they synapse with motor neurons or interneurons (Lemon, 2008).

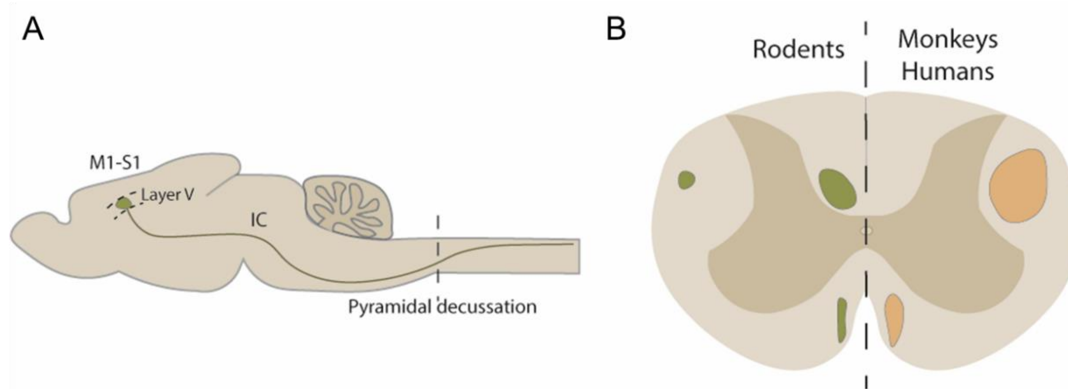

Figure 3. Schematic representation of the CST. A. Axons from CSMN in the layer V of the primary motor (M1) and somatosensory (S1) cortex reach the spinal cord via the internal capsule (IC). Adapted from (Welniarz et al., 2017). B. Comparison between the location of the CST in the spinal cord of rodents and monkeys/humans. Adapted from (Oudega and Perez, 2012).

Of note, the exact location of the tract inside the spinal cord differs among species: while in humans and monkeys the majority of the axons travel in the dorsal lateral region of the white matter in the spinal cord, with a minor component in the ventral medial region, in rodents the principal component of the CST can be found below the dorsal columns in the medial region, while the lateral and ventral components are less represented (Figure 3B) (Oudega and Perez, 2012). This divergence generates contrasting consequences of an injury of the same nature in different species, and has to be taken into account when using animal models. For example, while a contusion injury might affect only half of the CST in humans, it might transect almost completely the rodent CST.

The CST is a commonly affected tract after SCIs, which leads to severe motor deficits. Reestablishing the lost CST connections would represent an incredible leap in terms of life-quality in patients. However, CSMNs present very limited plasticity, evidenced by the scarcity of studies that have shown functional regeneration (Jin et al., 2015; Liu et al., 2010). In spite of that, several studies have observed CST reorganization after injury in rodents (Baltes et al., 2010; Fouad et al., 2001) and in monkeys (Rosenzweig et al., 2010), however, feasibility in humans remains to be elucidated (Kazim et al., 2021; Oudega and Perez, 2012).

3. Pathophysiology of the SCI

3.1. Succession of events after injury

After a SCI both a primary and a secondary injury take place. Primary injury refers to the physical damage generated as a direct consequence of (in general) the impact that initiates the injury. This impact fractures the vertebras which in turn crush the spinal cord and interrupt the blood flow (Ahuja et al., 2017). The secondary injury refers to the complex cascade of molecular and cellular events that occurs along time that follow and are initiated from the primary injury (Oyinbo, 2011). Temporally, three different phases have been described after a SCI: the acute phase (minutes to 48h), the subacute phase (48h to 2 weeks) and the chronic phase (2 weeks to years). The different events occurring in these phases are shown in Figure 4. Nevertheless, these phases have to be understood as a tool to facilitate the study of SCI progression, as they are a simplification of what happens in reality. As one can imagine, the transitions between these phases are continuous, as the events overlap and vary among injuries and individuals.

The events in the acute phase result mainly from the initial cell death and from the vascular disruption: the interruption of the blood flow originates ischemic injury, which, together with the initial cell death, triggers a vicious circle of ionic imbalance, excitotoxicity and increased cell

death (Alizadeh et al., 2019). Reactive oxygen species (ROS) production is also exaggerated after these events, increasing lipid peroxidation and leading to neurotoxic products (Hall, 2011). In response to the injury local inflammatory cells, neutrophils, as well as peripheral macrophages afterwards, are recruited during this phase (David et al., 2018; Mesquida-Veny et al., 2021). These cells release pro-inflammatory cytokines and are key in debris clearance.

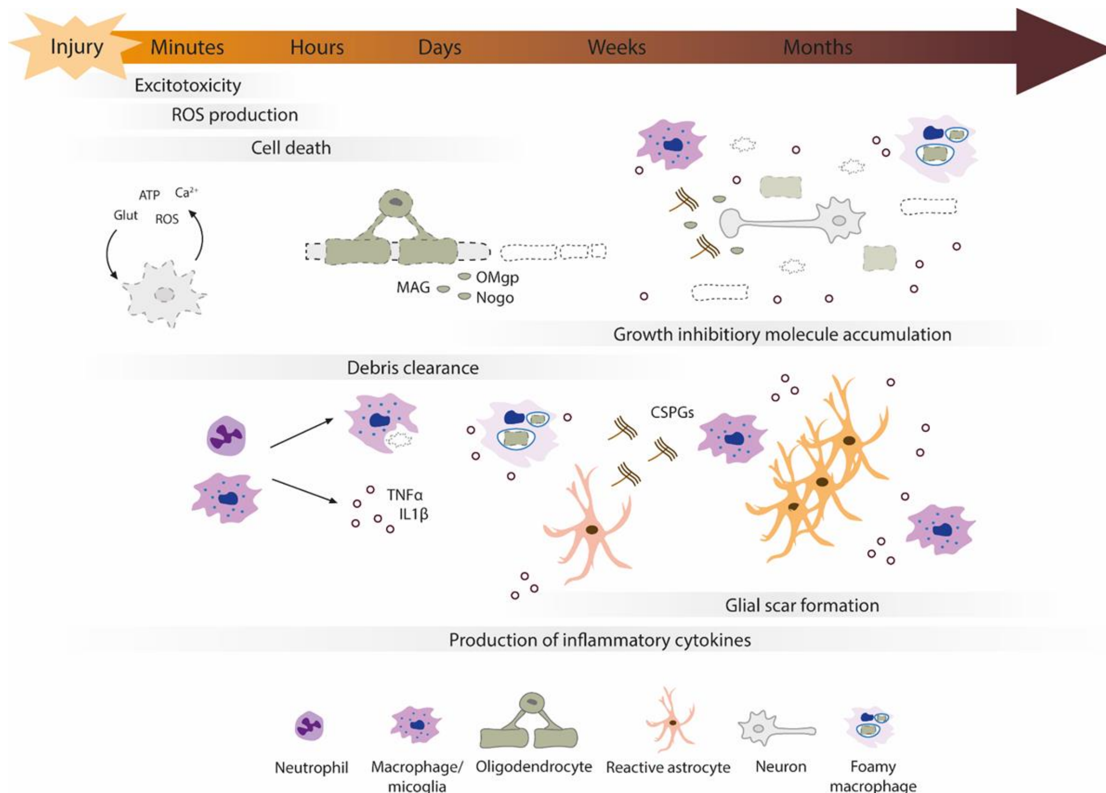

Figure 4. Timeline showing the different events occurring at each timepoint after a SCI. Many cellular components participate in the injury evolution. However, this progression results truncated as a result from the continuous production of inflammatory cytokines, leading to injury chronification. Moreover, the accumulation of growth-inhibitory molecules together with the glial scar impede regeneration. Adapted from (Mesquida-Veny et al., 2021).

The subacute phase derives from the conditions generated during the previous phase. As cell death persists, the loss of oligodendrocytes leads to demyelination and subsequent axonal loss as they remain unprotected to the generated hostile environment (Oyinbo, 2011). Besides, oligodendrocyte death also causes myelin-derived protein accumulation (Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp)), known to be inhibitory for axon growth (Filbin, 2003). Meanwhile the distal end of the severed axons suffer Wallerian degeneration (Rotshenker, 2011). During this phase, and in response to the previously commented injury signaling, multiple cell types (astrocytes, pericytes, fibroblasts, oligodendrocyte-progenitor cells (OPCs), as well as immune cells) proliferate and participate in the generation of an injury scar, a compact structure that surrounds the injury epicenter and avoids its expansion (Bradbury and Burnside, 2019). Activated astrocytes and other cells release

chondroitin sulfate proteoglycans (CSPGs), which are also inhibitory for axon growth (Galtrey and Fawcett, 2007; McKeon et al., 1999). While in other tissues at this time immune cells initiate a transition to pro-healing phenotypes and functions, after an injury in the CNS macrophages and microglia maintain a pro-inflammatory phenotype, leading to an unresolved and exaggerated inflammation (Mesquida-Veny et al., 2021). Notably, one of the factors contributing to this situation is myelin phagocytosis, which leads to the generation of the pro-inflammatory "foamy" macrophages, useless in debris clearance (Zhu et al., 2017).

In the CNS the chronic phase is basically an extension of the subacute phase. Inhibitory molecules (myelin-derived and CSPGs) as well as cell debris, further accumulate, intensifying the hostile environment and the inflammatory response (Bradbury and Burnside, 2019). During this phase, astrocytes, which have become reactive, align at the borders of the injury forming the glial scar which will compact and mature throughout the subsequent months (Burda and Sofroniew, 2014). Pericytes and fibroblasts migrate to the lesion core which becomes fibrotic as a result of their action (Alizadeh et al., 2019). In some mammals (like in humans, but not in mice), cystic cavities are formed in the lesion core as a result of the excessive tissue loss (Norenberg et al., 2004). Besides, during this phase inflammation perseveres, as macrophages and microglia lack the environmental cues to shift to pro-resolving phenotypes, therefore generating a permanent loop (Mesquida-Veny et al., 2021).

3.2. Extrinsic changes. The CNS environment

3.2.1. The glial scar and the inhibitory signalling

Some of the previously commented events after CNS injury lead to the generation of an adverse environment for axon regeneration (Figure 5). One of the clear examples is the formation of a non-resolving scar, composed of several cell types and extracellular matrix (ECM), which, as a whole as well as some of their individual elements, prevents neural plasticity and therefore recovery (Bradbury and Burnside, 2019). A key component of this scar are astrocytes, who hypertrophy and become reactive, enclosing the fibrotic injury core, forming a physical barrier that prevents regeneration, while avoiding injury expansion (Burda and Sofroniew, 2014; Silver, 2016). Moreover, astrocytes after injury express CSPGs (McKeon et al., 1999), which inhibit axonal growth through binding to different neuronal receptors. Nevertheless, research has shown that the formation of a proper astrocytic border is necessary for recovery after SCI, pointing towards a necessary role of these cells (Anderson et al., 2016; Faulkner et al., 2004; Wanner et al., 2013). Interestingly, both roles may be compatible as astrocytes in the injury are heterogeneous and can present different phenotypes depending on the environment (Khakh and Sofroniew, 2015; Liddelow et al., 2017; Okada et al., 2018; Sofroniew, 2020).

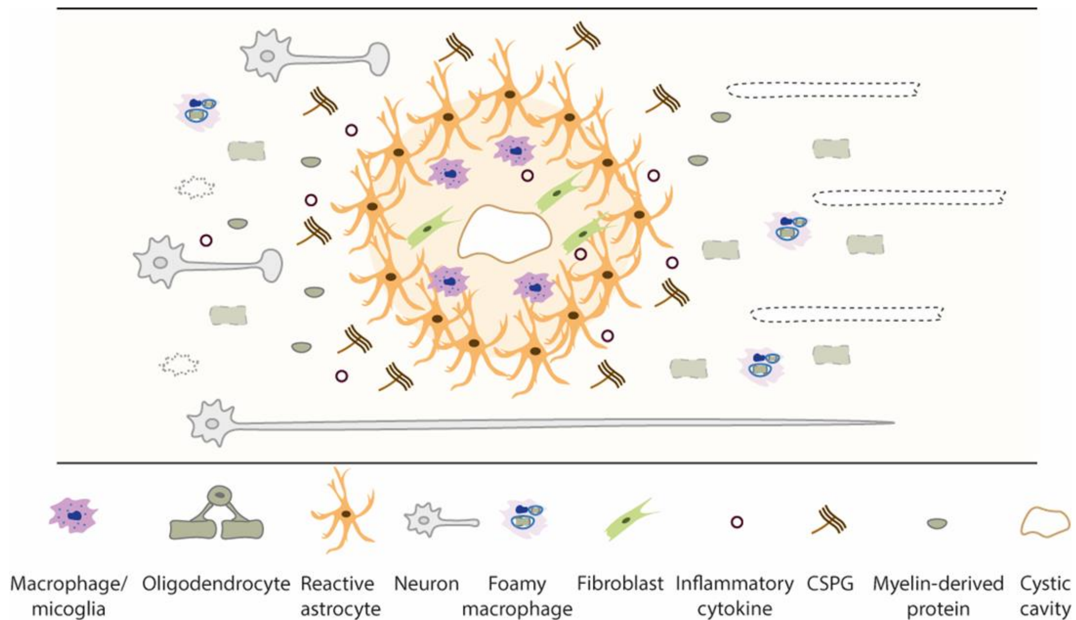

Figure 5. Formation of a glial scar and growth-inhibitory molecule deposition in chronic SCIs. The environment in a chronic SCI results from a complex interaction between several cell types and ECM components. Adapted from (Bradbury and Burnside, 2019; Mesquida-Veny et al., 2021).

Another component of the extrinsic barrier can be found in the ECM of the injury scar: the CSPGs. CSPGs such as neurocan, phosphacan or NG2 are expressed after injury by reactive astrocytes and by OPCs (Filous et al., 2014; McKeon et al., 1999), and their elimination through chondroitinase ABC (ChABC) addition, a bacterial enzyme which digests the glycosaminoglycan (GAG) chains, has been shown to promote recovery (Bradbury et al., 2002; Griffin and Bradke, 2020). These proteoglycans are composed of a central protein bonded to several negatively charged chondroitin sulfate GAG chains. Axons recognize CSPGs through different receptors: receptor protein tyrosine phosphatase sigma (RPTPσ), leukocyte common antigen-related (LAR) and Nogo receptor (NgR), an interaction which has repeatedly shown to prevent axon growth (Dickendesher et al., 2012; Fisher et al., 2011; Sami et al., 2020; Sharma et al., 2012; Shen et al., 2009) (Figure 6). The exact mechanism behind CSPG-mediated growth inhibition remains elusive, but several intracellular signaling pathways have been shown to be involved, such as Akt/glycogen synthase kinase 3-β (GSK-3β), protein kinase C (PKC) or Ras homolog gene family member A (RhoA) (Dill et al., 2008; Monnier et al., 2003; Sivasankaran et al., 2004). Another study proposed that CSPGs prevent axon growth not by direct repulsion but by strongly attaching to the axons, which remain captured by them (Filous et al., 2014).

CSPGs are not the only inhibitory molecules present in the ECM after injury. Myelin-associated inhibitors (specially Nogo, MAG and OMgp) are released following oligodendrocyte death and interact principally with paired immunoglobulin-like receptor B (PirB) (Atwal et al., 2008) or the complexes NgR-LINGO-1 (leucine-rich repeat and immunoglobulin-like domain-containing Nogo receptor-interacting protein 1)-p75 or NgR-LINGO-1-Troy (Mi et al., 2004; Park et al.,

2005; Wang et al., 2002) (Figure 6). The interaction with NgR complexes activates RhoA and Rho-associated kinase (ROCK) which eventually leads to growth cone stabilization through cytoskeleton alterations (Fournier et al., 2003; Fujita and Yamashita, 2014; Geoffroy and Zheng, 2014; Niederöst et al., 2002). In the case of PirB, growth inhibition is mediated by tropomyosin receptor kinase (Trk) inactivation, blocking neurotrophin-induced growth (Fujita et al., 2011).

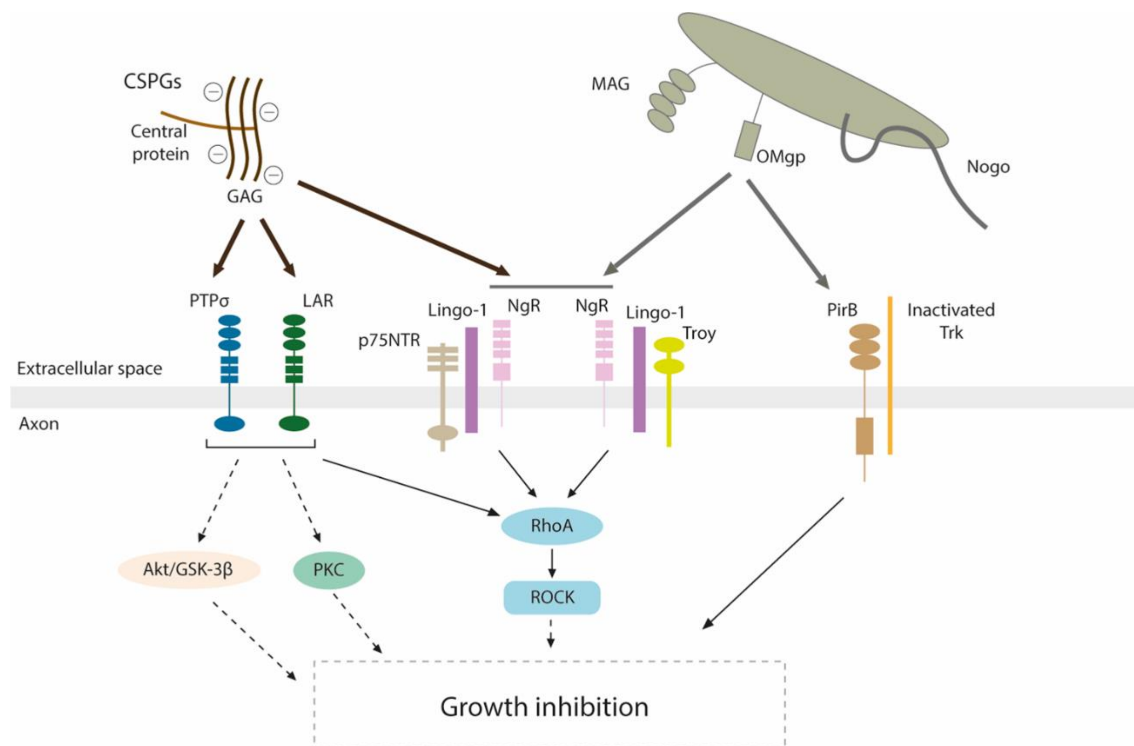

Figure 6. Growth-inhibitory signaling in the axon. Several receptors in the axonal membrane interact with CSPGs and myelin-associated inhibitors present in the injury activating intracellular pathways that have an impact on growth. Interestingly, some of these receptors and intracellular pathways are shared between both types of inhibitory molecules. Adapted from (Geoffroy and Zheng, 2014; McKerracher and Rosen, 2015; Sami et al., 2020).

3.2.2. Unresolved inflammation

A final extrinsic setback for axon regeneration in the CNS derives from the excessive and chronic inflammatory response generated by the maladaptive phenotype progression of the immune cells (Gensel and Zhang, 2015; Mesquida-Veny et al., 2021). These cells exert a great variety of functions after injury (Figure 7), but while they are essential to clear cellular debris and to prevent further tissue damage, as seen in several studies after their depletion (Bellver-Landete et al., 2019; Fu et al., 2020; Y. Li et al., 2020), their interrupted transition leads the lack of injury resolution (Mesquida-Veny et al., 2021).

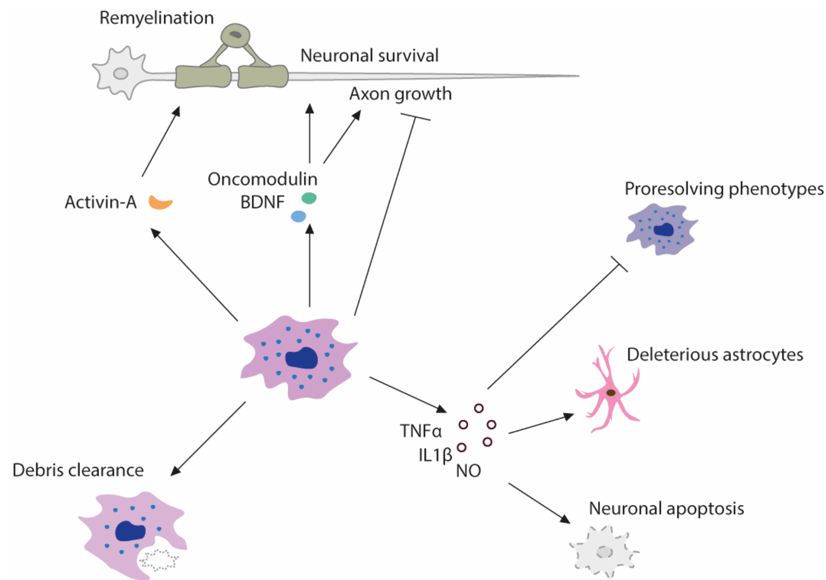

Figure 7. Summary of macrophage/microglial roles in a SCI. Immune cells are a double-edged sword after a SCI, as while they exert essential functions including debris clearance and secretion of pro-resolving factors, their sustained activation leads to excessive inflammation and lack of injury transition. Adapted from (Mesquida-Veny et al., 2021).

These immune cells present fundamental roles for proper healing, as they secrete factors promoting neuronal survival or axonal growth (such as in (Dougherty et al., 2000; Hervera et al., 2018; Kwon et al., 2015, 2013; Yin et al., 2006, 2003) and remyelination (Miron et al., 2013). Most importantly, they are crucial for their part in phagocyting myelin and other debris, a necessary step in all tissues for proper repair (Burda and Sofroniew, 2014; Novak and Koh, 2013; Takahashi et al., 2005). However, while in other tissues debris scavenging activates a transition towards pro-resolving phenotypes, this evolution is impeded in the CNS (Beck et al., 2010; Gensel and Zhang, 2015; Popovich et al., 1997; Sindrilaru and Scharffetter-Kochanek, 2013).

The environment in the CNS injury, marked by elevated tumor necrosis factor α (TNF-α) prevents the phenotypical conversion of macrophages. Additionally, repeated myelin phagocytosis triggers the generation of pro-inflammatory "foamy" macrophages which lack the ability to process myelin (Kong et al., 2020; Kroner et al., 2014; Zhu et al., 2017). Therefore, the initially high pro-inflammatory signaling is further potentiated, as macrophages and microglia continue releasing TNF-α, interleukin (IL)-1β and nitric oxide (NO), which directly increases neuronal apoptosis (Nesic et al., 2001; Satake et al., 2000; Yang et al., 2004) and promotes a deleterious phenotype in astrocytes (Liddelow et al., 2017). Meanwhile, this signaling further hampers the apparition of a phenotype in immune cells responsible for promoting tissue remodeling, angiogenesis and ECM matrix synthesis, among others (Gensel and Zhang, 2015; Krzyszczyk et al., 2018; Novak and Koh, 2013), which contributes to generate an unfavorable environment for axon growth. Lastly, macrophages and microglia also hinder directly axon growth via repulsive guidance molecule a

(RGMa) and through contact with axons (Busch et al., 2009; Hata et al., 2006; Horn et al., 2008; Kitayama et al., 2011).

3.2.3. Environment in the PNS

Several extrinsic elements contribute to the inhibition of axon growth in the CNS, however, these are slightly different in the PNS. First of all, the physical barrier that represents the glial scar in the CNS does not form in the PNS, instead satellite cells activate, proliferate and participate in regeneration promotion through peroxisome proliferator-activated receptor α (PPARα) signaling (Avraham et al., 2020; Woodham et al., 1989; Zigmond and Echevarria, 2019). Regarding the presence of inhibitory molecules, the myelinating cells of the PNS are Schwann cells, which do not present myelin-associated inhibitors on their membranes, with the exception of MAG, however, the high amount of laminin in the PNS counteracts its inhibitory activity (David et al., 1995; Qiu et al., 2000).

The presence of Schwann cells itself also represents a fundamental difference, as they are able to adopt a repairing phenotype after injury with a pro-regenerative secretome (Fontana et al., 2012; Hyung et al., 2019; Jessen et al., 2015; Lopez-Leal and Court, 2016; Nocera and Jacob, 2020). Thanks to Schwann cells and macrophages, debris clearance in PNS injuries is more efficient than in the CNS (Stoll et al., 1989; Zigmond and Echevarria, 2019). Finally, the inflammatory response after PNS injury seems to be properly orchestrated: during the first days phagocytic and pro-inflammatory phenotypes are predominant in the nerve, with recruiting and debris scavenging roles, while this population decreases over time in favor of a pro-resolving phenotype, with pro-regenerative secretomes and contributing to ECM synthesis (Hervera et al., 2018; Lee et al., 2018; Nadeau et al., 2011; Tomlinson et al., 2018; Wu et al., 2013; Zigmond and Echevarria, 2019).

3.3. Intrinsic changes. The intrinsic inability to regenerate

While CNS immature neurons are able to regenerate during development, these capacities are lost when they form synaptic connections (Geoffroy et al., 2016; Hilton and Bradke, 2017). In parallel, PNS adult neurons are also able to regenerate, and the existence of the conditioning effect implies that regrowth can be recapitulated in CNS adult neurons (Curcio and Bradke, 2018). Both of these paradigms have been useful to study the intrinsic mechanisms that allow axon regeneration, which are lacking in the CNS adult neurons (Mahar and Cavalli, 2018).

3.3.1. Injury signalling

Injury signalling is crucial for neurons to properly regenerate (Figure 8). The first injury signal to appear is a calcium influx, which results from membrane breaches and the consequent alterations of ionic channels and sodium/calcium (Na^{+}/Ca^{2+}) pump functioning, this is then propagated to the neuronal soma, where it triggers a great variety of processes (Rishal and Fainzilber, 2014).

Calcium-activated adenylate cyclase synthesizes cAMP, which in turn activates dual leucine zipper-bearing kinase (DLK) promoting axon regeneration (Hao et al., 2016). Calcium influx also activates PKCμ, triggering the nuclear export of histone deacetylase (HDAC) 5 promoting epigenetic pro-regenerative changes (Cho et al., 2013).

Retrograde transport of proteins also participates in injury signalling. In this regard, local protein translation plays an important role: vimentin and importin-β are locally synthesized after injury and allow phosphorylated ERK to bind to dynein and to be retrogradely transported, leading to Elk activation (Perlson et al., 2005). Other retrogradely transported proteins include locally translated and activated signal transducer and activator of transcription 3 (STAT3) and activated c-Jun N-terminal kinase (JNK), both of them DLK-dependent early after injury (Ben-Yaakov et al., 2012; Lindwall and Kanje, 2005; Shin et al., 2012). JNK will activate regenerative-associated transcription factors such as c-Jun and activating transcription factor 3 (ATF3) (Lindwall and Kanje, 2005).

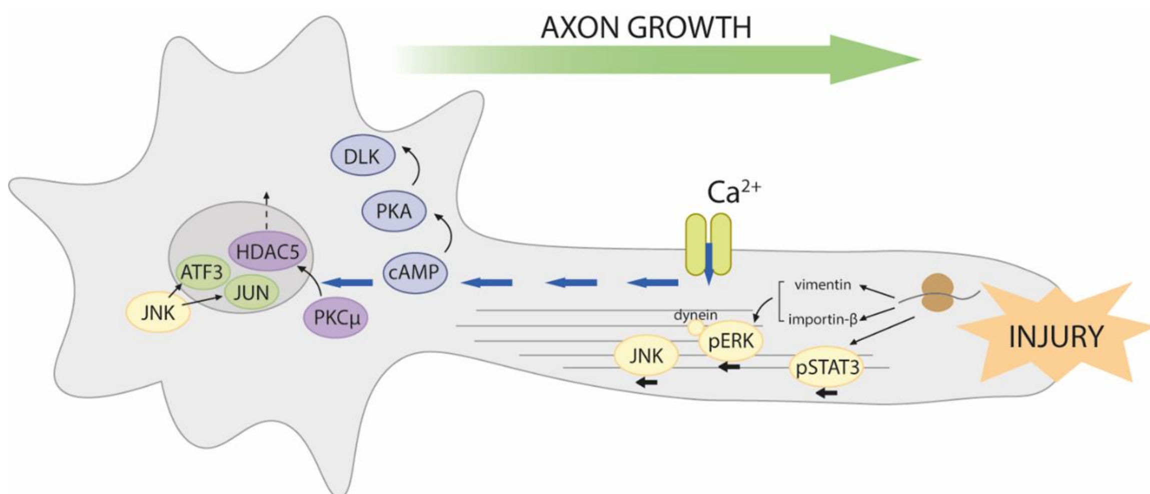

Figure 8. Injury signaling in regenerating neurons. After axonal injury, first Ca^{2+} and later transported proteins from the axon to the soma (pERK, pSTAT3 and JNK) are responsible for the initiation of a pro-regenerative response in the injured neuron. Adapted from (Mahar and Cavalli, 2018; Mar et al., 2014).

3.3.2. Cellular events following injury

Growth cone formation

After axonal injury the damaged membrane needs to be properly sealed in order to restore ionic equilibrium (Bradke et al., 2012). This process is highly dependent on Ca^{2+} concentration, and consists in the formation of a multivesicular patch at the injury site (Fishman and Bittner, 2003). At this time point the cytoskeleton in the axon starts to depolymerize as a result of high Ca^{2+} levels (He and Jin, 2016; Spira et al., 2003).

Next, injured axons need to form a highly motile structure at their tip, the growth cone (Bradke et al., 2012) (Figure 9). Growth cones are formed by a peripheral domain (P-domain) with a complex actin net, a central domain (C-domain) with highly organized microtubules and a transition zone (T-domain) where these two components interact (Coles and Bradke, 2015). The P-domain corresponds to a dynamic structure which will be continuously forming filopodia and lamellipodia as a consequence of actin polymerization (Dent and Gertler, 2003). Importantly, while microtubules in the rest of the axon are stabilized, microtubules reaching the axon tip will present higher dynamism (Blanquie and Bradke, 2018). The entrance of these microtubules in the growth cone is limited by the actomyosin ring in the T-domain, where myosin II interacts with actin (Blanquie and Bradke, 2018; Leite et al., 2021).

While injuries in the PNS result in the generation of growth cones, injured CNS axons are unable to do so and form retraction bulbs (Figure 9) (Ertürk et al., 2007). As opposed to growth cones, these structures lack their high microtubule organization, which instead remain depolymerized, as well as a proper compartmentalization of the different domains (Blanquie and Bradke, 2018; Ertürk et al., 2007). Moreover, resulting from cytoskeleton disorganization but preserved transport from the soma, retraction bulbs are enlarged overtime.

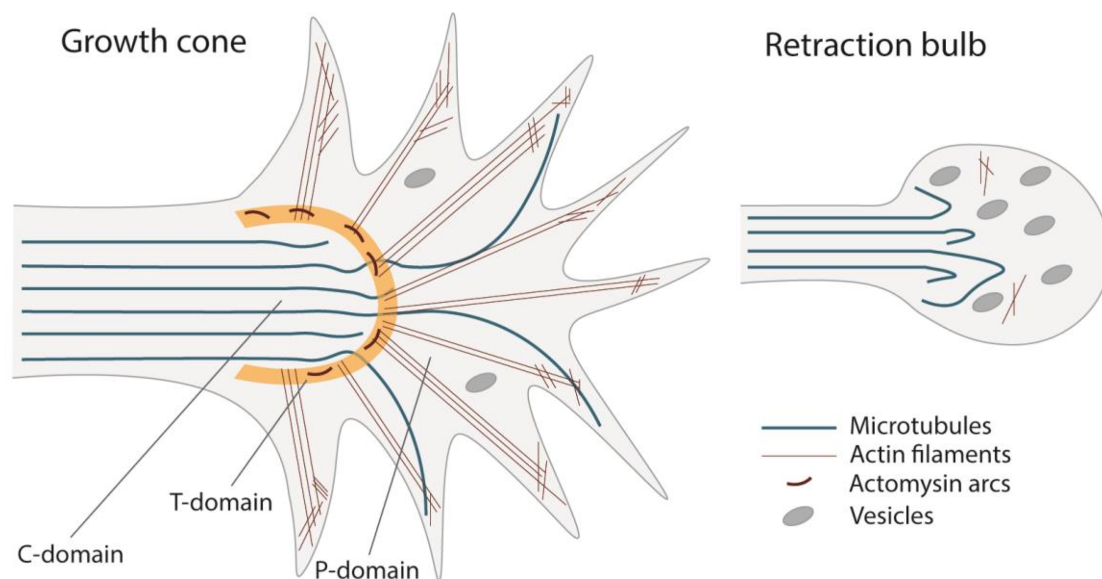

Figure 9. Growth cone and retraction bulb composition. Regenerating neurons form a highly structured growth cone. Microtubule organization (C-domain) and actin dynamics (P-domain), interacting in the T-domain are key elements in this structure and are fundamental for proper growth. Meanwhile, this clear organization is lost in the retraction bulb of non-regenerating neurons. Microtubules depolymerize and do not interact with the actin cytoskeleton, while vesicles accumulate. Adapted from (Curcio and Bradke, 2018).

Cytoskeleton in the growth cone

Several studies have investigated and manipulated the cytoskeleton in the growth cone in order to clarify its significance.

Microtubule-targeted drugs highlight the importance of this cytoskeletal component in the growth cone. Hence, nocodazole prevents regeneration in the PNS by depolymerizing microtubules while pharmacological microtubule stabilization with low doses of taxol or epothilone B or D promotes CNS axonal regeneration (Ertürk et al., 2007; Ruschel et al., 2015; Ruschel and Bradke, 2018; Sengottuvel et al., 2011).

Tubulin, the building block of microtubules, as any other protein, is subject to post-translational modifications, which can have a final effect on microtubule dynamics (Song and Brady, 2015). Acetylation and detyrosination are normally associated with microtubule stability, while tyrosination seems to affect mainly labile microtubules and is therefore enriched in growth cones, where deacetylated tubulin is also predominant (Blanquie and Bradke, 2018; Song and Brady, 2015). In relation to that, tubulin tyrosination has been shown to induce regeneration while increasing microtubule dynamism, which enables retrograde transport of the injury signals (Gobrecht et al., 2016; Song et al., 2015). Conversely, injury-triggered HDAC5 activation was found to be necessary for regeneration in the PNS by decreasing microtubule stability through microtubule deacetilation (Cho and Cavalli, 2012). In summary, it is now clear that microtubule organization needs to be tightly regulated for proper axon growth, and moreover, this regulation may also depend on the environment, as CNS axons seem to require higher microtubule stability, possibly to overcome the inhibitory signaling (Blanquie and Bradke, 2018; Cho and Cavalli, 2012).

In turn, a dynamic actin cytoskeleton is also important for proper axon growth (Leite et al., 2021). The actin cytoskeleton is continuously being remodeled: F-actin extends through faster monomer addition in the plus-end, while in the minus-end monomers are preferentially detached, a process called actin treadmilling, moreover, new branches can also be created in the filament (Pollard, 2016). A group of proteins, the actin-binding proteins (ABPs) interact with actin and regulate this remodeling. Thus, profilins promote actin polymerization, while cofilins, actin-depolymerizing factor (ADF) and gelsolin trigger filament shortening (Pollard, 2016). Furthermore, the final complexity of actin networks is achieved through the action of other ABPs, including the actin-related protein 2/3 (Arp 2/3) complex, which allow filaments to branch, formin, with a role in filament elongation, and filamin, promoting interactions between filaments (Pollard, 2016).

Some recent studies have started to elucidate how actin dynamics influence axon regeneration. Consistently, conditioning injury promotes actin treadmilling as well as ADF/cofilin and profilin1

activity, which were shown to be necessary for the conditioning effect (Pinto-Costa et al., 2020; Tedeschi et al., 2019). In addition, elevating their activity results in regeneration promotion (Frendo et al., 2019; Pinto-Costa et al., 2020; Tedeschi et al., 2019).

Inhibitory signaling in the growth cone

On another note, many components of the extrinsic barrier in the CNS exert their inhibitory effect through cytoskeleton alterations. CSPGs and myelin-associated inhibitors activate RhoA, however, the precise downstream mechanisms leading to growth inhibition were just recently described (Stern et al., 2021). In this study, induction of RhoA with inhibitory substrates hampered growth through myosin II activation via myosin light chain (MLC) phosphorylation. As a result, the actomyosin ring is contracted, preventing microtubule extension (Stern et al., 2021). These results are further supported by RhoA effects during development (Dupraz et al., 2019). Moreover, while RhoA activation leads to cofilin phosphorylation hence inactivation (RhoA-ROCK-LIM kinase (LIMK)-cofilin) as well as to profilin inactivation, also upon phosphorylation (RhoA-ROCK-profilin), in this paradigm RhoA did not seem to mediate these effects (Blanquie and Bradke, 2018; Leite et al., 2021; Stern et al., 2021).

All in all, it seems to be clear that the actin network in the growth cone plays a vital role during axon regeneration and, similarly to the microtubule cytoskeleton, might require precise modulation, in order to not trigger local unwanted effects (Loudon et al., 2006).

Energy requirements

The formation of a growth cone and the subsequent axon growth are high energy-dependent processes, however, mitochondria in injured axons are unable to maintain their membrane potential (Curcio and Bradke, 2018; Zhou et al., 2016). Therefore, enhancing mitochondrial motility through syntaphilin deletion or armadillo repeat containing X-linked 1 (Armcx1) overexpression results in axon regeneration (Cartoni et al., 2016; Han et al., 2020; Zhou et al., 2016). Moreover, and according to their lack of regenerative capacities, mature neurons display decreased mitochondrial dynamics, resulting from an increase in syntaphilin expression (Zhou et al., 2016).

3.3.3.Molecular pathways involved in regeneration

Studies comparing PNS neurons, CNS immature neurons and CNS adult neurons have led to the conclusion that while the first two are able to mount a pro-regenerative program elicited by the injury, CNS adult neurons remain irresponsive. This has allowed to identify a series of mechanisms involved in regeneration that remain silenced in these refractory to regenerate neurons (Curcio and Bradke, 2018) (Figure 10).

One of the milestones in axon regeneration was the discovery of phosphatase and tensin homolog (PTEN), an inhibitor of the mammalian target of rapamycin (mTOR) pathway, whose deletion leads to regeneration in the CNS (Liu et al., 2010; Park et al., 2008). While activated in the PNS after injury where it promotes regeneration through protein synthesis induction, the mTOR pathway is suppressed in CNS mature neurons, representing an age-dependent silencing of a pro-regenerative mechanism in this system (Abe et al., 2010; Liu et al., 2010; Park et al., 2008). Another highlighted pathway in axon regeneration, which remains inactive in the CNS, is the Janus kinase (JAK)/STAT pathway, initiated through gp130 signaling after IL-6, C-C motif ligand (CCL)2, ciliary neurotrophic factor (CNTF) or leukemia inhibitory factor (LIF) interaction (Cafferty et al., 2004; Leibinger et al., 2013; Niemi et al., 2016; Schwaiger et al., 2000). Consequently, STAT3 deletion impairs, while the lack of suppressor of cytokine signaling 3 (SOCS3) (an inhibitor of the JAK/STAT pathway), induces, regeneration (Bareyre et al., 2011; Smith et al., 2009). Of note, physiological activation of this pathway depends on the cytokine production from other cells, a reminder that intrinsic neuronal capacities may be triggered by its environment.

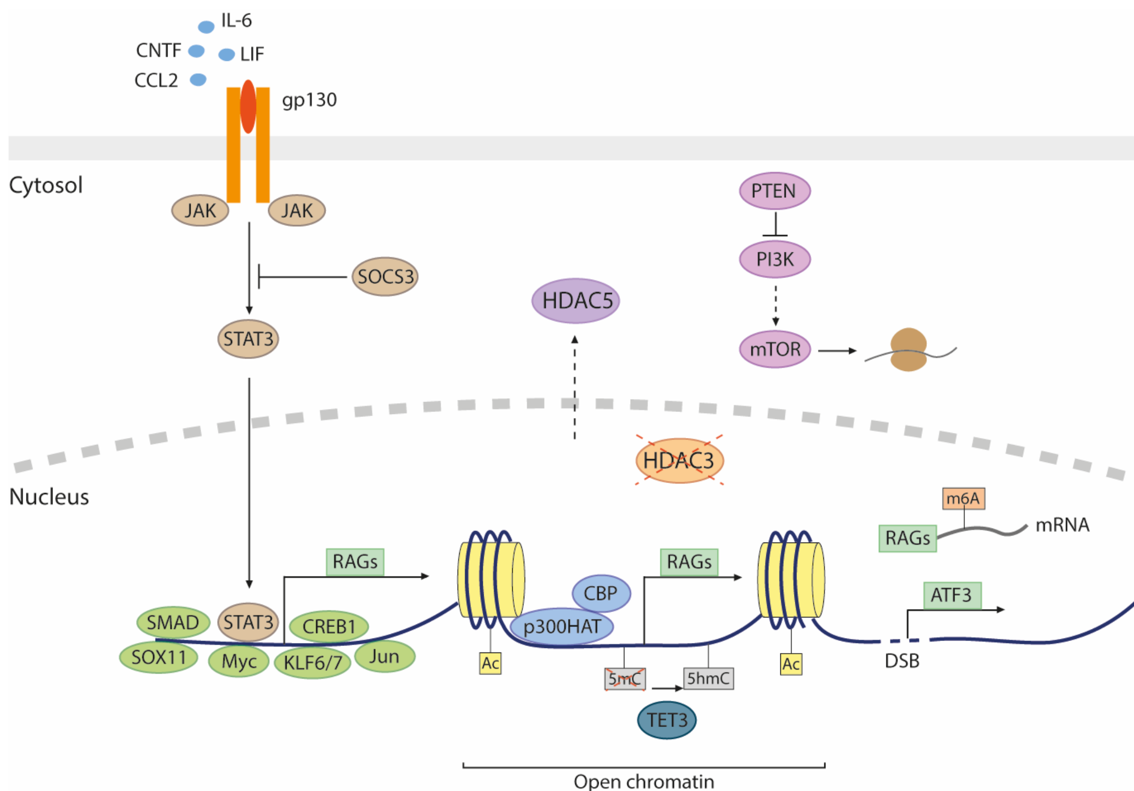

Figure 10. Summary of molecular pathways as well as epigenetic and other genomic alterations implicated in axonal regeneration. Axonal regeneration has shown to be regulated at several cellular levels, ranging from mTOR mediated protein synthesis, to physical DNA alterations (DSB) leading to the transcription of growth-promoting genes (ATF3). The acquisition of a pro-regenerative transcriptional state is tightly modulated by many other mechanisms, including the activation of specific transcription factors (STAT3, SMAD, SOX11…) to epigenetic modifications leading to open chromatin in specific growth-related regions. Adapted from (Curcio and Bradke, 2018; Mahar and Cavalli, 2018).

A great number of transcription factors have been also implicated in the pro-regenerative response after injury, such as c-Jun, SMAD, MYC, cAMP response element-binding protein (CREB) and SRY-related HMG-box 11 (SOX11) (Curcio and Bradke, 2018; Mahar and Cavalli, 2018). In general, they are responsible for the synthesis of a series of RAGs, known for their effects on axonal growth, which include: GAP43, ATF3, Sprr1a or Galanin, among many others (Mar et al., 2014; Qian and Zhou, 2020). Interestingly, some of these transcription factors suffer expression changes during development, and of them, the family of Krüppel-like factors (KLF), has received special attention, as KLF components that show raised expression throughout development (KLF4 or KLF9) hamper regeneration, and the ones that become downregulated (KLF6 or KLF7) enhance axon growth (Apara et al., 2017; Blackmore et al., 2012, 2010; Moore et al., 2009).

In line with these results, additional studies have further reinforced the existence of other age-regulated mechanisms that result in a reduction of growth capacities. RNA sequencing comparing regenerating with growth-restricted neurons led to the discovery of α2δ2, a subunit of voltage-gated calcium channels (VGCC), which represses growth in mature CNS neurons (Tedeschi et al., 2016). Similarly, a role for the presynaptic proteins Munc13 and Rab3 interacting molecule (RIM) in growth repression in mature neurons has just been uncovered (Hilton et al., 2021). Another study showed that PTEN deletion promotes regeneration in young mice, but this effect is far decreased in aged mice (Geoffroy et al., 2016). Strikingly, high levels of mTOR were maintained in PTEN-deleted aged animals, therefore, the lack of regeneration was attributed to a worsened response to the extrinsic component, which could be due to the observed degeneration of the environment, but also to an intrinsic increased refractory response (Geoffroy et al., 2016).

3.3.4. Epigenetic mechanisms involved in axon regeneration

Epigenetic mechanisms, which result in broad alterations of gene regulation, have also been described to modulate neuronal intrinsic growth capacities (Wahane et al., 2019) (Figure 10).

Some of these mechanisms, including histone modifications and DNA methylation have an impact on genomic accessibility, therefore altering gene expression. A recent study using sequencing techniques showed that while PNS injury triggered a relaxed and opened chromatin state, this changes were missing after CNS injury (Palmisano et al., 2019). Moreover, chromatin accessibility is decreased at regeneration-associated genomic regions after development in the CNS (Venkatesh et al., 2018, 2016).

An epigenetic mechanism that normally promotes chromatin accessibility, therefore allowing increased transcription, is histone acetylation (Wahane et al., 2019). H3 and H4 acetylation is further increased after peripheral injury (Finelli et al., 2013; Palmisano et al., 2019; Puttagunta et al., 2014). Accordingly, the activity of the histone acetyltransferase p300/CBP-associated factor

(PCAF) is necessary for the conditioning effect (Puttagunta et al., 2014). As previously stated, HDAC5 exits the nucleus after peripheral injury, reducing nuclear deacetylation and promoting regeneration (Cho et al., 2013). Furthermore, HDAC3 inhibition occurs upon PNS (but not CNS injury) allowing the activation of a previously repressed regenerating program (Hervera et al., 2019b). Histone deacetylation might therefore hamper regeneration, however, broad HDAC inhibition shows uneven effects, highlighting the need for more precise actuations (Gaub et al., 2010; Venkatesh et al., 2016).

Another epigenetic modification described to have an important role in axon regeneration regulation is DNA demethylation. Methylated DNA cytosines (5mC) are generally thought to repress expression. Ten eleven translocation 3 (TET3) activity, crucial in the demethylation process, is triggered after peripheral injury, leading to reduced DNA methylation and increased RAG expression, a process that does not occur in CNS injuries (Loh et al., 2017; Weng et al., 2017).

Finally, epigenetic RNA-related mechanisms are also starting to gain importance in the field of axon regeneration (Wahane et al., 2019). Several microRNAs (miR) such as miR-222 or 431 are altered after PNS injury, however, their role after CNS injuries remains to be elucidated (Wahane et al., 2019; Wu and Murashov, 2013; Zhou et al., 2012). In addition, an mRNA modification, the m6A methylation, was also demonstrated to play a role in axon regeneration, revealing yet another level of complexity present after axonal injury (Weng et al., 2018).

A special mention needs to be made for another non-epigenetic mechanism that may also be affecting gene expression: DNA double-strand breaks (DSB) were found to occur shortly after peripheral injury and to stimulate ATF3 expression, an important RAG (Cheng et al., 2021).

4. Therapeutic approaches for SCI

4.1. Strategies to promote recovery

Due to their nature, traumatic injuries are as varied and diverse as patients exist. The localization and size of the lesion, the proportion and type of the affected tracts as well as the first interventions, among others, determine their severity and ultimately, their outcome (Ahuja et al., 2017). In that direction, different injuries may require different strategies to promote recovery, which are mostly determined by the completeness of the injury. In incomplete injuries neuroplasticity may lead to recovery in several ways: regrowth of severed axons, most probably eluding the lesion core, sprouting of unaffected fibers or plasticity of existing spinal circuits

(O'Shea et al., 2017). On the other hand, in the case of complete SCIs, only two possibilities exist as a result of the lack of supraspinal connectivity: crossing the lesion-core or bypassing it with external devices (Courtine and Sofroniew, 2019; O'Shea et al., 2017).

As stated above, both extrinsic and intrinsic barriers to CNS regeneration need to be individually addressed. Depending on the aspect they target, several different strategies exist (Ahuja et al., 2017; Griffin and Bradke, 2020; Yang et al., 2020): neuroprotection, inhibitory signaling blockade, enhancement of intrinsic growth capacities, cell-based strategies, trophic factor addition and/or activity-based therapies (Figure 11). These strategies are further commented below, while Table 2 shows and exemplifies a selection of the most studied approaches.

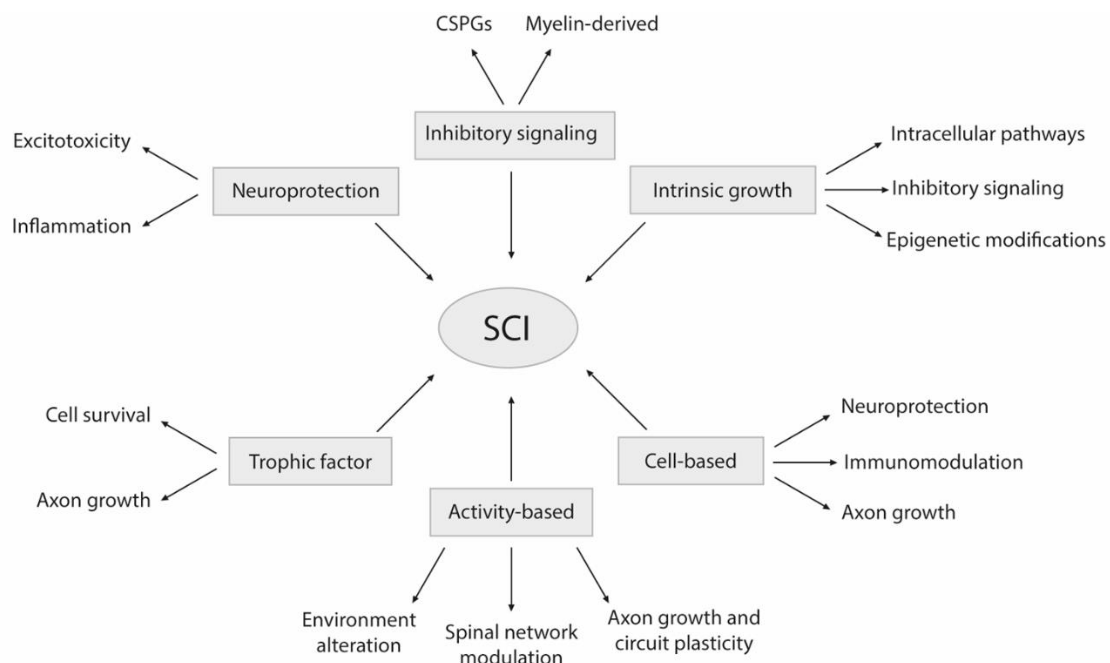

Figure 11. Therapeutic approaches in SCI and associated targets. Information from (Griffin and Bradke, 2020).

Neuroprotection: Neuroprotective treatments try to limit the secondary damage, normally by reducing excitotoxicity (such as with gacyclidine) or the inflammatory response (for example with methylprednisolone sodium succinate or minocycline), however, in terms of clinical trials these treatments have not obtained the expected success (Badhiwala et al., 2018; Griffin and Bradke, 2020). Besides, it is now known that completely abolishing the inflammatory response is detrimental for recovery, as inflammation is a necessary process for proper healing (Chio et al., 2021; Mesquida-Veny et al., 2021).

Inhibitory signaling blockade: As previously explained, after a SCI several growth inhibitory

molecules are secreted in the affected area. ChABC has been thoroughly used to promote CSPGs degradation with significant success in various experimentation animals (reviewed in (Griffin and Bradke, 2020)). Similarly, other researchers have tried to block the myelin-associated inhibitors, specially Nogo with an anti-Nogo-A antibody, or with the targeting of its receptor (Kucher et al., 2018; Li et al., 2004; Zörner and Schwab, 2010). However, the clinical efficacy of these treatments in humans remains to be elucidated.

Enhancement of intrinsic growth capacities: The conditioning-injury paradigm, as well as the fact that mature axons in the CNS lose their developemental regenerative capacities, have raised the question of whether this developing state could be retrieved in CNS neurons, and since then, diverse attempts have tried to unravel the nature of this switch, or a way to emulate it (He and Jin, 2016; Hilton and Bradke, 2017; Mar et al., 2014). Those attempts range from manipulating or blocking key molecular check-points that hamper growth (such as PTEN or SOCS3) (Park et al., 2008; Smith et al., 2009) as well as other intracellular inhibiting pathways, like RhoA (Dergham et al., 2002; Fehlings et al., 2018, 2011), to promote microtubule stabilization with epothilones or taxol (Hellal et al., 2011; Ruschel et al., 2015; Ruschel and Bradke, 2018; Sandner et al., 2018), and to finding epigenetic hallmarks that may affect the expression of several of these targets (Wahane et al., 2019). Although all these studies have been incredibly enlightening, the ubiquitous character of their targets and their fundamental roles in other cellular processes turn into a challenge the design of therapies without remarkable side effects.

Cell-based strategies: Cell-based strategies represent one of the most popular approaches to treat SCI, and to this day, a great variety of cell types have been assessed for transplant: neural stem cells (NSCs), Schwann cells, olfactory ensheating cells (OECs), OPCs, mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs), among others (Assinck et al., 2017). In general, cell transplants seem to exert their beneficial effects through neuroprotection and immunomodulation, and some appear to even induce axon growth (Assinck et al., 2017). While many of these attempts have shown some success in preclinical studies, important issues such as ectopic colony or even tumor formation or the lack of effectiveness in late stage patients remain to be addressed (Assinck et al., 2017). The lack of mechanistic insight of these approaches hinders as well the refinement of these therapies.

Trophic factor addition: One of the first designed strategies for SCI treatment was based on the supply of neurotrophins such as BDNF, neurotrophin-3 (NT-3) and nerve growth factor (NGF), however, further studies showed each of these neurotrophins presented divergent effects on different neuronal types (reviewed in (Keefe et al., 2017)). Additionally, these therapies have faced important administration concerns since sustained release of these factors is needed to induce recovery (Griffin and Bradke, 2020). Eventually, biomaterials appeared in the field to cope

with this last issue, as they allowed a long-term release of the trophic factors (Führmann et al., 2017). Other scaffolds or biomaterials are also being used in cell therapy, both for structural and trophic support (Assinck et al., 2017; Führmann et al., 2017). In a recent study, the combination of osteopontin, insulin-like growth factor 1 (IGF1) and CNTF overexpression with the supplementation of fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), and glial-derived growth factor (GDNF) in hydrogen depots induced regeneration over the injured area, while individual application of these approaches did not (Anderson et al., 2018). Moreover, this observed regeneration did not translate into functional recovery (Anderson et al., 2018). The complexity of this study together with its lack of functional improvements denotes the moderate efficacy of this type of treatments.

Activity-based therapies: Another broadly used strategy to treat SCI are activity-based therapies, which mostly refers to rehabilitation and/or electrical stimulation. Rehabilitation, which can be simulated in animal models through environmental enrichment, forced swimming or treadmill, among others (Loy and Bareyre, 2019) has repeatedly proved to induce recovery on them (i.e. (Asboth et al., 2018; Engesser-Cesar et al., 2007; Hutchinson et al., 2004; Hutson et al., 2019; Loy et al., 2018)). When applied to patients, rehabilitation yields only modest success, and has proven efficiency only in incomplete lesions, but to this day, it remains one of the leading therapies in terms of recovery, with consistent results (Griffin and Bradke, 2020). Besides, it can be easily combined with other strategies for better results (Griffin and Bradke, 2020). Electrical stimulation, which can be combined with rehabilitation or not, has also given relative great results, both clinically and in animal models (Carmel and Martin, 2014; Friel et al., 2014; Loy and Bareyre, 2019). Transcranial magnetic stimulation (TMS) has recently appeared as a less invasive alternative to electrical stimulation, inducing as well modest recovery in patients (Alexeeva and Calancie, 2016; Gomes-Osman and Field-Fote, 2015). Similarly, in cases with severe injuries with little supraspinal input, neuromodulation through both pharmacological and spatiotemporal electrical stimulation is able to engage lower circuits thus enabling control of previously lost functions (Courtine and Sofroniew, 2019; Wagner et al., 2018; Wenger et al., 2016). Importantly, these lastly commented therapies, which stimulate neuronal activity, succeed in engaging neuroplasticity mechanisms, which is a necessary event for recovery (Fouad and Tetzlaff, 2012; Hutson and Di Giovanni, 2019).

Lastly, a comment needs to be made for Brain-Computer-Interfaces (BCI), a technology that tries to detour the injury, instead of trying to repair it, by bypassing it (Borton et al., 2013; Courtine and Sofroniew, 2019). While these technologies have already accomplished movement restoration for some patients (Ajiboye et al., 2017; Bouton et al., 2016), and represent the preferred solution in complete injuries, to this day, they lack feasibility as they need to be

rigorously personalized (Borton et al., 2013).

Among all the commented strategies, it seems that activity-based therapies are the current leaders in terms of recovery as well as feasibility after SCI. However, much remains unknown about how these therapies act on the spinal cord networks and environment. Moreover, it is now clear that addressing one single aspect of the pathophysiology of CNS injuries will most likely not be sufficient to induce complete recovery in patients. Thus, combining strategies of different nature gives remarkable results, as clear examples result from the combination of ChABC or Anti-Nogo-A with rehabilitation (Chen et al., 2017; García-Alías et al., 2009; Shinozaki et al., 2016; D. Wang et al., 2011). In this regard, another key point is the optimal application of the therapies throughout time, as uncoordinated usage may result in poor or unfavorable effects (Maier et al., 2009). All in all, additional studies are required in order to optimize the application of activity-based therapies to SCI patient.

Strategy		Proposed mechanism	Clinical stage	Limitations	References
Neuroprotection	Methylprednisolone sodium succinate	Limits inflammatory response	Several clinical trials. Some show recovery. Generally adopted therapy	Immunosuppression. Terminates necessary inflammatory reactions.	(Badhiwala et al., 2018; Chio et al., 2021; Fehlings et al., 2017)
	Minocycline	Reduces microglial/macrophage pro-inflammatory response	Phase II completed, showing improvement in cervical patients	Lack of consistency in improvements.	(Badhiwala et al., 2019; Casha et al., 2012; Chio et al., 2021; Griffin and Bradke, 2020)
	Gacyclidine	Prevents excitotoxicity as a NMDA receptor antagonist	Phase II completed	Lack of statistical significance	(Badhiwala et al., 2018; Griffin and Bradke, 2020)
	Riluzole	Prevents excitotoxicity as a sodium-channel blocker	Phase I completed, showing increased recovery	Phase IIB/III unfinished	(Badhiwala et al., 2019; Griffin and Bradke, 2020; Grossman et al., 2014)
Inhibitory signaling interference	Anti-Nogo-A	Blocking of growth inhibitor	Phase I completed; ongoing phase II	Efficacy not demonstrated in humans	(Kucher et al., 2018; Zörner and Schwab, 2010)
Intrinsic modulation	Cethrin	Inhibitor of Rho signalling	Phase I/IIa completed showing increased recovery	Interrupted phase IIb/III, lack of efficacy	(Fehlings et al., 2011; Griffin and Bradke, 2020)
Cell-based	Autologous Schwann cells	Myelination, structural support and factor secretion	Phase I completed	Axons fail to surpass graft	(Assinck et al., 2017; Gant et al., 2021; Griffin and Bradke, 2020)
	OECs	Structural support and factor secretion	Phase I completed	Unclear efficiency	(Badhiwala et al., 2019; Li et al., 2015)
	Autologous MSCs	Immunomodulatory, factor secretion	Phase III completed	Very limited success rate	(Chio et al., 2021; Oh et al., 2016)
Combinatorial	Nerve graft and aFGF–fibrin glue	Structural support, permissive substrate and axon growth	Phase I/II completed showing recovery	Only modest recovery	(Cheng et al., 2004; Wu et al., 2011, 2008)
Electrical stimulation	Functional electrical stimulation	Increases cell excitability	Initiated phase III	Difficulties in parameter selection	(Badhiwala et al., 2019; Marquez-Chin and Popovic, 2020)

Table 2. Selection of the principal proposed therapies for SCI injury reaching clinical trials. In most of the trials patients also received or had previously received rehabilitation due to its widespread application (Griffin and Bradke, 2020). Most of the information about research stage was obtained from (Griffin and Bradke, 2020).

4.2. Activity-based therapies and underlying molecular mechanisms

Sensory experience can broadly modify CNS circuitry both during development and adulthood, a capacity termed neuroplasticity (Dunlop, 2008). During development, neuronal activity guides the formation of new connections, as well as the maintenance or elimination of others, while in the adult brain it also promotes reorganization (Carulli et al., 2011; Jamann et al., 2018). Importantly, while some degree of spontaneous plasticity occurs after CNS injury, this plasticity can be further reinforced with activity-based therapies, enhancing recovery in patients, and representing today the most effective treatment after SCI (Carulli et al., 2011; Griffin and Bradke, 2020). While not completely understood, the current knowledge on the molecular and cellular mechanisms responsible for the success of these therapies is summarized below.

4.2.1. Activity-dependent plasticity

Neuronal activity has long been known to regulate gene expression (Flavell and Greenberg, 2008; West and Greenberg, 2011), and neuronal activity-triggered plasticity may result partly from this regulation (Carulli et al., 2011; Hogan et al., 2020) (Figure 12).

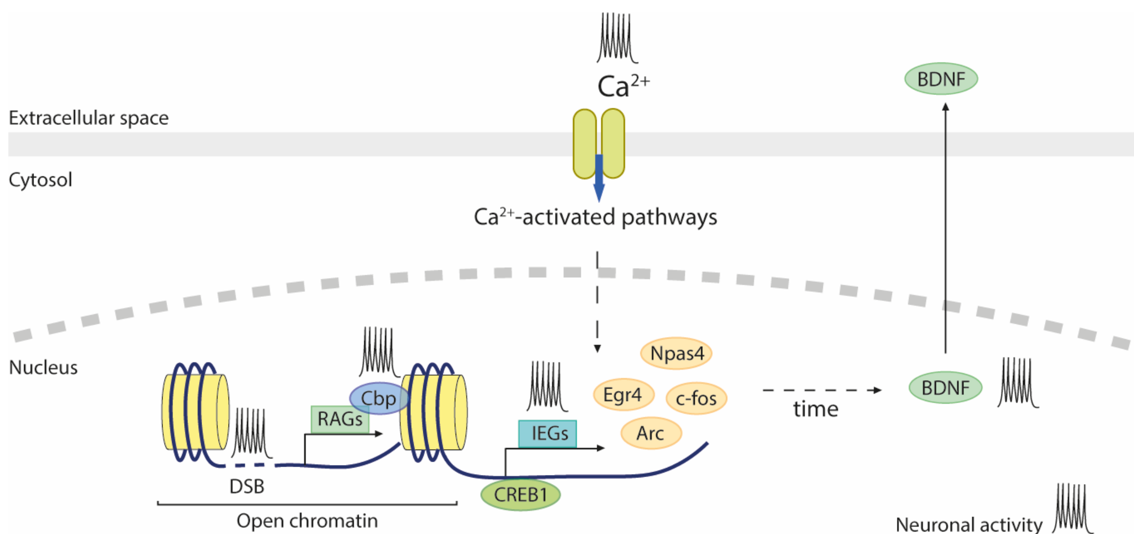

Figure 12. Activity-regulated molecular mechanisms. The transcription of several genes has been shown to be modulated by neuronal activity, which also induces important epigenetic changes, as well as DSBs, both of them further contributing to gene expression alterations upon neuronal activity.

During neuronal activity intracellular Ca^{2+} levels increase, which eventually promotes gene transcription due to the Ca^{2+}-activated molecular mechanisms (Carulli et al., 2011). A series of immediate early genes, including Arc, Npas4, Egr4 or c-Fos are expressed in response to these changes (Hogan et al., 2020). The activity-induced gene expression of these genes is mediated by other prevailing transcription factors, and of them, CREB has repeatedly been implied in plasticity (Carulli et al., 2011; Yap and Greenberg, 2018). Importantly, BDNF, one of the most described

neurotrophins to induce axon regeneration, is drastically up-regulated by neuronal activity (Flavell and Greenberg, 2008), which regulates not only its expression but also other processes such as its secretion (Hogan et al., 2020; Palomer et al., 2016; Wong et al., 2015; Zhou et al., 2006). Finally, neuronal activity also modulates processes that have a final effect on gene expression, as it impacts chromatin state (Su et al., 2017), and provokes DSBs, which induce transcription (Madabhushi et al., 2015). Moreover, a recent study showed that increased proprioceptor activity resulted in augmented histone acetylation via Creb-binding protein (Cbp), leading to a pro-regenerative state in these neurons (Hutson et al., 2019). Research has also shown that gene expression is differently regulated depending on the duration of neuronal activity (Tyssowski et al., 2018) as well as on its temporal cadence, including some genes associated with growth (Lee et al., 2017).

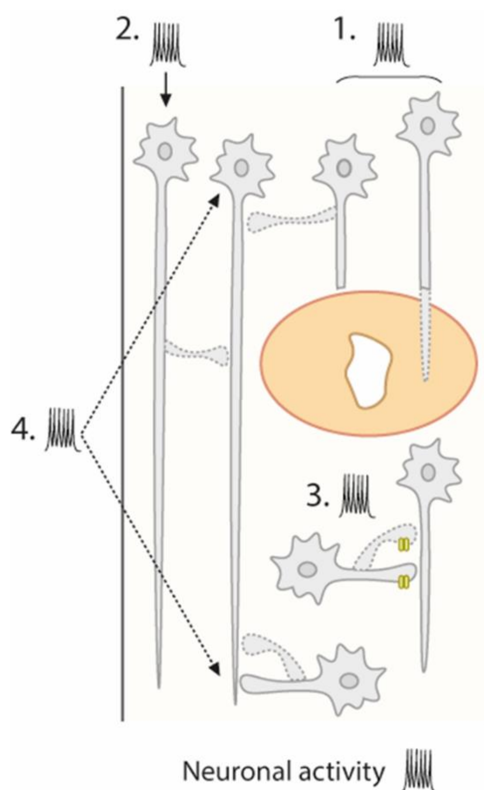

Figure 13. Different forms of plasticity may be elicited by activity-based therapies. Activity-based therapies may induce recovery through different forms of plasticity depending on the characteristics of the stimulation. The stimulation of injured fibers (1) can result in regeneration promotion or either in the generation of detour circuits, while activation of intact fibers (2) may promote their sprouting. In turn, the modulation of spinal circuits (3) seems to induce local circuit excitability and plasticity. Finally, paired stimulations (4) promote the strengthening of the precise connection. Adapted from (Courtine and Sofroniew, 2019; Jack et al., 2020).

Activity-based strategies try to activate these mechanisms in order to promote recovery in SCI, and the final success may result from several different forms of plasticity (Figure 13). Importantly, although activity-based therapies comprise different strategies, all of them with a final increase in neuronal activity, they differ on its specificity: while general strategies such as rehabilitation may be modulating other processes, chemogenetic or optogenetic techniques allow to selectively tailor neuronal activity with temporal and cellular specificities never achieved before (Deisseroth, 2015; Sternson and Roth, 2014). Therefore, despite greater success of the first therapies in

patients, these novel approaches grant to better identify the responsible mechanisms while allowing a fine tailoring of the same.

4.2.2.Rehabilitation

Rehabilitation strategies are based on increasing activity through physical activity, and while they have shown important success in patient recovery, the exact molecular basis are far from understood (Bilchak et al., 2021; Fouad and Tetzlaff, 2012; Harkema et al., 2012). Exercise contributes to the sprouting of serotonergic axons, resulting in increased recovery (Engesser-Cesar et al., 2007; Goldshmit et al., 2008). Moreover, exercise also contributes to recovery as it promotes the formation of contacts between injured corticospinal fibers and other intact fibers, generating detour circuits to overcome the injury (Loy et al., 2018). This was further confirmed with studies combining rehabilitation with pharmacological and electrical stimulation of the spinal cord in which the injured CST recovered its function by forming connections with uninjured tracts (Asboth et al., 2018; Van Den Brand et al., 2012).

Rehabilitation also seems to increment the plasticity of the local circuitry and the synapsis below the injury, as shown by increases in the synaptic markers synapsin I, synaptophysin, vesicular glutamate transporter 1 (vGlut1) and postsynaptic density protein-95 (PSD-95) (Goldshmit et al., 2008; Sánchez-Ventura et al., 2021; Wang et al., 2015; Ying et al., 2005). BDNF is increased by exercise in the injured spinal cord, and is a clear candidate to be mediating these effects, together with other forms of plasticity (Bilchak et al., 2021; Wang et al., 2015; Ying et al., 2005). CREB also experiences an exercise-induced increase, which may be a consequence of the BDNF interaction with its receptor, TrkB (X. Li et al., 2020; Ying et al., 2005). Exercise promotes increases in other trophic factors as well, such as NT-3 or GDNF, however, their role in promoting recovery remains unclear (Côté et al., 2011; Ying et al., 2005). Exercise has also been shown to modulate neurotransmission in the spinal networks, as it affects serotonergic receptor expression, increasing spinal cord excitability, and prevents the downregulation of the chloride transporter KCC2, aiding GABAergic and glycinergic neurotransmission (Beverungen et al., 2020; Bilchak et al., 2021).

Finally, rehabilitation has also shown to have an effect on the injury environment: exercise prevents a decrease in perineuronal nets (PNN), formed of extracellular matrix, and decreases astrogliosis and the immune cell response (Chhaya et al., 2019; Loy et al., 2018; Sánchez-Ventura et al., 2021; Sandrow-Feinberg et al., 2009).

While many studies make use of exercise to induce activity, enriched environment also results in increased sensorial activity, providing another tool to explore the effect of activity-based therapies in mice. Accordingly, enriched environment enhances recovery and regeneration through activity-

dependent epigenetic alterations, specifically, histone acetylation is increased as a result of Cbp activity (Berrocal et al., 2007; Hutson et al., 2019). Interestingly, the combination of enriched environment with a conditioning injury elicited a stronger regenerative state in neurons than both approaches individually, suggesting the different induced mechanisms act in an additive manner (De Virgiliis et al., 2020).

4.2.3.Electric stimulation

While rehabilitation allows to modulate only physiologically a very restricted number of neural networks, electrical stimulation permits a fine tuning of the stimulation parameters in a broad spectrum of fibers (Hogan et al., 2020). Electrical stimulation has been largely studied as a treatment for SCI, promoting different strategies depending on its use (Jack et al., 2020).

One of the most used strategies relies on modulating the spinal circuitry present beyond the injury through epidural stimulation, intraspinal microstimulation or transcutaneous stimulation (Jack et al., 2020). This approach has shown important success, especially when the stimulation is refined spatiotemporally with locomotor activity recordings (Capogrosso et al., 2016; Wenger et al., 2016, 2014). While not completely understood, the basis for this success likely depend on the induced plasticity in the spinal networks, which can result in reinforced proprioceptor-motor neuron connections, rearrangement of propriospinal networks and error correction circuitry adaptations (Eisdorfer et al., 2020).

Paired electrical stimulation has also been used after SCI, an approach that relies on activating separately but at the same time two neurons in the same circuit, intensifying the connection (Jack et al., 2020). This strategy has shown to promote recovery in rodents, primates and even in patients, and more importantly, these effects may persist weeks after the stimulation (Long et al., 2017; McPherson et al., 2015; Mishra et al., 2017; Wagner et al., 2018).

Conversely, other studies use electrical activity with the final goal of triggering regeneration-associated pathways and inducing sustained axonal growth, trying to emulate what happens in the PNS or during development (Jack et al., 2020; Jara et al., 2020). Electrical stimulation enhances regeneration of peripheral axons as well as it increases their GAP43, BDNF and TrkB expression (Ahlborn et al., 2007; Al-Majed et al., 2004, 2000a). Moreover, conditioning (prior to injury) stimulation of the peripheral nerve results in enhanced regeneration after injury (even in the central branch), increasing GAP43, cAMP, BDNF, and CREB levels, similar to the classic conditioning injury (Goganau et al., 2018; Senger et al., 2019, 2018; Udina et al., 2008). The observation that neuronal activity is important during development for the proper formation of neuronal connections (Martin et al., 1999) lead to evaluate the effect of electrical activity on CNS injuries. Since then, many studies have shown that electrical stimulation of the CST results in

functional recovery and increased sprouting of this tract (Carmel et al., 2013, 2010; Carmel and Martin, 2014). Importantly, in this paradigm axon sprouting is dependent on mTOR activity, while JAK/STAT guides synapse remodeling (Zareen et al., 2018).

Of note, electrical stimulation was also seen to increase glial fibrillary acidic protein (GFAP) levels, suggesting glial activation (Senger et al., 2019, 2018). These results may point towards non-neural effects of the electrical stimulation. These unspecific effects have stressed and given place to the creation and use of cell-specific strategies, such as optogenetics or chemogenetics.

4.2.4. Optogenetics and chemogenetics

Optogenetics and chemogenetics allow the modulation of neuronal activity in a cell-specific manner, using either light or the administration of a designer drug (Atasoy and Sternson, 2018; Deisseroth, 2015). Therefore, both of them are useful approaches to study activity-triggered molecular mechanisms implicated in recovery after an injury. Figure 14 shows the functioning of two of the most used optogenetic and chemogenetic proteins.

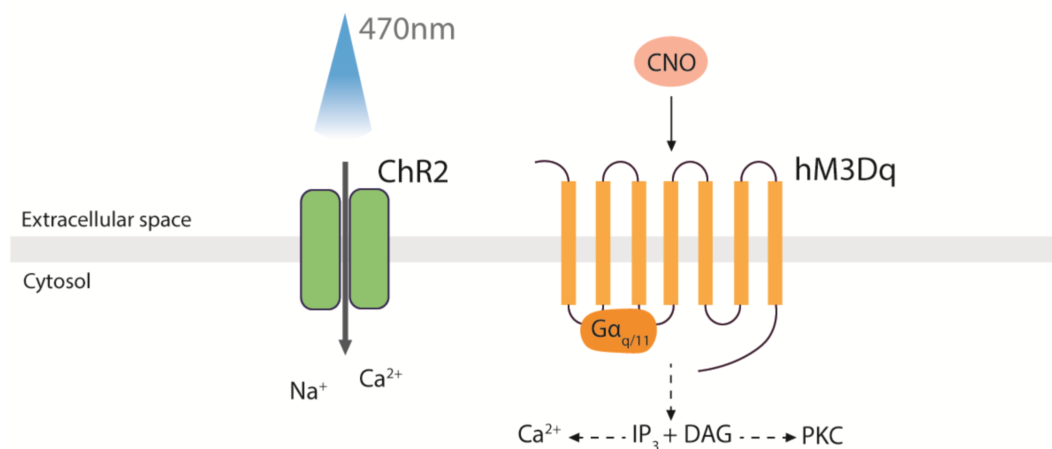

Figure 14. ChR2 and hM3Dq activation. Channelrhodopsin-2 (ChR2) is an optogenetic tool corresponding to a cationic channel that opens upon illumination with 470nm blue light, leading to Na^+ and Ca^{2+} entrance (Rost et al., 2017). In turn, hM3Dq is a designer receptor exclusively activated by a designer drug (DREADD), derived from a G-protein coupled receptors (GPCRs), that initiates $G_{q/11}$ intracellular responses upon interaction with its specific ligand, clozapine N-oxide (CNO) (Atasoy and Sternson, 2018). Neuronal expression of these proteins allows to specifically modulate neuronal activity as the activation of both of these proteins leads to neuronal depolarization. Importantly, as a result of light-triggered activation, ChR2 presents a higher temporal resolution (Rost et al., 2017).

After PNS injury, stimulating neuronal activity by optogenetic and/or chemogenetic means results in increased motor neuron regeneration and recovery (Jaiswal and English, 2017; Sala-Jarque et al., 2020; Ward et al., 2016). Similarly, optogenetic stimulation of the spinal neurons induced recovery of the respiratory function after SCI (Alilain et al., 2008) and motor function was likewise ameliorated after optogenetic stimulation of the motor cortex, while NGF, BDNF and GAP43 were increased (Deng et al., 2021). Several studies have also seen recovery after

optogenetic stimulation in stroke models, coupled with neurotrophin (BDNF, NGF and NT-3) and GAP43 increases, sprouting and synapse formation (Cheng et al., 2014; Tennant et al., 2017; Wahl et al., 2017). Accordingly, one of the first studies using optogenetic stimulation to induce growth in DRG neurons *in vitro* also showed increased expression of the assessed neurotrophins (NGF and BDNF) (Park et al., 2015).

Neuronal activity seems therefore to promote regeneration regardless of the injury or cell-types, as assessed with optogenetic and chemogenetic tools in other models: neuronal activity was found to be essential in CST network formation after injury (Bradley et al., 2019), and increasing neuronal activity in RGC (retinal ganglion cells) induced regeneration after injury (Li et al., 2016; Lim et al., 2016), an effect also observed in DRG neurons after dorsal root crush (in combination with ChABC) (Wu et al., 2020). In this last study *in vitro* experiments showed neuronal activity did not induce growth over CSPG-coated substrates, which had to be digested using ChABC. Moreover, they observed that neuronal activity reduced microtubule stability at the axon tip and induced mTOR activity in the soma (Wu et al., 2020).

5. Cell-to-cell communication after injury

5.1. Cellular dialogues in SCIs

As previously stated, many cell types react after a CNS injury and participate in its pathophysiology. These cells modulate each other through their interactions and molecular exchanges, thus paving the way for injury progression to other phases (Bradbury and Burnside, 2019; Greenhalgh et al., 2020; Mesquida-Veny et al., 2021) (Figure 15). We recently reviewed some of the most important cellular interplays in neuronal injury, particularly the ones involving macrophages, as well as the effect of neuronal secretion on microglial and macrophagic phenotypes (Mesquida-Veny et al., 2021).

Macrophages and microglia present very plastic phenotypes which shift and adjust depending on the environment (Milich et al., 2019; Murray et al., 2014), and are thus fundamental players in cell-to-cell communication after injury. These two cells interact and influence each other, as microglia increase phagocytosis in macrophages, but instead macrophages reduce this capacity in microglia (Greenhalgh et al., 2018). Another cell type which has recently described to adjust its phenotype are astrocytes (Khakh and Sofroniew, 2015; Okada et al., 2018; Sofroniew, 2020). Indeed, microglia and macrophages modulate astrocytic reactivity, triggering a different bias depending on their phenotype (Haan et al., 2015; Liddelow et al., 2017; Song et al., 2019; Sonn

et al., 2019). Moreover, microglial cells are necessary for proper glial scar formation (Bellver-Landete et al., 2019).

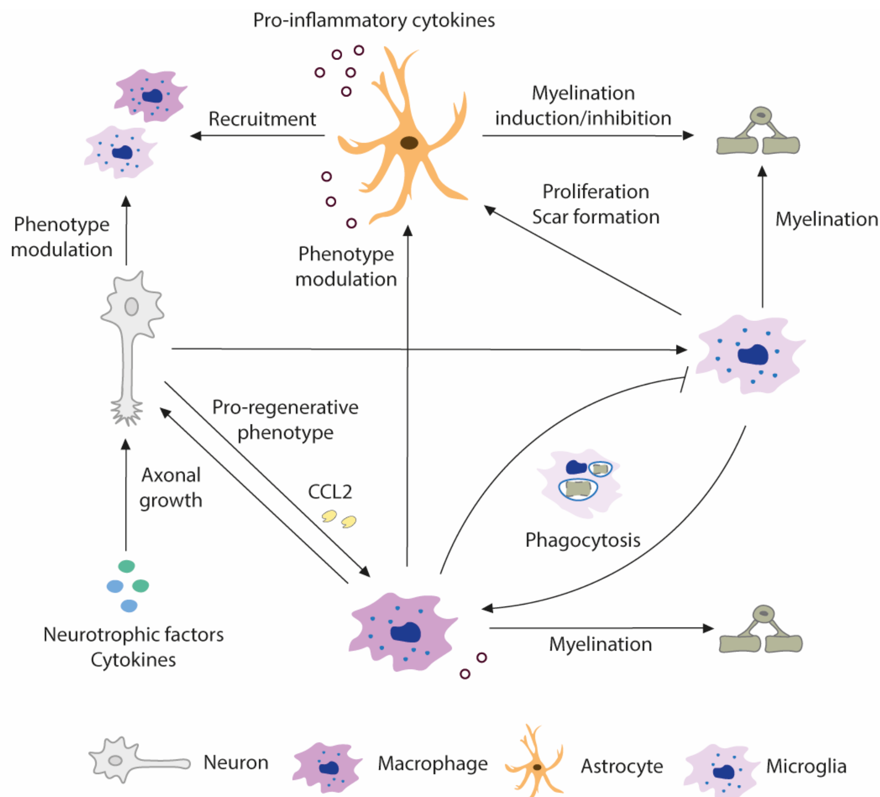

Figure 15. Summary of relevant cell-to-cell dialogues after CNS injury. Cell communication seems to have a role in orchestrating the injury progression. However, while some of these dialogues induce repair, including the production of growth-promoting neurotrophic factors or cytokines, as well as remyelination induction, others contribute to chronify the injury response. Moreover, the complexity of cellular interactions occurring after a SCI is increased as a result of the existence of cells with varying phenotypes (macrophages, microglia and astrocytes), meaning the same cell type may result in opposing functions, as seen by the astrocytic effect on myelination.

At the same time, activated astrocytes induce increased inflammatory responses through cytokine secretion while they also have a role in inducing immune cell migration to the injury core, in order to isolate it from the remaining spared tissue (Faulkner et al., 2004; Okada et al., 2006; Yoshizaki et al., 2021). Different astrocytic phenotypes have also opposing effects on myelination (Nash et al., 2011; Y. Wang et al., 2011), while myelination is both enhanced by TNF-α and by pro-resolving macrophage/microglia phenotypes (Arnett et al., 2001; Miron et al., 2013). Signals from other cells can also have a direct effect on axonal growth. Therefore, neurotrophic factors (Dougherty et al., 2000; Pöyhönen et al., 2019) as well as growth promoting-cytokines (activating the JAK-STAT pathway) are expressed after injury by other cell types, increasing axon regeneration (Gensel and Zhang, 2015; Leibinger et al., 2016; Yang et al., 2012; Zigmond, 2012). In that sense, macrophages were found to be fundamental for the conditioning effect in DRGs, an

effect that depended on the neuronal chemokine CCL2, triggering a pro-regenerative phenotype in macrophages (Biber and Boddeke, 2014; Kwon et al., 2015, 2013; Niemi et al., 2016).

Neurons express several molecules that signal to other cells, specifically neurotrophins, cytokines, chemokines, neurotransmitters, second messengers, reactive species and neuropeptides (Szepesi et al., 2018; Tian et al., 2012). The majority of these factors have an effect on modulating the glial or immune response, as seen with the cytokine transforming growth factor β (TGF-β), an important regulator of microglial function, similar to the observed effects of NGF and BDNF (Kerschensteiner et al., 2009; Tian et al., 2012). After injury, neurons adapt their secretome, mostly seen by alterations in neurotransmitter and chemokine release levels (Biber et al., 2007; Mesquida-Veny et al., 2021). Glutamate and the purines ATP and UDP are released from neurons after injury and function as danger signals to microglia, eliciting different responses (Inoue, 2006; Tsuda et al., 2005). In example, while the purine receptor P2Y6 increases phagocytic responses, the P2X7 prevents this increase (Fang et al., 2009; Koizumi et al., 2007). However, the most important messengers from neurons after injury are neuronal chemokines, which will be further addressed below, as well as their effects.

All these interactions have a final effect on the injury environment and on axon regeneration, which means they can be modulated to induce recovery. In that direction, activity-based therapies may also play an important role, as they may be affecting the injury evolution by modifying the cellular dialogues. Indeed, electrical stimulation induced a repairing program in macrophages (McLean and Verge, 2016), however, much remains unknown regarding the underlying effects of these therapies on cell-to-cell communication and its consequences on the repair program.

5.2. Neuronal chemokines

Chemokines are small secreted proteins classically known for their role in migration promotion (Hughes and Nibbs, 2018). However, since their discovery, these molecules have been described to participate in many other cellular processes, and are thus fundamental in cell-to-cell communication in several systems, including the CNS (De Haas et al., 2007; Mesquida-Veny et al., 2021). Chemokines are divided in 4 subfamilies: CC, CXC, CX_3C and (X)C, a division based on the position of the two pairs of cysteines that maintain the chemokine structure due to the formation of disulfide bonds (Zlotnik and Yoshie, 2012) (Figure 16). This division is also the basis for chemokine nomenclature, but many chemokines also have alternative names, as they were discovered before the establishment of this systematic nomenclature (Hughes and Nibbs, 2018; Zlotnik and Yoshie, 2000). Importantly, as in order to perform their functions chemokines need to be immobilized close to cells, the interaction of a specific chemokine with GAGs present in the environment will define spatially and temporally its effects (Monneau et al., 2016).

Chemokine function is also dependent on trimming and post-translational modifications (Hughes and Nibbs, 2018).

Chemokines interact with two types of receptors, both of them with seven transmembrane domains: conventional chemokine receptors (cCKRs) and atypical chemokine receptors (ACKRs) (Hughes and Nibbs, 2018) (Figure 16). While cCKRs are G-protein coupled receptors (GPCRs) that induce intracellular signaling upon chemokine interaction, ACKRs are, despite their structural similarity with the previous ones, unable to trigger this same signaling (Ulvmar et al., 2011).

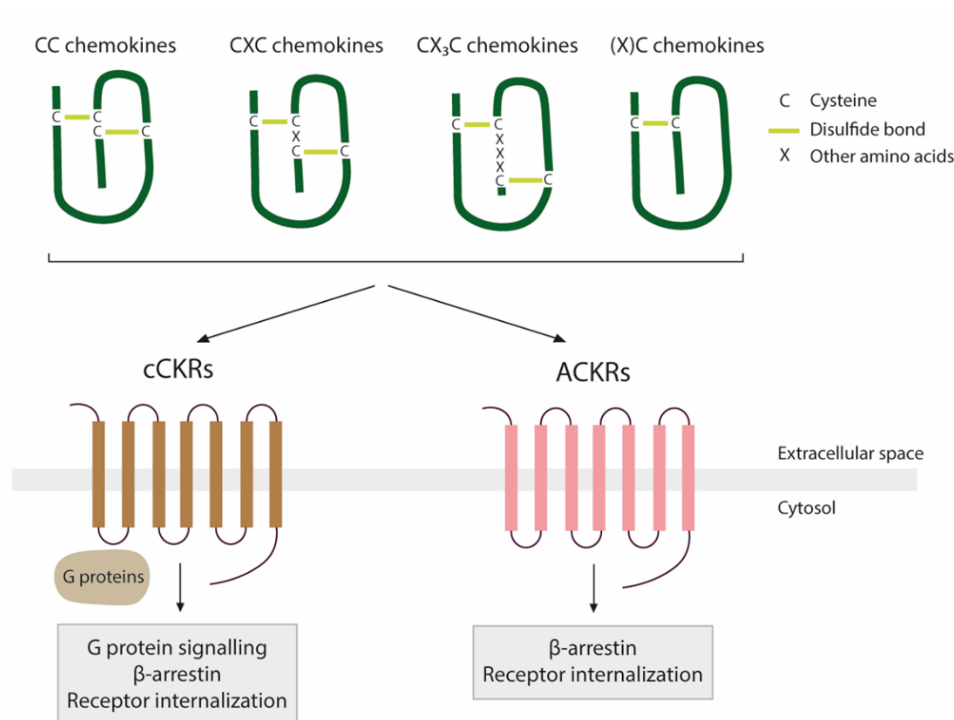

Figure 16. Chemokines and their receptors. Schematic representation of the different chemokine families depending on the position of their disulfide bonds. Adapted from (Xue et al., 2019). Chemokine interactions with cCKRs induce GPCR signaling while ACKRs are best known for their role in chemokine scavenging. Adapted from (Cancellieri et al., 2013).

Neurons express a great variety of chemokines both during normal conditions and after injury or insult (De Haas et al., 2007). Indeed, neuronal CX_3CL1 (also termed fractalkine) regulates microglial function in homeostatic conditions (Williams et al., 2014). After injury, the expression of several neuronal chemokines is altered with subsequent roles in the pathophysiology of the injury (De Haas et al., 2007; Mesquida-Veny et al., 2021) (Figure 17).

5.3. Neuronal chemokines after injury

Unfortunately, several injury-up-regulated neuronal chemokines derive in neuropathic pain, a pathologic condition originated from injuries in the somatosensory system (Baron et al., 2010; Mesquida-Veny et al., 2021). These chemokines include CCL1, CCL3 and CXCL10 which activate microglia triggering pro-inflammatory molecule secretion in the spinal cord (Akimoto et al., 2013; Chen et al., 2019; Ha et al., 2015; Knerlich-Lukoschus et al., 2011; Sun et al., 2016; Zychowska et al., 2017). Similarly, CCL7 as well as CXCL13 are also increased in neurons after injury and trigger astrocyte activation and neuropathic pain (Jiang et al., 2016; Ke et al., 2016; Wang et al., 2018; Zhang et al., 2016). CXCL13 also participates in inflammatory pain through increasing neuronal excitability (Wu et al., 2016).

Recent work has elucidated that injured neurons release CCL2, CCL7 and CCL12 in a SARM1-DLK-cJun/STAT3 dependent manner in response to injury (Hu et al., 2019; Wang et al., 2018). This mechanism derived in immune cell recruitment and activation as well as neuropathic pain (Hu et al., 2019; Wang et al., 2018). CXCL12 (also named stromal cell-derived factor 1 (SDF1)) also results increased upon neuronal injury, where it has shown to present desirable functions including axonal regeneration promotion, however, whether these effects derive from neuronal expression or not remains controversial (Bai et al., 2016; Liu et al., 2019; Luo et al., 2016; Opatz et al., 2009; Trettel et al., 2019; Wang et al., 2012).

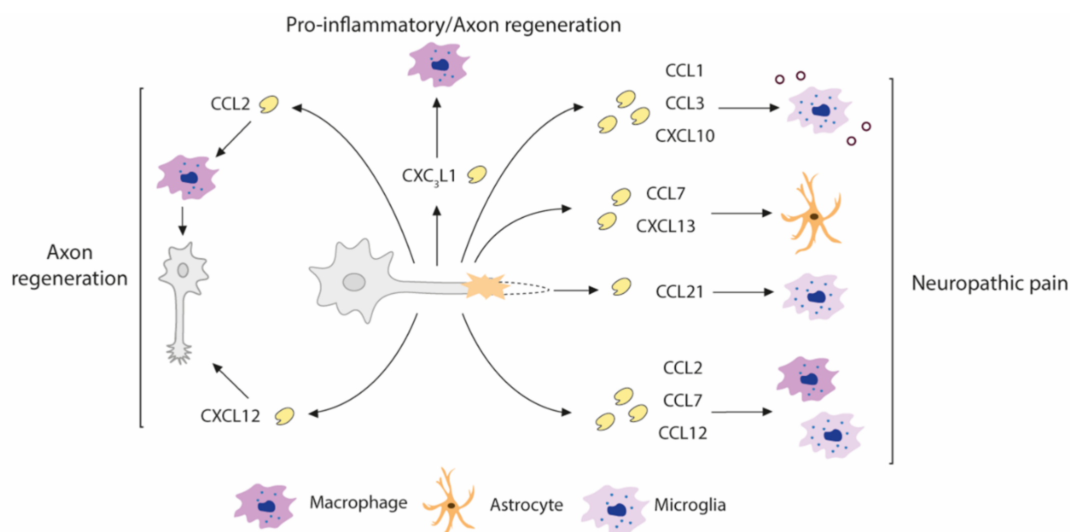

Figure 17. Upregulated neuronal chemokines after injury. Neurons express a great number of chemokines after injury, which subsequently affect other cell types present in the injury inducing mainly neuropathic pain. Despite that, some of these secreted chemokines have been shown to be involved in axon regeneration.

Another chemokine with important though debatable repercussions after injury is CX_3CL1. While CX_3CL1 is normally expressed in neurons and expression alterations after injury remain

uncertain, the physiologic signalling of this chemokine seems to be disrupted in these situations (Poniatowski et al., 2017; Sheridan and Murphy, 2013). CX_3CL1 is recognized by the receptor CX_3CR1, both in soluble or membrane-bound forms. In general, CX_3CL1 seems to promote pro-inflammatory signalling after SCI (Donnelly et al., 2011; Kwon et al., 2015). Nevertheless, neuronal peripheral CX_3CL1 recruits CX_3CR1 macrophages after peripheral injury, which produce NADPH oxidase 2 (NOX2)-containing vesicles that activate regeneration-associated pathways in neurons (Hervera et al., 2018). However, it is still unclear whether this chemokine is beneficial after SCI, as studies have shown both worsening and improvement in recovery after the deletion of its receptor (Blomster et al., 2013; Donnelly et al., 2011).

Finally, as previously commented, CCL2 is secreted by neurons after peripheral injury, where it is essential for the conditioning injury (Kwon et al., 2015, 2013; Niemi et al., 2016). Mechanistically, and according to its known role, neuronal CCL2 recruits macrophages and then it shifts them towards a phenotype that, in turn, contributes to neuronal intrinsic pro-regenerative signalling (Kwon et al., 2015, 2013; Niemi et al., 2016). Importantly, the final effect on neurons seems to be mediated by neuronal STAT3 (Niemi et al., 2016).

Of note, CCL21 is also upregulated in neurons in response to injury, however the mechanism and their effects will be further addressed later (Biber et al., 2011; Biber and Boddeke, 2014).

5.3.1. CCL21 and molecular signaling

CCL21 (also named secondary lymphoid-tissue chemokine (SLC), chemokine with 6 cysteines (6Ckine) or Exodus-2) is an atypical chemokine: while the majority of chemokines present two pairs of cysteines, CCL21 has 3 pairs in its sequence; moreover, CCL21 exhibits a rarely elongated C-terminus (Love et al., 2012).

CCL21 is a chemokine best known for its role in immune cell recruitment to lymphoid organs, where it is constitutively generated (Förster et al., 2008). This process is mediated by the classical CCL21 receptor: CCR7, expressed in a several immune cells especially in some dendritic cell (DC) and T cell subpopulations (Comerford et al., 2013). According to its function, CCR7 is a GPCR that activates intracellular signaling cascades mainly related to cell migration, but other cellular processes are also affected by this receptor, such as cell adhesion and survival (Hauser and Legler, 2016; Rodríguez-Fernández and Criado-García, 2020).

Many studies have tried to elucidate the molecular mechanisms triggered by CCR7 activation (Figure 18), most of them in leukocytes (Hauser and Legler, 2016; Rodríguez-Fernández and Criado-García, 2020). Upon CCR7 interaction with the ligand, the mitogen-activated protein kinase (MAPK) pathway leads to ERK 1/2 and p38 activation, promoting chemotaxis (Riol-

Blanco et al., 2005). CCR7-induced ERK1/2 may also slightly increment cellular survival (López-Cotarelo et al., 2015). Meanwhile, survival promotion after CCR7 activation mostly results from phosphoinositide-3-kinase (PI3K)/Akt inhibition of apoptotic pathways (Escribano et al., 2009; Sánchez-Sánchez et al., 2004). CCR7 has also a major role in actin cytoskeleton rearrangement, which underlies its migratory properties (Rodríguez-Fernández and Criado-García, 2020). In that case, RhoA is activated, inducing proline-rich tyrosine kinase 2 (Pyk2)- and LIMK-mediated cofilin inactivation (via phosphorylation), with final effects on actin cytoskeleton dynamics and polymerization (Bardi et al., 2003; Riol-Blanco et al., 2005; Svitkina, 2018; Torres-Bacete et al., 2015). Importantly, in this paradigm RhoA may also modulate actin compaction through activation of myosin light chain (MLC) via ROCK (Agarwal and Zaidel-Bar, 2019; Rodríguez-Fernández and Criado-García, 2020; Torres-Bacete et al., 2015).

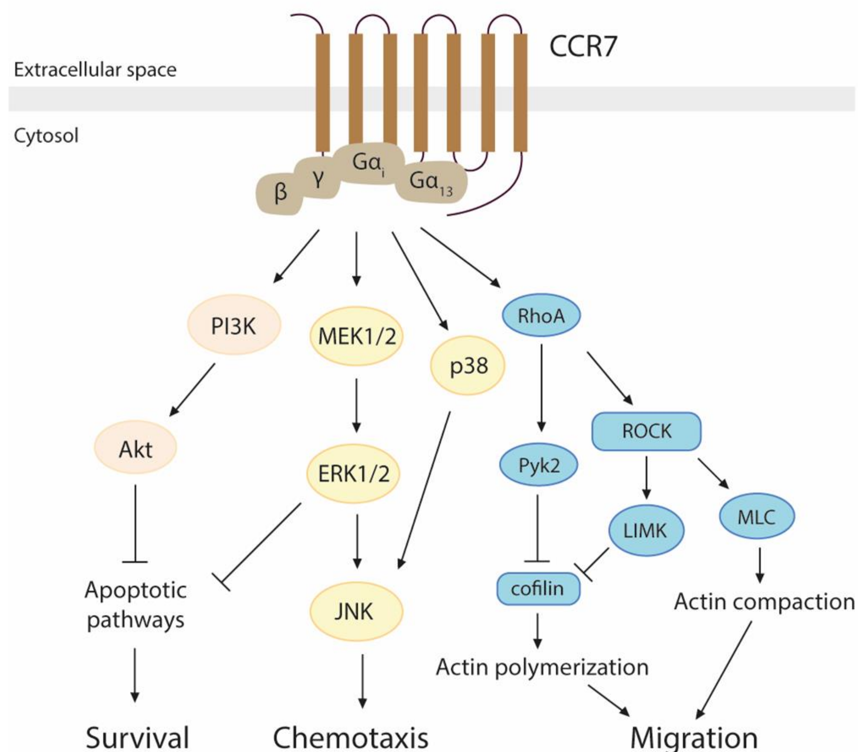

Figure 18. CCR7-mediated intracellular signaling. CCR7 activation in leucocytes has been described to activate the PI3K/Akt pathway promoting cell survival, the MEK/ERK pathway inducing chemotaxis and affecting as well cell survival, and finally, to modulate the actin cytoskeleton via RhoA and its subsequent molecular signaling, eventually inducing migration. Adapted from (Rodríguez-Fernández and Criado-García, 2020).

Importantly, the chemokine CCL19 is also recognized by CCR7 and presents similar functions to CCL21 (Förster et al., 2008). Still, these chemokines present different availability, as CCL21 is preferentially retained to GAG due to its charged C-terminal tail which can also be trimmed to increase its solubility (Schumann et al., 2010). This characteristic dictates the temporal effect of

these chemokines: while CCL19 rapidly diffuses, CCL21 is retained and has a prolonged effect, which also becomes transient after cleavage (Jørgensen et al., 2018). However, the differences between CCL21 and CCL19 lie beyond their availability, as they induce a slightly different signaling upon CCR7 activation. In summary, CCL19 seems to induce more robust GPCR responses and elicit β-arrestin activation and receptor internalization, while CCL21 results in diminished effects on the same mechanisms, however, results are not consistent among studies, probably resulting from slight differences in the distinct cell types used (Hauser and Legler, 2016; Jørgensen et al., 2018).

Finally, CCL21 has also been shown to interact with microglial CXCR3 (Biber et al., 2001; Rappert et al., 2002) and with the scavenging receptor ACKR4 (also known as CCRL1) (Ulvmar et al., 2014). CXCR3 is a GPCR that can modulate several intracellular pathways, including the MAPK and PI3K/Akt, which may lead to cell migration (Billottet et al., 2013). Consistently, CCL21 promotes chemotaxis in microglial cells through CXCR3 (Biber et al., 2001; Rappert et al., 2002). Meanwhile, ACKR4 binds to CCL21 and induces its degradation, thereby diminishing its extracellular content generating gradients for migration (Bastow et al., 2021; Ulvmar et al., 2014).

5.3.2. Neuronal CCL21

CCL21, which can be carried through axons inside vesicles, is highly expressed in neurons after injury, as shown after ischemia, nerve injury and SCI (Biber et al., 2011, 2001; De Jong et al., 2008, 2005; Zhao et al., 2007). Specifically, small-diameter nociceptors (TRPV1$^+$ (transient receptor potential vanilloid receptor 1)) have been shown to be responsible for this secretion (Biber et al., 2011). Accordingly, activation of the nociceptive spinothalamic tract also results in elevated CCL21 expression (Zhao et al., 2007).

CCL21 release results in neuropathic pain promotion, as a result of microglial activation and upregulation of P2X4 (Biber et al., 2011; Piotrowska et al., 2018). Still, while both CXCR3 and CCR7 can be expressed by microglial cells it is unclear whether they are responsible for neuropathic pain induction or if this effect results from another possible receptor (Biber and Boddeke, 2014; Weering et al., 2010). Interestingly, CCL19-CCL21 deficient mice (*plt* mice) show reduced numbers of pro-inflammatory immune cell phenotypes as well as decreased TNF-α after SCI, further contributing to a plausible role of CCL21 in neuroinflammation (Honjoh et al., 2019).

Of note, as another example of cell-to-cell communication, in the presence of tumor cells neurons release CCL21 which induces migration in these cells as well as cancer-associated pain which relates to increased neuronal plasticity (Hirth et al., 2020).

However, to this day much remains unknown about the role of CCL21 in the nervous system. Figure 19 depicts the described CCL21 functions in this paradigm, as well as others of interest.

5.3.3. CCL21 on other paradigms

As commented, the most studied role of CCL21 remains to induce migration of immune cells, which has raised interest to study the effect of this chemokine in autoimmune diseases. Accordingly, CCL21 is decisive in the course of diseases such as multiple sclerosis and rheumatoid arthritis, affecting both the generation of pathogenic T cells as well as in promoting a detrimental phenotype in macrophages (Kuwabara et al., 2009; Van Raemdonck et al., 2020). Also in relation to its classical role, CCL21 is implicated in lymph node metastasis, as tumoral cells may be recruited by this chemokine, however, protective effects have also been observed due to its capacity to mobilize immune cells (Poeta et al., 2019; Rizeq and Malki, 2020).

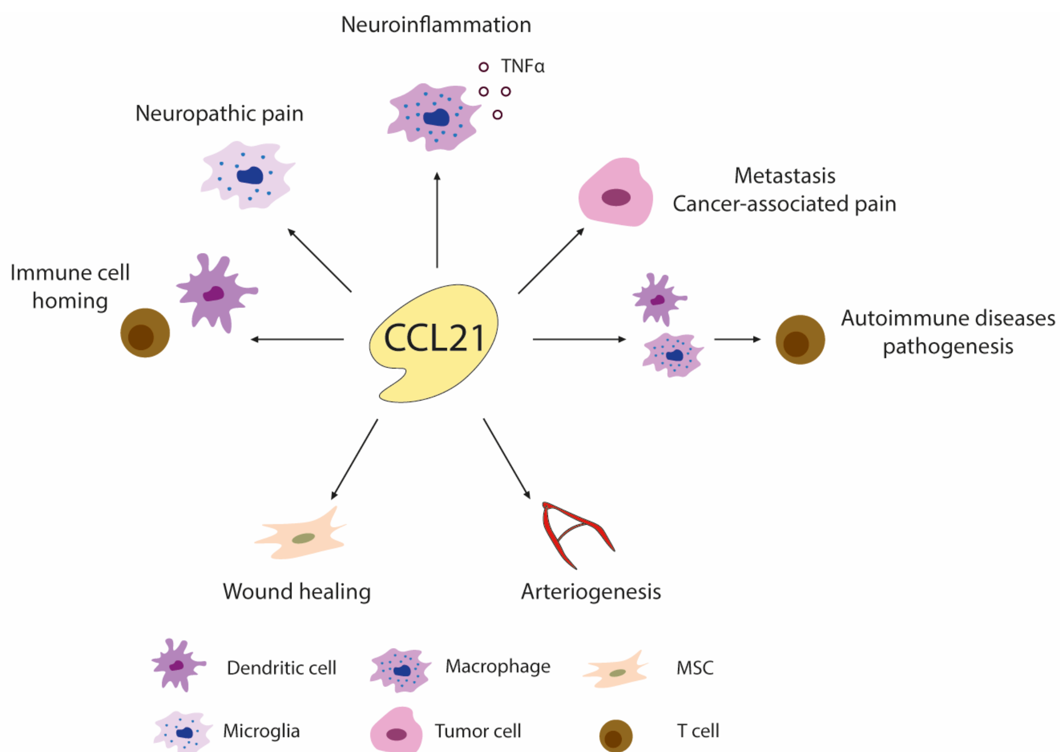

Figure 19. Summary of relevant described roles for CCL21. CCL21 is expressed in a great variety of tissues, where it can interact with different cells and exert differential functions.

However, beneficial effects have also been described, as administration of CCL21 has been shown to promote wound healing after transplant of MSC in skin (Sasaki et al., 2008), and after cartilage injury, an event that occurs spontaneously in young animals (Joutoku et al., 2019). Similarly, CCR7 and its ligands promote arteriogenesis (Nossent et al., 2017).

Altogether, these studies highlight the plethora of different roles the chemokine CCL21 presents in distinct paradigms and cell types. Nevertheless, despite the existence of several reports

describing CCL21 increases after neuronal injury (Biber et al., 2011, 2001; De Jong et al., 2005; Zhao et al., 2007), and the presence of its receptors in the nervous system (Biber et al., 2001; Kuwabara et al., 2009; Rappert et al., 2002), the effect of this chemokine on neurons has not received much attention.

Objectives

Despite many efforts, treatments for SCI still yield poor advances in terms of recovery. This fact, together with the heterogeneity of spinal cord lesions, as well as their subsequent different outcomes in patients, further stresses the need for a better molecular and cellular characterization of the injury and their distinct therapies, as research has shown customized-therapies may be key in this field.

In that direction, while activity-based therapies have become one of the most common strategies due to their relative success as well as their viability, a fine description of their working mechanisms as well as their physiological consequences is still missing. As previously stated, some studies with electrical stimulation and/or exercise have started to elucidate the rationale behind these therapies (as in (Al-Majed et al., 2004; Goganau et al., 2018; Goldshmit et al., 2008; Wang et al., 2015; Ying et al., 2005) among others), however, the lack of spatial and cellular specificity of these strategies derives in an impossibility to discern the effects of the different circuits and cellular actors.

It is now well established that regeneration failure in the adult CNS, and therefore, after a SCI, is originated by both an intrinsic neuronal growth-brake, as well as extrinsic adverse conditions (Bradbury and Burnside, 2019; Mahar and Cavalli, 2018; Mesquida-Veny et al., 2021). Interestingly, neuronal activity, the foundation of activity-based therapies, may affect both.

Neuronal activity has been shown to broadly modulate gene expression at several levels (Flavell and Greenberg, 2008; Hogan et al., 2020). Meanwhile, other approaches inducing profound transcriptional and epigenetic alterations, such as the conditioning injury, have shown to robustly promote a recapitulation of the neuronal growth capacities (Curcio and Bradke, 2018). In parallel, as neuronal activity-triggered molecular modifications may also affect neuronal cues (Bilchak et al., 2021; Chivet et al., 2014; Wong et al., 2015), molecular dialogues between neurons and other cells might be affected as well. In this regard, as cell-to-cell communication is fundamental in the evolution of the injury (Bradbury and Burnside, 2019; Greenhalgh et al., 2020; Mesquida-Veny et al., 2021), neuronal activity may also be affecting this process.

Having said that, the general objective of this thesis was to study the role of neuronal activity in SCI in order to understand the neuronal-specific mechanisms triggered by activity-based therapies. Particularly, we hypothesized that neuronal activity was the main actor in activity-based therapies, and that it could promote ***1) axonal growth in the directly stimulated neurons, recapitulating a pro-regenerative state,*** and ***2) alterations in neuronal communication with consequences in the injury environment and in the final regeneration***. In that direction, we used optogenetic and chemogenetic tools to manipulate neuronal activity with cellular specificity and to determine its impact regarding these two premises. The different aspects of our hypothesis are investigated in the two chapters of this thesis:

- Chapter 1. Effects of neuronal activity modulation on axonal growth

- Chapter 2. Role of neuronal activity in cell-to-cell communication after injury.

In this chapters, the specific objectives of this thesis were:

- **Chapter 1:**

  -*Objective 1.* To study the direct effect of neuronal activity modulation on axonal growth in regenerating-capable systems.

  -*Objective 2*. To study the direct effect of neuronal activity modulation on axonal growth in non-regenerating systems.

- **Chapter 2:**

  -*Objective 1*. To study the role of neuronal activity in the modulation of the injury environment.

  -*Objective 2.* To study an indirect effect of neuronal activity on regeneration: CCL21 and its neuronal impact.

Materials and methods

Mice

Wild type (WT) C57BL/6J and OF1 pregnant females were purchased from Charles River Laboratories and B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J (Thy1-ChR2) (Arenkiel et al., 2007) were purchased from Jackson Laboratories. PV-Cre/Ai27D/CSP-Flox (PV-ChR2-mCherry) mice were obtained from Dr. Rafael Fernández Chacón (Instituto de Biomedicina de Sevilla). Animals were used at the age of 6-10 weeks. In all surgeries mice were anaesthetized with inhaled isofluorane (5% induction, 2% maintenance). Animal work was performed under the approval of the Ethics Committee on Animal Experimentation (CEEA) of the University of Barcelona, under the procedures C-0007, 276/16, 47/20, and OB41/21.

Dorsal root ganglia (DRG) neuronal culture

C57BL/6J or Thy1-ChR2 adult animals were used in these experiments. The vertebral column of sacrificed mice was exposed ventrally and then removed, revealing the spinal cord and the dorsal roots. The DRGs were then isolated, soaked in ice-cold Hank's balanced salt solution (HBSS) (ThermoFisher Scientific), and incubated for 45min in a solution containing 5mg/ml Dispase II (Merck) and 2.5mg/ml Collagenase Type II (ThermoFisher Scientific) in DMEM (Dulbecco's Modified Eagle Medium) (ThermoFisher Scientific) at 37ºC. The enzymatic digestion was inactivated by removal of the solution and posterior addition of fresh media containing FBS (Fetal Bovine Serum) (ThermoFisher Scientific) (DMEM/F-12 media (ThermoFisher Scientific) + 10% FBS and 1X B27 (ThermoFisher Scientific)). The DRGs were then manually disgregated by pipetting. Single cells were centrifuged and resuspended in DMEM/F-12 media with 1x B27 and penicillin/streptomycin (P/S) (ThermoFisher Scientific), and seeded in glass-coverslips, in 48-well plates (3000-4000 cells/well-coverslip), or in 24-well plates (6000-8000 cells/well). Cell substrates had been previously coated with poly-D-lysine (Merck) (0.1mg/ml) for 2h at 37ºC and laminin (Merck) (2 µg/ml) at RT (room temperature) O/N (over-night). When needed, coverslips were incubated with 20 µg/ml chondroitin sulfate proteoglycans (CSPGs) (Merck) for 2h at 37ºC after the laminin coating. Cells were maintained at 37ºC in a 5% CO_2 atmosphere and fixed at 24h unless specified.

Neuronal cortical culture

OF1 pregnant females at embryonic day E16.5 (E16.5) were acquired and primary cortical neurons were obtained from mice embryos at that embryonic day. The brain was dissected in ice-cold 0.1M phosphate-buffered saline (PBS) (ThermoFisher Scientific) + 6.5 mg/ml glucose (Merck) and the meninges were removed. Cortices were isolated, chopped at 300µm and incubated with trypsin (ThermoFisher Scientific) at 37ºC 15min. Normal horse serum (NHS)

(ThermoFisher Scientific) was added for inactivation and the cell suspension was then mechanically digested in 0.1 M PBS with 0.025% DNAse (Roche). The cells were then plated in poly-D-lysine (0.1mg/ml, 2h 37ºC) pre-coated plate-bonded microfluidic devices (200.000 cells/device) in culture medium (Neurobasal medium (ThermoFisher Scientific), 2mM glutamine (ThermoFisher Scientific), 6.5mg/ml glucose, $NaHCO_3$ (Merck), P/S, 1X B27 and NHS 5%). Neuronal cultures were kept at 37ºC in a 5% CO_2 atmosphere. Media was renovated every 2-3 days and NHS was added only during the first day *in vitro* (DIV) to avoid proliferation of non-neuronal cells.

Microfluidic devices

For microfluidic device production, a master of the design was fabricated at IBEC Microfab Space facilities using standard photolithography. Soft lithography allowed to produce poly(dimethylsiloxane) (PDMS) (Dow) devices, which were then coupled to glass-bottom substrates by oxygen plasma treatment. The PDMS chips allowed to isolate axons from the neuronal bodies as a result of their design, which was an adaptation of a previously published device (Taylor et al., 2005). In the device, four 7mm diameter exterior wells allowed to introduce cells and media and were communicated in pairs through two long compartments. These two compartments corresponded to the cell body and the axonal compartments respectively. 100 annexing microchannels (10µm x 10µm x 900µm) allowed the axons to cross from the cell body to the axonal compartments. Axotomies were performed by vacuum aspiration of the axonal compartment.

***In vitro* viral infection**

For ChR2 expression, primary cortical neurons were infected with either laboratory-generated LV-EF1α-hChR2(H134R)-EYFP-WPRE (LV-ChR2), or commercial AAV9-hSyn-hChR2(H134R)-EYFP (AAV-ChR2) (Addgene). In the case of LV-ChR2, the LV was added to the media, at 4 DIV and 24h later media was changed. The exact viral volume varied among production batches, and had to be determined on each of them. Infection levels were detected by YFP fluorescence, and were high at 7DIV. For AAV-ChR2 infection, cells were infected with 1µl of AAV-ChR2 at 1DIV for 72h. High fluorescence levels could also be observed at 7DIV.

***In vitro* optical stimulation**

For *in vitro* optogenetic stimulations DRG neurons were obtained from adult Thy1-ChR2 animals, while primary cortical neurons were obtained from WT embryos and infected with either LV-

ChR2 or AAV-ChR2 as previously stated. The optogenetic illumination system consisted of a homemade (Sala-Jarque et al., 2020) LED array of 470nm (LuxeonRebelTM), powered by a Driver LED (FemtoBuck, SparkFun) of 600mA. Pulses were constructed by a pulse generator (PulsePal, OpenEphys) (Siegle et al., 2017). 20Hz frequency pulses (5-45ms pulses) (unless specified) in trains of 1s ON-1s OFF during 1h were used as stimulation pattern. Cortical neurons were illuminated at a certain day of culture or at different post-axotomy times while DRG neurons were illuminated 2h after plating.

Drugs and other administered compounds

CNO (Tocris, 5mg/kg/day i.p.), capsaicin (Merck, 100µg/ml i.pl.), CCL21 (Peprotech, 1nM, 10nM, 50nM *in vitro*; 100ng/sciatic nerve *in vivo*/*ex vivo*), NBI-74330 (Tocris, 20µg/sciatic nerve *ex vivo*), αCCR7 blocking antibody (R&D systems, 2-5µg/ml), immunoglobulin (Ig) G Rat (Merck, 2-5µg/ml), CCL19 (Peprotech,1 nM, 10nM, 50nM), Pertussis toxin (Merck, 50ng/ml), U0126 (Promega, 1µM), Wiskostatin (Merck, 1 µM).

For *in vitro* experiments, all compounds were added the day of culture (2h after seeding) and cells were fixed 24h later unless specified. The CCR7-blocking antibody as well as the other inhibitors were added 30min before CCL21 to ensure their effect.

Sciatic nerve crush (SNC)

The skin was precisely perforated in the lower back area and the sciatic nerve was exposed after breaching the gluteus maximus and the biceps femoralis by blunt dissection. The nerve was hooked, immobilized and crushed using thin tweezers 2x10s orthogonally. 24h after the SNC the sciatic nerve and the sciatic DRGs (L4, L5, L6) were extracted and processed as needed, unless specified.

***In vivo* chemogenetic stimulation**

WT C57BL/6J mice received injection of 1µl of the commercial adeno-associated virus AAV5-hSynhM3D(Gq)-mCherry or the control virus AAV5-hSyn-mCherry (Addgene) in the sciatic nerve and 4-5 weeks were waited for viral expression prior to the experiment. Chemogenetic stimulations consisted of two intraperitoneal (i.p.) injections of CNO (Clozapine N-oxide) at 5mg/kg per day. In peripheral injury experiments, CNO injections were performed before the injury and lasted 4 days. The last day of injection coincided with the injury day. In the case of central injury, the stimulation period was from 3 days post-injury (DPI) until 9DPI.

CST and dorsal columns axotomy

Mice were anaesthetized and their thoracic vertebral column was exposed upon an incision between the shoulder blades. The T9 vertebrae and the dura mater were removed. To axotomize the CST, a lateral injury was performed in the spinal cord with a scalpel leaving approximately 25% of the tissue intact laterally. For dorsal column axotomy (DCA), fine forceps were used to hemisect dorsally the spinal cord until the central canal. Animals were allowed to recover for 7d after CST and 3d after DCA before starting stimulation protocols.

***In vivo* optical stimulation**

Thy1-ChR2 transgenic mice were used for optogenetic stimulation experiments. A region of the motor cortex (M1) responsible for hindlimb innervation was selected and tested for effective motor activation prior to stimulation experiments. In the resulting coordinates (–1mm antero-posterior and 1.5mm lateral of bregma), a cannula containing an optic fiber (1.25mm, 0.22 NA, Thorlabs) was inserted at 0,5mm deep and was attached to the skull with two screws and dental cement. The optic fiber was connected to a LED source (Thorlabs) and to a pulse generator (Pulse Pal, Open ephys) (Siegle et al., 2017) which allowed for precise frequency and train delivery. 470nm blue light was delivered for 1h at 10Hz (10-90ms pulses), in 1sON-4sOFF periods on a daily basis for optical stimulation. This procedure was repeated from days 7 to 11 DPI and from 14 to 18 DPI. Animals were then allowed to recover and their sensorimotor function evolution was assessed. Non-stimulated animals underwent surgery for fiber-optic implantation but did not receive illumination.

Behavioral assessment of sensorimotor function: gridwalk and Basso Mouse Scale (BMS) tests

BMS assessments consisted in grading mice while freely moving using the BMS scale (Basso et al., 2006). Using this scale, the final punctuation depends on, in brief, their hindlimb ankle movement, paw placing, stepping, coordination, trunk stability and tail position, and is ranged from 0 (totally impaired locomotion) to 9 (healthy mice). For the gridwalk test, animals were filmed while crossing a 50cm-long plastic grid of 1 x 1 cm holes, three times on each time-point. The number of missteps and the total number of steps was blindly computed. Sensorimotor evaluations were performed at -1, 1, 7, 14, 21, 28 and 35 DPI for CST injuries and at -1, 1, 3, 7, 10, 21 and 28 DPI for DCA.

Anterograde tracing of injured spinal cords

Animals with injured CST received injection of a fluorescent tracer (10% Dextran AlexaFluor 594, ThermoFisher Scientific) at 35DPI into the motor cortex to trace the stimulated CST, using the same coordinates than for optical stimulation. A total of 1µl was injected at a 0.2 µl/minute rate, and 5 additional minutes were waited before needle extraction to avoid liquid spillage. Mice were perfused 5 days later.

Injured CST retrotracing and tissue processing for flow cytometry

At 35DPI mice were anesthetized, their injured spinal cord was exposed and, in order to stain the regenerating neurons, a fluorescent retrotracer (FluoroGold (2%, 1 µl) (Santa Cruz) or Cholera Toxin Subunit B AlexaFluor 647 (CTB-647) (2 µg/µl; 1 µl) (ThermoFisher Scientific) was injected into the spinal cord using a stereotaxic frame 0.3mm below the injury site, in the centre of the spinal cord at 470µm deep. 5 days after the injection these animals were cervically dislocated and their brains extracted. The stimulated area in the motor cortex was dissected using a 2mm diameter punch in ice-cold EBSS (ThermoFisher Scientific), and the inferior white matter was discarded. Two samples from different mice were pooled at this point in order to obtain enough tissue. Samples were processed following an adaptation and combination of previous protocols (Arlotta et al., 2005; Catapano et al., 2001; Saxena et al., 2012). Samples were enzymatically digested in 0.05mM (2R)-amino-5-phosphonovaleric acid (APV) (Merck), 0.8mM kynurenic acid, 5% trehalose, 15mg/ml papain and 0.016µg/µl L-cys-HCl in EBSS for 15min at 37ºC. DNase was then added for 5 more minutes (37ºC). After that time, a solution containing 0.05mM APV, 0.8mM kynurenic acid (Merck), 5% trehalose (Merck) and 10mg/ml ovomucoid (Merck) in EBSS was added to stop the digestion. The samples were then centrifuged at 100g for 10min, the supernatant discarded and the pellet resuspended with the same solution than in the previous step. After dissociating mechanically by pipetting, the debris was eliminated using a 40µm strainer, and DMEM/F-12 media containing 5% of trehalose, 0.05mM APV and 0.8mM kynurenic acid was added to the solution. This mixture was poured on a 20% Percoll solution (Merck) and centrifuged 10min at 600g. The resulting pellet was resuspended in supplemented DMEM/F-12 media, centrifuged again to eliminate Percoll and finally resuspended in the same media.

Fluorescence activated cell sorting (FACS) of regenerating neurons

Cell suspensions were stained with Hoechst and propidium iodide. Gating strategies were previously optimized and allowed to select non-aggregated nucleated live cells. ChR2-eYFP$^+$/CTB-647$^+$ labelled cells were finally collected using a FacsAria Fusion sorter. These

experiments were performed at the Centres Científics i Tecnològics Universitat de Barcelona (CCiTUB).

Cytokinome quantification

The levels of 40 cytokines in stimulated and non-stimulated injured spinal cords were detected using the Proteome Profiler Mouse Cytokine Array Kit, Panel A (R&D). For that, Thy1-ChR2 mice were injured and stimulated with the previously explained protocol since day 7 post-injury, for a total of 5 days. The day of the last stimulation (corresponding to 11DPI), their injured spinal cords were dissected, sliced and homogenized. The resulting samples were processed as specified by the suppliers of the kit.

The same Array kit was used for cytokine detection in the case of CCL21-treated naïve and injured sciatic nerves. CCL21 (100ng) or vehicle were injected into the sciatic nerve (injured or sham) and 24h later the nerves were dissected and processed as specified by the suppliers.

Intensity measurements were obtained with the help of the Protein Array Analyzer for ImageJ (macro: http://rsb.info.nih.gov/ij/macros/toolsets/Protein Array Analyzer.txt; G. Carpentier (2010)), then normalized with each intrinsic reference spot and finally to the non-stimulated or sham vehicle group respectively. Inconsistent as well as almost non-detectable cytokines were excluded from the analysis.

Chemokinome quantification

The levels of 25 chemokines were measured from stimulated and non-stimulated DRG culture media using a Mouse Chemokine Array C1 (RayBiotech). Thy1-ChR2 DRG neurons were cultured as previously explained and optically stimulated 2h after seeding. 24h later the media was recovered and processed as specified in the array instructions.

The Protein Array Analyzer for ImageJ (macro: http://rsb.info.nih.gov/ij/macros/toolsets/Protein Array Analyzer.txt; G. Carpentier (2010)) was used for intensity measurements, which were normalized to each reference spot and then to the non-stimulated group. Variable as well as really-low expressed chemokines were not included in the analysis.

Capsaicin administration

10µl of capsaicin (a stock solution of 0.1g/ml in ethanol was diluted 1:1000 in PBS + 10% ethanol) or vehicle were injected intraplantally (i.pl.) into WT mice. 2h later the DRGs of these animals were dissected and fixed for posterior analysis.

Local delivery to the sciatic nerve

Same procedure used in SNC was used to immobilize the sciatic nerve for local administration of compounds or viruses to the sciatic nerve. 1 or 2 µl depending on the solution were then injected into the nerve using a 32G Hamilton syringe. When combined with SNC, the crush was performed prior to the injection, and the injection was performed 5mm rostrally to the crush site.

Ex vivo DRG cell culture

For *ex vivo* DRG cultures, a solution was injected *in vivo* in the sciatic nerve as explained and 24h later the sciatic DRGs (L4, L5 and L6) were obtained and digested for DRG neuronal culture as previously detailed.

Bone-marrow derived macrophages (BMDM) culture

The bone marrow from femurs of WT mice was extracted after washing the inside of the bone with HBSS inserted through a 25G needle. The resulting bone marrow was then mechanically disgregated and centrifuged at 600g for 10min. BMDM cells were seeded in non-treated plastic petri dishes and maintained in DMEM/F-12 supplemented with 10% FBS, 1% glutamine, 1% P/S at 37ºC in a 5% CO_2 atmosphere. The necessary macrophage-colony stimulating factor (M-CSF) for differentiation was supplied through addition of 20% of L929-cell conditioned media (LCCM). Culture media was changed every two days and cell differentiation was completed at 7DIV. The cells were then detached, replated for the necessary experiments and maintained without LCCM.

LCCM production

L-929 (ATCC) cells were maintained in DMEM/F-12 supplemented with 10% FBS, 1% glutamine and 1% P/S. When confluent, media was recovered and cell debris was eliminated through centrifugation. The resulting media corresponded to LCCM used at 20% in BMDM cultures.

Conditioned media experiments

BMDM were treated with CCL21 50nM or vehicle for 24h. Media was then changed, and the cells were allowed to grow for 24 additional hours. The media was then recovered and concentrated using 10k Amicon® Ultra 0.5 mL Centrifugal Filters (Merck). Resulting

concentrated media were then pooled in pairs and added to DRG cultured neurons 2h after seeding. Neurite outgrowth was analysed 24h later.

Quantitative real-time PCR

RNA was extracted from both cultured cells and fresh tissues using the RNeasy Mini Kit (Qiagen) according to the kit instructions. 600-1000ng of RNA were then used for cDNA production using the SuperScriptTM II Reverse Transcriptase (ThermoFisher Scientific). LightCycler SYBR Green (Roche) was used for real-time qPCRs, which were conducted using a StepOnePlus real-time PCR (Applied Biosystems). Technical replicates were performed for each sample. The Cts and posteriorly the $2^{-\Delta\Delta Ct}$ were determined for each gene, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (housekeeping gene) was used for normalization in each sample. Used primers are shown in Table 4.

Immunocytochemistry

Ice-cold 4% paraformaldehyde (PFA) was added to the cells for fixation and was incubated 15 min at 4ºC. The fixative was discarded and, after several PBS washes, blocking solution (1% bovine serum albumin (BSA), 0.25% Triton X-100 in PBS 0.1M (PBS-0.25% Tx)) was added for 1h at RT. The cells were then incubated O/N at 4ºC with primary antibody in the same solution and the following day, washed with PBS-0.25% Tx and incubated 1h with fluorescence-conjugated secondary antibodies at RT. Hoechst was used to stain cell nuclei when needed.

Tissue processing for immunohistochemistry

To obtain IHC samples, mice were transcardially perfused with cooled 4% PFA. The brain, the spinal cord, sciatic nerves and/or the DRGs were extracted and post-fixed for 24 more hours in PFA 4% at 4ºC. The DRGs and the sciatic nerves were directly extracted and fixed in cooled 4% PFA for 2h whenever only these tissues were needed. Tissues were then transferred to 30% sucrose in PBS for cryoprotection. Spinal cords, sciatic nerves and DRGs were frozen within blocks of tissue freezing medium and 10 µm (for DRGs) or 18µm (for spinal cord) slices were obtained using a cryostat (Leica CM 1900). Brains were frozen without prior embedding and 30µm slices were obtained using a freezing microtome (Leica SM 2000R) and kept in cryoprotection solution (30% glycerol, 30% ethylene glycol, 40% PBS). In the case of whole-mount staining, the cryoprotection step was skipped and the tissues were maintained in PBS 0.1M.

Immunohistochemistry

Free floating brain slices were washed in PBS 0.1M and blocked for 1h in 0.2% gelatin, 10% FBS, 0.2M glycine and 0.5% Triton X-100 in PBS 0.1M at RT. A solution with 0.2% gelatin, 5% FBS and 0.5% Triton X-100 in PBS 0.1M was used for primary antibody incubation, O/N at 4ºC. After washing with PBS0.1M-0.5% Triton X-100, fluorescent secondary antibodies were incubated in the previous solution. Slices were then washed and incubated with Hoechst in PBS 0.1M and mounted with Mowiol™.

Cryostat DRG, sciatic nerve and spinal cord slices mounted on slides were washed in TTBS (TBS (Tris-buffered saline) + 1% Tween to eliminate the freezing medium. Blocking solution containing 8% BSA and 0.3% Triton-X-100 and 1/150 mαIgG (Jackson Immuno Research) in TBS was added for 1h at RT. Primary antibody was incubated in 2% BSA and 0.2% Triton-X-100 in TBS O/N at 4ºC. Washes with TTBS were followed by secondary antibody together with Hoechst incubation in the same solution for 1h at RT. The slides were washed in TTBS and mounted with Mowiol™.

For whole-mount staining, sciatic nerves or DRGs were blocked in 8% BSA, 1% Triton X-100 and 1/150 mαIgG in TBS for 2h at RT. Primary antibody was added in a solution containing 2% BSA, 0.3% Triton X-100 in TBS and was left for 3 days at 4ºC. After washing with TTBS, secondary antibodies were added in the same previous solution O/N at 4ºC. Stained tissues were washed in TTBS and mounted with Mowiol™.

Lentiviral production

293-FT (ATCC) cells maintained in Advanced DMEM (ThermoFisher Scientific) supplemented with 10% FBS, 1% P/S and 0.5% glutamine were used for LV production. The media was changed for Opti-MEM media (ThermoFisher Scientific) and the cells were transfected with three plasmids at the same time (pMD2.G (VSV-G envelope expressing plasmid), psPAX2 (lentiviral packaging plasmid), and the plasmid of interest which will be expressed by the LV (in this case: pLenti-EF1α-hChR2(H134R)-EYFP-WPRE) for 6h using Lipofectamine 2000 Transfection Reagent (ThermoFisher Scientific). The media containing the resulting LVs was collected at 48h and 72h post-transfection. After centrifugation at 1200g and posterior filtering of the supernatant, the media was introduced into conical tubes (Beckman Coulter) with a sucrose cushion (20%). These tubes were then centrifuged at 26.000rpm for 2h at 4ºC, the media and the sucrose cushion were discarded and the collected viruses were resuspended in PBS-1% BSA, aliquoted and kept at -80ºC for future applications.

Image acquisition and analysis:

All images were acquired using either an inverted Olympus microscope IX71 with an ORCA Flash 4 camera (Hamamatsu), an Olympus BX61 and a DP12L cooled camera or a LSM 800 confocal microscope (Zeiss) and an AxioCam 503c camera (Zeiss).

Fluorescence intensity

Mean fluorescence intensity (MFI) was determined using ImageJ and each image was normalized to the background fluorescence level. Exposure time was preserved in all images in the same experiment.

Neurite length analysis

Images of neuron-specific class III β-tubulin (Tuj-1) immunostained cultures were obtained using an inverted Olympus microscope IX71 at 10x magnification. 3 fields per well were captured. Neurite length measurements were blindly performed using the Neuron J plugin for ImageJ (Meijering et al., 2004). Only large-diameter cells (diameter>35µm) were measured and mean neurite length per cell was determined.

Axonal growth analysis

Microfluidic devices were immunostained for green fluorescent protein (GFP)-yellow fluorescent protein (YFP) and the entire surface covered by axon growth in the axonal compartment was imaged using an inverted Olympus microscope IX71 at 10x magnification. Axonal growth was measured using the Neuron J plugin for ImageJ (Meijering et al., 2004). The mean total axon growth per field in growth experiments and the individual axon length in axotomy experiments was determined. In the case of WT cultures, the axonal growth area per microchannel of Tuj-1 immunostainined devices was quantified using Image J.

Quantification of axonal regeneration, sprouting and synapse formation

SCG-10 (superior cervical ganglion-10 protein) was used as a marker of regenerating sensory axons in injured sciatic nerves. Whole-mount images were obtained using a LSM 800 confocal microscope (Zeiss). Several images (5x magnification) were necessary to cover all the nerve. A collage with all the XY images and then a Z projection using the maximum fluorescence intensity was obtained with all the stack images. The number of SCG-10^{+} axons at each distance from the injury was blindly quantified.

Sprouting in the injured spinal cord was measured based on the number of dextran^{+} axons in fluorescence images acquired with an Olympus microscope IX71. For sprouting reanalysis, the mean fluorescence intensity of these axons was determined in separate compartments: the white matter and the grey matter.

Presynaptic vGlut1 immunostaining images were obtained from the ventral region of the spinal cord at 20x magnification with a LSM 800 confocal microscope (Zeiss). The total number of vGlut1^{+} neurons as well as co-localization with dextran^{+} axons was computed.

Statistical analysis

GraphPad Prism was used for statistical analysis and graphical representation. Results are plotted as the mean±s.e.m (standard error of the mean) unless indicated. Normality of the distributions was verified with the Shapiro-Wilk test or assumed when the sample size was too reduced. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ denote significant differences in Student's t-test or ANOVA with Bonferroni post hoc test for normal distributions or Mann–Whitney U-test or Kruskal-Wallis test for non-parametric distributions with Dunn's post hoc test.

Antibodies

AlexaFluor 488, 568, 594 or 405-conjugated antibodies (ThermoFisher Scientific) were used as secondary antibodies.

Antigen	Concentration	Experimental application	Source/Reference
c-Fos (9F6)	1:200	ICC	Cell signaling/#2250
ChR2	1:500	ICC	Progen/651180
Tubulin β-III (clone Tuj1)	1:1000 1:1000	ICC ICC	Biolegend/801201 Biolegend/802001
GFP	1:500 1:500	ICC IHC	ThermoFisher Scientific/A-11122
SCG-10/ Stathmin-2	1:1000	IHC	Novus Biologicals/NBP1-49461
vGlut1	1:200	IHC	Synaptic systems/ 135 302
GFAP	1:500	IHC	Agilent Dako/ Z0334
CCL21	1:200	IHC	Peprotech/500-P114
TRPV1/VR1	1:200	IHC	Santa Cruz/sc-398417

CXCR3	1:200	IHC	Abcam/ ab64714
CD11b/c	1:200	IHC	Novus Biologicals/ NB110-40766
CCR7	1:200 1:200	IHC/ICC	Abcam/ ab32527 R&D systems/ MAB3477
NFH	1:1000	ICC	Merck/N0142

Table 3. Antibodies used in our experiments and the specific concentrations. ICC: immunocytochemistry. IHC: immunohistochemistry.

Primers

Gene	Forward	Reverse
Gapdh	5'-ACCCTGTTGCTGTAGCCGTATCA-3'	5'-TCAACAGCAACTCCCACTCTCCA-3'
Atf3	5'-GAGGATTTTGCTAACCTGACACC-3'	5'-TTGACGGTAACTGACTCCAGC-3'
Bdnf	5’-AGTCTCCAGGACAGCAAAGC-3’	5’-TCGTCAGACCTCTCGAACCT-3’
Cap23	5'-GGGAGAGAGAGAGCCTTTGC-3'	5'-CTTCGGCCTTCTTGTCTTTG-3'
Galanin	5’-GTGACCCTGTCAGCCACTCT-3’	5’-GGTCTCCTTTCCTCCACCTC-3’
Sprr1a	5'-CCCCTCAACTGTCACTCCAT-3'	5'-CAGGAGCCCTTGAAGATGAG-3'
Gap43	5'-TCTGCTACTACCGATGCAGC-3'	5'-TGGAGGACGGGGAGTTATCAG-3'
Ngf	5'-CAAGGACGCAGTTTCTATACTG-3'	5'-CTTCAGGGACAGAGTCTCCTTCT-3'

Table 4. Primers used for quantitative real-time PCR experiments.

Results

Chapter 1: Effects of neuronal activity modulation on axonal growth

1.1. Effects of neuronal activity on regenerating-capable systems

1.1.1. Neuronal activity induces increased neurite outgrowth in *in vitro* DRG neurons

The main hypothesis of this thesis stated that neuronal activity could promote axon regeneration and growth. Therefore, to first approach it, and as a proof of principle, we tested *in vitro* whether the outgrowth of DRG neurons was affected by the modulation of neuronal activity. These neurons represent an interesting model to study regeneration as their axons bifurcate to form a regenerating peripheral branch in the sciatic nerve, and a non-regenerating central branch in the spinal cord (He and Jin, 2016). Hence, they allow to test the impact of a determined strategy in both of these systems within the same neuron. Specifically, we focused on large-diameter neurons (diameter>35µm), which include proprioceptors and mechanoreceptors, and are a well-established model for the study of axon regeneration.

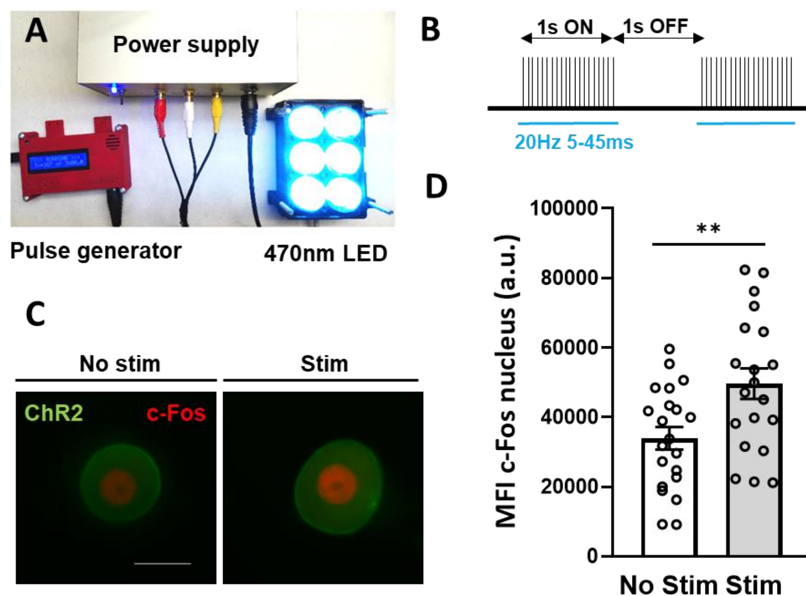

Figure 20. Neuronal activation upon delivery of the selected optogenetic stimulation protocol. A. Image of the homemade optogenetic illumination system. B. Schematic diagram of the selected optical stimulation pattern. C. DRG large-diameter neurons from Thy1-ChR2 mice express ChR2. c-Fos (red) and ChR2 (green) immunofluorescence representative images. Scale bar: 50µm. D. c-Fos levels in the cell nucleus of $ChR2^+$ cells are incremented after optogenetic stimulation. MFI: mean fluorescence intensity; a.u.: arbitrary units. Data are expressed as mean nuclear fluorescence intensity±s.e.m in arbitrary units (n=20-25 cells). $^{**}p < 0.01$ denotes significant differences in Student's t-test.

As a tool to modulate neuronal activity, we used optogenetics, which allows for precise manipulations of specific patterns of neuronal activity at a cellular level. DRG neurons were therefore obtained from B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J (Thy1-ChR2) adult mice, and large-diameter DRG neuron expression of ChR2 was verified (Figure 20C). Then, we tested

whether our illumination system (Figure 20A) and optogenetic stimulation pattern successfully induced neuronal activation. The selected stimulation protocol was obtained from a previous study using DRG neurons (Park et al., 2015) and consisted in 1h of 470nm illumination at 20Hz of frequency with 5ms pulses, in periods 1s ON-1s OFF periods (Figure 20B). ChR2 channel opens upon 470nm blue light illumination, allowing cations to cross and thus causing neuronal depolarization. We then used c-Fos immunostaining of stimulated and non-stimulation neurons at the end of the stimulation as a readout to confirm neuronal activation with our system and optical protocol (Figure 20C-D) (Student's t-test p= 0.0058).

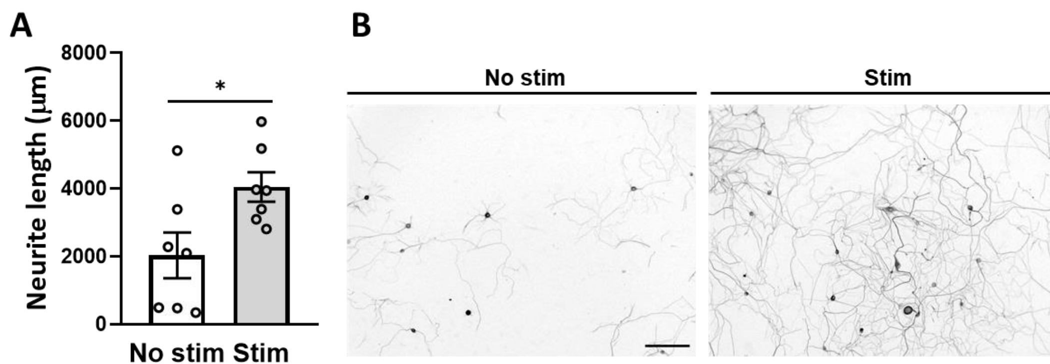

Figure 21. Neuronal activity boosts neurite outgrowth in large-diameter DRG neurons. A. Optogenetically stimulated cells showed a significant increase in neurite growth when compared to the non-stimulated. Mean neurite length per cell was quantified (n=7 wells). Data are expressed as mean±s.e.m. *p < 0.05 denotes significant differences in Student's t-test. B. Illustrative images of Tuj-1 immunofluorescence used in neurite length analysis. Scale bar: 500µm.

Then, we aimed to determine whether this stimulation protocol would promote neurite outgrowth in these cells. Optogenetic stimulation was applied to Thy1-ChR2 DRG neurons 2h after seeding, and 22h later (total of 24h *in vitro*) the cells were fixed, immunostained for Tuj-1 and their neuritic outgrowth was quantified. Only large-diameter neurons were included in the analysis. In line with our hypothesis, optogenetic stimulation induced a significant increase in neurite outgrowth in DRG large-diameter neurons (Figure 21) (Student's t-test p= 0.0267). These results show that increasing neuronal activity increments neuritic growth.

1.1.2. Modulation of neuronal activity in *in vitro* cortical neurons results in different outcomes depending on its time of application and characteristics.

Next we asked two questions: whether this effect was maintained in other neuronal types, and later, if it also occurred after axonal injury. To address both questions at the same time, we took

advantage of another *in vitro* model: embrionary (E16.5) cortical neurons cultured in microfluidic devices.

As cortical neurons from Thy1-ChR2 embryos did not show ChR2 expression at the developmental stage (E16.5) they were harvested, we obtained these neurons from WT embryos and infected them with LV-EF1α-hChR2(H134R)-EYFP-WPRE (LV-ChR2) that we produced in our laboratory. These LV-ChR2 effectively infected cells yielding high ChR2 expression levels at 7 days *in vitro* (DIV) (Figure 22A). ChR2 expression was found mainly in neurons, despite the use a constitutive promoter (EF1α), and could even be observed at the neuritic/axonal level. Infection efficiency was never significantly different among batches.

Our microfluidic devices contain two separate compartments connected by microchannels that allowed to spatially separate the cell bodies and the axons (further explained in the Materials and methods section) (Figure 22B), which facilitated axon growth studies as well as it allowed to perform standardized axotomies. Moreover, these devices allowed to study axons alone without the confounding presence of neurites, which do not cross the length of the microchannels (900µm) (Taylor et al., 2005). Cortical neurons were thus cultured in microfluidic devices, infected with LV-ChR2, and at 7DIV, both ChR2 expression and axonal crossing to the axonal compartment could be observed, indicating experiments could be initiated, and axotomies and stimulations performed (Figure 22B).

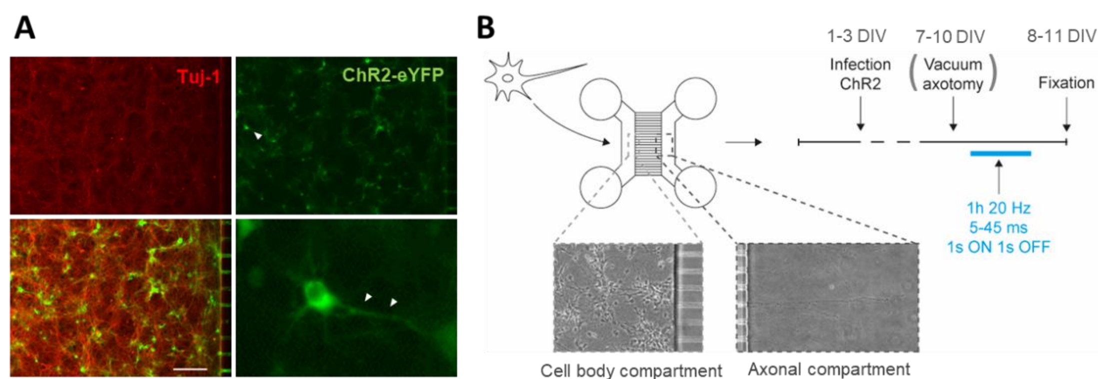

Figure 22. ChR2⁺ cortical neuron culture, axotomy and optogenetic stimulation in microfluidic devices. A. Immunocytochemistry depicting ChR2 expression levels (YFP in green) in LV-ChR2 infected cortical neurons (Tuj-1 in red). White arrows in a high magnification inset show ChR2 expression in neural prolongations. Scale bar: 250µm. B. Schematic diagram of the protocol used to analyze the effect of optogenetic stimulation on axon growth/regrowth in cortical neurons.

We first wanted to test whether our optogenetic stimulation increased the axonal growth of cortical neurons, without prior injury. In this case the stimulation was not delivered in trains, but continuously. Axonal growth in the axon compartment was quantified 24h after the stimulation, and a non-significant increase in axonal growth could be observed in the stimulated condition

(Figure 23A-B) (Mann Whitney test p= 0.1320). Importantly, when illumination was delivered to WT non-infected cortical neurons, axon growth did not differ among conditions (Figure 23C) (Student's t-test p= 0.9259), reinforcing a specific effect of the optogenetic stimulation on axon growth.

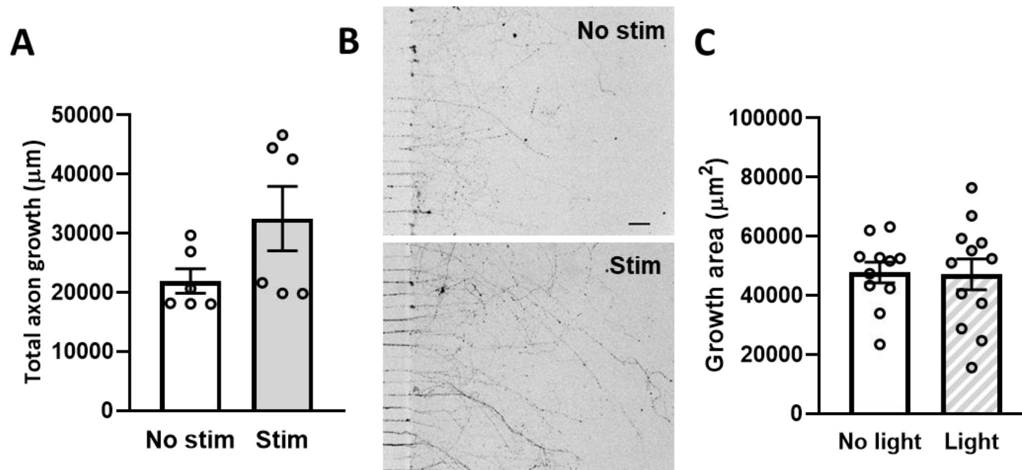

Figure 23. Optogenetic stimulation induces a slight increase in axonal growth of cortical neurons. A. Optically stimulated ChR2+ axons showed non-significant growth increments. Total ChR2+ axon length per field in an image was computed (n=6 images). B. Illustrative images of GFP/YFP immunofluorescence used in ChR2+ axon growth analysis. Scale bar: 200µm. C. WT illuminated axons (Light) did not present growth differences when compared to non-illuminated ones (No Light). Total growth area/microchannel was computed (n=11-12 images). Data are expressed as mean±s.e.m.

Moving to axotomy experiments, we found that when the optogenetic stimulation was applied 30 minutes after the axotomy, the axonal regrowth of stimulated axons was robustly reduced (Figure 24A) (Student's t-test p= 0.0001). Conversely, when the optogenetic stimulation was applied 6h after the axotomy, stimulated axons regenerated almost significantly longer distances than non-stimulated axons (Figure 24B-C) (Student's t-test p= 0.0759). Again, no changes were observed when illuminating WT axotomized axons (Figure 24D) (Student's t-test p= 0.8476). Finally, we assessed the repercussion of other stimulation frequencies on axonal regeneration: 10Hz and 50 Hz. Results showed that 50Hz stimulation had detrimental consequences on axon regeneration (Figure 24E) (ANOVA followed by Bonferroni test, no stim vs 10Hz p= 0.1717; no stim vs 50Hz p= 0.0115). Commercial AAV-ChR2 viruses were used in these experiments due to LV-ChR2 availability issues, but efficiency of infection as well as expression levels were similar between viruses.

Altogether, these results with *in vitro* cortical neurons indicate that a determined pattern of neuronal activity can promote axonal growth and regeneration *in vitro* regardless of the cell type.

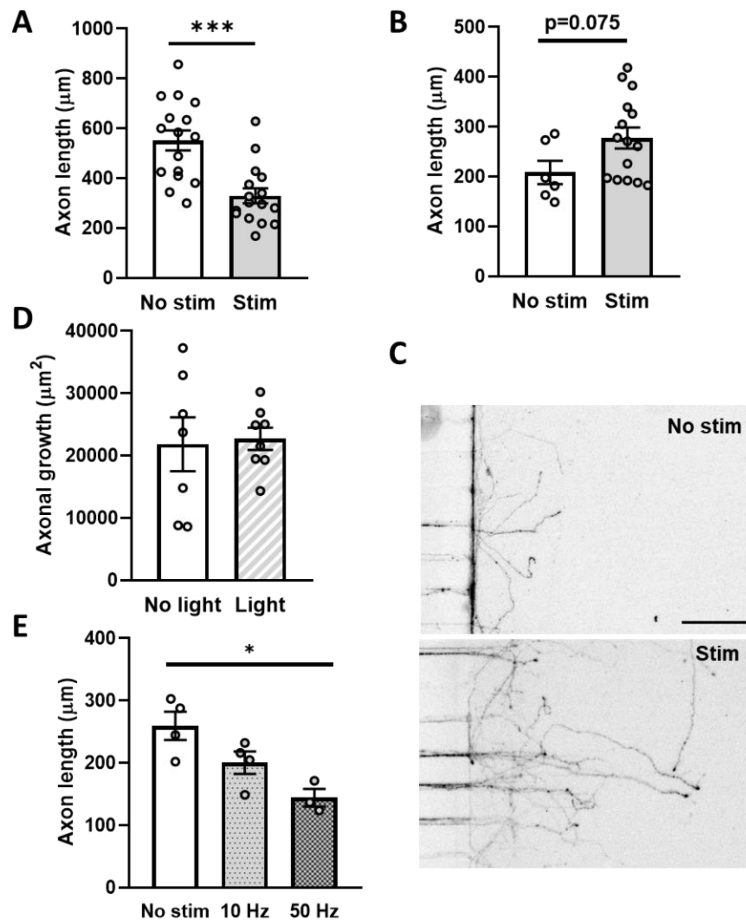

Figure 24. Increasing neuronal activity after axotomy results in different effects depending on the moment and frequency of stimulation. A. Stimulated axons regenerated significantly shorter distances when the stimulation was applied 30min after the axotomy. Individual $ChR2^+$ axon lengths were quantified (n=16 images). B. Optogenetic stimulation 6h after axotomy promoted an almost significant increase in axonal regrowth in the stimulated axons. $ChR2^+$ axon lengths were determined (n=7-15 images). C. Illustrative images of GFP/YFP immunofluorescence used in $ChR2^+$ axonal regrowth quantification at 6h post-axotomy. Scale bar: 200µm. D. WT illuminated axons (Light) did not regrowth differently from non-illuminated ones (No Light). Growth area/microchannel was quantified (n=7-8 images). E. Other assessed stimulation frequencies (10 and 50Hz) had different effects on regrowth after axotomy. Individual $ChR2^+$ axon lengths were quantified (n=3-5 devices). Data are expressed as mean±s.e.m. ***$p < 0.001$ denotes significant differences in Student's t-test. *$p < 0.05$ denotes significant differences in ANOVA followed by Bonferroni test.

1.1.3.Neuronal activity enhances axonal regeneration in peripheral nerve injury.

The encouraging *in vitro* results motivated us to move to *in vivo* models of axon injury. As a first approach and to test our hypothesis in yet another regenerating-capable system, we focused on the peripheral branch of the DRGs. In the following experiments neuronal activity was modulated using the chemogenetic tool hM3Dq, as the anatomical location of the DRGs impeded the implantation of the optic fiber necessary for optogenetic stimulations.

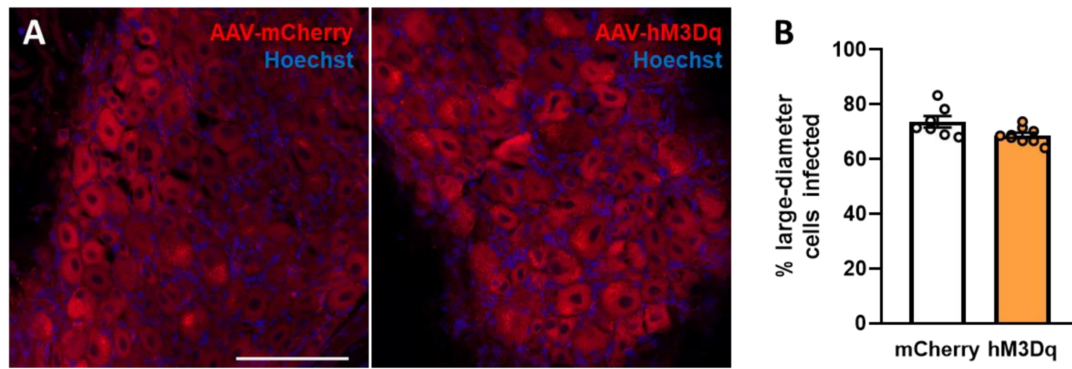

Figure 25. AAV-mCherry and AAV-hM3Dq successfully infect large-diameter DRG neurons 4-5 weeks after sciatic injection. A. Representative images of AAV-mCherry and AAV-hM3Dq expression levels. Scale bar: 100µm. B. Both virus infected predominantly large-diameter DRG cells. The proportion of large-diameter neurons respect the total of infected cells was quantified (n=7-9 DRGs). Data are expressed as mean±s.e.m.

AAV5-hSynhM3D(Gq)-mCherry (AAV-hM3Dq) or its control virus AAV5-hSyn-mCherry (AAV-mCherry) were injected into the sciatic nerves of adult C57BL/6J mice and 4-5 weeks later hM3Dq as well as mCherry expression was observed in the sciatic DRGs (Figure 25A). Importantly, large-diameter neurons (diameter>35µm) were preferentially transduced by both viruses, as infected large-diameter neurons corresponded to approximately the 70% of infected cells (Figure 25B).

hM3Dq and mCherry infected animals received two daily CNO injections during 4 days prior to injury (including the injury day), and then their sciatic nerves were crushed (Figure 26A). 24h after the SNC sciatic nerves were fixed and immunostained for SCG10, a marker of regenerating sensory axons. Quantification of labelled axons showed that chemogenetic stimulation enhanced regeneration in the stimulated nerves (hM3Dq-CNO), which was maintained throughout all the assessed distances and was robust at long distances (Figure 26B-C) (two-way ANOVA followed by Bonferroni test; interaction p= 0.0101 ; 1000µm p= 0.0163; 1250µm p= 0.0261; 1500µm p= 0.0259).

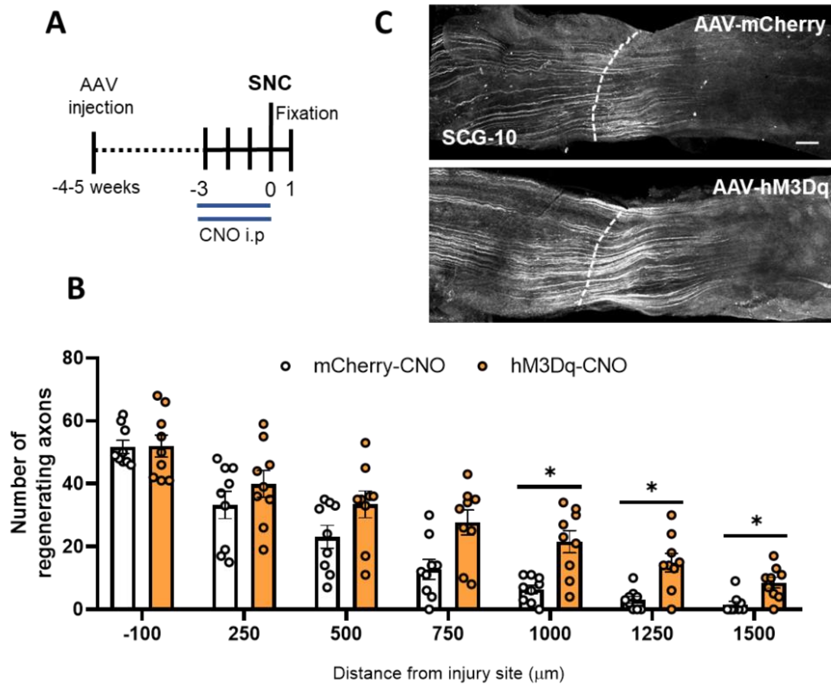

Figure 26. Chemogenetic stimulation promotes peripheral regeneration. A. Diagram depicting the timeline of the experiment. B. Stimulated axons (hM3Dq-CNO) regenerated longer distances than the non-stimulated (mCherry-CNO) as seen by increased number of regenerating axons (SCG-10^{+}) at all assessed distances, with statistical significance at greater lengths. Data are expressed as mean SCG-10^{+} axons ±s.e.m. at each distance from the crush (n=9 sciatic nerves). *p < 0.05 denotes significant differences in two-way ANOVA followed by Bonferroni test. C. Representative images of injured sciatic nerves immunostained for SCG-10. Dotted lines indicate the SNC. Scale bar: 200µm

These results show that increasing neuronal activity promotes regeneration in the injured PNS. Previous studies have suggested that neuronal activity induces BDNF expression as well as it activates an intrinsic pro-regenerative state (such as in (Al-Majed et al., 2000a; Hutson et al., 2019; Senger et al., 2019, 2018)). In an attempt to characterize the mechanism responsible for our results, we analyzed the gene expression of several known RAGs by qPCR: *Bdnf, Atf3, Galanin, Sprr1a* and *Cap23*. We obtained the DRGs of mice treated as previously explained (virus administration, CNO injections and posterior SNC), and after gene expression analysis, we did not observe changes in any of these genes among stimulated (hM3Dq-CNO) and non-stimulated (mCherry-CNO) DRGs (Figure 27).

All in all, our work in regenerating-capable systems evidences that neuronal activity effectively triggers axonal growth.

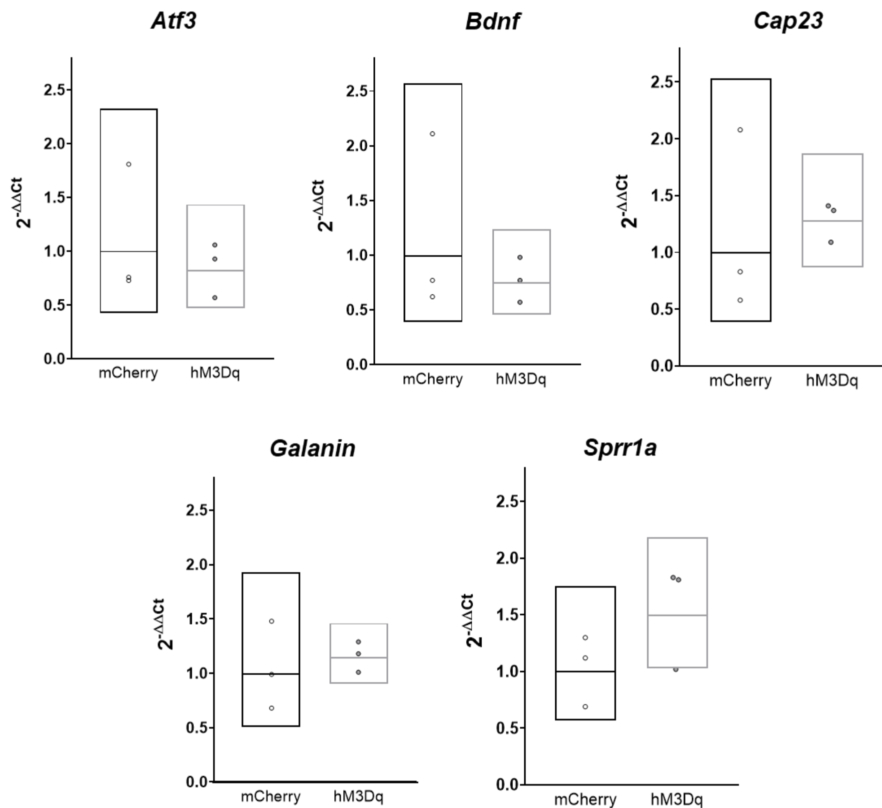

Figure 27. The gene expression of several known RAGs is not altered after chemogenetic stimulation, 24h after injury. Data are expressed as $2^{-\Delta\Delta Ct}$ ±upper ($2^{-(\Delta\Delta Ct+SD)}$)/lower limits ($2^{-(\Delta\Delta Ct-SD)}$) (n=3 biological replicates).

1.2. Effects of neuronal activity on non-regenerating systems

1.2.1. Neuronal activity is insufficient to promote recovery after SCI

While *in vitro* and peripheral injury models are important tools to understand and characterize axonal regeneration, the real challenge is to overcome the CNS failure to regenerate. Hence, we proceeded to investigate the effect of neuronal activity after SCI.

We first focused on the CST, as it is one of the principal targets in terms of patient recovery after SCI due to its major contribution to voluntary motor function. Again, Thy1-ChR2 animals were used in these experiments, as, whenever possible, optogenetics was the preferred method for neuronal activity modulation for its higher temporal resolution. ChR2 expression in layer V cortical neurons (where the CST is originated), was corroborated prior to the experiments (Figure 28A), and the stereotaxic coordinates that allowed illumination of the corticospinal motor neurons (CSMNs) responsible for hindlimb innervation were then determined by bibliographic search and experimental validation.

Next, the CST of Thy1-ChR2 mice was transected and optogenetic stimulation was delivered (in the selected coordinates) from days 7 to 11 and from 14 to 18 DPI (Figure 28B). In this case, the optogenetic stimulation paradigm consisted in 1h of 10Hz illumination in 1sON-4sOFF trains. The 20Hz applied in *in vitro* experiments had to be discarded for *in vivo* experiments due to seizure induction in some mice. Sensorimotor evaluation analysis using the BMS and gridwalk tests showed that the optogenetic stimulation (ChR2 Light) did not ameliorate functional recovery in CST injured mice (Figure 28C-D).

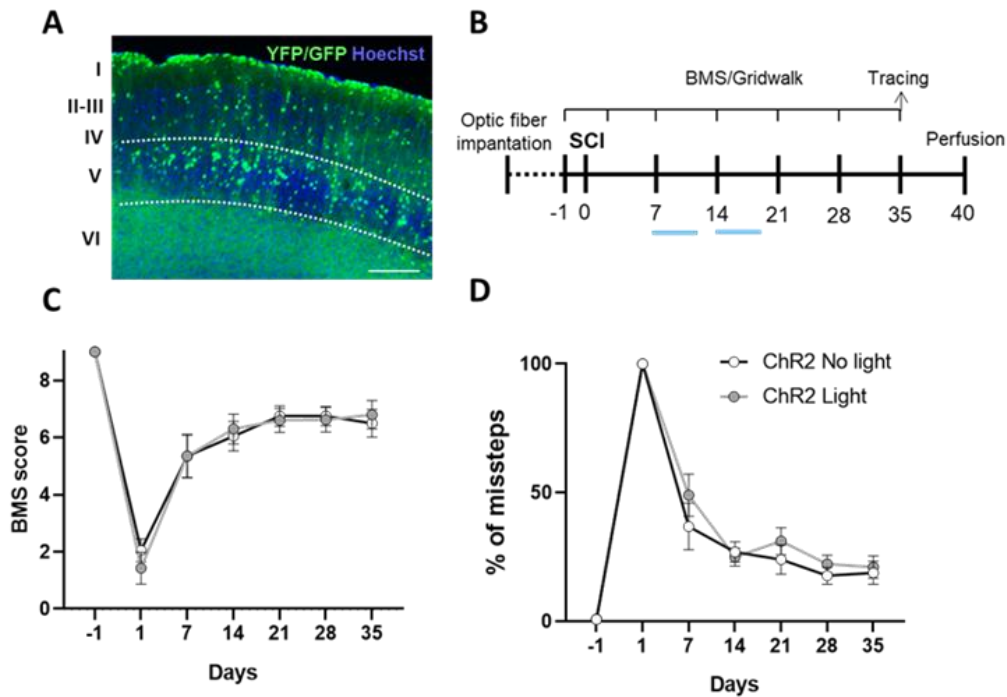

Figure 28. Increasing neuronal activity has no effect on recovery after CST injury. A. ChR2-YFP immunohistochemistry of the cortex of a Thy1-ChR2 mice. ChR2 was mainly expressed by layer V neurons, although expression could also be observed at some layer II-III neurons. Scale bar: 100µm B. Schematic timeline of the experiment. C and D. BMS (C) and gridwalk (D) sensorimotor evaluations showed no differences in recovery among stimulated (ChR2 Light) and non-stimulated (ChR2 No light) groups after CST injury. Results correspond to the BMS score (C) (n=10 mice) and % of missteps for the gridwalk (D) (n=8-9 mice). Data are expressed as mean±s.e.m.

In order to further examine the role of neuronal activity after SCI, we moved to another model: injury to the dorsal columns, as neuronal activity had shown a more robust impact in large-diameter DRG neurons (whose central axons form the dorsal columns in the spinal cord) in our previous experiments. WT mice were infected with the AAV-hM3Dq or AAV-mCherry control virus and 4-5 weeks later they underwent surgery for dorsal hemisection of the spinal cord (Figure 29A). Chemogenetic stimulation was delivered for 7 continuous days after injury, starting at 3DPI, and recovery was then evaluated with the BMS and the gridwalk tests. Again, no differences were observed in recovery between experimental groups (stimulated: hM3Dq-CNO;

non-stimulated: mCherry-CNO, mCherry-veh) (Figure 29B-C). Results from both CNS injury models indicate that increasing neuronal activity does not translate into sensorimotor function amelioration after a SCI.

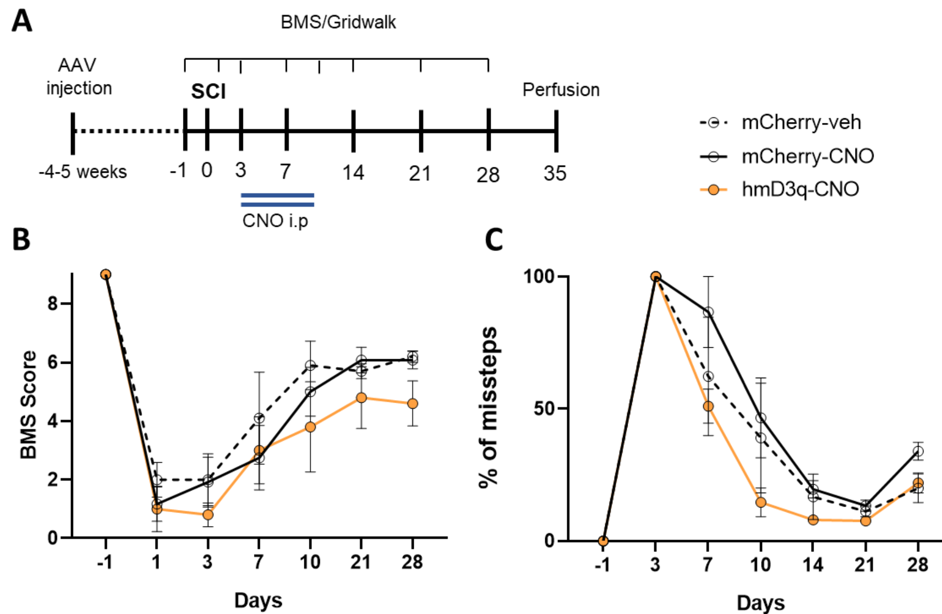

Figure 29. Neuronal activity modulation of the injured dorsal columns does not ameliorate sensorimotor function. A. Schematic timeline of the experiment. B and C. BMS (B) and gridwalk (C) results evidence similar recovery patterns in chemogenetically stimulated (hM3Dq-CNO) mice vs non-stimulated (mCherry-CNO, m-Cherry-Veh). Data in the graphics represent the BMS score (B) (n=5-6 mice) and the % of missteps in the gridwalk (D) (n=3-5 mice). Data are expressed as mean±s.e.m.

1.2.2. Axon growth is induced by neuronal activity only in growth-permissive environments.

In order to study the role of neuronal activity in the histological axonal plasticity in the injured spinal cord, the injured CSTs of Thy1-ChR2 mice were traced with a fluorescent tracer (Dextran-594) at 35DPI. Axonal growth (Figure 30A) and synapse formation (Figure 30B-C) examination did not reveal differences among the stimulated and non-stimulated conditions (axonal growth: Student's t-test p= 0.6520; synapse formation: vGlut1^{+}: Student's t-test p= 0.4430, dextran^{+}/ vGlut1^{+}: Student's t-test p= 0.2856).

At this moment we had observed that neuronal activity induced growth *in vitro* and in the injured PNS, but failed to do so in the injured CNS. As regeneration in the CNS is prevented due to both extrinsic and intrinsic factors, we intended to determine which of these barriers was hindering the effects of neuronal activity in this system. Our experiments in the PNS showed that the expression of several RAGs remained unchanged (Figure 27) after stimulation, suggesting our paradigm was

not affecting the intrinsic neuronal properties. Therefore we hypothesized that extrinsic factors in the CNS may be blocking the growth-promoting effect of neuronal activity.

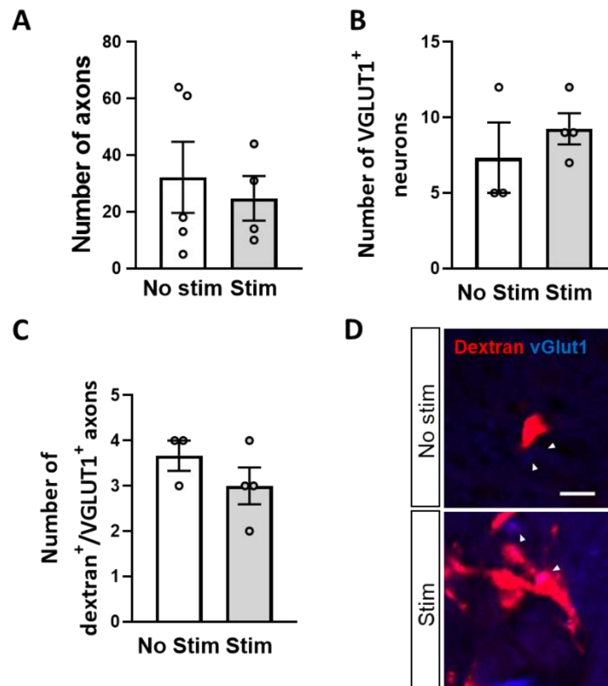

Figure 30. Optogenetically stimulated mice show similar histologic outcomes to their controls. A. Comparable number of traced (dextran$^+$) axons were found in stimulated and non-stimulated mice at 1.5mm post-injury. Data are expressed as mean number of axons±s.e.m (n=4-5 spinal cords). B and C. Quantification of the total number of vGlut1$^+$ neurons (B) and dextran$^+$ axons co-localizing with vGlut1$^+$ neurons (C) (n=3-4 spinal cords). Data are expressed as mean±s.e.m. D. Illustrative images of vGlut1 immunofluorescence of dextran-traced spinal cords. Scale bar: 10µm

To test this hypothesis, we cultured Thy1-ChR2 DRG neurons in growth-permissive (laminin-coated) and in growth-inhibitory (CSPG-coated) substrates and assessed the consequences of optogenetic stimulation on each of them. We found that while the stimulation triggered augmented neurite outgrowth in permissive substrates, this augment was not observed in growth-inhibitory substrates (Figure 31A-B) (laminin: Student’s t-test p= 0.0128; CSPG: Student’s t-test p= 0.4381).

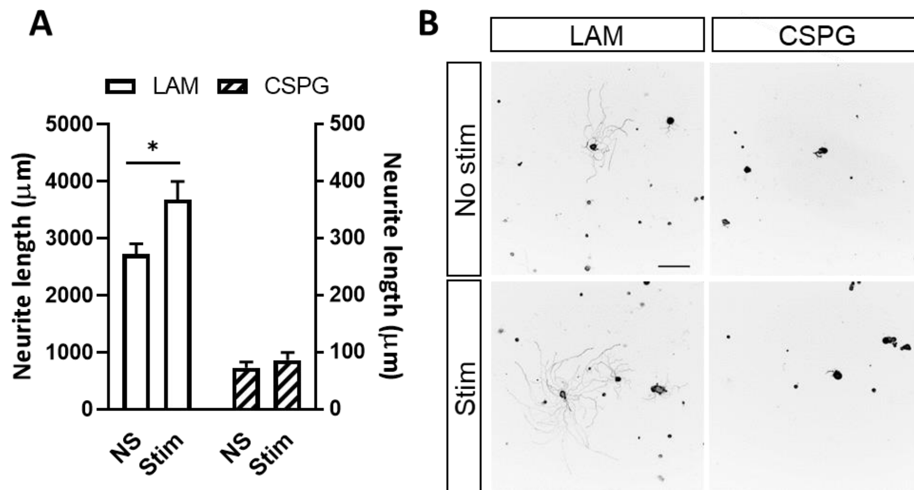

Figure 31. Neuronal activity promotes neurite outgrowth on permissive substrates but not on inhibitory substrates. A. Neurite length analysis demonstrated growth increases after stimulation on laminin-coated (LAM) substrates, but not on growth inhibitory CSPG-coated substrates. Data are expressed as mean neurite length per cell±s.e.m. (n=11-23 images). *p < 0.05 denotes significant differences in Student's t-test. B. Illustrative images of Tuj-1 immunofluorescence used in neurite length analysis. Scale bar: 200µm. NS: no stimulation

Finally, we asked whether similar events could be occurring *in vivo* in the CNS. Accordingly, after reanalysis of injured Thy1-ChR2 spinal cords we observed increased axonal sprouting in stimulated mice, restricted to pre-injury levels, reaching significance in grey matter regions (Figure 32) (WM-Pre-injury: Student's t-test p= 0.082; WM-Post-injury: Student's t-test p= 0.325; GM-Pre-injury: Student's t-test p= 0.001; GM-Post-injury: Student's t-test p= 0.303; GM: grey matter; WM: white matter).

Altogether, we have provided proof that while neuronal activity enhances axonal growth, this mechanism is impeded in the presence of growth-inhibitory molecules. These results suggest that activity-based therapies successfully promote axonal regeneration, but in order to be effective they must be combined with other strategies targeting the inhibitory environment after injury.

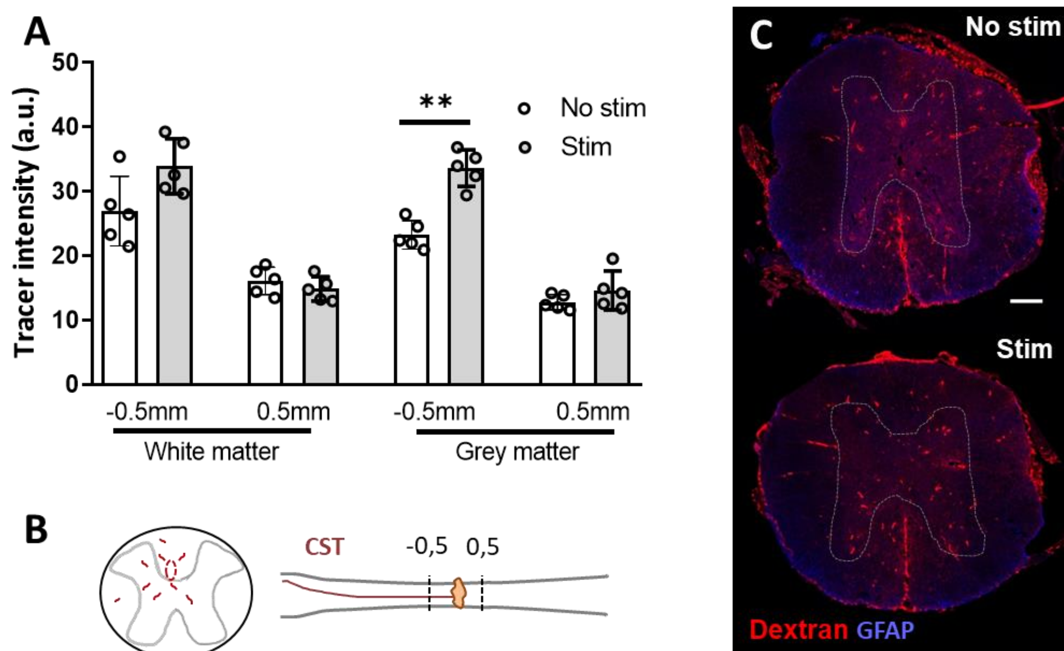

Figure 32. Neuronal activity induces axonal sprouting in pre-lesion regions after SCI. A. Histologic reanalysis of injured CST showed significant axonal sprouting promotion only in grey matter pre-injury regions. Data are expressed as mean tracer intensity (a.u.: arbitrary units)±s.e.m (n=5 spinal cords). **p < 0.01 denotes significant differences in Student's t-test. B. Anatomical diagram of the quantification. C. Illustrative images of analyzed spinal cords (-0.5mm). Scale bar: 150µm

1.2.3.Isolation of regenerating CSMN for molecular studies

The general objective of this project was to study the role of neuronal activity in spinal cord injuries, and initially we had hypothesized that intrinsic epigenetic and transcriptional changes would be induced as a result of our actuations. In order to be able to describe these presumed changes, we established a system that allowed us to isolate stimulated regenerating neurons, which could be subsequently submitted to high throughput molecular analysis (Figure 33).

To do that, with the help of a modified stereotaxic instrument, we injected a fluorescent retrotracer into the spinal cord at the T9 level, in the center and at 470µm deep (Figure 33A). These coordinates allowed to properly retrotrace the intact CST, as shown in Figure 33B. To trace injured CSTs, the retrotracer (in this case Cholera Toxin Subunit B (CTB-A647)) was injected at 0.3mm below the lesion. As a result of the injection site, only the axons regenerating past the injury would be labelled.

Next, we established a protocol to properly isolate these labelled neurons adapting and combining previously described protocols (Arlotta et al., 2005; Catapano et al., 2001; Saxena et al., 2012). As our intention was to use this protocol for optogenetically stimulated mice, we dissected the area of the motor cortex that had been illuminated, which was then submitted to gentle enzymatic

digestion (Figure 33A). The selection of the digestion solution was delicate, as other assessed methods (trypsin, other papain concentrations and digestion times) resulted in excessive cell death. Trehalose was added to the solutions in order to preserve cell survival (Saxena et al., 2012). Enzymatic digestion was followed by mechanical dissociation and then by several steps to eliminate debris (strainer and Percoll centrifugation).

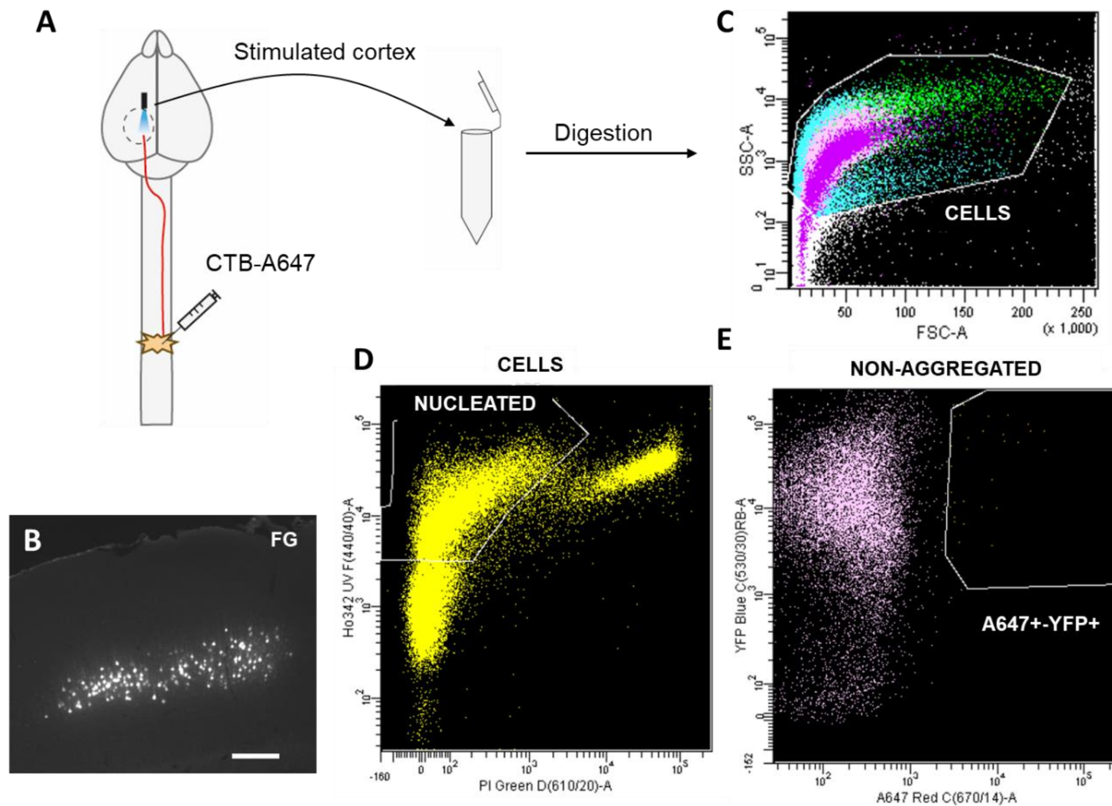

Figure 33. Establishment of a system to isolate regenerating neurons. A. Schematic experimental diagram. B. Corticospinal motor neurons retrotraced with FluoroGold (FG) injection to the spinal cord. Scale bar: 250µm. C-D-E. Gating strategy to isolate regenerating CST neurons (A647^{+}-YFP^{+}). Cells are selected among all the debris, then only the nucleated, and finally, from the non-aggregated cells, YFP^{+} neurons with high A647^{+} fluorescence (from CTB-A647-traced regenerating neurons), and thus, regenerating neurons, are isolated.

Finally, the resulting cell suspension was separated using fluorescence-activated cell sorting (FACS). Several gating steps had to be applied in order to avoid exaggerated debris, benefiting from the use of viability markers (Figure 33C-D-E). Briefly, live cells were selected from the total cell whole, then only the nucleated ones were selected and the aggregates were discarded. From this selection, YFP^{+}-CTB-A647^{+} double labelled cells were isolated, as they corresponded to the cells of interest: YFP labelling indicated potentially stimulated neurons and CTB-A647 labelling indicated regenerating cells. Around 250 YFP^{+}-CTB-647^{+} cells from each sample (2 pooled mice) could be recovered using this protocol.

Chapter 2: Role of neuronal activity in cell-to-cell communication after injury

2.1. Neuronal activity in the modulation of the injury environment

2.1.1. The glial scar and the cytokinome in a SCI are not altered by neuronal activity

In order to better understand the mechanisms underlying activity-based therapies in the SCI paradigm, we broadened our research and intended to investigate the impact neuronal activity could have in the environment after the injury.

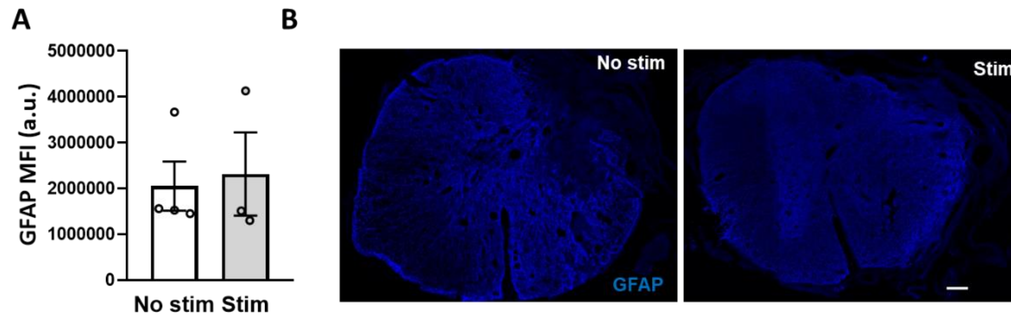

Figure 34. Optogenetic stimulation does not affect astrocytic activation. A. The GFAP+-glial scar of stimulated mice and non-stimulated mice did not differ. Data are expressed as mean fluorescence intensity of the GFAP+ region on each spinal cord ±s.e.m (n=3-4 spinal cords). MFI: mean fluorescence intensity. a.u.: arbitrary units B. Illustrative images of GFAP immunostaining. Scale bar: 100µm

The glial scar and a pro-inflammatory milieu are the two main hallmarks after a SCI, so we focused on examining these features in injured stimulated Thy1-ChR2 mice. GFAP was used as a marker for glial activation, and GFAP+- scar examination at 40DPI showed resembling glial scars in stimulated mice compared to the non-stimulated groups (Figure 34) (Mann–Whitney U-test p= 0.8571).

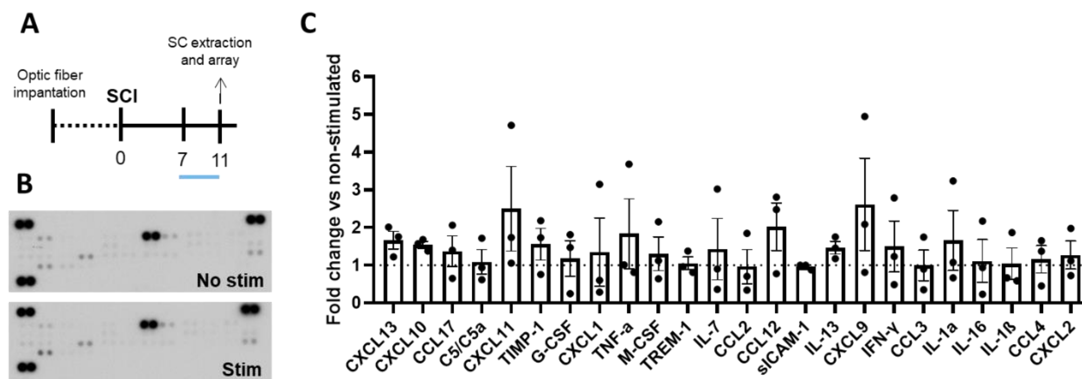

Figure 35. Cytokinome analysis of injured stimulated spinal cords. A. Schematic timeline of the experiment. B. Representative images of cytokine arrays. C. Cytokine expression levels remained unaltered upon optogenetic stimulation. Data are expressed as mean fold change of array spots compared to non-stimulated±s.e.m (n=3-4 spinal cords).

To explore whether neuronal activity could influence the inflammatory conditions after injury, Thy1-ChR2 mice with SCI were stimulated for 5 days and the day of the last stimulation the cytokine levels in the injured spinal cord were assessed using a Mouse Cytokine Array (Figure 35A). However, no significant changes were observed in any of the examined cytokines, despite slight increases in the chemokines CXCL11 and CXCL9 (Figure 35B-C).

Taken together, these results indicate that the predominant injury environment remains unaffected after neuronal activity modulation.

2.1.2. Nociceptor activation triggers neuronal chemokine CCL21 expression

The lack of observed differences in previous experiments could result from an earlier excessive imbalance, therefore, we sought for alterations at a more specific level with *in vitro* experiments. Chemokines have been shown to guide many cellular dialogues, including neuronal chemokines after axonal injury, and are thus great candidates to orchestrate the injury response (De Haas et al., 2007; Mesquida-Veny et al., 2021), for this reason we investigated whether neuronal activity had an effect on chemokine expression on cultured DRG neurons.

Thy1-ChR2 DRG neuronal cultures were optically stimulated and their media was recovered 24h later and used to measure chemokine secretion using a Mouse Chemokine Array. Surprisingly, this exploratory experiment showed a remarkable increase in CCL21 levels (Figure 36).

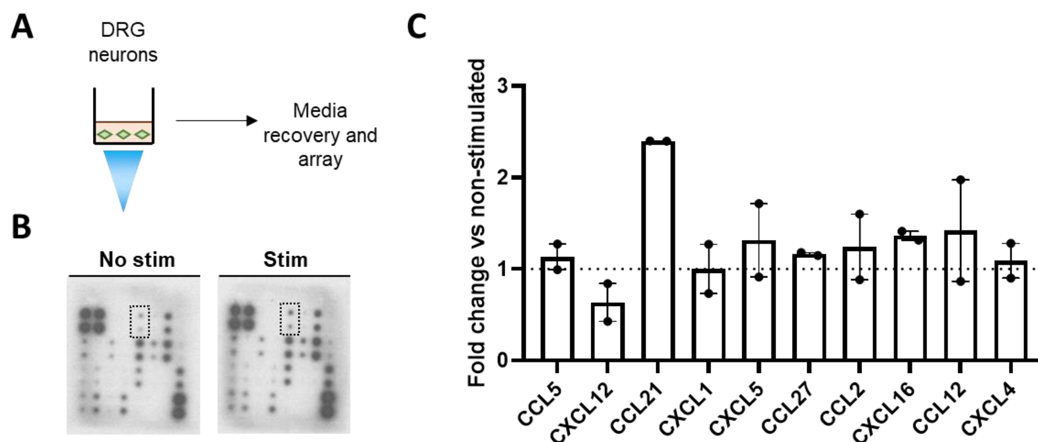

Figure 36. Chemokinome analysis of stimulated DRG neurons. A. Schematic diagram of the experiment. B. Representative images of chemokine arrays. The selection indicates the position of CCL21. C. CCL21 expression was increased after optogenetic stimulation. Data are expressed as mean fold change of array spots ±s.e.m (n=2 wells). Statistical analysis was not performed due to low sample size.

Previous studies had reported CCL21 expression specifically in nociceptors (Biber et al., 2011; Zhao et al., 2007), so we hypothesized this neuronal type could be responsible for the CCL21 increase as nociceptors are also present in DRG cultures. Accordingly, CCL21 expression was increased in TRPV1^{+} nociceptors 2h after capsaicin (a TRPV1 agonist) injection (Figure 37) (Student's t-test p= 0.0347).

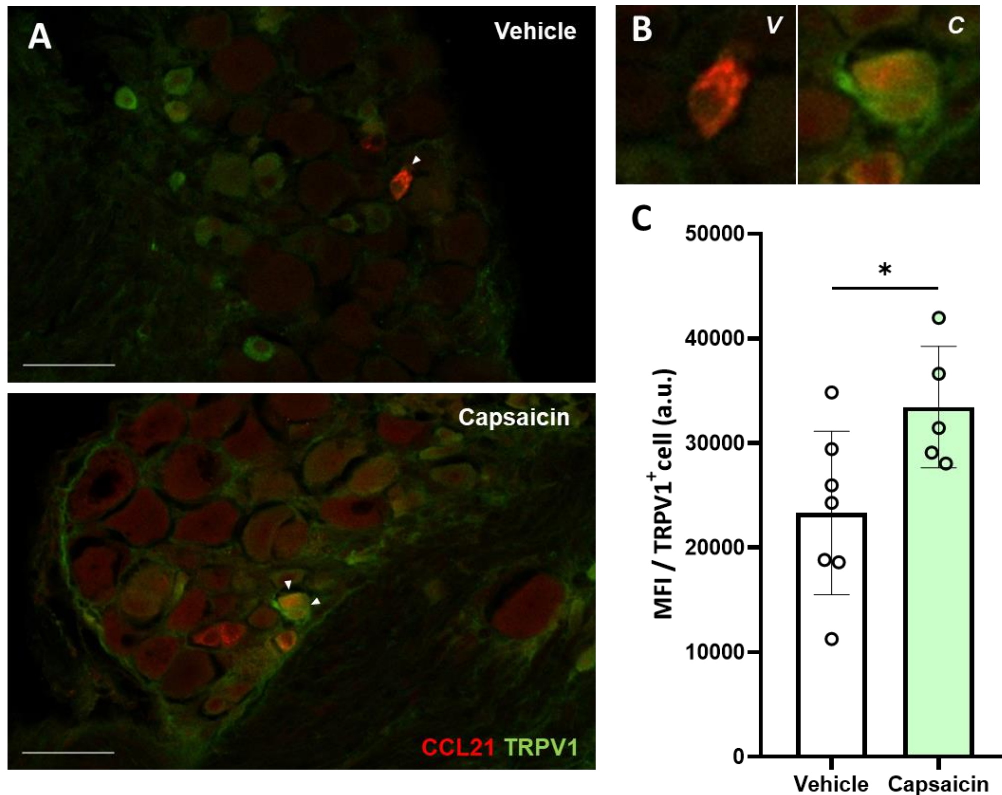

Figure 37. Capsaicin administration upregulates CCL21 expression specifically in TRPV1^{+} neurons. A. Immunostaining depicting CCL21 (red) and TRPV1 (green) co-localization after vehicle of capsaicin administrarion. White arrows indicate high magnification regions. Scale bar: 50μm. B. Higher magnification of the CCL21-stained neurons. V: vehicle; C: capsaicin. C. Quantification of CCL21 mean fluorescence intensity (MFI) in TRPV1^{+} cells shows increased CCL21 expression after capsaicin injection. a.u.: arbitrary units. Data are expressed as mean±s.e.m. (n=5-7 images). *$p < 0.05$ denotes significant differences in Student's t-test.

2.1.3. Non-neuronal impact of CCL21 in the sciatic nerve

According to our data that indicates that nociceptor activation promotes CCL21 secretion, this mechanism may be triggered upon activity-based therapies, so we next examined which role this CCL21 could exert after injury.

We used a peripheral injury model (SNC) to characterize this influence. First, we evaluated whether CCL21 affected axonal regeneration. However, CCL21 local injection into the sciatic

nerve did not result in significant nerve regeneration alterations (Figure 38A-B). Then, as a second approach to explore the role of CCL21 in the sciatic nerve, we examined cytokine alterations in sham or injured sciatic nerves after CCL21 administration. While some of the assessed cytokines remained unaltered (soluble intercellular adhesion molecule-1 (sICAM1), IL-1 receptor antagonist (IL1ra), CCL2 and tissue inhibitor of metalloproteinase 1 (TIMP1)), a general decrease in several chemokine levels was observed both in sham and injured conditions in CCL21-treated mice, as seen in CXCL13, CXCL10, CXCL1, CCL3, CCL4, CCL5 (Figure 38C).

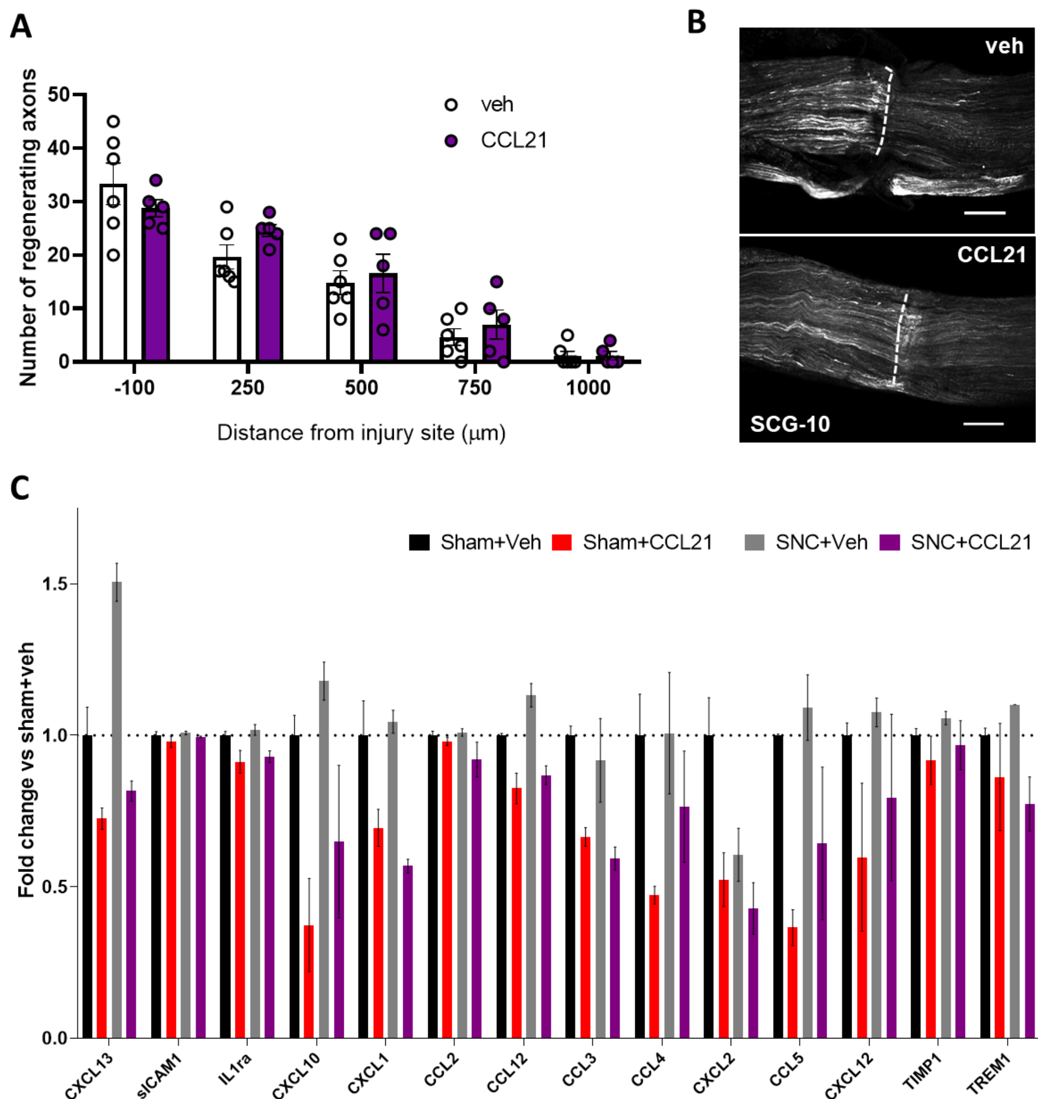

Figure 38. CCL21 local administration into the sciatic nerve does not improve axon regeneration but affects the cytokinome. A. CCL21- and vehicle-treated sciatic nerves presented similar regenerating capacities. Data are expressed as mean number of regenerating axons (SCG-10^{+})±s.e.m. at different distances from the injury (n=5-6 sciatic nerves). B. SCG-10 immunostaining of representative sciatic nerves. Dotted lines indicate the SNC. Scale bar: 250µm C. Cytokinome analysis of sham and injured sciatic nerves 24h after the administration of CCL21 revealed reduction of several cytokines. Data are expressed as mean fold change of array spots compared to sham vehicle group±s.e.m (n=2 mice). Statistical analysis was not performed due to low sample size.

In other pathologies macrophages have been described to mediate CCL21-induced effects (Van Raemdonck et al., 2020), so we asked whether these cells could be responding to CCL21 in our paradigm. Moreover, CCL21 has been described to interact with microglial CXCR3 (Biber et al., 2001; Rappert et al., 2002), a receptor also expressed by macrophages (Butler et al., 2017). In line with this, we found CXCR3 expression in CD11b/c^{+} cells in the sciatic nerve (Figure 39A). Meanwhile neuronal expression of CXCR3 was not observed in the DRG (Figure 39B).

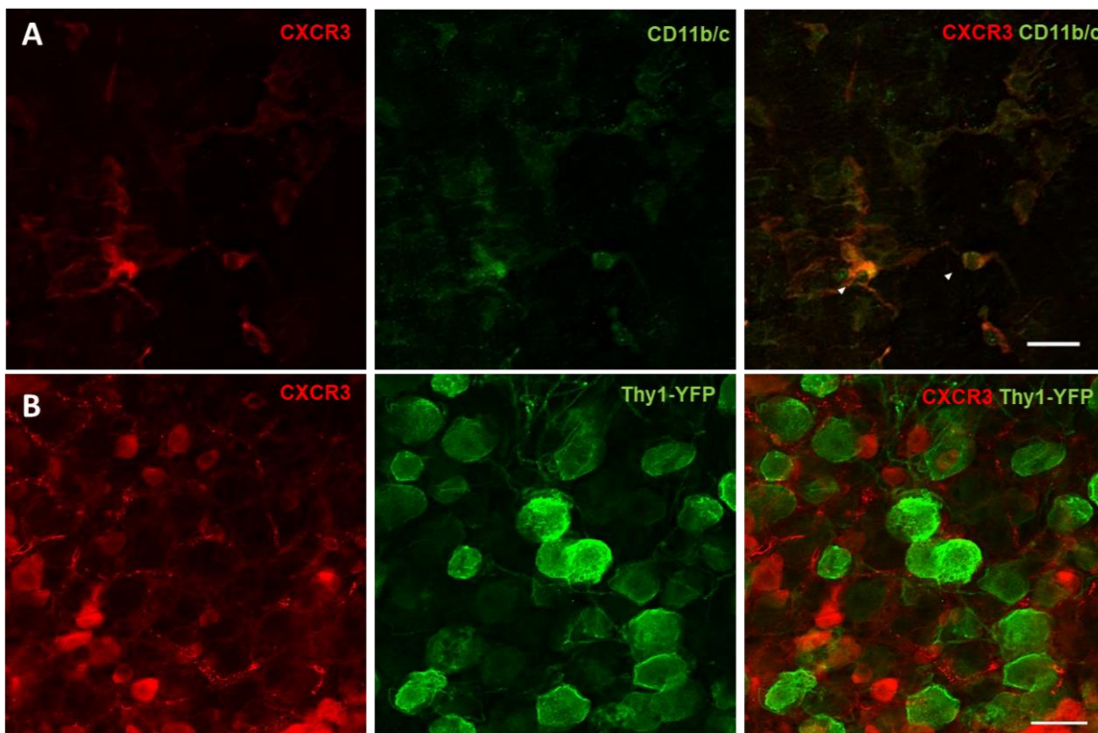

Figure 39. CXCR3 is expressed in CD11b/c^{+} cells but not in neurons. A. Sciatic nerves immunostained for CXCR3 (red) and CD11b/c (green). Yellow indicates co-localization. Scale bar: 25µm. B. Thy1-YFP (green) DRG neurons did not show CXCR3 (red) expression. Scale bar: 50µm.

In order to understand the consequences of CXCR3 activation we administered a CXCR3 pharmacological inhibitor, NBI-74330, in the injured sciatic nerve, and processed for *ex vivo* cell culture the corresponding sciatic DRGs 24h later. The SNC was performed in order to elevate CCL21 levels, as described in (Biber et al., 2011). Remarkably, neurite length analysis showed CXCR3 inhibition promoted neurite outgrowth *ex vivo* (Figure 40A-B) (Student's t-test p= 0.0494). These data suggest that CCL21 inhibits axon regeneration via non-neuronal CXCR3 activation. A direct role of CXCR3 on axonal growth was excluded as this receptor is not expressed in neurons (Figure 39B).

To analyze whether growth inhibition resulted from differential macrophage secretions prompted by CCL21, we cultured DRG neurons in conditioned media (CM) from CCL21-treated BMDM (bone marrow-derived macrophages) (Figure 40C). However, DRG neurons cultured with CM

from CCL21-treated BMDM did not present differences in their neurite outgrowth capacities when compared to the control group (Figure 40D) (Student's t-test p= 0.9810).

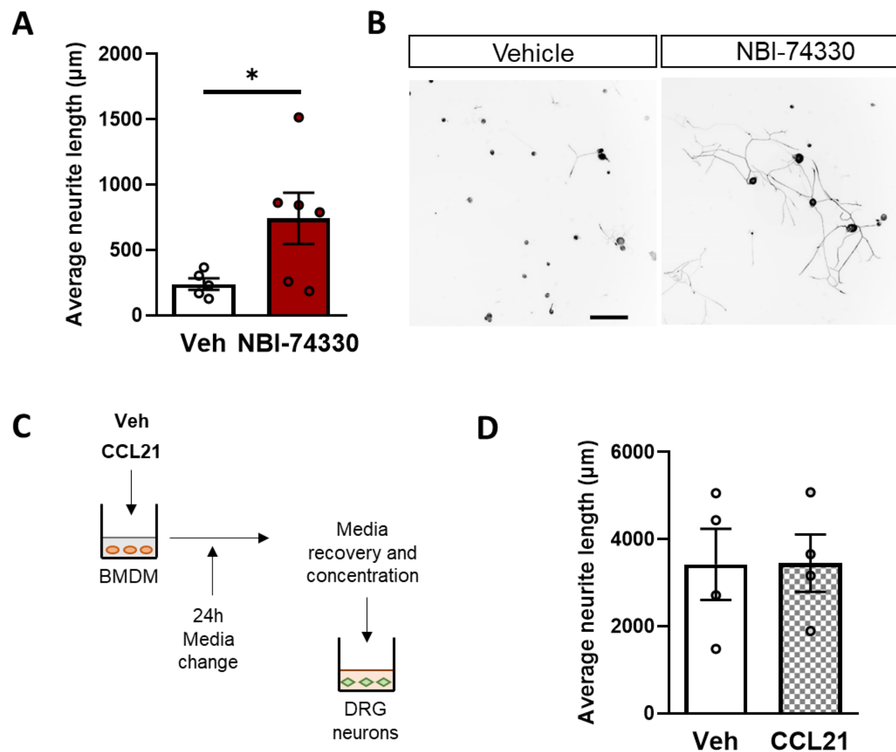

Figure 40. CCL21 blocks axon growth in a CXCR3-mediated manner but independently from the macrophage secretome. A. *Ex vivo* administration of the CXCR3-inhibitor NBI-74330 induced increased neurite outgrowth. Data are expressed as mean neurite length per cell±s.e.m (n=5-6 sciatic nerves) *p < 0.05 denotes significant differences in Student's t-test C. Representative images of Tuj-1 immunofluorescence used in neurite length analysis. Scale bar: 200µm. C. Schematic diagram of conditioned media experiments. D. Conditioned media from CCL21-treated BMDM did not have an effect on the neurite growth of DRG neurons. Data are expressed as mean neurite length per cell±s.e.m (n=4 wells).

These results indicate that neuronal activity promotes nociceptor secretion of CCL21, which may inhibit axon regeneration via non-neuronal CXCR3 activation. However, this mechanism is not directly induced by macrophage secretome. Moreover, CCL21 diminishes the expression of several chemokines, however, whether the lack of these chemokines affects axonal regeneration remains to be elucidated.

2.2. Neuronal activity indirectly affects regeneration: CCL21-mediated growth promotion

2.2.1. CCL21 enhances neurite outgrowth

Our initial results suggested that CCL21 blocks axonal regeneration, however, *in vivo* CCL21 administration into the sciatic nerve alone did not result in regeneration inhibition. We therefore asked whether this chemokine could also influence axonal growth through a different mechanism.

To unravel this issue, we injected CCL21 into the sciatic nerve and obtained the sciatic DRGs 24h later for *ex vivo* culture. Interestingly, we observed an increment in the neuritic outgrowth of *ex vivo* CCL21-treated DRG neurons (Figure 41A-B) (Student's t-test p= 0.0046). Moreover, the administration of different CCL21 doses (1nM, 10nM, 50nM) resulted in a dose-dependent increase in neurite outgrowth in DRG neuronal cultures (Figure 41C-D) (ANOVA followed by Bonferroni test; Veh vs 50nM p= 0.0129; 1nM vs 50nM p= 0.0440). These data indicate that CCL21 exerts a direct effect on neurite growth.

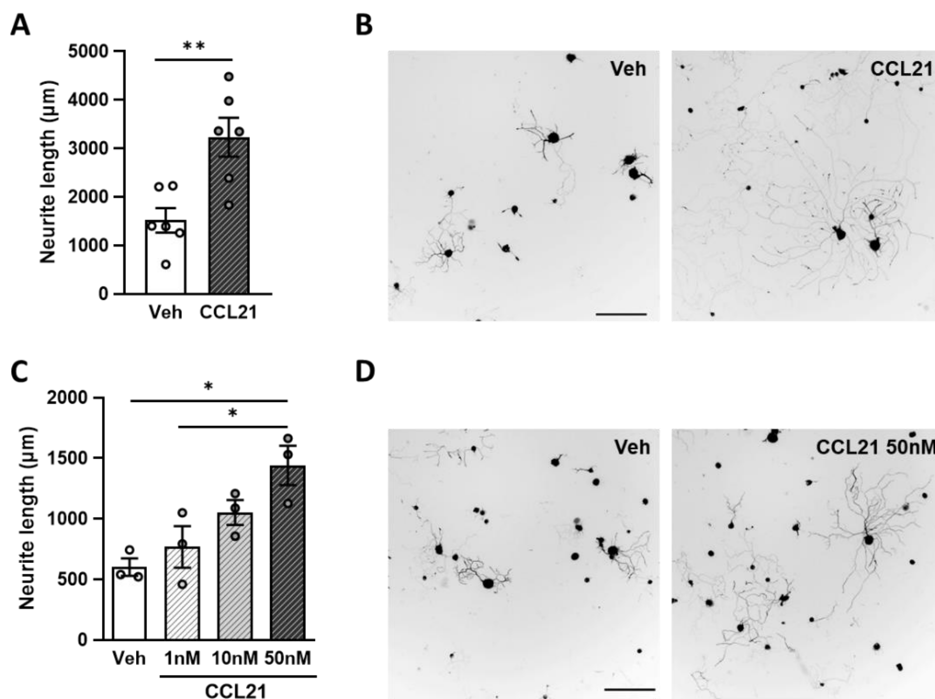

Figure 41. ***Ex vivo*** **and** ***in vitro*** **CCL21 administration promotes neurite outgrowth.** A. CCL21 injection into the sciatic nerve resulted in increased neurite length in *ex vivo* DRG cultures. Data are expressed as mean neurite length per cell±s.e.m (n=6 sciatic nerves). **p < 0.01 denotes significant differences in Student's t-test. C. CCL21 induced neurite outgrowth in a dose-dependent manner in *in* vitro large-diameter DRG neurons. Mean neurite length per cell was quantified (n=3 wells). Data are expressed as mean±s.e.m. *p < 0.05 denotes significant differences in ANOVA followed by Bonferroni test. B-D. Illustrative images of Tuj-1 immunofluorescence used in neurite length analysis (B: *ex vivo* D: *in vitro* experiments). Scale bar: 250µm.

2.2.2. Molecular insights into the growth-promoting mechanism triggered by CCL21

Next, we intended to identify the receptor responsible for the CCL21-dependent growth induction. As we had not observed neuronal expression of CXCR3 (Figure 39B), we inspected for neuronal expression of CCR7, the canonical CCL21 receptor. Indeed, CCR7 expression was found in DRG neurons, including in parvalbumin^{+} (PV) cells (a marker of proprioceptors) (Figure 42A). Moreover, CCR7-blocking antibody co-administration to DRG cultures suppressed the CCL21 effects on neurite outgrowth (Figure 42B-C) (two-way ANOVA followed by Bonferroni test; interaction p= 0.0233; IgG-veh vs IgG-CCL21 p= 0.0055; IgG-CCL21 vs αCCR7 2µg-veh p= 0.0040; IgG-CCL21 vs αCCR7 2µg-CCL21 p= 0.0355; IgG-CCL21 vs αCCR7 5µg-veh p= 0.0037; IgG-CCL21 vs αCCR7 5µg-CCL21 p= 0.0079). These observations suggest that CCL21 triggers growth via the CCR7 receptor.

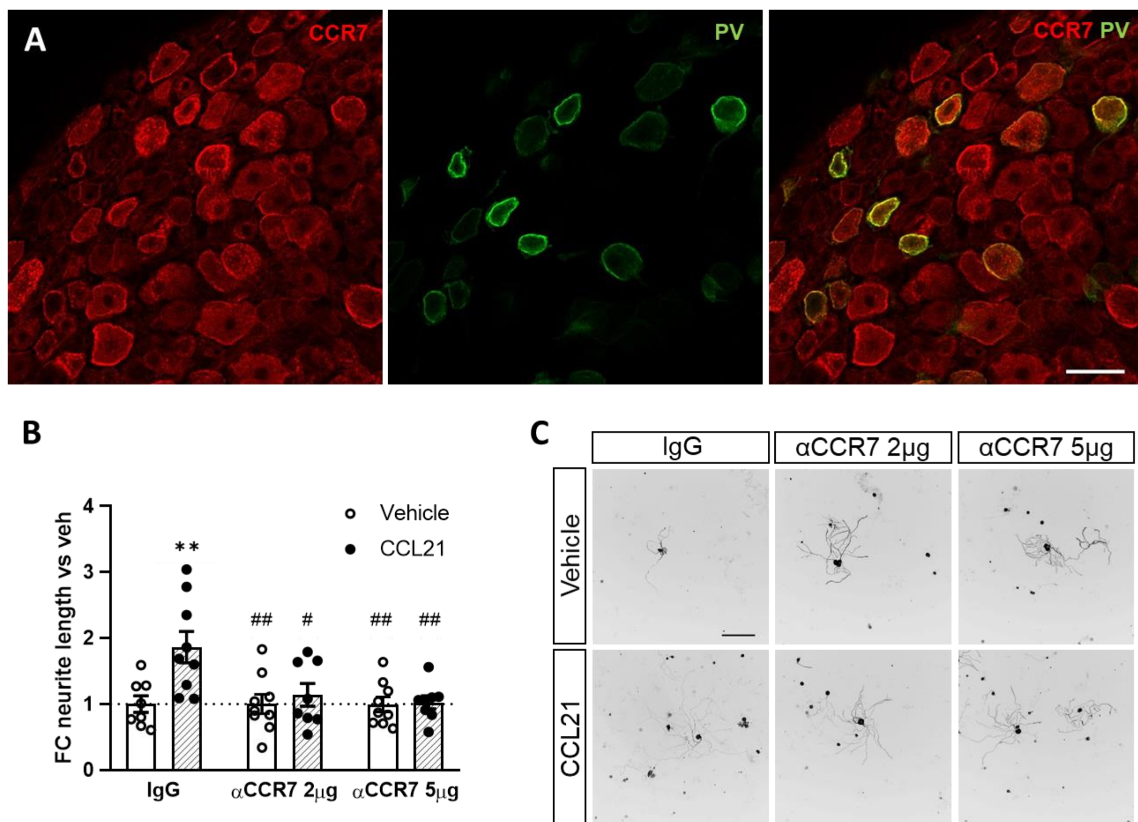

Figure 42. CCL21 effects on neurite growth are dependent on the neuronal receptor CCR7. A. CCR7 (red) immunostaining showed neuronal expression and colocalization with PV^{+} (green) cells. Scale bar: 50µm. B. CCR7-blocking antibody addition prevented the growth promotion induced by CCL21. Data are expressed as mean fold change compared to each respective vehicle group±s.e.m (n=8-9 images). **p < 0.01 denotes significant differences in two-way ANOVA followed by Bonferroni test (compared to IgG-veh); #p < 0.05; ##p < 0.01 denote significant differences in two-way ANOVA followed by Bonferroni test (compared to IgG-CCL21). C. Representative images of Tuj-1 immunostainings used for neurite length quantification. Scale bar: 250µm.

To further inspect the CCL21-CCR7 growth-promoting mechanism, we asked whether CCL19, the other canonic CCR7 ligand, would have the same impact on neurons. However, CCL19 addition to DRG neuronal cultures did not result in increased neurite outgrowth (Figure 43A) (Kruskal-Wallis test followed by Dunn's test; Veh vs 50nM $p > 0.9999$), suggesting exclusivity of CCL21 in this particular CCR7 biased mechanism.

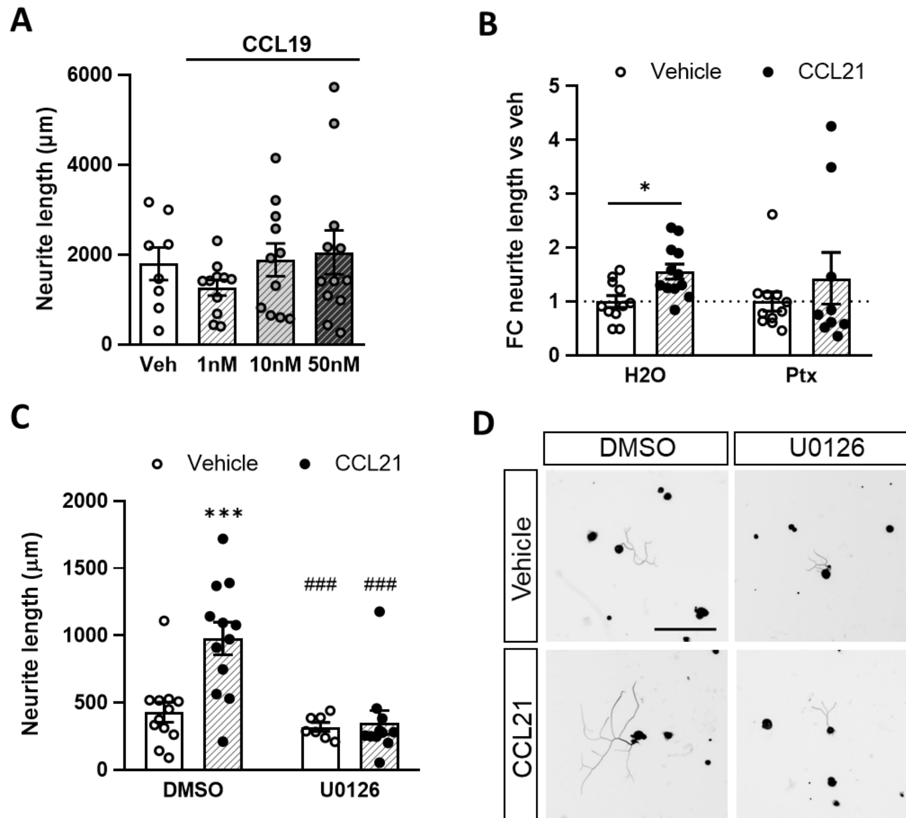

Figure 43. CCL21-induced growth promotion is exclusive in the CCR7 ligands and dependent of the MEK pathway. A. CCL19, the other CCR7 ligand did not affect neurite outgrowth. Data are expressed as mean neurite length per cell±s.e.m. (n=8-12 images). B. Pertussis toxin (Ptx), a G_i inhibitor, did not eliminate the growth-promoting effect of CCL21. Data are expressed as mean fold change (FC) compared to each vehicle ±s.e.m. (n=9-12 images).). *$p < 0.05$ denotes significant differences in Student's t-test. C. U0126, a MEK inhibitor, suppressed the CCL21-mediated growth induction. Data are expressed as mean neurite length per cell ±s.e.m. (n=7-12 images). ***$p < 0.001$ denotes significant differences in two-way ANOVA followed by Bonferroni test (compared to veh-DMSO); ###$p < 0.001$ denotes significant differences in two-way ANOVA followed by Bonferroni test (compared to CCL21-DMSO). D. Representative images of Tuj-1 immunostainings used for neurite length quantification. Scale bar: 250μm.

Then, in an attempt to identify the intracellular molecular pathway activated by the CCL21-CCR7 axis in our paradigm, we targeted with pharmacological inhibitors two known downstream actuators of CCR7, the MEK pathway and the G_i protein, both of them described to participate in axon growth and regeneration (as in (Chierzi et al., 2005; Hasegawa et al., 2004; Perlson et al., 2005)). Interestingly, inhibiting the MEK pathway with U0126 effectively abolished the CCL21-triggered increase in neurite outgrowth (Figure 43C-D) (two-way ANOVA followed by

Bonferroni test; interaction p= 0.0117; DMSO-Veh vs DMSO-CCL21 p= 0.0005; DMSO-CCL21 vs U0126-Veh p= 0.0004; DMSO-CCL21 vs U0126-CCL21 p= 0.0001). Meanwhile, pertussis toxin (Ptx) (G_i inhibitor) did not abrogate the effect of CCL21, although a slight reduction was observed (Figure 43B) (two-way ANOVA followed by Bonferroni test; interaction p= 0.7961; Student's t-test w/o (without) Ptx: p= 0.0115; with Ptx: p= 0.7458). These data points out that the MEK pathway is required for the CCL21-induced axonal growth.

The MEK-ERK pathway has been described to enhance regeneration via RAG expression promotion (Puttagunta et al., 2014), to this aim, we inspected the expression of different RAGs, some of them known to be MEK-dependent, after CCL21 addition through qPCR, however, we did not find significant expression alterations in any of the assessed genes (*Gap43, Cap23, Sprr1a, Bdnf, Ngf*) (Figure 44).

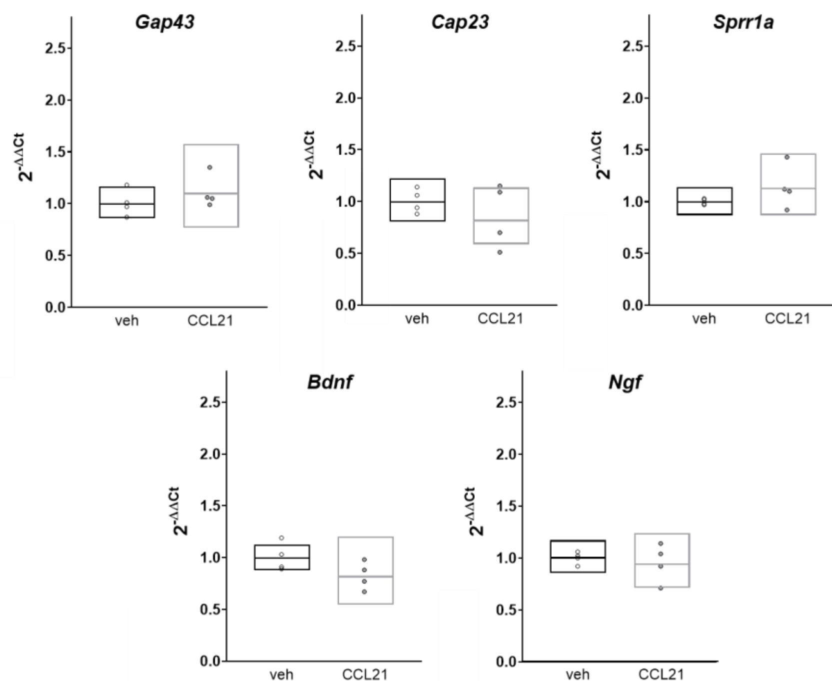

Figure 44. CCL21 administration does not affect RAG expression at 4h. Data are expressed as $2^{-\Delta\Delta Ct}$ ±upper ($2^{-(\Delta\Delta Ct+SD)}$)/lower limits ($2^{-(\Delta\Delta Ct-SD)}$) (n=4 biological replicates).

2.2.3. CCL21 affects the growth cone locally

As CCL21 did not seem to trigger an important transcriptional switch, we explored for alternative mechanisms. Closer examination to DRG cultures lead to the observation that CCL21-treated neurites seemed to present larger growth cones (Figure 45A). Furthermore, CCR7 expression was found to be especially high in the growth cone (Figure 45B).

These data led us to hypothesize CCL21 was exerting a local effect in this structure. As a result from the observed growth cone morphologies, we expected increased actin branching after CCL21 treatment, for this reason we combined CCL21 administration with wiskostatin, a neural Wiskott-Aldrich syndrome protein (N-WASP) inhibitor (which leads to prevented Arp2/3 activation). As predicted, the combination of these two compounds greatly reduced the CCL21 growth induction (Figure 45C) (two-way ANOVA followed by Bonferroni test; interaction p= 0.0825; one-way ANOVA followed by Bonferroni test; DMSO-Veh vs DMSO-CCL21 p= 0.0207; DMSO-CCL21 vs Wiskostatin-Veh p= 0.0202). These results suggest that the effects of CCL21 on axonal growth are partially a result of increased actin branching in the growth cone.

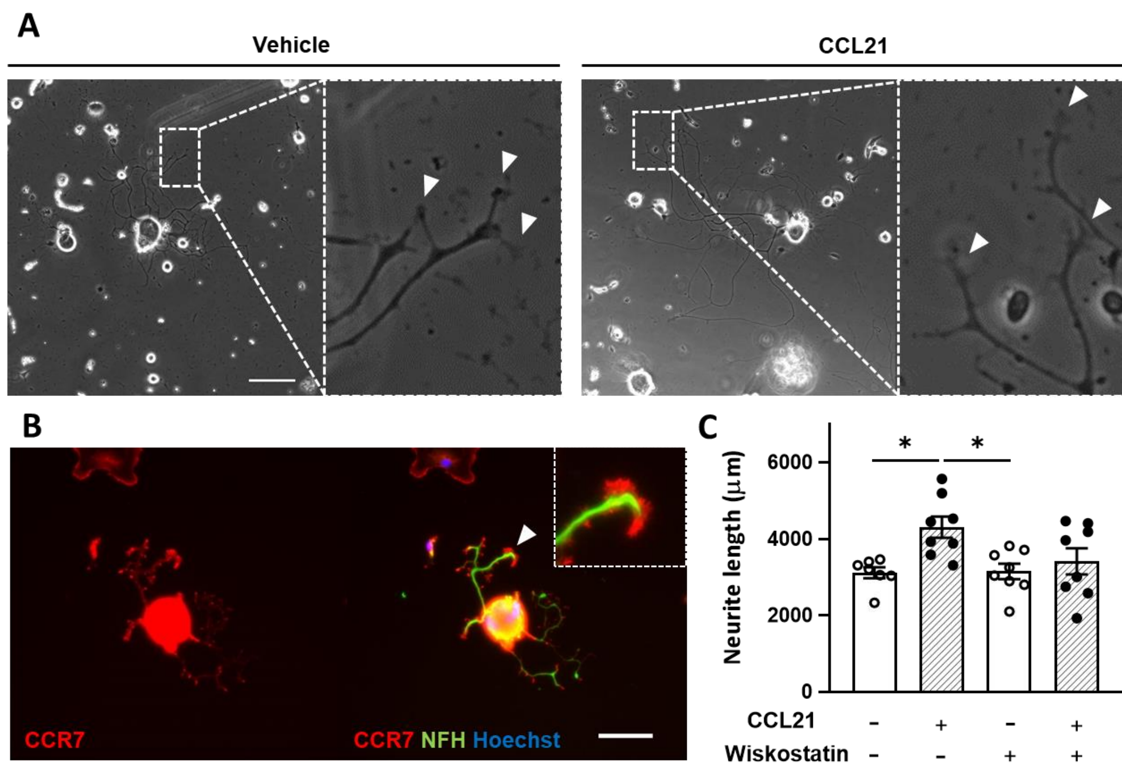

Figure 45. CCL21 acts locally on the growth cone. A. CCL21-treated growth cones seemed to protrude more than the vehicle-treated ones. Arrows in the magnified images indicate growth cones. Scale bar: 100µm. B. CCR7 (red) immunostaining showed high expression in the growth cone. Scale bar: 50µm. C. Wiskostatin, an inhibitor of actin branching, partially abolished the effect of CCL21. Data are expressed as mean neurite length per cell ±s.e.m. (n=7-8 wells). *p < 0.05 denotes significant differences in one-way ANOVA followed by Bonferroni test.

In summary, our data show that neuronal activity modulates the environment after injury by specific mechanisms which may be neutralized as a result of excessive inflammation and inhibitory substrates. Additionally, we have described how nociceptor activity triggers CCL21 secretion with subsequent differential roles in axon regeneration (Figure 46). In one hand, CCL21 has shown to enhance regeneration locally, through growth cone dynamics stimulation, upon

CCR7-MEK activation (Figure 47), while on the other, this regeneration is prevented as a result of CCL21 interaction with CXCR3.

Discussion

SCIs are severe lesions that generally lead to functional impairments. Moreover, as a result of their etiology, these injuries normally occur at relatively young ages, meaning patients will suffer their consequences for many years (Ahuja et al., 2017). Neurological deficits in this type of injury derive from the lack of regeneration of adult CNS axons, which fail to overcome both extrinsic and intrinsic barriers to regeneration in this system (Bradbury and Burnside, 2019; Mahar and Cavalli, 2018; Mesquida-Veny et al., 2021).

Activity-based therapies are among the leading clinical approaches for SCI patients. These therapies stimulate neuronal activity in order to promote activity-dependent plasticity (Carulli et al., 2011; Hogan et al., 2020). However, the mechanisms behind their success are not completely understood. Hence, while a proper molecular description is still lacking, fine-tuning of these therapies remains challenging as it relies on trial and error. With this in mind, the general objective of this thesis was to investigate the specific impact of neuronal activity in SCIs, as a way to better characterize the underlying molecular bases for activity-based therapies. For that, we focused on two different aspects: the direct effect of neuronal activity on axonal growth, as well as in cellular communication.

For the first part, we observed that specifically modulating neuronal activity at a cellular level effectively promoted axonal growth, however, and more importantly, this was prevented in the presence of growth inhibitory substrates. These results suggested that these discrete manipulations result in local alterations, with a positive effect in growth but not in a global intrinsic reprogramming of the regenerative capacities.

Secondly, we observed that cellular specific manipulations of neuronal activity did not result in significant changes in the injury inflammatory or glial environments in the CNS. However, detailed examination of acute neuronal secretion alterations revealed the existence of a complex cellular dialogue centered on CCL21. Specifically, increased activity in DRG nociceptors induced the secretion of this chemokine that in turn derived in subsequent differential consequences on regeneration: while it promotes growth in proprioceptors, regeneration *in vivo* is impaired due to a CCL21-linked mechanism in non-neuronal cells.

Our findings imply functional recovery induced by current clinical activity-based therapies may derive from the synergy of different mechanisms, in a more holistic manner.

1. Effects of neuronal activity modulation on axonal growth in regenerating and non-regenerating systems

While it is generally established that activity-based therapies rely on neuronal activity-triggered plasticity to promote recovery (Carulli et al., 2011; Hogan et al., 2020), this principle mostly

derives from work using exercise or electrical stimulation (as in (Carmel et al., 2013, 2010; Carmel and Martin, 2014; Engesser-Cesar et al., 2007; Goldshmit et al., 2008; Sánchez-Ventura et al., 2021)). Still, while these strategies boost neuronal activity, they lack cellular specificity and are therefore not best suited to characterize the events underlying this increased neuronal activity.

In relation to that, we first analyzed the effect of neuronal activity on axonal growth at the cellular level. Importantly, neuronal activity alters gene expression by promoting transcriptional, epigenetic and other structural changes (as summarized in Figure 12) (Flavell and Greenberg, 2008; Hogan et al., 2020; Madabhushi et al., 2015; Su et al., 2017; Tyssowski et al., 2018). With this in mind, we initially hypothesized that neuronal activity could trigger an intrinsic reprograming that induced a long-lasting pro-regenerative state in the specifically stimulated neurons. This hypothesis implied that the primordial mechanism for the success in activity-based therapies would be the increased neuronal activity itself that would generate a switch in individual neurons.

Therefore, we performed a series of experiments in which we benefitted from optogenetics and chemogenetics (Deisseroth, 2015; Sternson and Roth, 2014) to stimulate neuronal activity in specific neurons and examined its consequences on their axonal growth. The characterization of these particular manipulations in different neuronal models demonstrated that neuronal activity enhanced growth, but only in the absence of growth-inhibitory molecules.

1.1. Neuronal activity promotes growth in regenerating capable systems

In line with previous reports including (Jaiswal and English, 2017; Park et al., 2015; Sala-Jarque et al., 2020; Ward et al., 2016), our *in vitro* results together with PNS injury experiments revealed that increasing neuronal activity enhanced axonal growth in regenerating capable models. Moreover, also in accordance to another study, we showed these effects are maintained among cell types (Ward et al., 2018), in our case in both adult DRG neurons as well as embryonic cortical neurons. We believe the lack of statistical significance in cortical neuron experiments is caused by the intrinsic variability and heterogeneity of these cultures and should not disparage the observed differences, as the use of proper controls clearly denotes an impact of neuronal activity. For instance, high growing capacities at early stages in these embrionary neurons lead to overgrown cultures that hamper the quantification and could be diluting a greater impact of our manipulations.

Of note, a fine adjustment of the optogenetic paradigm was crucial in terms of axonal growth promotion, as this effect was highly dependent on the time of the stimulation after injury as well as its frequency. With a first bibliographic research we initially selected a 20Hz pulse frequency stimulation pattern, which had been successfully used in other studies using electrical stimulations (Al-Majed et al., 2000b; Udina et al., 2008), as well as in one with optogenetic stimulation (Park

et al., 2015), and later proved to be successful also in our experiments. Meanwhile, stimulating cortical neurons as early as 30 min after axotomy or with a 50Hz pulse frequency resulted in a sturdy reduction of axonal growth. Membrane disruption after axonal injury leads to Ca^{2+} influx, necessary for many processes in the generation of a growth cone and in the posterior regeneration (Bradke et al., 2012). However, exaggerate Ca^{2+} entrance and maintained neuronal depolarization leads to the activation of some VGCCs that inhibit growth (Enes et al., 2010; Goganau et al., 2018; Hilton et al., 2021; Tedeschi et al., 2016). This may likely be the cause of the decrease in axonal growth observed in the commented stimulation paradigms, as both of them result in excessive intracellular Ca^{2+}.

These data further demonstrate that neuronal activity needs to be tightly modulated to trigger the expected outcome, in accordance to previous studies showing differential gene expression (Lee et al., 2017; Miyasaka and Yamamoto, 2021; Tyssowski et al., 2018) and plasticity (reviewed in (Fauth and Tetzlaff, 2016)). In contrast to our findings, a previous study (Park et al., 2015), showed that frequencies above 20Hz could still induce axonal growth. These differences may result from the use of an uninjured model: whole DRG explants, that also contain trophic supporting cells such as satellite glia and Schwann cells, which may contribute to proper cationic homeostasis in the neighboring neurons. Unfortunately, we were not able to test the effects of neuronal activity in *in vitro* injured DRGs due to the limited amount of neurons obtained in DRG cultures (~100.000cells/animal) compared to our microfluidic device needs (200.000cells/device).

To avoid the commented variable outcomes of differential activity modulations, we leaned towards the technique yielding greater temporal resolution to manipulate neuronal activity: optogenetics (Rost et al., 2017). However, the anatomical location of the DRGs implied extremely invasive surgical procedures to insert the optical fiber for illumination, in prejudice of the welfare of the animals. Consequently, we used chemogenetics to tackle this inconvenience, which had a comparable impact on axonal growth than the observed *in vitro* with optogenetics, meaning *in vivo* chemogenetic stimulation in a model of peripheral regeneration also resulted in regeneration promotion. Noticeably, in this case we applied the chemogenetic stimulation prior to injury (preconditioning), yielding similar outcomes to those from other studies using a preconditioning approach with electrical stimulation (Goganau et al., 2018; Senger et al., 2018; Udina et al., 2008) or enriched environment (Hutson et al., 2019).

Therefore, we and others have shown that manipulating neuronal activity at the cellular level (Jaiswal and English, 2017; Ward et al., 2016) yields similar regeneration responses than electrical stimulations (Ahlborn et al., 2007; Al-Majed et al., 2004), indicating that neuronal activity *per se* might contribute to the growth-promoting effects of activity-based therapies.

1.2. The growth-promoting impact of neuronal activity is impaired by inhibitory environments

We then tested our findings in SCI mouse models, however, stimulating neuronal activity at the cellular level did not enhance functional recovery, as opposed to other studies with different activity-based strategies such as electrical stimulation (Carmel et al., 2013, 2010; Carmel and Martin, 2014), or physical activity (Engesser-Cesar et al., 2007; Goldshmit et al., 2008; Loy et al., 2018). Whether longer stimulation periods would have led to improvements remains however unanswered.

Nonetheless, in an attempt to elucidate whether regeneration was not induced in this model, or whether it did not translate into improved sensorimotor performance, we performed additional analysis. The leap to a CNS system entails the existence of intrinsic and extrinsic barriers for restoration (Bradbury and Burnside, 2019; Mahar and Cavalli, 2018; Mesquida-Veny et al., 2021), hence, we examined whether the lack of recovery could be a consequence of one of them. *In vitro* experiments showed that optogenetic stimulation promoted growth in laminin-coated substrates, but not in CSPG-coated substrates. Similarly, we did not observe increased axon regeneration beyond the injury site, however, when we analyzed spinal segments rostral to the injury, we did observe that stimulated animals presented an increased amount of regenerating axons, particularly in grey matter regions. These data revealed that while axonal growth is specifically increased in stimulated neurons, this gain-of-function is suppressed in the presence of growth-inhibitory molecules.

In accordance to our findings, recent work from another laboratory also found a lack of axonal growth in the presence of inhibitory molecules after specific increases in neuronal activity (Wu et al., 2020). In the same study, sensorimotor performance improvements after CNS injury were only observed after more than a month of daily neuronal activity modulation with chemogenetics in association with the administration of the CSPG-digesting enzyme, ChABC (Wu et al., 2020). Moreover, combining rehabilitation with therapies that eliminate CSPGs, with ChABC (García-Alías et al., 2009; D. Wang et al., 2011) or a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) (Griffin et al., 2020) results in magnified improvements, even when delivered at a delayed time-point (D. Wang et al., 2011). All these reports, together with our results, indicate that the extrinsic inhibitory barrier in the CNS reduces the efficiency of activity-based therapies, as well as they support the idea that combinatorial therapies are needed in order to approach the different features of the SCI pathophysiology (Griffin and Bradke, 2020). Still, the mechanisms behind each therapy as well as their impact need to be properly described in order to further optimize them and elicit their full potential, especially when combined, as, for

example, simultaneous treatment with anti-Nogo and exercise resulted in detrimental outcomes (Maier et al., 2009).

1.3. The role of specific neuronal activity in growth-promotion

As previously stated, our initial hypothesis was that neuronal activity itself was the main responsible for functional improvements in activity-based therapies, via induction of an intrinsic switch in the activated neurons, similar to what happens in DRGs after conditioning injury (Curcio and Bradke, 2018). However, the results obtained imply that activity *per se* has a rather moderate effect on intrinsic growing capacities.

First of all, modulating specifically neuronal activity did not promote recovery *in vivo*, as opposite to other activity-based therapies (such as in (Asboth et al., 2018; Carmel et al., 2010; Hutchinson et al., 2004; Hutson et al., 2019; Loy et al., 2018). This observation suggests that the success of activity-based therapies is beyond the specific intracellular mechanisms that neuronal activity by itself promotes.

Then, a large transcriptional or epigenetic switch would potentiate growth over the extrinsic barriers, as seen after the conditioning injury (Curcio and Bradke, 2018), HDAC3 inhibition (Hervera et al., 2019b) or PCAF overexpression (Puttagunta et al., 2014). Instead, neurons with increased neuronal activity were unable to overcome this inhibitory signaling.

Importantly, while neuronal activity robustly enhanced peripheral regeneration, we did not observe increased RAG expression (ATF3, BDNF, Cap23, Galanin and Sprr1a) at 24h after the last stimulation. These results suggest a lack of long-lasting neuronal reprogramming. Therefore, while RAG expression might be induced by the stimulation at shorter time-points, as seen after optogenetic stimulation of DRGs, when BDNF increases only from 2 to 5h after stimulation (Park et al., 2015), these levels are not maintained throughout time. Interestingly, this possibility is further supported by our *in vitro* experiments in which growth was promoted within one day after the optogenetic stimulation, while electric stimulation was unable to promote growth at that time point, which was attributed to a necessary time latency to induce a pro-regenerative state (Goganau et al., 2018; Udina et al., 2008).

Therefore, the success of activity-based therapies does not derive from specific activity modulations leading to a subsequent pro-regenerative state. Instead, other restricted mechanisms might underlie growth increases when modulating activity at a cellular level. Neuronal activity may still be altering gene regulation, as largely described (Flavell and Greenberg, 2008; Yap and Greenberg, 2018), and some of the modulated genes could be exerting an effect on growth, however, their impact seems to be rather punctual. Nevertheless, the effects of activity on growth may also result from other cellular consequences of neuronal activity, including increased

glycolysis with associated lipid incorporation to the membrane (Bas-Orth et al., 2017; Segarra-Mondejar et al., 2018). As a result of its nature, neuronal activity also modifies the distribution and the activation of several ionic channels (Enes et al., 2010; Misonou et al., 2004; Romer et al., 2016). This alteration can affect growth through ionic modulations and their subsequent consequences (Shim and Ming, 2010; Tedeschi et al., 2016), or even due to altered gene expression (Enes et al., 2010). Additionally, cytoskeleton dynamics have been previously linked to activity, for instance, microtubule polymerization in spines is increased by neuronal activity (Hu et al., 2008). In fact, neuronal activity stimulation led to boosted microtubule dynamics in the growth cone, as seen by increased labile microtubules (Wu et al., 2020). Using pharmacological inhibitors, the authors concluded that a reduction of acetylated tubulin was necessary for activity-mediated growth induction, while increasing tyrosination phenocopied the effects of the chemogenetic stimulation (Wu et al., 2020).

As a result of all the commented, activity-based therapies need to target activity in a holistic manner, raising the excitability of several networks at the same time, probably leading to plasticity and circuit reorganization (Courtine and Sofroniew, 2019). This is further evidenced by the fact that strategies targeting activity in a general manner, such as enriched environment, exert a more robust outcome than specific stimulations alone, as in the case of chemogenetic stimulation (Hutson et al., 2019). Remarkably, less specific stimulation paradigms activate spinal networks leading to residual circuit rearrangement, moreover, in recent studies, first in primates and later in patients, the combination of epidural stimulation with functional rehabilitation has surprisingly led to restoration of function even without stimulation (Asboth et al., 2018; Capogrosso et al., 2016; Wagner et al., 2018). In agreement to that, recovery in several studies using activity-based therapies has been found to derive from network reorganization, even after optogenetic stimulation leading to breathing restoration (Alilain et al., 2008; Eisdorfer et al., 2020; Loy et al., 2018). Sprouting, another form of plasticity, also underlies recovery after exercise or electrical stimulation (Carmel et al., 2013, 2010; Carmel and Martin, 2014; Engesser-Cesar et al., 2007; Goldshmit et al., 2008). Of note, many of these strategies rely on sprouting of spared fibers, but not of the injured ones. Accordingly, the level of recovery in these strategies is always greater as more incomplete are the injuries, presenting more spared tissue; for instance, contusion injuries need stronger stimulations than unilateral injuries (Carmel et al., 2014; Martin, 2016). The type of injury might also explain why in our hands optogenetic stimulation of the motor cortex failed to induce sensorimotor improvements, in contrast to others with a comparable stimulating approach (Deng et al., 2021), as compression injury leaves more uninjured fibers than our axotomy of the CST. Similarly, another study showed strong regeneration after optic nerve crush when applying visual or chemogenetic stimulations (Lim et al., 2016). This discrepancy may also

originate from a less invasive injury model. Apart from that, extrinsic barriers in complete injuries are magnified, hindering plasticity and reorganization of all systems in the spinal cord.

In parallel, and since our aim was to clarify the precise impact of neuronal activity in activity-based therapies, presuming it would lead to robust regeneration, we designed and optimized a system that allowed us to recover specifically a population of stimulated regenerating neurons using retrotracing and FACS. For the development of this system, we adapted the neuronal isolation protocol from previous studies (Arlotta et al., 2005; Catapano et al., 2001) to our needs. Importantly, and in contrast to these studies, we faced the challenge to isolate adult cortical neurons, which are extremely fragile, meaning they had to be processed with stricter delicacy, and even despite that, the proportion of recovered neurons would be lower. To minimize this loss, the sugar trehalose was incorporated to the cell suspension to preserve membrane integrity and increase cell viability (Saxena et al., 2012). Debris was also greater as a consequence of using adult brains, that contain greater amounts of myelin and a more complex ECM, so additional separation steps together with viability stainings had to be included. Unfortunately, since our paradigm did not induce relevant regeneration, at the end we were only able to successfully recover approximately 250 stimulated regenerating neurons per sample. However, we have successfully generated a system to isolate regenerating neurons which will surely be useful in other projects, since it could be applied to future studies were the manipulation leads to stronger regeneration.

All in all, we found that neuronal activity manipulation at the cellular level promoted growth only in the absence of adverse environments. Moreover, as stated, this strategy fails to reach the promising outcomes of other non-specific approximations. These findings, together with several other previous studies indicate that the success in activity-based therapies relies on restricted alterations in specifically modulated neurons (instead of on a recapitulation of their regenerating capacities), together with a raised excitability and plasticity of several networks in the spinal cord.

2. Role of neuronal activity in cell-to-cell communication after injury

In line with our main objective, we intended to extend our knowledge about the effects of specifically modulating neuronal activity in SCIs, so we evaluated whether it could alter the neuronal dialogue with other cells. In that line, research has shown that in many healing tissues, the key for injury resolution is cellular communication, as these interactions orchestrate the different injury phase transitions (Gurtner et al., 2008; Wynn and Vannella, 2016). However, after CNS injury this process is disrupted causing chronic inflammation (Mesquida-Veny et al., 2021). Neuronal activity may lead to secretome alterations, as seen by increased exosome and BDNF

release (Chivet et al., 2014; Wong et al., 2015). Therefore, we considered the possibility that neuronal activity modulation would be affecting cell-to-cell communication after injury.

Importantly, while we did not observe changes neither in the scar nor in the chronic environment of the injury after modulating neuronal activity, it allowed us to identify a specific activity-triggered mechanism in nociceptor cells with a bivalent effect on regeneration. Consequently, we identified two new roles of CCL21, the mediator of these effects, depending on the subsequently activated receptor and cell type.

2.1. Neuronal activity does not modify the general environment after injury

In a first approach, we evaluated the final impact of activity on two of the fundamental characteristics of the SCI environment: the glial scar and the inflammatory conditions. While other studies had observed reduced glial scar in exercised animals (Loy et al., 2018; Sandrow-Feinberg et al., 2009), our optogenetic stimulation paradigm did not yield the same results, a discrepancy which may derive again from more global repercussions of exercise, such as release of neurotrophins and pro-resolving cytokines by non-neuronal cells (reviewed in (Bilchak et al., 2021; Cerqueira et al., 2020). However, deeper examination is required in order to exclude transient changes, already observed after exercise (Loy et al., 2018). Similarly, cytokine levels in the injured spinal cord remained unaffected after the optogenetic stimulation. In this case, we altered the time-point of analysis (11DPI), which was selected to allow at least a round of 5 day stimulation, and to analyze the injury environment before chronification of the inflammation, as at this approximate time-point CNS injuries start to differ from healing ones, failing to start the resolving phase (Mesquida-Veny et al., 2021; Novak and Koh, 2013). In line with our results, enriched environment did not alter the cytokinome of the injured spinal cord (Hutson et al., 2019).

Neuronal activity modulates microglial actions during development and homeostasis (Ferro et al., 2021). Microglia respond to increased neuronal activity with alterations in Ca^{2+} levels and process dynamics (Ferro et al., 2021; Tremblay et al., 2010; Umpierre et al., 2020). Interestingly, IL-33 was found to contribute to activity-mediated neuron-microglia communication (Nguyen et al., 2020). Moreover, exercise reduced microglial activity after SCI (Chhaya et al., 2019), while electrical stimulation increased the numbers of pro-resolving macrophages in demyelinated nerves (McLean and Verge, 2016). These studies suggest that activity-based interventions can reshape cellular dialogues even after injury, however, as we have observed, punctual interactions are insufficient to restore balance and avoid SCI chronification, due to the excessive production of pro-inflammatory molecules (Kroner et al., 2014; Mesquida-Veny et al., 2021).

2.2. Involvement of CCL21 in activity-triggered cell communication with subsequent effects on axonal regeneration

As stated, it is clear that neuronal activity can alter the neuronal signaling to other cells. In relation to that, our experiments show an induction in the expression and release of the chemokine CCL21 upon neuronal stimulation. Importantly, as previously described, we showed that specific nociceptor activation derived in CCL21 production (Zhao et al., 2007). Nevertheless, the contribution of other neuronal types to CCL21 production in less specific stimulations cannot be excluded.

In order to describe how this activity-triggered mechanism of cell communication could influence the injury environment or even axonal growth, we further studied the impact of CCL21 in these paradigms. This neuronal chemokine had already been described for its role in neuropathic pain induction, as injured nociceptors also release this chemokine (Biber et al., 2011; De Jong et al., 2005), however, its role in axon regeneration had never been described. Surprisingly, our results revealed a bivalent role of CCL21 in axon regeneration (Figure 46), as it induces growth in proprioceptor neurons in a CCR7-MEK-ERK-dependent manner, with a final effect in growth cone dynamics, as observed by our *in vitro* results, but these effects are counteracted by CXCR3 activation in non-neuronal cells.

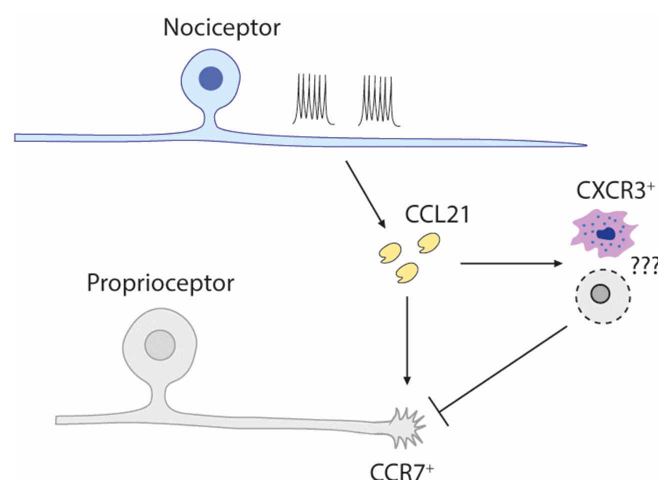

Figure 46. Proposed mechanism for nociceptor-derived CCL21 in axonal injuries. Neuronal activity induces the release of CCL21 in nociceptors. This CCL21 enhances axonal regeneration in the CCR7-expressing growth cones of proprioceptors, while CXCR3^{+} cells respond to the CCL21 secretion suppressing this induced axonal growth.

2.2.1. CXCR3-mediated growth inhibition

To this day, three receptors have been described to interact with CCL21: CCR7, CXCR3 and ACKR4. CCR7 is the canonical receptor of CCL21 and mediates its largely role known in

leukocyte attraction to lymphoid organs (Comerford et al., 2013; Förster et al., 2008). Moreover, it has been shown that CCL21 can attract microglia via the CXCR3 receptor (Biber et al., 2001; Rappert et al., 2002), while the scavenging receptor ACKR4 participates in CCL21 clearance from the extracellular space (Bastow et al., 2021; Ulvmar et al., 2014). Motivated by the presence of CXCR3 in the nervous system (Goldberg et al., 2001; Zhou et al., 2019), as well as the observation of CXCR3 expression in macrophages, in accordance to previous findings (Butler et al., 2017; Pandey et al., 2021), we analyzed the role of this receptor in axonal regeneration. CXCR3 inhibition after sciatic nerve injury, which has been reported to induce the expression and release of CCL21 (Biber et al., 2011) resulted in a clear promotion of neuritic growth in *ex vivo* cultures, pointing towards a detrimental effect of CXCR3 on growth after injury. In agreement with these results, deletion of CXCR3 leads to enhanced functional improvements after SNC (Jeub et al., 2020).

We next checked the expression pattern of this receptor in the DRG, and we did not observe any neuronal expression, and while another study showed that nerve injury triggers its neuronal expression, this occurs at much later time-points after injury (Kong et al., 2021). We then targeted macrophages as the most likely candidates to mediate the CCL21 effects, as already described in other pathologies (Van Raemdonck et al., 2020). Moreover, it has been shown that interaction of CXCR3 with its ligands can induce macrophage activation while driving their phenotype (Hasegawa et al., 2021; Pandey et al., 2021).

Still, the precise mechanism behind the CCL21-CXCR3-dependent growth blockade remains to be elucidated. Our experiments using macrophage-derived conditioned-media showed that macrophage secretome and/or their extracellular vesicles (EVs) were not responsible for altering the regeneration capacities in neurons, which was the case for other previous studies (Hervera et al., 2018; Kigerl et al., 2009; Kwon et al., 2015). We cannot exclude however another kind of neuron-macrophage interaction driving these effects, such as direct contact or the participation of a third mediator. Indeed, macrophages have been described to establish physical interactions with damaged neurons (Iwai et al., 2021) and besides, a direct contact is required for a macrophage/microglia-mediated growth restriction (Hata et al., 2006; Kitayama et al., 2011) and dystrophic axon dieback (Busch et al., 2009; Horn et al., 2008). Meanwhile, mechanisms involving several steps of communication have also been described, highlighting the importance of complex dialogues with more than two different actors, for instance, neuronal CX_3CL1 has been shown to induce CXCL16 expression both in microglia and astrocytes, promoting CCL2 production in this last cell-type, and eventually increasing neuronal survival (Rosito et al., 2014, 2012). Nonetheless, our experiments using concentrated conditioned media do not allow to discard an effect of small molecules, as this media contained predominantly molecules above 10kDa, as a result of the concentration protocol used.

On the other hand, CXCR3 presents other described ligands: CXCL9, CXCL10 and CXCL11 (Karin, 2020). Therefore, it is possible that, instead of CCL21, the CXCR3-mediated growth blockade is activated by one of these other ligands. Nerve injury elevates neuronal CCL21 expression already from early time points (12-24h post-injury) (Biber et al., 2011). Meanwhile, CXCL9, CXCL10 and CXCL11 alterations seem to occur at later time-points (Kong et al., 2021; Piotrowska et al., 2018). According to that, an influence of these chemokines is rather unlikely, as our *ex vivo* culture was performed at 24h after the SNC. Moreover, CCL21 administration after SNC did not alter regeneration, which we believe results from the CXCR3-mediated inhibition counterbalanced by a CCR7-dependent growth induction, which will be later further discussed. Nevertheless, further experiments combining the pharmacological inhibitor NBI-74330 with genetic deletion of CCL21 and/or the other ligands of CXCR3 would help to corroborate our hypothesis.

Additionally, we cannot exclude the participation of other supporting cells such as satellite glia, Schwann cells or even less abundant leukocytes in the mounting of these inhibitory effects, either directly or through interaction with other non-neuronal cells. To this aim a thorough characterization of the different CXCR3^{+} subpopulations, and the relative presence of migratory leukocytes after CCL21 using state-of-the-art separation and molecular characterization techniques is needed, in order to unveil the precise mechanisms behind these effects.

On another note, CCL21 administration *in vivo* lowered the levels of several chemokines. Importantly, some of these chemokines, specifically CCL3 and CXCL10 have been shown to induce microglial activation and pro-inflammatory molecule secretion (Ha et al., 2015; Sun et al., 2016). Therefore, at a first glance, CCL21 would seem to reduce the inflammatory response, which contrasts to previous findings showing reduced pro-inflammatory cytokines in CCL21-deficient mice after SCI at later timepoints (Honjoh et al., 2019). However, these results might be useful to explain the presence or absence at early time-points of other migratory leukocytes that might be involved in the triggering of the underlying effects. Still, as above mentioned, further exploration is needed in order to determine the precise interactions and whether these findings are linked to the subsequent regeneration blockade.

2.2.2. CCR7-mediated growth enhancement

The observation that *ex vivo* neurons are primed to regenerate after CXCR3 inhibition indicated a final effect on the intrinsic growth capacities of neurons, and while we cannot exclude other chemokines to be involved, we thought that CCL21 might be responsible, at least to part of this additional growth, moreover, direct administration of this chemokine did not reduce regeneration after SNC. These observations led us to hypothesize that CCL21 could be promoting axonal regeneration through a different mechanism. Consistently, *ex vivo* as well as *in vitro*

administration of this chemokine resulted in increased neuritic growth in large-diameter proprioceptive neurons. Importantly, the observed growth induction appears to be dose-dependent, and specifically neuronal.

Interestingly, similar roles have already been described for other chemokines, as CCL7, CCL20, and CXCL2 induce growth *in vitro* (Bhardwaj et al., 2013) and CXCL12 has been shown to promote growth even after a SCI in rodents (Opatz et al., 2009; Pujol et al., 2005). As commented, CCL21 was first described for its role in recruiting leucocytes, and therefore has a primordial role in stimulating the migration of dendritic cells as well as several T cell subpopulations (Britschgi et al., 2010; Förster et al., 2008; Scandella et al., 2004; Shannon et al., 2010), but can also induce migration in other cell types such as tumorigenic cells or MSC (Joutoku et al., 2019; Li et al., 2014; Mo et al., 2015; Sasaki et al., 2008; Xiong et al., 2017). Remarkably, migration and axonal growth are processes that share many characteristics and molecular mechanisms, and indeed, the leading edge of migratory cells and the axonal growth cone are very similar cellular structures (Aberle, 2019; Cooper, 2013). Therefore, it is not surprising to find molecules promoting both processes, such as what happens with CXCL12 (Borrell and Marín, 2006; Doitsidou et al., 2002; Opatz et al., 2009; Pujol et al., 2005).

In our particular case, we found that CCL21 was exerting its function through the CCR7 receptor. Additionally, expression of this receptor was reliably found in proprioceptor neurons. In accordance, neuronal expression of CCR7 had also been described before in other studies, specifically in peripheral nerves as well as in the hippocampus (Hirth et al., 2020; Liu et al., 2007). However, these studies did not provide a proper description of its role. Our findings regarding the implication of CCR7 in axonal growth match the already defined role of the CCL21-CCR7 axis in cell migration (Comerford et al., 2013; Li et al., 2014; Mo et al., 2015; Shannon et al., 2010; Xiong et al., 2017).

Interestingly, the other ligand of CCR7, CCL19, was not found to induce axonal growth in our experiments. While this result might seem controversial at first, as CCL19 presents a similar role to CCL21 in immune cell homing to lymph nodes (Förster et al., 2008), these chemokines have shown to trigger slightly divergent activations in CCR7 (Hauser and Legler, 2016; Jørgensen et al., 2018). This biased activations are believed to result from the impact each of the ligands exerts on the conformation of the receptor, and has been recently described to depend on variations in the core region of these chemokines (Hauser and Legler, 2016; Jørgensen et al., 2019). In that line, the general hypothesis is that both chemokines induce shared signaling pathways, but CCL19 does so in a more robust manner (Hauser and Legler, 2016; Jørgensen et al., 2018). Despite that, some particular mechanisms seem to be activated preferentially by CCL21, an effect that seems

to differ depending on the cell type, as seen for example in the case of ERK1/2 activation (Corbisier et al., 2015; Hjortø et al., 2016; Kohout et al., 2004; Shannon et al., 2012).

As a GPCR, CCR7 modulates several signaling pathways (Rodríguez-Fernández and Criado-García, 2020). Pharmacological inhibition of MEK-ERK pathway, one of the described mediators of CCR7, was able to inhibit the effect of CCL21 in our experiments, indicating a CCR7-MEK-ERK-dependent mechanism. This finding is in accordance with previous studies describing a role of this pathway in the CCL21-CCR7-mediated chemotaxis (Riol-Blanco et al., 2005; Shannon et al., 2010). These studies also showed that G_i protein signaling was regulating the MEK-ERK pathway activation (Riol-Blanco et al., 2005; Shannon et al., 2010). While in our experiments inhibiting the G_i protein did not completely abrogate the CCL21 effect, a similar regulation could still be occurring, as the lack of growth-suppression could derive from other opposing pathways activated by the G_i inhibition.

Our work has therefore identified a mechanism that promotes growth in a MEK-ERK-dependent manner upon the CCL21-CCR7 interaction. Previous work has already demonstrated an implication of the MEK-ERK pathway in axon regeneration (as in (Chierzi et al., 2005; Perlson et al., 2005; Puttagunta et al., 2014). Particularly, ERK has been described to be phosphorylated after peripheral injury and to function as a retrograde signal to the cell soma, leading to transcription alterations, including increased RAG expression ((Perlson et al., 2005; Puttagunta et al., 2014). Nevertheless, in our model we did not observe any change in RAG expression after CCL21 addition to neurons, indicating our mechanism was not increasing their regenerative capacity through a transcriptional reprogramming.

In addition to its impact on gene expression, the MEK-ERK pathway also directly regulates other cellular mechanisms (Tanimura and Takeda, 2017). Accordingly, several studies have shown an impact of this pathway on actin polymerization as a result of WAVE2, cortactin and Rac1 phosphorylation (Danson et al., 2007; Martinez-Quiles et al., 2004; Mendoza et al., 2011; Nakanishi et al., 2007; Tong et al., 2013), as well as in cellular adhesion, resulting from the phosphorylation of other proteins, including FAK and paxillin (Hunger-Glaser et al., 2003; Ishibe et al., 2004).

In an attempt to further clarify the mechanism underlying the growth promoting effect of CCL21, we focused on the growth cone, where we found CCR7 expression to be particularly elevated. Actin polymerization is a key process in this structure, and, as stated, it can be modulated by the downstream effectors of CCL21-CCR7, namely the MEK-ERK pathway (Leite et al., 2021; Tanimura and Takeda, 2017). Pharmacological inhibition of N-WASP, one of the regulators of actin polymerization, almost completely prevented CCL21 effects. This protein is a well-known Arp2/3 activator, which is in turn an actin-binding protein responsible for actin filament branching

(Pollard, 2016). In accordance to our results, other studies previously revealed a requirement for Arp2/3 for growth cone expansion after treatment with several growth stimulating cues (San Ruiz-Miguel and Letourneau, 2014; Schlau et al., 2018). Moreover, ERK has been shown to phosphorylate cortactin, allowing it to bind to N-WASP, ultimately mediating its activation (Martinez-Quiles et al., 2004). These discoveries suggest a feasible mechanism linking CCL21-dependent CCR7 activation and actin branching stimulation (Figure 47): CCL21 locally interacts with its receptor CCR7 at the axonal tip, leading to ERK activation, which in turn enables cortactin (via its phosphorylation) to bind to N-WASP, with a final effect on Arp2/3 complex activation. Eventually, this pathway would trigger actin filament ramification with subsequent increased neuritic growth.

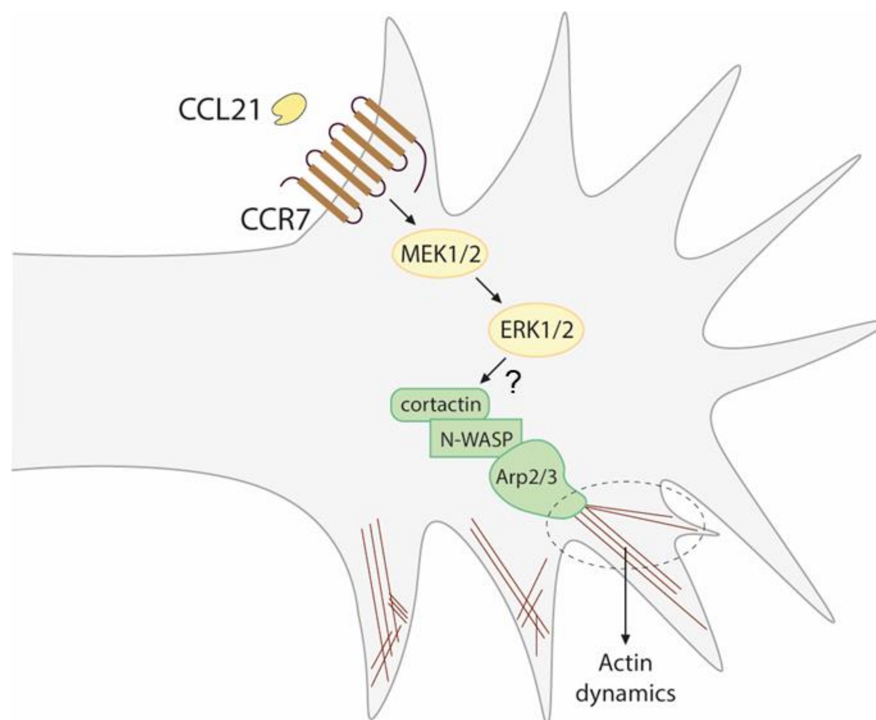

Figure 47. Proposed mechanism underlying CCL21-induced growth. In proprioceptors, CCR7 in the growth cone activates a pathway that modulates the Arp2/3 function, probably via ERK phosphorylation of some of their interacting components. CCL21-induced growth seems therefore to depend on actin branching promotion.

Altogether, our findings provide evidence for a role of CCL21 in cytoskeleton rearrangements in the growth cone. Nevertheless, more work would be needed in order to further characterize this working model. Indeed, the lack of interaction significance in the N-WASP inhibition experiments probably derives from the action of CCL21 on other cytoskeletal effectors, as described in other models (Riol-Blanco et al., 2005; Rodríguez-Fernández and Criado-García, 2020; Torres-Bacete et al., 2015), further highlighting the need for additional experiments to better characterize all the mechanisms involved. For instance, in dendritic cells, downstream effectors of CCR7 inactivate cofilin, leading to actin polymerization while actin contraction is

also affected as a result of MLC activation alterations (Riol-Blanco et al., 2005; Rodríguez-Fernández and Criado-García, 2020; Torres-Bacete et al., 2015). As we have not excluded the possibility that these other CCR7-activated pathways may be contributing to our observations, it would be very interesting to test their possible influence in our model.

All in all, we have identified the chemokine CCL21 as a cue for regeneration of proprioceptive neurons, expanding its previously known role in regeneration in other tissues including skin, cartilage and vascular remodeling (Joutoku et al., 2019; Nossent et al., 2017; Sasaki et al., 2008).

On another note, some issues have to be discussed regarding the therapeutic implications of our findings. Our *in vivo* experiments show that the sole application of CCL21 does not induce axon regeneration, a fact that we attributed to its growth-inhibitory effects via CXCR3 activation. Experiments combining CCL21 administration with the use of CXCR3 inhibitors after injury are needed in order to further corroborate this hypothesis and to verify its efficacy as a treatment for axon regeneration. Moreover, while CCL21 has been shown to induce neuropathic pain (Biber and Boddeke, 2014), CXCR3 might be the mediator for these effects, and its inhibition contributes to attenuate it (Biber and Boddeke, 2014; Piotrowska et al., 2018). However, as a complete description of the effects of this chemokine on the other cells in the injury is still missing, with our current knowledge, the most optimal strategy would be to directly stimulate the impact of this chemokine on the growth cone.

2.3. A complex pain-dependent mechanism promoting regeneration

During the course of this thesis we have described a new neuron-neuron dialogue, with a final effect on growth. First of all, this mechanism involves a paracrine communication system between two different neuronal types: nociceptors and proprioceptors. While neurons are highly interconnected through synapses, little is known about their other forms of communication (Herrmann and Broihier, 2018). Growth factors, such as BDNF or vascular endothelial growth factor (VEGF), have been implicated in both paracrine and autocrine neuronal dialogues (Acheson et al., 1995; Cheng et al., 2011; Froger et al., 2020; Ogunshola et al., 2002). Whether these particular interplays are accentuated by neuronal activity requires further exploration, as some studies claim to observe activity-triggered neurotrophin increases, while others present contrasting results (Hutson et al., 2019; Keeler et al., 2012; Molteni et al., 2004; Park et al., 2015). Remarkably, we have described a chemokine as a messenger to mediate neuron-neuron signaling. Chemokines were classically described for their role in immune cell communication, however, growing research is now showing their implications in other cell type dialogues (De Haas et al., 2007; Jaerve and Müller, 2012; Mesquida-Veny et al., 2021). In parallel to our findings, another chemokine, CCL2 has also been proposed to be released by neurons and directly act on them (reviewed in (Biber and Boddeke, 2014)). These data encourage the idea that chemokines might

represent a fundamental form of communication between neurons, especially after injury, and therefore, the role of other neuronal chemokines should be studied in this context.

Then, and even more remarkably, we have elucidated a novel role for nociception in sensory axon regeneration. Nociception and pain normally occur upon injury to any tissue (Baliki and Apkarian, 2015; Woller et al., 2017), and while it is not surprising that a signal triggered by the injury promotes regeneration, similarly to what happens in normally healing tissues, after nerve injury nociceptor activation is often attributed to pathological maladaptive neuropathic pain. Indeed, several injury signals including a Ca^{2+} wave, ROS production and mechanotransduction contribute to healing via leucocyte recruitment inducing a subsequent inflammatory phase, as well as they may directly stimulate tissue regeneration (Enyedi and Niethammer, 2015; Hervera et al., 2019a). However, the implication of nociception is only beginning to be elucidated. Meanwhile, the contrary relationship has received more attention, as undesired plasticity upon injury often leads to neuropathic pain (Costigan et al., 2009).

Our experiments show that increased nociceptor activity leads to the secretion of a growth-promoting chemokine, CCL21. In line with this, other studies have also suggested a role for nociception in tissue regeneration, as nociceptor activation has been shown to lead to adipose tissue regeneration, as a result of CGRP (calcitonin gene-related peptide) production (Rabiller et al., 2021), angiogenesis via the effects of Substance-P (Amadesi et al., 2012), and skin healing, in this case through an impact on immune cells (Wei et al., 2020). Meanwhile, another study showed reduced recovery after pain induction in a model of SCI (Turtle et al., 2018), however, adverse consequences in this case might be related to excessive nociceptive induction, which can lead to neuronal death (Pecze et al., 2013). Contrarily, as stated, a physiological-like activation seems to play an important role for regeneration. Pain may therefore be a more complex evolutionary mechanism than we had previously anticipated, as it seems to orchestrate and promote repair beyond its mere warning function against hazardous situations. This idea also raises a question about the adequacy of the use of strong analgesics such as opioids after injury, in a similar manner to what happened with the use of indiscriminate immunosuppressive drugs after SCIs (Donnelly and Popovich, 2008). Therefore, as the analgesic effect might be hindering regeneration at the same time, a better description of the impact of these drugs on healing at different stages after injury would help to optimize their applications and tailor current therapies (Berthézène et al., 2021).

3. New mechanisms underlying activity-based therapies

The term activity-based therapy refers to a set of strategies, from methodologically simpler ones like rehabilitation, to really complex strategies that provide spatiotemporally tailored stimulations, all of them deriving in a final raise of neuronal activity (Courtine and Sofroniew,

2019). While these strategies have shown promising results after SCI, their degree of success remains limited, which is why many research efforts are trying to understand how they work, and why they fail. Understanding their physiological and molecular bases is key in order to generate finely tailored strategies, as well as to design new combinatorial therapies that counteract the pitfalls of current individual ones (Griffin and Bradke, 2020).

Over the years, many studies have tried to shed light on this particular issue, however, little work has focused on the particular effect of neuronal activity, as the selected strategies did not allow for cellular-specific studies. During the course of this thesis, we have investigated various mechanisms underlying activity-based therapies upon discrete manipulations of neuronal activity, taking advantage of optogenetic and chemogenetic approaches.

First of all, we have uncovered the fact that neuronal activity alone, despite displaying a growth-promoting effect, does not seem to trigger a potent own neuronal reprogramming by itself. In turn, this indicates that the success of activity-based strategies relies on both specifically locally-guided alterations, as well as on a general increase on plasticity promoted by a holistic state of neuronal activation. Then, and in accordance to that, we identified a new mechanism of neuronal communication, initiated by neuronal activity, with a final effect on growth.

Therefore, we propose that neuronal activity promotes growth not only in the activated neurons, but also on other neurons as a result of their communication, increasing plasticity in a broader way. This idea is reinforced by the fact that increasing activity in a more general manner exponentially increments excitability and neuronal plasticity in comparison to other more specific interventions (Courtine and Sofroniew, 2019): as more neurons are modulated, their signaling reaches and engages a larger number of receptor neurons. In parallel with our results, another study showed that optogenetic stimulation of DRGs enhanced growth in adjacent non-stimulated DRGs (Park et al., 2015). While we only investigated chemokine alterations, and the influence of neurotrophins remains controversial in this context (Hutson et al., 2019; Keeler et al., 2012; Molteni et al., 2004; Park et al., 2015), it would be also interesting to search for other neuronal-secreted molecules that could be mediating this plasticity, including cytokines, neuropeptides, other growth factors and secreted proteins or even EVs.

Finally, regarding this particular issue, it is also worth commenting that the configuration of neurons and tracts, and the discovery of neuron-neuron forms of communication with an influence on growth would open the possibility to target areas with low anatomical accessibility.

In addition, the discovery of this particular cellular dialogue reinforces the idea that the success of activity-based therapies results from the synergy of many individual mechanisms that affect excitability and plasticity. In a similar manner, increased plasticity may also be aided by ECM modifications, as neuronal activity seems to increase the action of metalloproteases, responsible

for CSPG cleavage, among others (Foscarin et al., 2011; Michaluk et al., 2007). Finally, neurons may also signal to other cells, also paving the way for increased repair, as indeed, several studies have shown neuronal activity boosts myelination (Gibson et al., 2014; Mitew et al., 2018; Ortiz et al., 2019).

On the other hand, the mechanism behind CCL21-growth promotion has further revealed the importance of cytoskeleton alterations for axonal growth. While these local modulations do not seem to be as effective as targeting a growth-modulating gene, they should not be underestimated, as they present less off-targets as well as increased safety. In line with that, other cytoskeleton-targeted drugs have already shown efficacy in the treatment for SCI, specifically the microtubule stabilizing drugs epothilones B and D have resulted in functional amelioration in rodents (Ruschel et al., 2015; Ruschel and Bradke, 2018). Importantly, and as stated, neuronal activity seems to promote cytoskeletal dynamics (Hu et al., 2008; Wu et al., 2020). Thus, examining our results from the first chapter, we observe many coincidences with the effect of CCL21. These observations lead us to propose increased cytoskeleton dynamics as a reasonable mechanism underlying activity-mediated growth induction. Moreover, this mechanism would match with the lack of grow of stimulated neurons on CSPGs, as a recent study has demonstrated CSPGs impair growth by RhoA-induced actomyosin compaction, impeding microtubule extension (Stern et al., 2021). Therefore, even though activity promotes microtubule dynamism, their elongation is physically and biochemically impeded by actin contraction.

Finally, our results also contribute to the idea that cytoskeletal dynamics can also prime neurons to recapitulate their growing capacities (Tedeschi et al., 2019), a mechanism that had been attributed solely to a pro-regenerative transcriptional switch. In this regard, recent work has shown that the conditioning injury paradigm leads to and requires increased actin dynamics (Pinto-Costa et al., 2020; Tedeschi et al., 2019). Similarly, we found that *ex vivo* CCL21 administration reactivated a neuronal growing state, presumably via actin cytoskeleton modulation. Again, a comparable circumstance was also found when increasing neuronal activity prior to PNS injury, as we did not observe RAG expression alterations but the regeneration capacities were already primed. These similarities further evidence the possible role of cytoskeletal alterations upon long term activity-triggered growth.

Conclusions

1. Neuronal activity promotes growth in stimulated neurons

2. Specific short-term stimulations of neuronal activity do not promote recovery after SCI

3. Activity-induced growth does not result from a pro-regenerative reprogramming

4. Activity-induced growth cannot overcome the inhibitory signaling induced by growth inhibitory molecules

5. The combination of spinal retrotracing with subsequent FACS of corticospinal motor neurons allows to successfully recover regenerating neurons for specific molecular studies.

6. Specific short-term modulations of neuronal activity are insufficient to alter the general injury environment after SCI

7. Nociception promotes the release of CCL21 with bivalent effects in axonal regeneration

8. Non-neuronal CXCR3 blocks axonal regeneration affecting intrinsic growth capacities of neurons

9. The CCL21-CCR7-MEK-ERK axis enhances growth in proprioceptor neurons through modulation of the cytoskeleton in the growth cone

10. Activity-based therapies rely on the synergy of several mechanisms and circuits for their success

References

Abe, N., Borson, S.H., Gambello, M.J., Wang, F., Cavalli, V., 2010. Mammalian Target of Rapamycin (mTOR) activation increases axonal growth capacity of injured peripheral nerves. J. Biol. Chem. 285, 28034–28043. https://doi.org/10.1074/jbc.M110.125336

Aberle, H., 2019. Axon guidance and collective cell migration by substrate-derived attractants. Front. Mol. Neurosci. 12, 1–7. https://doi.org/10.3389/fnmol.2019.00148

Acheson, A., Conover, J.C., Fandl, J.P., DeChiara, T.M., Russell, M., Thadani, A., Squinto, S.P., Yancopoulos, G.D., Lindsay, R.M., 1995. A BDNF autocrine loop in adult sensory neurons prevents cell death. Nat. 374, 450–453. https://doi.org/10.1038/374450a0

Agarwal, P., Zaidel-Bar, R., 2019. Principles of Actomyosin Regulation In Vivo. Trends Cell Biol. 29, 150–163. https://doi.org/10.1016/j.tcb.2018.09.006

Ahlborn, P., Schachner, M., Irintchev, A., 2007. One hour electrical stimulation accelerates functional recovery after femoral nerve repair. Exp. Neurol. 208, 137–144. https://doi.org/10.1016/j.expneurol.2007.08.005

Ahuja, C.S., Wilson, J.R., Nori, S., Kotter, M.R.N., Druschel, C., Curt, A., Fehlings, M.G., 2017. Traumatic spinal cord injury. Nat. Rev. Dis. Prim. 3. https://doi.org/10.1038/nrdp.2017.18

Ajiboye, A.B., Willett, F.R., Young, D.R., Memberg, W.D., Murphy, B.A., Miller, J.P., Walter, B.L., Sweet, J.A., Hoyen, H.A., Keith, M.W., Peckham, P.H., Simeral, J.D., Donoghue, J.P., Hochberg, L.R., Kirsch, R.F., 2017. Restoration of reaching and grasping movements through brain-controlled muscle stimulation in a person with tetraplegia: a proof-of-concept demonstration. Lancet (London, England) 389, 1821–1830. https://doi.org/10.1016/S0140-6736(17)30601-3

Akimoto, N., Honda, K., Uta, D., Beppu, K., Ushijima, Y., Matsuzaki, Y., Nakashima, S., Kido, M.A., Imoto, K., Takano, Y., Noda, M., 2013. CCL-1 in the spinal cord contributes to neuropathic pain induced by nerve injury. Cell Death Dis. https://doi.org/10.1038/cddis.2013.198

Al-Ali, H., Beckerman, S.R., Bixby, J.L., Lemmon, V.P., 2017. In vitro models of axon regeneration. Exp. Neurol. 287, 423–434. https://doi.org/10.1016/j.expneurol.2016.01.020

Al-Majed, A.A., Brushart, T.M., Gordon, T., 2000a. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. Eur. J. Neurosci. 12, 4381–4390.

Al-Majed, A.A., Neumann, C.M., Brushart, T.M., Gordon, T., 2000b. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. J. Neurosci. 20, 2602–2608. https://doi.org/10.1523/jneurosci.20-07-02602.2000

Al-Majed, A.A., Tam, S.L., Gordon, T., 2004. Electrical Stimulation Accelerates and Enhances Expression of Regeneration-Associated Genes in Regenerating Rat Femoral Motoneurons. Cell. Mol. Neurobiol. 24, 379–402. https://doi.org/10.1023/b:cemn.0000022770.66463.f7

Alexeeva, N., Calancie, B., 2016. Efficacy of QuadroPulse rTMS for improving motor function after spinal cord injury: Three case studies. J. Spinal Cord Med. 39, 50–57. https://doi.org/10.1179/2045772314Y.0000000279

Alilain, W.J., Li, X., Horn, K.P., Dhingra, R., Dick, T.E., Herlitze, S., Silver, J., 2008. Light-induced rescue of breathing after spinal cord injury. J. Neurosci. 28, 11862–11870. https://doi.org/10.1523/JNEUROSCI.3378-08.2008

Alizadeh, A., Dyck, S.M., Karimi-Abdolrezaee, S., 2019. Traumatic spinal cord injury: An overview of pathophysiology, models and acute injury mechanisms. Front. Neurol. 10, 1–25. https://doi.org/10.3389/fneur.2019.00282

Amadesi, S., Reni, C., Katare, R., Meloni, M., Oikawa, A., Beltrami, A.P., Avolio, E., Cesselli, D., Fortunato, O., Spinetti, G., Ascione, R., Cangiano, E., Valgimigli, M., Hunt, S.P., Emanueli, C., Madeddu, P., 2012. Role for substance P-based nociceptive signaling in progenitor cell activation and angiogenesis during ischemia in mice and in human subjects. Circulation 125, 1774–1786. https://doi.org/10.1161/CIRCULATIONAHA.111.089763

Anderson, M.A., Burda, J.E., Ren, Y., Ao, Y., O'Shea, T.M., Kawaguchi, R., Coppola, G., Khakh, B.S., Deming, T.J., Michael, S. V, 2016. Astrocyte scar formation aids CNS axon regeneration. Nature 532, 195–200. https://doi.org/10.1038/nature17623.Astrocyte

Anderson, M.A., O'Shea, T.M., Burda, J.E., Ao, Y., Barlatey, S.L., Bernstein, A.M., Kim, J.H., James, N.D., Rogers, A., Kato, B., Wollenberg, A.L., Kawaguchi, R., Coppola, G., Wang, C., Deming, T.J., He, Z., Courtine, G., Sofroniew, M. V., 2018. Required growth facilitators propel axon regeneration across complete spinal cord injury. Nature 561, 396–400. https://doi.org/10.1038/s41586-018-0467-6

Apara, A., Galvao, J., Wang, Y., Blackmore, M., Trillo, A., Iwao, K., Brown, D.P., Fernandes, K.A., Huang, A., Nguyen, T., Ashouri, M., Zhang, X., Shaw, P.X., Kunzevitzky, N.J., Moore, D.L., Libby, R.T., Goldberg, J.L., 2017. KLF9 and JNK3 interact to suppress axon regeneration in the adult CNS. J. Neurosci. 37, 9632–9644. https://doi.org/10.1523/JNEUROSCI.0643-16.2017

Arenkiel, B.R., Peca, J., Davison, I.G., Feliciano, C., Deisseroth, K., Augustine, G.J.J., Ehlers, M.D., Feng, G., 2007. In Vivo Light-Induced Activation of Neural Circuitry in Transgenic Mice Expressing Channelrhodopsin-2. Neuron 54, 205–218. https://doi.org/10.1016/j.neuron.2007.03.005

Arlotta, P., Molyneaux, B.J., Chen, J., Inoue, J., Kominami, R., MacKlis, J.D., 2005. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron 45, 207–221. https://doi.org/10.1016/j.neuron.2004.12.036

Arnett, H.A., Mason, J., Marino, M., Suzuki, K., Matsushima, G.K., Ting, J.P.Y., 2001. TNFα promotes proliferation of oligodendrocyte progenitors and remyelination. Nat. Neurosci. https://doi.org/10.1038/nn738

Asboth, L., Friedli, L., Beauparlant, J., Martinez-Gonzalez, C., Anil, S., Rey, E., Baud, L., Pidpruzhnykova, G., Anderson, M.A., Shkorbatova, P., Batti, L., Pagès, S., Kreider, J., Schneider, B.L., Barraud, Q., Courtine, G., 2018. Cortico-reticulo-spinal circuit reorganization enables functional recovery after severe spinal cord contusion. Nat. Neurosci. 21, 576–588. https://doi.org/10.1038/s41593-018-0093-5

Assinck, P., Duncan, G.J., Hilton, B.J., Plemel, J.R., Tetzlaff, W., 2017. Cell transplantation therapy for spinal cord injury. Nat. Neurosci. 20, 637–647. https://doi.org/10.1038/nn.4541

Atasoy, D., Sternson, S.M., 2018. CHEMOGENETIC TOOLS FOR CAUSAL CELLULAR AND NEURONAL BIOLOGY 391–418. https://doi.org/10.1152/physrev.00009.2017

Attwell, C.L., van Zwieten, M., Verhaagen, J., Mason, M.R.J., 2018. The Dorsal Column Lesion Model of Spinal Cord Injury and Its Use in Deciphering the Neuron-Intrinsic Injury Response. Dev. Neurobiol. 78, 926–951. https://doi.org/10.1002/dneu.22601

Atwal, J.K., Pinkston-Gosse, J., Syken, J., Stawicki, S., Wu, Y., Shatz, C., Tessier-Lavigne, M., 2008. PirB is a functional receptor for myelin inhibitors of axonal regeneration. Science (80-.). 322, 967–970. https://doi.org/10.1126/science.1161151

Avraham, O., Deng, P.Y., Jones, S., Kuruvilla, R., Semenkovich, C.F., Klyachko, V.A., Cavalli, V., 2020. Satellite glial cells promote regenerative growth in sensory neurons. Nat. Commun. 11, 1–17. https://doi.org/10.1038/s41467-020-18642-y

Badhiwala, J.H., Ahuja, C.S., Fehlings, M.G., 2019. Time is spine: A review of translational

advances in spinal cord injury. J. Neurosurg. Spine 30, 1–18. https://doi.org/10.3171/2018.9.SPINE18682

Badhiwala, J.H., Wilson, J.R., Kwon, B.K., Casha, S., Fehlings, M.G., 2018. A Review of Clinical Trials in Spinal Cord Injury Including Biomarkers. J. Neurotrauma 35, 1906–1917. https://doi.org/10.1089/neu.2018.5935

Bai, L., Wang, X., Li, Z., Kong, C., Zhao, Y., Qian, J.L., Kan, Q., Zhang, W., Xu, J.T., 2016. Upregulation of Chemokine CXCL12 in the Dorsal Root Ganglia and Spinal Cord Contributes to the Development and Maintenance of Neuropathic Pain Following Spared Nerve Injury in Rats. Neurosci. Bull. https://doi.org/10.1007/s12264-015-0007-4

Baliki, M.N., Apkarian, A.V., 2015. Nociception, Pain, Negative Moods, and Behavior Selection. Neuron 87, 474–491. https://doi.org/10.1016/j.neuron.2015.06.005

Baltes, C., Rudin, M., Sydekum, E., Ghosh, A., Schwab, M.E., Weber, B., Haiss, F., Schneider, R., Mueggler, T., Wyss, M.T., Gullo, M., 2010. Rewiring of hindlimb corticospinal neurons after spinal cord injury. Nat. Neurosci. 13, 97–104. https://doi.org/10.1038/nn.2448

Bardi, G., Niggli, V., Loetscher, P., 2003. Rho kinase is required for CCR7-mediated polarization and chemotaxis of T lymphocytes. FEBS Lett. 542, 79–83. https://doi.org/10.1016/S0014-5793(03)00351-X

Bareyre, F.M., Garzorz, N., Lang, C., Misgeld, T., Büning, H., Kerschensteiner, M., 2011. In vivo imaging reveals a phase-Specific role of stat3 during central and peripheral nervous system axon regeneration. Proc. Natl. Acad. Sci. U. S. A. 108, 6282–6287. https://doi.org/10.1073/pnas.1015239108

Baron, R., Binder, A., Wasner, G., 2010. Neuropathic pain: Diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. 9, 807–819. https://doi.org/10.1016/S1474-4422(10)70143-5

Bas-Orth, C., Tan, Y.W., Lau, D., Bading, H., 2017. Synaptic activity drives a genomic program that promotes a neuronal warburg effect. J. Biol. Chem. 292, 5183–5194. https://doi.org/10.1074/jbc.M116.761106

Basso, D.M., Fisher, L.C., Anderson, A.J., Jakeman, L.B., McTigue, D.M., Popovich, P.G., 2006. Basso mouse scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. J. Neurotrauma 23, 635–659. https://doi.org/10.1089/neu.2006.23.635

Bastow, C.R., Bunting, M.D., Kara, E.E., McKenzie, D.R., Caon, A., Devi, S., Tolley, L., Mueller, S.N., Frazer, I.H., Harvey, N., Condina, M.R., Young, C., Hoffmann, P., McColl, S.R., Comerford, I., 2021. Scavenging of soluble and immobilized CCL21 by ACKR4 regulates peripheral dendritic cell emigration. Proc. Natl. Acad. Sci. U. S. A. 118, 1–9. https://doi.org/10.1073/pnas.2025763118

Beck, K.D., Nguyen, H.X., Galvan, M.D., Salazar, D.L., Woodruff, T.M., Anderson, A.J., 2010. Quantitative analysis of cellular inflammation after traumatic spinal cord injury: Evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain. https://doi.org/10.1093/brain/awp322

Bellver-Landete, V., Bretheau, F., Mailhot, B., Vallières, N., Lessard, M., Janelle, M.E., Vernoux, N., Tremblay, M.È., Fuehrmann, T., Shoichet, M.S., Lacroix, S., 2019. Microglia are an essential component of the neuroprotective scar that forms after spinal cord injury. Nat. Commun. https://doi.org/10.1038/s41467-019-08446-0

Ben-Yaakov, K., Dagan, S.Y., Segal-Ruder, Y., Shalem, O., Vuppalanchi, D., Willis, D.E., Yudin, D., Rishal, I., Rother, F., Bader, M., Blesch, A., Pilpel, Y., Twiss, J.L., Fainzilber, M., 2012. Axonal transcription factors signal retrogradely in lesioned peripheral nerve.

EMBO J. 31, 1350–1363. https://doi.org/10.1038/emboj.2011.494

Berrocal, Y., Pearse, D.D., Singh, A., Andrade, C.M., McBroom, J.S., Puentes, R., Eaton, M.J., 2007. Social and environmental enrichment improves sensory and motor recovery after severe contusive spinal cord injury in the rat. J. Neurotrauma 24, 1761–1772. https://doi.org/10.1089/neu.2007.0327

Berthézène, C.D., Rabiller, L., Jourdan, G., Cousin, B., Pénicaud, L., Casteilla, L., Lorsignol, A., 2021. Tissue regeneration: The dark side of opioids. Int. J. Mol. Sci. 22, 1–11. https://doi.org/10.3390/ijms22147336

Beverungen, H., Klaszky, S.C., Klaszky, M., Côté, M.P., 2020. Rehabilitation Decreases Spasticity by Restoring Chloride Homeostasis through the Brain-Derived Neurotrophic Factor-KCC2 Pathway after Spinal Cord Injury. J. Neurotrauma 37, 846–859. https://doi.org/10.1089/neu.2019.6526

Bhardwaj, D., Náger, M., Camats, J., David, M., Benguria, A., Dopazo, A., Cantí, C., Herreros, J., 2013. Chemokines induce axon outgrowth downstream of hepatocyte growth factor and TCF/ß-catenin signaling. Front. Cell. Neurosci. 7, 1–10. https://doi.org/10.3389/fncel.2013.00052

Biber, K., Boddeke, E., 2014. Neuronal CC chemokines: The distinct roles of CCL21 and CCL2 in neuropathic pain. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2014.00210

Biber, K., Neumann, H., Inoue, K., Boddeke, H.W.G.M., 2007. Neuronal “On” and “Off” signals control microglia. Trends Neurosci. https://doi.org/10.1016/j.tins.2007.08.007

Biber, K., Sauter, A., Brouwer, N., Copray, S.C.V.M., Boddeke, H.W.G.M., 2001. Ischemia-induced neuronal expression of the microglia attracting chemokine secondary lymphoid-tissue chemokine (SLC). Glia. https://doi.org/10.1002/glia.1047

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. https://doi.org/10.1038/emboj.2011.89

Bilchak, J.N., Caron, G., Côté, M.P., 2021. Exercise-induced plasticity in signaling pathways involved in motor recovery after spinal cord injury. Int. J. Mol. Sci. 22. https://doi.org/10.3390/ijms22094858

Billottet, C., Quemener, C., Bikfalvi, A., 2013. CXCR3, a double-edged sword in tumor progression and angiogenesis. Biochim. Biophys. Acta - Rev. Cancer 1836, 287–295. https://doi.org/10.1016/j.bbcan.2013.08.002

Blackmore, M.G., Moore, D.L., Smith, R.P., Goldberg, J.L., Bixby, J.L., Lemmon, V.P., 2010. High content screening of cortical neurons identifies novel regulators of axon growth. Mol. Cell. Neurosci. 44, 43–54. https://doi.org/10.1016/j.mcn.2010.02.002

Blackmore, M.G., Wang, Z., Lerch, J.K., Motti, D., Zhang, Y.P., Shields, C.B., Lee, J.K., Goldberg, J.L., Lemmon, V.P., Bixby, J.L., 2012. Krüppel-like Factor 7 engineered for transcriptional activation promotes axon regeneration in the adult corticospinal tract. Proc. Natl. Acad. Sci. U. S. A. 109, 7517–7522. https://doi.org/10.1073/pnas.1120684109

Blanquie, O., Bradke, F., 2018. Cytoskeleton dynamics in axon regeneration. Curr. Opin. Neurobiol. 51, 60–69. https://doi.org/10.1016/j.conb.2018.02.024

Blomster, L. V., Brennan, F.H., Lao, H.W., Harle, D.W., Harvey, A.R., Ruitenberg, M.J., 2013. Mobilisation of the splenic monocyte reservoir and peripheral CX3CR1 deficiency adversely affects recovery from spinal cord injury. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2013.05.002

Borrell, V., Marín, O., 2006. Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. Nat. Neurosci. 9, 1284–1293. https://doi.org/10.1038/nn1764

Borton, D., Micera, S., Del R. Millán, J., Courtine, G., 2013. Personalized neuroprosthetics. Sci. Transl. Med. 5. https://doi.org/10.1126/scitranslmed.3005968

Bouton, C.E., Shaikhouni, A., Annetta, N. V., Bockbrader, M.A., Friedenberg, D.A., Nielson, D.M., Sharma, G., Sederberg, P.B., Glenn, B.C., Mysiw, W.J., Morgan, A.G., Deogaonkar, M., Rezai, A.R., 2016. Restoring cortical control of functional movement in a human with quadriplegia. Nature 533, 247–250. https://doi.org/10.1038/nature17435

Bradbury, E.J., Burnside, E.R., 2019. Moving beyond the glial scar for spinal cord repair. Nat. Commun. https://doi.org/10.1038/s41467-019-11707-7

Bradbury, E.J., Moon, L.D.F., Popat, R.J., King, V.R., Bennett, G.S., Patel, P.N., Fawcett, J.W., McMahon, S.B., 2002. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416, 636–640. https://doi.org/10.1038/416636a

Bradke, F., Fawcett, J.W., Spira, M.E., 2012. Assembly of a new growth cone after axotomy: The precursor to axon regeneration. Nat. Rev. Neurosci. 13, 183–193. https://doi.org/10.1038/nrn3176

Bradley, P.M., Denecke, C.K., Aljovic, A., Schmalz, A., Kerschensteiner, M., Bareyre, F.M., 2019. Corticospinal circuit remodeling after central nervous system injury is dependent on neuronal activity. J. Exp. Med. 216, 2503–2514. https://doi.org/10.1084/jem.20181406

Britschgi, M.R., Favre, S., Luther, S.A., 2010. CCL21 is sufficient to mediate DC migration, maturation and function in the absence of CCL19. Eur. J. Immunol. 40, 1266–1271. https://doi.org/10.1002/eji.200939921

Burda, J.E., Sofroniew, M. V., 2014. Reactive gliosis and the multicellular response to CNS damage and disease. Neuron. https://doi.org/10.1016/j.neuron.2013.12.034

Busch, S.A., Horn, K.P., Silver, D.J., Silver, J., 2009. Overcoming macrophage-mediated axonal dieback following CNS injury. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.1151-09.2009

Butler, K.L., Clancy-Thompson, E., Mullins, D.W., 2017. CXCR3 + monocytes/macrophages are required for establishment of pulmonary metastases. Sci. Rep. 7, 1–9. https://doi.org/10.1038/srep45593

Cafferty, W.B.J., Gardiner, N.J., Das, P., Qiu, J., McMahon, S.B., Thompson, S.W.N., 2004. Conditioning Injury-Induced Spinal Axon Regeneration Fails in Interleukin-6 Knock-Out Mice. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2245-02.2004

Cancellieri, C., Caronni, N., Vacchini, A., Savino, B., Borroni, E.M., Locati, M., Bonecchi, R., 2013. Review: Structure-function and biological properties of the atypical chemokine receptor D6. Mol. Immunol. 55, 87–93. https://doi.org/10.1016/j.molimm.2012.08.003

Capogrosso, M., Milekovic, T., Borton, D., Wagner, F., Moraud, E.M., Mignardot, J.B., Buse, N., Gandar, J., Barraud, Q., Xing, D., Rey, E., Duis, S., Jianzhong, Y., Ko, W.K.D., Li, Q., Detemple, P., Denison, T., Micera, S., Bezard, E., Bloch, J., Courtine, G., 2016. A brain-spine interface alleviating gait deficits after spinal cord injury in primates. Nature 539, 284–288. https://doi.org/10.1038/nature20118

Carmel, J.B., Berrol, L.J., Brus-Ramer, M., Martin, J.H., 2010. Chronic electrical stimulation of the intact corticospinal system after unilateral injury restores skilled locomotor control and promotes spinal axon outgrowth. J. Neurosci. 30, 10918–10926. https://doi.org/10.1523/JNEUROSCI.1435-10.2010

Carmel, J.B., Kimura, H., Berrol, L.J., Martin, J.H., 2013. Motor cortex electrical stimulation promotes axon outgrowth to brain stem and spinal targets that control the forelimb impaired by unilateral corticospinal injury. Eur. J. Neurosci. 37, 1090–1102. https://doi.org/10.1111/ejn.12119

Carmel, J.B., Kimura, H., Martin, J.H., 2014. Electrical stimulation of motor cortex in the uninjured hemisphere after chronic unilateral injury promotes recovery of skilled locomotion through ipsilateral control. J. Neurosci. 34, 462–466. https://doi.org/10.1523/JNEUROSCI.3315-13.2014

Carmel, J.B., Martin, J.H., 2014. Motor cortex electrical stimulation augments sprouting of the corticospinal tract and promotes recovery of motor function. Front. Integr. Neurosci. 8, 1–11. https://doi.org/10.3389/fnint.2014.00051

Cartoni, R., Norsworthy, M.W., Bei, F., Wang, C., Li, S., Zhang, Y., Gabel, C. V., Schwarz, T.L., He, Z., 2016. The Mammalian-Specific Protein Armcx1 Regulates Mitochondrial Transport during Axon Regeneration. Neuron 92, 1294–1307. https://doi.org/10.1016/j.neuron.2016.10.060

Carulli, D., Foscarin, S., Rossi, F., 2011. Activity-Dependent Plasticity and Gene Expression Modifications in the Adult CNS. Front. Mol. Neurosci. 4, 1–12. https://doi.org/10.3389/fnmol.2011.00050

Casha, S., Zygun, D., McGowan, M.D., Bains, I., Yong, V.W., John Hurlbert, R., 2012. Results of a phase II placebo-controlled randomized trial of minocycline in acute spinal cord injury. Brain 135, 1224–1236. https://doi.org/10.1093/brain/aws072

Catapano, L.A., Arnold, M.W., Perez, F.A., Macklis, J.D., 2001. Specific neurotrophic factors support the survival of cortical projection neurons at distinct stages of development. J. Neurosci. 21, 8863–8872. https://doi.org/10.1523/jneurosci.21-22-08863.2001

Cerqueira, É., Marinho, D.A., Neiva, H.P., Lourenço, O., 2020. Inflammatory Effects of High and Moderate Intensity Exercise—A Systematic Review. Front. Physiol. 10, 1–14. https://doi.org/10.3389/fphys.2019.01550

Chen, K., Marsh, B.C., Cowan, M., Al'Joboori, Y.D., Gigout, S., Smith, C.C., Messenger, N., Gamper, N., Schwab, M.E., Ichiyama, R.M., 2017. Sequential therapy of anti-Nogo-A antibody treatment and treadmill training leads to cumulative improvements after spinal cord injury in rats. Exp. Neurol. 292, 135–144. https://doi.org/10.1016/j.expneurol.2017.03.012

Chen, Y., Yin, D., Fan, B., Zhu, X., Chen, Q., Li, Y., Zhu, S., Lu, R., Xu, Z., 2019. Chemokine CXCL10/CXCR3 signaling contributes to neuropathic pain in spinal cord and dorsal root ganglia after chronic constriction injury in rats. Neurosci. Lett. https://doi.org/10.1016/j.neulet.2018.11.021

Cheng, H., Liao, K.-K., Liao, S.-F., Chuang, T.-Y., Shih, Y.-H., 2004. Spinal cord repair with acidic fibroblast growth factor as a treatment for a patient with chronic paraplegia. Spine (Philadelphia, Pa. 1976) 29, E284-288.

Cheng, M.Y., Wang, E.H., Woodson, W.J., Wang, S., Sun, G., Lee, A.G., Arac, A., Fenno, L.E., Deisseroth, K., Steinberg, G.K., 2014. Optogenetic neuronal stimulation promotes functional recovery after stroke. Proc. Natl. Acad. Sci. U. S. A. 111, 12913–12918. https://doi.org/10.1073/pnas.1404109111

Cheng, P.L., Song, A.H., Wong, Y.H., Wang, S., Zhang, X., Poo, M.M., 2011. Self-amplifying autocrine actions of BDNF in axon development. Proc. Natl. Acad. Sci. U. S. A. 108, 18430–18435. https://doi.org/10.1073/pnas.1115907108

Cheng, Y.C., Snavely, A., Barrett, L.B., Zhang, X., Herman, C., Frost, D.J., Riva, P., Tochitsky,

I., Kawaguchi, R., Singh, B., Ivanis, J., Huebner, E.A., Arvanites, A., Oza, V., Davidow, L., Maeda, R., Sakuma, M., Grantham, A., Wang, Q., Chang, A.N., Pfaff, K., Costigan, M., Coppola, G., Rubin, L.L., Schwer, B., Alt, F.W., Woolf, C.J., 2021. Topoisomerase I inhibition and peripheral nerve injury induce DNA breaks and ATF3-associated axon regeneration in sensory neurons. Cell Rep. 36, 109666. https://doi.org/10.1016/j.celrep.2021.109666

Chhaya, S.J., Quiros-Molina, D., Tamashiro-Orrego, A.D., Houlé, J.D., Detloff, M.R., 2019. Exercise-Induced Changes to the Macrophage Response in the Dorsal Root Ganglia Prevent Neuropathic Pain after Spinal Cord Injury. J. Neurotrauma 36, 877–890. https://doi.org/10.1089/neu.2018.5819

Chierzi, S., Ratto, G.M., Verma, P., Fawcett, J.W., 2005. The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. Eur. J. Neurosci. 21, 2051–2062. https://doi.org/10.1111/j.1460-9568.2005.04066.x

Chio, J.C.T., Xu, K.J., Popovich, P., David, S., Fehlings, M.G., 2021. Neuroimmunological therapies for treating spinal cord injury: Evidence and future perspectives. Exp. Neurol. 341, 113704. https://doi.org/10.1016/j.expneurol.2021.113704

Chivet, M., Javalet, C., Laulagnier, K., Blot, B., Hemming, F.J., Sadoul, R., 2014. Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. J. Extracell. Vesicles 3. https://doi.org/10.3402/jev.v3.24722

Cho, Y., Cavalli, V., 2012. HDAC5 is a novel injury-regulated tubulin deacetylase controlling axon regeneration. EMBO J. 31, 3063–3078. https://doi.org/10.1038/emboj.2012.160

Cho, Y., Sloutsky, R., Naegle, K.M., Cavalli, V., 2013. XInjury-Induced HDAC5 nuclear export is essential for axon regeneration. Cell 155, 894. https://doi.org/10.1016/j.cell.2013.10.004

Chong, M.S., Reynolds, M.L., Irwin, N., Coggeshall, R.E., Emson, P.C., Benowitz, L.I., Woolf, C.J., 1994. GAP-43 expression in primary sensory neurons following central axotomy. J. Neurosci. 14, 4375–4384.

Coles, C.H., Bradke, F., 2015. Coordinating Neuronal Actin-Microtubule Dynamics. Curr. Biol. 25, R677–R691. https://doi.org/10.1016/j.cub.2015.06.020

Comerford, I., Harata-Lee, Y., Bunting, M.D., Gregor, C., Kara, E.E., McColl, S.R., 2013. A myriad of functions and complex regulation of the CCR7/CCL19/CCL21 chemokine axis in the adaptive immune system. Cytokine Growth Factor Rev. 24, 269–283. https://doi.org/10.1016/j.cytogfr.2013.03.001

Cooper, J.A., 2013. Mechanisms of cell migration in the nervous system. J. Cell Biol. 202, 725–734. https://doi.org/10.1083/jcb.201305021

Corbisier, J., Galès, C.L., Huszagh, A., Parmentier, M., Springael, J.Y., 2015. Biased signaling at chemokine receptors. J. Biol. Chem. 290, 9542–9554. https://doi.org/10.1074/jbc.M114.596098

Costigan, M., Scholz, J., Woolf, C.J., 2009. Neuropathic pain: A maladaptive response of the nervous system to damage. Annu. Rev. Neurosci. 32, 1–32. https://doi.org/10.1146/annurev.neuro.051508.135531

Côté, M.P., Azzam, G.A., Lemay, M.A., Zhukareva, V., Houlé, J.D., 2011. Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after spinal cord injury. J. Neurotrauma 28, 299–309. https://doi.org/10.1089/neu.2010.1594

Courtine, G., Sofroniew, M. V., 2019. Spinal cord repair: advances in biology and technology. Nat. Med. 25. https://doi.org/10.1038/s41591-019-0475-6

Curcio, M., Bradke, F., 2018. Axon Regeneration in the Central Nervous System: Facing the Challenges from the Inside. Annu. Rev. Cell Dev. Biol. https://doi.org/10.1146/annurev-cellbio-100617-062508

Danson, C.M., Pocha, S.M., Bloomberg, G.B., Cory, G.O., 2007. Phosphorylation of WAVE2 by MAP kinases regulates persistent cell migration and polarity. J. Cell Sci. 120, 4144–4154. https://doi.org/10.1242/jcs.013714

David, S., Braun, P.E., Jackson, D.L., Kottis, V., McKerracher, L., 1995. Laminin overrides the inhibitory effects of PNS and CNS myelin- derived inhibitors of neurite growth. J Neurosci 42, 594–602. https://doi.org/10.1002/jnr.490420417

David, S., Kroner, A., Greenhalgh, A.D., Zarruk, J.G., López-Vales, R., 2018. Myeloid cell responses after spinal cord injury. J. Neuroimmunol. https://doi.org/10.1016/j.jneuroim.2018.06.003

De Haas, A.H., Van Weering, H.R.J., De Jong, E.K., Boddeke, H.W.G.M., Biber, K.P.H., 2007. Neuronal chemokines: Versatile messengers in central nervous system cell interaction. Mol. Neurobiol. https://doi.org/10.1007/s12035-007-0036-8

De Jong, E.K., Dijkstra, I.M., Hensens, M., Brouwer, N., Van Amerongen, M., Liem, R.S.B., Boddeke, H.W.G.M., 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. https://doi.org/10.1523/JNEUROSCI.1019-05.2005

De Jong, E.K., Vinet, J., Stanulovic, V.S., Meijer, M., Wesseling, E., Sjollema, K., Boddeke, H.W.G.M., Biber, K., 2008. Expression, transport, and axonal sorting of neuronal CCL21 in large dense-core vesicles. FASEB J. https://doi.org/10.1096/fj.07-101907

De Virgiliis, F., Hutson, T.H., Palmisano, I., Amachree, S., Miao, J., Zhou, L., Todorova, R., Thompson, R., Danzi, M.C., Lemmon, V.P., Bixby, J.L., Wittig, I., Shah, A.M., Di Giovanni, S., 2020. Enriched conditioning expands the regenerative ability of sensory neurons after spinal cord injury via neuronal intrinsic redox signaling. Nat. Commun. 11, 1–16. https://doi.org/10.1038/s41467-020-20179-z

Deisseroth, K., 2015. Optogenetics : 10 years of microbial opsins in neuroscience 18, 1213–1225. https://doi.org/10.1038/nn.4091

Deng, W.W., Wu, G.Y., Min, L.X., Feng, Z., Chen, H., Tan, M.L., Sui, J.F., Liu, H.L., Hou, J.M., 2021. Optogenetic Neuronal Stimulation Promotes Functional Recovery After Spinal Cord Injury. Front. Neurosci. 15, 1–10. https://doi.org/10.3389/fnins.2021.640255

Dent, E.W., Gertler, F.B., 2003. Cytoskeletal dynamics and transport in growth cone motility and guidance. Neuron 40, 209–227. https://doi.org/10.1016/S0896-6273(03)00633-0

Dergham, P., Ellezam, B., Essagian, C., Avedissian, H., Lubell, W.D., McKerracher, L., 2002. Rho Signaling Pathway Targeted to Promote Spinal Cord Repair. J. Neurosci. 22, 6570–6577. https://doi.org/10.1523/JNEUROSCI.22-15-06570.2002

Deumens, R., Koopmans, G.C., Joosten, E.A.J., 2005. Regeneration of descending axon tracts after spinal cord injury. Prog. Neurobiol. 77, 57–89. https://doi.org/10.1016/j.pneurobio.2005.10.004

Dickendesher, T.L., Baldwin, K.T., Mironova, Y.A., Koriyama, Y., Raiker, S.J., Askew, K.L., Wood, A., Geoffroy, C.G., Zheng, B., Liepmann, C.D., Katagiri, Y., Benowitz, L.I., Geller, H.M., Giger, R.J., 2012. NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. Nat. Neurosci. 15, 703–712. https://doi.org/10.1038/nn.3070

Dill, J., Wang, H., Zhou, F., Li, S., 2008. Inactivation of glycogen synthase kinase 3 promotes axonal growth and recovery in the CNS. J. Neurosci. 28, 8914–8928.

https://doi.org/10.1523/JNEUROSCI.1178-08.2008

Doitsidou, M., Reichman-Fried, M., Stebler, J., Köprunner, M., Dörries, J., Meyer, D., Esguerra, C. V., Leung, T.C., Raz, E., 2002. Guidance of primordial germ cell migration by the chemokine SDF-1. Cell 111, 647–659. https://doi.org/10.1016/S0092-8674(02)01135-2

Donnelly, D.J., Longbrake, E.E., Shawler, T.M., Kigerl, K.A., Lai, W., Amy Tovar, C., Ransohoff, R.M., Popovich, P.G., 2011. Deficient CX3CR1 signaling promotes recovery after mouse spinal cord injury by limiting the recruitment andactivation of Ly6Clo/iNOS+ macrophages. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2114-11.2011

Donnelly, D.J., Popovich, P.G., 2008. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2007.06.009

Dougherty, K.D., Dreyfus, C.F., Black, I.B., 2000. Brain-derived neurotrophic factor in astrocytes, oligodendrocytes, and microglia/macrophages after spinal cord injury. Neurobiol. Dis. https://doi.org/10.1006/nbdi.2000.0318

Dunlop, S.A., 2008. Activity-dependent plasticity: implications for recovery after spinal cord injury. Trends Neurosci. 31, 410–418. https://doi.org/10.1016/j.tins.2008.05.004

Dupraz, S., Hilton, B.J., Husch, A., Santos, T.E., Coles, C.H., Stern, S., Brakebusch, C., Bradke, F., 2019. RhoA Controls Axon Extension Independent of Specification in the Developing Brain. Curr. Biol. 29, 3874-3886.e9. https://doi.org/10.1016/j.cub.2019.09.040

Eisdorfer, J.T., Smit, R.D., Keefe, K.M., Lemay, M.A., Smith, G.M., Spence, A.J., 2020. Epidural Electrical Stimulation: A Review of Plasticity Mechanisms That Are Hypothesized to Underlie Enhanced Recovery From Spinal Cord Injury With Stimulation. Front. Mol. Neurosci. 13, 1–12. https://doi.org/10.3389/fnmol.2020.00163

Enes, J., Langwieser, N., Ruschel, J., Carballosa-Gonzalez, M.M., Klug, A., Traut, M.H., Ylera, B., Tahirovic, S., Hofmann, F., Stein, V., Moosmang, S., Hentall, I.D., Bradke, F., 2010. Electrical activity suppresses axon growth through Cav1.2 channels in adult primary sensory neurons. Curr. Biol. 20, 1154–1164. https://doi.org/10.1016/j.cub.2010.05.055

Engesser-Cesar, C., Ichiyama, R.M., Nefas, A.L., Hill, M.A., Edgerton, V.R., Cotman, C.W., Anderson, A.J., 2007. Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. Eur. J. Neurosci. 25, 1931–1939. https://doi.org/10.1111/j.1460-9568.2007.05469.x

Enyedi, B., Niethammer, P., 2015. Mechanisms of epithelial wound detection. Trends Cell Biol. 25, 398–407. https://doi.org/10.1016/j.tcb.2015.02.007

Ertürk, A., Hellal, F., Enes, J., Bradke, F., 2007. Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. J. Neurosci. 27, 9169–9180. https://doi.org/10.1523/JNEUROSCI.0612-07.2007

Escribano, C., Delgado-Martín, C., Rodríguez-Fernández, J.L., 2009. CCR7-Dependent Stimulation of Survival in Dendritic Cells Involves Inhibition of GSK3β. J. Immunol. 183, 6282–6295. https://doi.org/10.4049/jimmunol.0804093

Falgairolle, M., O'Donovan, M.J., 2019. Feedback regulation of locomotion by motoneurons in the vertebrate spinal cord. Curr. Opin. Physiol. 8, 50–55. https://doi.org/10.1016/j.cophys.2018.12.009

Fang, K.M., Yang, C.S., Sun, S.H., Tzeng, S.F., 2009. Microglial phagocytosis attenuated by short-term exposure to exogenous ATP through P2X7 receptor action. J. Neurochem. https://doi.org/10.1111/j.1471-4159.2009.06409.x

Faulkner, J.R., Herrmann, J.E., Woo, M.J., Tansey, K.E., Doan, N.B., Sofroniew, M. V., 2004. Reactive Astrocytes Protect Tissue and Preserve Function after Spinal Cord Injury. J. Neurosci. 24, 2143–2155. https://doi.org/10.1523/JNEUROSCI.3547-03.2004

Fauth, M., Tetzlaff, C., 2016. Opposing effects of neuronal activity on structural plasticity. Front. Neuroanat. 10, 1–18. https://doi.org/10.3389/fnana.2016.00075

Fehlings, M.G., Kim, K.D., Aarabi, B., Rizzo, M., Bond, L.M., McKerracher, L., Vaccaro, A.R., Okonkwo, D.O., 2018. Rho Inhibitor VX-210 in Acute Traumatic Subaxial Cervical Spinal Cord Injury: Design of the SPinal Cord Injury Rho INhibition InvestiGation (SPRING) Clinical Trial. J. Neurotrauma 35, 1049–1056. https://doi.org/10.1089/neu.2017.5434

Fehlings, M.G., Theodore, N., Harrop, J., Maurais, G., Kuntz, C., Shaffrey, C.I., Kwon, B.K., Chapman, J., Yee, A., Tighe, A., McKerracher, L., 2011. A phase I/IIa clinical trial of a recombinant Rho protein antagonist in acute spinal cord injury. J. Neurotrauma 28, 787–796. https://doi.org/10.1089/neu.2011.1765

Fehlings, M.G., Wilson, J.R., Tetreault, L.A., Aarabi, B., Anderson, P., Arnold, P.M., Brodke, D.S., Burns, A.S., Chiba, K., Dettori, J.R., Furlan, J.C., Hawryluk, G., Holly, L.T., Howley, S., Jeji, T., Kalsi-Ryan, S., Kotter, M., Kurpad, S., Kwon, B.K., Marino, R.J., Martin, A.R., Massicotte, E., Merli, G., Middleton, J.W., Nakashima, H., Nagoshi, N., Palmieri, K., Skelly, A.C., Singh, A., Tsai, E.C., Vaccaro, A., Yee, A., Harrop, J.S., 2017. A Clinical Practice Guideline for the Management of Patients With Acute Spinal Cord Injury: Recommendations on the Use of Methylprednisolone Sodium Succinate. Glob. Spine J. 7, 203S-211S. https://doi.org/10.1177/2192568217703085

Ferreira, L.M.R., Floriddia, E.M., Quadrato, G., Di Giovanni, S., 2012. Neural regeneration: Lessons from regenerating and non-regenerating systems. Mol. Neurobiol. 46, 227–241. https://doi.org/10.1007/s12035-012-8290-9

Ferro, A., Auguste, Y.S.S., Cheadle, L., 2021. Microglia, Cytokines, and Neural Activity: Unexpected Interactions in Brain Development and Function. Front. Immunol. 12, 1–14. https://doi.org/10.3389/fimmu.2021.703527

Filbin, M.T., 2003. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat. Rev. Neurosci. 4, 1019. https://doi.org/10.1038/nrn1195

Filous, A.R., Tran, A., James Howell, C., Busch, S.A., Evans, T.A., Stallcup, W.B., Kang, S.H., Bergles, D.E., Lee, S. Il, Levine, J.M., Silver, J., 2014. Entrapment via synaptic-like connections between NG2 proteoglycan + cells and dystrophic axons in the lesion plays a role in regeneration failure after spinal cord injury. J. Neurosci. 34, 16369–16384. https://doi.org/10.1523/JNEUROSCI.1309-14.2014

Finelli, M.J., Wong, J.K., Zou, H., 2013. Epigenetic regulation of sensory axon regeneration after spinal cord injury. J. Neurosci. 33, 19664–19676. https://doi.org/10.1523/JNEUROSCI.0589-13.2013

Fisher, D., Xing, B., Dill, J., Li, H., Hoang, H.H., Zhao, Z., Yang, X.L., Bachoo, R., Cannon, S., Longo, F.M., Sheng, M., Silver, J., Li, S., 2011. Leukocyte common antigen-related phosphatase is a functional receptor for chondroitin sulfate proteoglycan axon growth inhibitors. J. Neurosci. 31, 14051–14066. https://doi.org/10.1523/JNEUROSCI.1737-11.2011

Fishman, H.M., Bittner, G.D., 2003. Vesicle-mediated restoration of a plasmalemmal barrier in severed axons. News Physiol. Sci. 18, 115–118. https://doi.org/10.1152/nips.01429.2002

Flavell, S.W., Greenberg, M.E., 2008. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu. Rev. Neurosci. 31, 563–590. https://doi.org/10.1146/annurev.neuro.31.060407.125631

Fontana, X., Hristova, M., Da Costa, C., Patodia, S., Thei, L., Makwana, M., Spencer-Dene, B., Latouche, M., Mirsky, R., Jessen, K.R., Klein, R., Raivich, G., Behrens, A., 2012. C-Jun in Schwann cells promotes axonal regeneration and motoneuron survival via paracrine signaling. J. Cell Biol. https://doi.org/10.1083/jcb.201205025

Förster, R., Davalos-Misslitz, A.C., Rot, A., 2008. CCR7 and its ligands: Balancing immunity and tolerance. Nat. Rev. Immunol. 8, 362–371. https://doi.org/10.1038/nri2297

Foscarin, S., Ponchione, D., Pajaj, E., Leto, K., Gawlak, M., Wilczynski, G.M., Rossi, F., Carulli, D., 2011. Experience-dependent plasticity and modulation of growth regulatory molecules at central synapses. PLoS One 6. https://doi.org/10.1371/journal.pone.0016666

Fouad, K., Pedersen, V., Schwab, M.E., Brösamle, C., 2001. Cervical sprouting of corticospinal fibers after thoracic spinal cord injury accompanies shifts in evoked motor responses. Curr. Biol. 11, 1766–1770. https://doi.org/10.1016/s0960-9822(01)00535-8

Fouad, K., Tetzlaff, W., 2012. Rehabilitative training and plasticity following spinal cord injury. Exp. Neurol. 235, 91–99. https://doi.org/10.1016/j.expneurol.2011.02.009

Fournier, A.E., Takizawa, B.T., Strittmatter, S.M., 2003. Rho kinase inhibition enhances axonal regeneration in the injured CNS. J. Neurosci. 23, 1416–1423. https://doi.org/10.1523/jneurosci.23-04-01416.2003

Frendo, M.E., da Silva, A., Phan, K.D., Riche, S., Butler, S.J., 2019. The cofilin/limk1 pathway controls the growth rate of both developing and regenerating motor axons. J. Neurosci. 39, 9316–9327. https://doi.org/10.1523/JNEUROSCI.0648-19.2019

Friel, K.M., Williams, P.T.J.A., Serradj, N., Chakrabarty, S., Martin, J.H., 2014. Activity-Based Therapies for Repair of the Corticospinal System Injured during Development. Front. Neurol. 5, 1–11. https://doi.org/10.3389/fneur.2014.00229

Froger, N., Matonti, F., Roubeix, C., Forster, V., Ivkovic, I., Brunel, N., Baudouin, C., Sahel, J.A., Picaud, S., 2020. VEGF is an autocrine/paracrine neuroprotective factor for injured retinal ganglion neurons. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-68488-z

Fu, H., Zhao, Y., Hu, D., Wang, S., Yu, T., Zhang, L., 2020. Depletion of microglia exacerbates injury and impairs function recovery after spinal cord injury in mice. Cell Death Dis. 11. https://doi.org/10.1038/s41419-020-2733-4

Führmann, T., Anandakumaran, P.N., Shoichet, M.S., 2017. Combinatorial Therapies After Spinal Cord Injury: How Can Biomaterials Help? Adv. Healthc. Mater. 6, 1–21. https://doi.org/10.1002/adhm.201601130

Fujita, Y., Endo, S., Takai, T., Yamashita, T., 2011. Myelin suppresses axon regeneration by PIR-B/SHP-mediated inhibition of Trk activity. EMBO J. 30, 1389–1401. https://doi.org/10.1038/emboj.2011.55

Fujita, Y., Yamashita, T., 2014. Axon growth inhibition by RhoA/ROCK in the central nervous system. Front. Neurosci. 8, 1–12. https://doi.org/10.3389/fnins.2014.00338

Galtrey, C.M., Fawcett, J.W., 2007. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res. Rev. 54, 1–18. https://doi.org/10.1016/j.brainresrev.2006.09.006

Gant, K.L., Guest, J.D., Palermo, A.E., Vedantam, A., Jimsheleishvili, G., Bunge, M.B., Brooks, A.E., Anderson, K.D., Thomas, C.K., Santamaria, A.J., Perez, M.A., Curiel, R., Nash, M.S., Saraf-Lavi, E., Pearse, D.D., Widerström-Noga, E., Khan, A., Dietrich, W.D., Levi, A.D., 2021. Phase 1 Safety Trial of Autologous Human Schwann Cell Transplantation in Chronic Spinal Cord Injury. J. Neurotrauma 15, 1–15. https://doi.org/10.1089/neu.2020.7590

García-Alías, G., Barkhuysen, S., Buckle, M., Fawcett, J.W., 2009. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat. Neurosci. 12, 1145–1151. https://doi.org/10.1038/nn.2377

Gaub, P., Tedeschi, A., Puttagunta, R., Nguyen, T., Schmandke, A., Di Giovanni, S., 2010. HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation. Cell Death Differ. 17, 1392–1408. https://doi.org/10.1038/cdd.2009.216

Gensel, J.C., Zhang, B., 2015. Macrophage activation and its role in repair and pathology after spinal cord injury. Brain Res. https://doi.org/10.1016/j.brainres.2014.12.045

Geoffroy, C.G., Hilton, B.J., Tetzlaff, W., Zheng, B., 2016. Evidence for an Age-Dependent Decline in Axon Regeneration in the Adult Mammalian Central Nervous System. Cell Rep. 15, 238–246. https://doi.org/10.1016/j.celrep.2016.03.028

Geoffroy, C.G., Zheng, B., 2014. Myelin-associated inhibitors in axonal growth after CNS injury. Curr. Opin. Neurobiol. 27, 31–38. https://doi.org/10.1016/j.conb.2014.02.012

Gibson, E.M., Purger, D., Mount, C.W., Goldstein, A.K., Lin, G.L., Wood, L.S., Inema, I., Miller, S.E., Bieri, G., Zuchero, J.B., Barres, B.A., Woo, P.J., Vogel, H., Monje, M., 2014. Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. Sci. (American Assoc. Adv. Sci. 344, 487. https://doi.org/10.1126/science.1252304

Giovanni, S. Di, 2009. Molecular targets for axon regeneration: Focus on the intrinsic pathways. Expert Opin. Ther. Targets 13, 1387–1398. https://doi.org/10.1517/14728220903307517

Gobrecht, P., Andreadaki, A., Diekmann, H., Heskamp, A., Leibinger, M., Fischer, D., 2016. Promotion of functional nerve regeneration by inhibition of microtubule detyrosination. J. Neurosci. 36, 3890–3902. https://doi.org/10.1523/JNEUROSCI.4486-15.2016

Goganau, I., Sandner, B., Weidner, N., Fouad, K., Blesch, A., 2018. Depolarization and electrical stimulation enhance in vitro and in vivo sensory axon growth after spinal cord injury. Exp. Neurol. 300, 247–258. https://doi.org/10.1016/j.expneurol.2017.11.011

Goldberg, S.H., Van Der Meer, P., Hesselgesser, J., Jaffer, S., Kolson, D.L., Albright, A. V., González-Scarano, F., Lavi, E., 2001. CXCR3 expression in human central nervous system diseases. Neuropathol. Appl. Neurobiol. 27, 127–138. https://doi.org/10.1046/j.1365-2990.2001.00312.x

Goldshmit, Y., Lythgo, N., Galea, M.P., Turnley, A.M., 2008. Treadmill training after spinal cord hemisection in mice promotes axonal sprouting and synapse formation and improves motor recovery. J. Neurotrauma 25, 449–465. https://doi.org/10.1089/neu.2007.0392

Gomes-Osman, J., Field-Fote, E.C., 2015. Improvements in Hand Function in Adults With Chronic Tetraplegia Following a Multiday 10-Hz Repetitive Transcranial Magnetic Stimulation Intervention Combined With Repetitive Task Practice. J. Neurol. Phys. Ther. 39, 23–30. https://doi.org/10.1097/NPT.0000000000000062

Greenhalgh, A.D., David, S., Bennett, F.C., 2020. Immune cell regulation of glia during CNS injury and disease. Nat. Rev. Neurosci. https://doi.org/10.1038/s41583-020-0263-9

Greenhalgh, A.D., Zarruk, J.G., Healy, L.M., Baskar Jesudasan, S.J., Jhelum, P., Salmon, C.K., Formanek, A., Russo, M. V., Antel, J.P., McGavern, D.B., McColl, B.W., David, S., 2018. Peripherally derived macrophages modulate microglial function to reduce inflammation after CNS injury. PLoS Biol. https://doi.org/10.1371/journal.pbio.2005264

Griffin, J.M., Bradke, F., 2020. Therapeutic repair for spinal cord injury: combinatory approaches to address a multifaceted problem. EMBO Mol. Med. 12, 1–29. https://doi.org/10.15252/emmm.201911505

Griffin, J.M., Fackelmeier, B., Clemett, C.A., Fong, D.M., Mouravlev, A., Young, D., O'Carroll, S.J., 2020. Astrocyte-selective AAV-ADAMTS4 gene therapy combined with hindlimb rehabilitation promotes functional recovery after spinal cord injury. Exp. Neurol. 327. https://doi.org/10.1016/j.expneurol.2020.113232

Grossman, R.G., Fehlings, M.G., Frankowski, R.F., Burau, K.D., Chow, D.S.L., Tator, C., Teng, A., Toups, E.G., Harrop, J.S., Aarabi, B., Shaffrey, C.I., Johnson, M.M., Harkema, S.J., Boakye, M., Guest, J.D., Wilson, J.R., 2014. A prospective, multicenter, Phase i matched-comparison group trial of safety, pharmacokinetics, and preliminary efficacy of riluzole in patients with traumatic spinal cord injury. J. Neurotrauma 31, 239–255. https://doi.org/10.1089/neu.2013.2969

Gurtner, G.C., Werner, S., Barrandon, Y., Longaker, M.T., 2008. Wound repair and regeneration. Nature. https://doi.org/10.1038/nature07039

Ha, Y., Liu, H., Xu, Z., Yokota, H., Narayanan, S.P., Lemtalsi, T., Smith, S.B., Caldwell, R.W., Caldwell, R.B., Zhang, W., 2015. Endoplasmic reticulum stress-regulated CXCR3 pathway mediates inflammation and neuronal injury in acute glaucoma. Cell Death Dis. https://doi.org/10.1038/cddis.2015.281

Haan, N., Zhu, B., Wang, J., Wei, X., Song, B., 2015. Crosstalk between macrophages and astrocytes affects proliferation, reactive phenotype and inflammatory response, suggesting a role during reactive gliosis following spinal cord injury. J. Neuroinflammation. https://doi.org/10.1186/s12974-015-0327-3

Hall, E.D., 2011. Antioxidant Therapies for Acute Spinal Cord Injury. Neurotherapeutics 8, 152–167. https://doi.org/10.1007/s13311-011-0026-4

Han, Q., Xie, Y., Ordaz, J.D., Huh, A.J., Huang, N., Wu, W., Liu, N., Chamberlain, K.A., Sheng, Z.H., Xu, X.M., 2020. Restoring Cellular Energetics Promotes Axonal Regeneration and Functional Recovery after Spinal Cord Injury. Cell Metab. 31, 623-641.e8. https://doi.org/10.1016/j.cmet.2020.02.002

Hao, Y., Frey, E., Yoon, C., Wong, H., Nestorovski, D., Holzman, L.B., Giger, R.J., DiAntonio, A., Collins, C., 2016. An evolutionarily conserved mechanism for cAMP elicited axonal regeneration involves direct activation of the dual leucine zipper kinase DLK. Elife 5, 1–18. https://doi.org/10.7554/eLife.14048

Harkema, S., Behrman, A., Barbeau, H., 2012. Evidence-based therapy for recovery of function after spinal cord injury, 1st ed, Handbook of Clinical Neurology. Elsevier B.V. https://doi.org/10.1016/B978-0-444-52137-8.00016-4

Hasegawa, T., Venkata Suresh, V., Yahata, Y., Nakano, M., Suzuki, Shigeto, Suzuki, Shigeki, Yamada, S., Kitaura, H., Mizoguchi, I., Noiri, Y., Handa, K., Saito, M., 2021. Inhibition of the CXCL9-CXCR3 axis suppresses the progression of experimental apical periodontitis by blocking macrophage migration and activation. Sci. Rep. 11, 1–15. https://doi.org/10.1038/s41598-021-82167-7

Hasegawa, Y., Fujitani, M., Hata, K., Tohyama, M., Yamagishi, S., Yamashita, T., 2004. Promotion of axon regeneration by myelin-associated glycoprotein and Nogo through divergent signals downstream of Gi/G. J. Neurosci. 24, 6826–6832. https://doi.org/10.1523/JNEUROSCI.1856-04.2004

Hata, K., Fujitani, M., Yasuda, Y., Doya, H., Saito, T., Yamagishi, S., Mueller, B.K., Yamashita, T., 2006. RGMa inhibition promotes axonal growth and recovery after spinal cord injury. J. Cell Biol. https://doi.org/10.1083/jcb.200508143

Hauser, M.A., Legler, D.F., 2016. Common and biased signaling pathways of the chemokine receptor CCR7 elicited by its ligands CCL19 and CCL21 in leukocytes. J. Leukoc. Biol. 99,

869–882. https://doi.org/10.1189/jlb.2mr0815-380r

Hawryluk, G.W.J., Ruff, C.A., Fehlings, M.G., 2012. Development and maturation of the spinal cord. implications of molecular and genetic defects, 1st ed, Handbook of Clinical Neurology. Elsevier B.V. https://doi.org/10.1016/B978-0-444-52137-8.00001-2

He, Z., Jin, Y., 2016. Intrinsic Control of Axon Regeneration. Neuron. https://doi.org/10.1016/j.neuron.2016.04.022

Hellal, F., Hurtado, A., Ruschel, J., Flynn, K., Laskowski, C., Umlauf, M., Kapitein, L., Strikis, D., Lemmon, V., Bixby, J., Hoogenraad, C., Bradke, F., 2011. Microtubule Stabilization Reduces Scarring and Causes Axon Regeneration After Spinal Cord Injury. Sci. (American Assoc. Adv. Sci. 331, 928–931. https://doi.org/10.1126/science.1201148

Herrmann, K.A., Broihier, H.T., 2018. What neurons tell themselves: autocrine signals play essential roles in neuronal development and function. Curr. Opin. Neurobiol. 51, 70–79. https://doi.org/10.1016/j.conb.2018.03.002

Hervera, A., De Virgiliis, F., Palmisano, I., Zhou, L., Tantardini, E., Kong, G., Hutson, T., Danzi, M.C., Perry, R.B.-T., Santos, C.X.C., Kapustin, A.N., Fleck, R.A., Del Río, J.A., Carroll, T., Lemmon, V., Bixby, J.L., Shah, A.M., Fainzilber, M., Di Giovanni, S., 2018. Reactive oxygen species regulate axonal regeneration through the release of exosomal NADPH oxidase 2 complexes into injured axons. Nat. Cell Biol. 20. https://doi.org/10.1038/s41556-018-0039-x

Hervera, A., Santos, C.X., De Virgiliis, F., Shah, A.M., Di Giovanni, S., 2019a. Paracrine Mechanisms of Redox Signalling for Postmitotic Cell and Tissue Regeneration. Trends Cell Biol. https://doi.org/10.1016/j.tcb.2019.01.006

Hervera, A., Zhou, L., Palmisano, I., McLachlan, E., Kong, G., Hutson, T.H., Danzi, M.C., Lemmon, V.P., Bixby, J.L., Matamoros-Angles, A., Forsberg, K., De Virgiliis, F., Matheos, D.P., Kwapis, J., Wood, M.A., Puttagunta, R., del Río, J.A., Di Giovanni, S., 2019b. PP4-dependent HDAC3 dephosphorylation discriminates between axonal regeneration and regenerative failure. EMBO J. 38, 1–20. https://doi.org/10.15252/embj.2018101032

Hilton, B., Husch, A., Schaffran, B., Lin, T., Burnside, E., Dupraz, S., Schelski, M., Kim, J., Müller, J.A., Schoch, S., Imig, C., Brose, N., Bradke, F., 2021. An active vesicle priming machinery suppresses axon regeneration upon adult CNS injury. Neuron 51–69. https://doi.org/10.1016/j.neuron.2021.10.007

Hilton, B.J., Bradke, F., 2017. Can injured adult CNS axons regenerate by recapitulating development? Dev. 144, 3417–3429. https://doi.org/10.1242/dev.148312

Hirth, M., Gandla, J., Höper, C., Gaida, M.M., Agarwal, N., Simonetti, M., Demir, A., Xie, Y., Weiss, C., Michalski, C.W., Hackert, T., Ebert, M.P., Kuner, R., 2020. CXCL10 and CCL21 Promote Migration of Pancreatic Cancer Cells Toward Sensory Neurons and Neural Remodeling in Tumors in Mice, Associated With Pain in Patients. Gastroenterology 159, 665-681.e13. https://doi.org/10.1053/j.gastro.2020.04.037

Hjortø, G.M., Larsen, O., Steen, A., Daugvilaite, V., Berg, C., Fares, S., Hansen, M., Ali, S., Rosenkilde, M.M., 2016. Differential CCR7 targeting in dendritic cells by three naturally occurring CC-chemokines. Front. Immunol. 7, 1–15. https://doi.org/10.3389/fimmu.2016.00568

Hoffman, P.N., 2010. A conditioning lesion induces changes in gene expression and axonal transport that enhance regeneration by increasing the intrinsic growth state of axons. Exp. Neurol. 223, 11–18. https://doi.org/10.1016/j.expneurol.2009.09.006

Hogan, M.K., Hamilton, G.F., Horner, P.J., 2020. Neural Stimulation and Molecular Mechanisms of Plasticity and Regeneration: A Review. Front. Cell. Neurosci. 14, 1–16.

https://doi.org/10.3389/fncel.2020.00271

Honjoh, K., Nakajima, H., Hirai, T., Watanabe, S., Matsumine, A., 2019. Relationship of Inflammatory Cytokines From M1-Type Microglia/Macrophages at the Injured Site and Lumbar Enlargement With Neuropathic Pain After Spinal Cord Injury in the CCL21 Knockout (plt) Mouse. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2019.00525

Horn, K.P., Busch, S.A., Hawthorne, A.L., Van Rooijen, N., Silver, J., 2008. Another barrier to regeneration in the CNS: Activated macrophages induce extensive retraction of dystrophic axons through direct physical interactions. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2488-08.2008

Hu, X., Viesselmann, C., Nam, S., Merriam, E., Dent, E.W., 2008. Activity-dependent dynamic microtubule invasion of dendritic spines. J. Neurosci. 28, 13094–13105. https://doi.org/10.1523/JNEUROSCI.3074-08.2008

Hu, Z., Deng, N., Liu, K., Zeng, W., 2019. DLK mediates the neuronal intrinsic immune response and regulates glial reaction and neuropathic pain. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2019.113056

Hughes, C.E., Nibbs, R.J.B., 2018. A guide to chemokines and their receptors. FEBS J. 285, 2944–2971. https://doi.org/10.1111/febs.14466

Hunger-Glaser, I., Salazar, E.P., Sinnett-Smith, J., Rozengurt, E., 2003. Bombesin, lysophosphatidic acid, and epidermal growth factor rapidly stimulate focal adhesion kinase phosphorylation at Ser-910: Requirement for ERK activation. J. Biol. Chem. 278, 22631–22643. https://doi.org/10.1074/jbc.M210876200

Hutchinson, K.J., Gómez-Pinilla, F., Crowe, M.J., Ying, Z., Basso, D.M., 2004. Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. Brain (London, Engl. 1878) 127, 1403–1414. https://doi.org/10.1093/brain/awh160

Hutson, T.H., Di Giovanni, S., 2019. The translational landscape in spinal cord injury: focus on neuroplasticity and regeneration. Nat. Rev. Neurol. 15, 732–745. https://doi.org/10.1038/s41582-019-0280-3

Hutson, T.H., Kathe, C., Palmisano, I., Bartholdi, K., Hervera, A., De Virgiliis, F., McLachlan, E., Zhou, L., Kong, G., Barraud, Q., Danzi, M.C., Medrano-Fernandez, A., Lopez-Atalaya, J.P., Boutillier, A.L., Sinha, S.H., Singh, A.K., Chaturbedy, P., Moon, L.D.F., Kundu, T.K., Bixby, J.L., Lemmon, V.P., Barco, A., Courtine, G., Di Giovanni, S., 2019. Cbp-dependent histone acetylation mediates axon regeneration induced by environmental enrichment in rodent spinal cord injury models. Sci. Transl. Med. 11, eaaw2064-. https://doi.org/10.1126/scitranslmed.aaw2064

Hyung, S., Im, S.K., Lee, B.Y., Shin, J., Park, J.C., Lee, C., Suh, J.K.F., Hur, E.M., 2019. Dedifferentiated Schwann cells secrete progranulin that enhances the survival and axon growth of motor neurons. Glia. https://doi.org/10.1002/glia.23547

Inoue, K., 2006. The function of microglia through purinergic receptors: Neuropathic pain and cytokine release. Pharmacol. Ther. https://doi.org/10.1016/j.pharmthera.2005.07.001

Ishibe, S., Joly, D., Liu, Z.X., Cantley, L.G., 2004. Paxillin serves as an ERK-regulated scaffold for coordinating FAK and Rac activation in epithelial morphogenesis. Mol. Cell 16, 257–267. https://doi.org/10.1016/j.molcel.2004.10.006

Iwai, H., Ataka, K., Suzuki, H., Dhar, A., Kuramoto, E., Yamanaka, A., Goto, T., 2021. Tissue-resident M2 macrophages directly contact primary sensory neurons in the sensory ganglia after nerve injury. J. Neuroinflammation 18, 1–15. https://doi.org/10.1186/s12974-021-02283-z

Jack, A.S., Hurd, C., Martin, J., Fouad, K., 2020. Electrical Stimulation as a Tool to Promote Plasticity of the Injured Spinal Cord. J. Neurotrauma 37, 1933–1953. https://doi.org/10.1089/neu.2020.7033

Jaerve, A., Müller, H.W., 2012. Chemokines in CNS injury and repair. Cell Tissue Res. https://doi.org/10.1007/s00441-012-1427-3

Jaiswal, P.B., English, A.W., 2017. Chemogenetic enhancement of functional recovery after a sciatic nerve injury. Eur. J. Neurosci. 45, 1252–1257. https://doi.org/10.1111/ejn.13550

Jamann, N., Jordan, M., Engelhardt, M., 2018. Activity-dependent axonal plasticity in sensory systems. Neuroscience 368, 268–282. https://doi.org/10.1016/j.neuroscience.2017.07.035

Jara, J.S., Agger, S., Hollis, E.R., 2020. Functional Electrical Stimulation and the Modulation of the Axon Regeneration Program. Front. Cell Dev. Biol. 8, 1–12. https://doi.org/10.3389/fcell.2020.00736

Jessen, K.R., Mirsky, R., Lloyd, A.C., 2015. Schwann cells: Development and role in nerve repair. Cold Spring Harb. Perspect. Biol. 7, 1–15. https://doi.org/10.1101/cshperspect.a020487

Jeub, M., Siegloch, P.A., Nitsch, L., Zimmermann, J., Mueller, M.M., 2020. Reduced inflammatory response and accelerated functional recovery following sciatic nerve crush lesion in CXCR3-deficient mice. Neuroreport 672–677. https://doi.org/10.1097/WNR.0000000000001468

Jiang, B.C., Cao, D.L., Zhang, X., Zhang, Z.J., He, L.N., Li, C.H., Zhang, W.W., Wu, X.B., Berta, T., Ji, R.R., Gao, Y.J., 2016. CXCL13 drives spinal astrocyte activation and neuropathic pain via CXCR5. J. Clin. Invest. https://doi.org/10.1172/JCI81950

Jin, D., Liu, Y., Sun, F., Wang, X., Liu, X., He, Z., 2015. Restoration of skilled locomotion by sprouting corticospinal axons induced by co-deletion of PTEN and SOCS3. Nat. Commun. https://doi.org/10.1038/ncomms9074

Jørgensen, A.S., Larsen, O., Uetz-von Allmen, E., Lückmann, M., Legler, D.F., Frimurer, T.M., Veldkamp, C.T., Hjortø, G.M., Rosenkilde, M.M., 2019. Biased Signaling of CCL21 and CCL19 Does Not Rely on N-Terminal Differences, but Markedly on the Chemokine Core Domains and Extracellular Loop 2 of CCR7. Front. Immunol. 10. https://doi.org/10.3389/fimmu.2019.02156

Jørgensen, A.S., Rosenkilde, M.M., Hjortø, G.M., 2018. Biased signaling of G protein-coupled receptors – From a chemokine receptor CCR7 perspective. Gen. Comp. Endocrinol. 258, 4–14. https://doi.org/10.1016/j.ygcen.2017.07.004

Joutoku, Z., Onodera, T., Matsuoka, M., Homan, K., Momma, D., Baba, R., Hontani, K., Hamasaki, M., Matsubara, S., Hishimura, R., Iwasaki, N., 2019. CCL21/CCR7 axis regulating juvenile cartilage repair can enhance cartilage healing in adults. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-41621-3

Karin, N., 2020. CXCR3 Ligands in Cancer and Autoimmunity, Chemoattraction of Effector T Cells, and Beyond. Front. Immunol. 11, 1–9. https://doi.org/10.3389/fimmu.2020.00976

Kazim, S.F., Bowers, C.A., Cole, C.D., Varela, S., Karimov, Z., Martinez, E., Ogulnick, J. V., Schmidt, M.H., 2021. Corticospinal Motor Circuit Plasticity After Spinal Cord Injury: Harnessing Neuroplasticity to Improve Functional Outcomes. Mol. Neurobiol. https://doi.org/10.1007/s12035-021-02484-w

Ke, B.C., Huang, X.X., Li, Y., Li, L.Y., Xu, Q.X., Gao, Y., Liu, Y., Luo, J., 2016. Neuronal-derived Ccl7 drives neuropathic pain by promoting astrocyte proliferation. Neuroreport. https://doi.org/10.1097/WNR.0000000000000625

Keefe, K.M., Sheikh, I.S., Smith, G.M., 2017. Targeting neurotrophins to specific populations of neurons: NGF, BDNF, and NT-3 and their relevance for treatment of spinal cord injury. Int. J. Mol. Sci. 18, 1–17. https://doi.org/10.3390/ijms18030548

Keeler, B.E., Liu, G., Siegfried, R.N., Zhukareva, V., Murray, M., Houlé, J.D., 2012. Acute and prolonged hindlimb exercise elicits different gene expression in motoneurons than sensory neurons after spinal cord injury. Brain Res. 1438, 8–21. https://doi.org/10.1016/j.brainres.2011.12.015

Kerschensteiner, M., Meinl, E., Hohlfeld, R., 2009. Neuro-immune crosstalk in CNS diseases. Neuroscience. https://doi.org/10.1016/j.neuroscience.2008.09.009

Khakh, B.S., Sofroniew, M. V, 2015. Diversity of astrocyte functions and phenotypes in neural circuits Introduction and historical perspective. Nat. Neurosci. 18, 942–952. https://doi.org/10.1038/nn.4043.Diversity

Kigerl, K.A., Gensel, J.C., Ankeny, D.P., Alexander, J.K., Donnelly, D.J., Popovich, P.G., 2009. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.3257-09.2009

Kitayama, M., Ueno, M., Itakura, T., Yamashita, T., 2011. Activated microglia inhibit axonal growth through RGMa. PLoS One. https://doi.org/10.1371/journal.pone.0025234

Knerlich-Lukoschus, F., Von Der Ropp-Brenner, B., Lucius, R., Mehdorn, H.M., Held-Feindt, J., 2011. Spatiotemporal CCR1, CCL3(MIP-1α), CXCR4, CXCL12(SDF-1α) expression patterns in a rat spinal cord injury model of posttraumatic neuropathic pain: Laboratory investigation. J. Neurosurg. Spine. https://doi.org/10.3171/2010.12.SPINE10480

Koch, S.C., Acton, D., Goulding, M., 2018. Spinal Circuits for Touch, Pain, and Itch. Annu. Rev. Physiol. 80, 189–217. https://doi.org/10.1146/annurev-physiol-022516-034303

Kohout, T.A., Nicholas, S.L., Perry, S.J., Reinhart, G., Junger, S., Struthers, R.S., 2004. Differential desensitization, receptor phosphorylation, β-arrestin recruitment, and ERK1/2 activation by the two endogenous ligands for the CC chemokine receptor 7. J. Biol. Chem. 279, 23214–23222. https://doi.org/10.1074/jbc.M402125200

Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., Joshi, B. V., Jacobson, K.A., Kohsaka, S., Inoue, K., 2007. UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature. https://doi.org/10.1038/nature05704

Kong, F.Q., Zhao, S.J., Sun, P., Liu, H., Jie, J., Xu, T., Xu, A. Di, Yang, Y.Q., Zhu, Y., Chen, J., Zhou, Z., Qian, D.F., Gu, C.J., Chen, Q., Yin, G.Y., Zhang, H.W., Fan, J., 2020. Macrophage MSR1 promotes the formation of foamy macrophage and neuronal apoptosis after spinal cord injury. J. Neuroinflammation. https://doi.org/10.1186/s12974-020-01735-2

Kong, Y.F., Sha, W.L., Wu, X.B., Zhao, L.X., Ma, L.J., Gao, Y.J., 2021. CXCL10/CXCR3 Signaling in the DRG Exacerbates Neuropathic Pain in Mice. Neurosci. Bull. 37, 339–352. https://doi.org/10.1007/s12264-020-00608-1

Kroner, A., Greenhalgh, A.D., Zarruk, J.G., PassosdosSantos, R., Gaestel, M., David, S., 2014. TNF and Increased Intracellular Iron Alter Macrophage Polarization to a Detrimental M1 Phenotype in the Injured Spinal Cord. Neuron. https://doi.org/10.1016/j.neuron.2014.07.027

Krzyszczyk, P., Schloss, R., Palmer, A., Berthiaume, F., 2018. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Front. Physiol. 9, 1–22. https://doi.org/10.3389/fphys.2018.00419

Kucher, K., Johns, D., Maier, D., Abel, R., Badke, A., Baron, H., Thietje, R., Casha, S., Meindl,

R., Gomez-Mancilla, B., Pfister, C., Rupp, R., Weidner, N., Mir, A., Schwab, M.E., Curt, A., 2018. First-in-man intrathecal application of neurite growth-promoting anti-nogo- a antibodies in acute spinal cord injury. Neurorehabil. Neural Repair 32, 578–589. https://doi.org/10.1177/1545968318776371

Kuwabara, T., Ishikawa, F., Yasuda, T., Aritomi, K., Nakano, H., Tanaka, Y., Okada, Y., Lipp, M., Kakiuchi, T., 2009. CCR 7 Ligands Are Required for Development of Experimental Autoimmune Encephalomyelitis through Generating IL-23-Dependent Th17 Cells. J. Immunol. 183, 2513–2521. https://doi.org/10.4049/jimmunol.0800729

Kwon, M.J., Kim, J., Shin, H., Jeong, S.R., Kang, Y.M., Choi, J.Y., Hwang, D.H., Kim, B.G., 2013. Contribution of macrophages to enhanced regenerative capacity of dorsal root ganglia sensory neurons by conditioning injury. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.0278-13.2013

Kwon, M.J., Shin, H.Y., Cui, Y., Kim, H., Le Thi, A.H., Choi, J.Y., Kim, E.Y., Hwang, D.H., Kim, B.G., 2015. CCL2 mediates neuron-macrophage interactions to drive proregenerative macrophage activation following preconditioning injury. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.1924-15.2015

Le Pichon, C.E., Chesler, A.T., 2014. The functional and anatomical dissection of somatosensory subpopulations using mouse genetics. Front. Neuroanat. 8, 1–18. https://doi.org/10.3389/fnana.2014.00021

Lee, D.H., Lee, J.K., 2013. Animal models of axon regeneration after spinal cord injury. Neurosci. Bull. 29, 436–444. https://doi.org/10.1007/s12264-013-1365-4

Lee, P.R., Cohen, J.E., Iacobas, D.A., Iacobas, S., Douglas Fields, R., 2017. Gene networks activated by specific patterns of action potentials in dorsal root ganglia neurons. Sci. Rep. 7, 1–14. https://doi.org/10.1038/srep43765

Lee, S.H., Shi, X.Q., Fan, A., West, B., Zhang, J., 2018. Targeting macrophage and microglia activation with colony stimulating factor 1 receptor inhibitor is an effective strategy to treat injury-triggered neuropathic pain. Mol. Pain. https://doi.org/10.1177/1744806918764979

Leibinger, M., Andreadaki, A., Diekmann, H., Fischer, D., 2013. Neuronal STAT3 activation is essential for CNTF- and inflammatory stimulation-induced CNS axon regeneration. Cell Death Dis. 4, e805-8. https://doi.org/10.1038/cddis.2013.310

Leibinger, M., Andreadaki, A., Gobrecht, P., Levin, E., Diekmann, H., Fischer, D., 2016. Boosting central nervous system axon regeneration by circumventing limitations of natural cytokine signaling. Mol. Ther. https://doi.org/10.1038/mt.2016.102

Leite, S.C., Pinto-Costa, R., Sousa, M.M., 2021. Actin dynamics in the growth cone: a key player in axon regeneration. Curr. Opin. Neurobiol. 69, 11–18. https://doi.org/10.1016/j.conb.2020.11.015

Lemon, R.N., 2008. Descending Pathways in Motor Control. Annu. Rev. Neurosci. 31, 195–218. https://doi.org/10.1146/annurev.neuro.31.060407.125547

Li, F., Zou, Z., Suo, N., Zhang, Z., Wan, F., Zhong, G., Qu, Y., Ntaka, K.S., Tian, H., 2014. CCL21/CCR7 axis activating chemotaxis accompanied with epithelial–mesenchymal transition in human breast carcinoma. Med. Oncol. 31, 1–10. https://doi.org/10.1007/s12032-014-0180-8

Li, L., Adnan, H., Xu, B., Wang, J., Wang, C., Li, F., Tang, K., 2015. Effects of transplantation of olfactory ensheathing cells in chronic spinal cord injury: a systematic review and meta-analysis. Eur. spine J. 24, 919–930.

Li, S., Liu, B.P., Budel, S., Li, M., Ji, B., Walus, L., Li, W., Jirik, A., Rabacchi, S., Choi, E.,

Worley, D., Sah, D.W.Y., Pepinsky, B., Lee, D., Relton, J., Strittmatter, S.M., 2004. Blockade of Nogo-66, Myelin-Associated Glycoprotein, and Oligodendrocyte Myelin Glycoprotein by Soluble Nogo-66 Receptor Promotes Axonal Sprouting and Recovery after Spinal Injury. J. Neurosci. 24, 10511–10520. https://doi.org/10.1523/JNEUROSCI.2828-04.2004

Li, S., Yang, C., Zhang, L., Gao, X., Wang, X., Liu, W., Wang, Y., Jiang, S., Wong, Y.H., Zhang, Y., Liu, K., 2016. Promoting axon regeneration in the adult CNS by modulation of the melanopsin/GPCR signaling. Proc. Natl. Acad. Sci. U. S. A. 113, 1937–1942. https://doi.org/10.1073/pnas.1523645113

Li, X., Wang, Q., Ding, J., Wang, S., Dong, C., Wu, Q., 2020. Exercise training modulates glutamic acid decarboxylase-65/67 expression through TrkB signaling to ameliorate neuropathic pain in rats with spinal cord injury. Mol. Pain 16. https://doi.org/10.1177/1744806920924511

Li, Y., He, X., Kawaguchi, R., Zhang, Y., Wang, Q., Monavarfeshani, A., Yang, Z., Chen, B., Shi, Z., Meng, H., Zhou, S., Zhu, J., Jacobi, A., Swarup, V., Popovich, P.G., Geschwind, D.H., He, Z., 2020. Microglia-organized scar-free spinal cord repair in neonatal mice. Nature 587, 613–618. https://doi.org/10.1038/s41586-020-2795-6

Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.S., Peterson, T.C., Wilton, D.K., Frouin, A., Napier, B.A., Panicker, N., Kumar, M., Buckwalter, M.S., Rowitch, D.H., Dawson, V.L., Dawson, T.M., Stevens, B., Barres, B.A., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. https://doi.org/10.1038/nature21029

Lim, J.H.A., Stafford, B.K., Nguyen, P.L., Lien, B. V., Wang, C., Zukor, K., He, Z., Huberman, A.D., 2016. Neural activity promotes long-distance, target-specific regeneration of adult retinal axons. Nat. Neurosci. 19, 1073–1084. https://doi.org/10.1038/nn.4340

Lindwall, C., Kanje, M., 2005. Retrograde axonal transport of JNK signaling molecules influence injury induced nuclear changes in p-c-Jun and ATF3 in adult rat sensory neurons. Mol. Cell. Neurosci. 29, 269–282. https://doi.org/10.1016/j.mcn.2005.03.002

Liu, J.X., Cao, X., Tang, Y.C., Liu, Y., Tang, F.R., 2007. CCR7, CCR8, CCR9 and CCR10 in the mouse hippocampal CA1 area and the dentate gyrus during and after pilocarpine-induced status epilepticus. J. Neurochem. 100, 1072–1088. https://doi.org/10.1111/j.1471-4159.2006.04272.x

Liu, K., Lu, Y., Lee, J.K., Samara, R., Willenberg, R., Sears-Kraxberger, I., Tedeschi, A., Park, K.K., Jin, D., Cai, B., Xu, B., Connolly, L., Steward, O., Zheng, B., He, Z., 2010. PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat. Neurosci. 13, 1075–1081. https://doi.org/10.1038/nn.2603

Liu, Z.Y., Song, Z.W., Guo, S.W., He, J.S., Wang, S.Y., Zhu, J.G., Yang, H.L., Liu, J.B., 2019. CXCL12/CXCR4 signaling contributes to neuropathic pain via central sensitization mechanisms in a rat spinal nerve ligation model. CNS Neurosci. Ther. https://doi.org/10.1111/cns.13128

Loh, Y.H.E., Koemeter-Cox, A., Finelli, M.J., Shen, L., Friedel, R.H., Zou, H., 2017. Comprehensive mapping of 5-hydroxymethylcytosine epigenetic dynamics in axon regeneration. Epigenetics 12, 77–92. https://doi.org/10.1080/15592294.2016.1264560

Long, J., Federico, P., Perez, M.A., 2017. A novel cortical target to enhance hand motor output in humans with spinal cord injury. Brain 140, 1619–1632. https://doi.org/10.1093/brain/awx102

López-Cotarelo, P., Escribano-Díaz, C., González-Bethencourt, I.L., Gómez-Moreira, C., Deguiz,

M.L., Torres-Bacete, J., Gómez-Cabañas, L., Fernández-Barrera, J., Delgado-Martín, C., Mellado, M., Regueiro, J.R., Miranda-Carús, M.E., Rodríguez-Fernández, J.L., 2015. A novel MEK-ERK-AMPK signaling axis controls chemokine receptor CCR7-dependent survival in human mature dendritic cells. J. Biol. Chem. 290, 827–840. https://doi.org/10.1074/jbc.M114.596551

Lopez-Leal, R., Court, F.A., 2016. Schwann Cell Exosomes Mediate Neuron–Glia Communication and Enhance Axonal Regeneration. Cell. Mol. Neurobiol. https://doi.org/10.1007/s10571-015-0314-3

Loudon, R.P., Silver, L.D., Yee, H.F., Gallo, G., 2006. RhoA-kinase and myosin II are required for the maintenance of growth cone polarity and guidance by nerve growth factor. J. Neurobiol. 66, 847–867. https://doi.org/10.1002/neu.20258

Love, M., Sandberg, J.L., Ziarek, J.J., Gerarden, K.P., Rode, R.R., Jensen, D.R., McCaslin, D.R., Peterson, F.C., Veldkamp, C.T., 2012. Solution structure of CCL21 and identification of a putative CCR7 binding site. Biochemistry 51, 733–735. https://doi.org/10.1021/bi201601k

Loy, K., Bareyre, F.M., 2019. Rehabilitation following spinal cord injury: How animal models can help our understanding of exercise-induced neuroplasticity. Neural Regen. Res. 14, 405–412. https://doi.org/10.4103/1673-5374.245951

Loy, K., Schmalz, A., Hoche, T., Jacobi, A., Kreutzfeldt, M., Merkler, D., Bareyre, F.M., 2018. Enhanced Voluntary Exercise Improves Functional Recovery following Spinal Cord Injury by Impacting the Local Neuroglial Injury Response and Supporting the Rewiring of Supraspinal Circuits. J. Neurotrauma 35, 294–2915. https://doi.org/10.1089/neu.2017.5544

Luo, X., Tai, W.L., Sun, L., Pan, Z., Xia, Z., Chung, S.K., Cheung, C.W., 2016. Crosstalk between astrocytic CXCL12 and microglial CXCR4 contributes to the development of neuropathic pain. Mol. Pain. https://doi.org/10.1177/1744806916636385

Madabhushi, R., Gao, F., Pfenning, A.R., Pan, L., Yamakawa, S., Seo, J., Rueda, R., Phan, T.X., Yamakawa, H., Pao, P.C., Stott, R.T., Gjoneska, E., Nott, A., Cho, S., Kellis, M., Tsai, L.H., 2015. Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes. Cell 161, 1592–1605. https://doi.org/10.1016/j.cell.2015.05.032

Mahar, M., Cavalli, V., 2018. Intrinsic mechanisms of neuronal axon regeneration. Nat. Rev. Neurosci. https://doi.org/10.1038/s41583-018-0001-8

Maier, I.C., Ichiyama, R.M., Courtine, G., Schnell, L., Lavrov, I., Edgerton, V.R., Schwab, M.E., 2009. Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. Brain 132, 1426–1440. https://doi.org/10.1093/brain/awp085

Mar, F.M., Bonni, A., Sousa, M.M., 2014. Cell intrinsic control of axon regeneration. EMBO Rep. 15, 254–263. https://doi.org/10.1002/embr.201337723

Marquez-Chin, C., Popovic, M.R., 2020. Functional electrical stimulation therapy for restoration of motor function after spinal cord injury and stroke: A review. Biomed. Eng. Online 19, 1–25. https://doi.org/10.1186/s12938-020-00773-4

Martin, J.H., 2016. Harnessing neural activity to promote repair of the damaged corticospinal system after spinal cord injury. Neural Regen. Res. 11, 1389–1391. https://doi.org/10.4103/1673-5374.191199

Martin, J.H., Kably, B., Hacking, A., 1999. Activity-dependent development of cortical axon terminations in the spinal cord and brain stem. Exp. Brain Res. 125, 184–199. https://doi.org/10.1007/s002210050673

Martinez-Quiles, N., Ho, H.-Y.H., Kirschner, M.W., Ramesh, N., Geha, R.S., 2004. Erk/Src

Phosphorylation of Cortactin Acts as a Switch On-Switch Off Mechanism That Controls Its Ability To Activate N-WASP. Mol. Cell. Biol. 24, 5269–5280. https://doi.org/10.1128/mcb.24.12.5269-5280.2004

McKeon, R.J., Jurynec, M.J., Buck, C.R., 1999. The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. J. Neurosci. 19, 10778–10788. https://doi.org/10.1523/jneurosci.19-24-10778.1999

McKerracher, L., Rosen, K.M., 2015. MAG, myelin and overcoming growth inhibition in the CNS. Front. Mol. Neurosci. 8, 1–6. https://doi.org/10.3389/fnmol.2015.00051

McLean, N.A., Verge, V.M.K., 2016. Dynamic impact of brief electrical nerve stimulation on the neural immune axis—polarization of macrophages toward a pro-repair phenotype in demyelinated peripheral nerve. Glia 64, 1546–1561. https://doi.org/10.1002/glia.23021

McPherson, J.G., Miller, R.R., Perlmutter, S.I., Poo, M.M., 2015. Targeted, activity-dependent spinal stimulation produces long-lasting motor recovery in chronic cervical spinal cord injury. Proc. Natl. Acad. Sci. U. S. A. 112, 12193–12198. https://doi.org/10.1073/pnas.1505383112

Meijering, E., Jacob, M., Sarria, J.C.F., Steiner, P., Hirling, H., Unser, M., 2004. Design and Validation of a Tool for Neurite Tracing and Analysis in Fluorescence Microscopy Images. Cytom. Part A 58, 167–176. https://doi.org/10.1002/cyto.a.20022

Mendoza, M.C., Er, E.E., Zhang, W., Ballif, B.A., Elliott, H.L., Danuser, G., Blenis, J., 2011. ERK-MAPK Drives Lamellipodia Protrusion by Activating the WAVE2 Regulatory Complex. Mol. Cell 41, 661–671. https://doi.org/10.1016/j.molcel.2011.02.031

Mesquida-Veny, F., Del Río, J.A., Hervera, A., 2021. Macrophagic and microglial complexity after neuronal injury. Prog. Neurobiol. 200. https://doi.org/10.1016/j.pneurobio.2020.101970

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R.L., McCoy, J.M., Pepinsky, R.B., 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat. Neurosci. 7, 221–228. https://doi.org/10.1038/nn1188

Michaluk, P., Kolodziej, L., Mioduszewska, B., Wilczynski, G.M., Dzwonek, J., Jaworski, J., Gorecki, D.C., Ottersen, O.P., Kaczmarek, L., 2007. β-Dystroglycan as a target for MMP-9, in response to enhanced neuronal activity. J. Biol. Chem. 282, 16036–16041. https://doi.org/10.1074/jbc.M700641200

Milich, L.M., Ryan, C.B., Lee, J.K., 2019. The origin, fate, and contribution of macrophages to spinal cord injury pathology. Acta Neuropathol. https://doi.org/10.1007/s00401-019-01992-3

Miron, V.E., Boyd, A., Zhao, J.W., Yuen, T.J., Ruckh, J.M., Shadrach, J.L., Van Wijngaarden, P., Wagers, A.J., Williams, A., Franklin, R.J.M., Ffrench-Constant, C., 2013. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat. Neurosci. https://doi.org/10.1038/nn.3469

Mishra, A.M., Pal, A., Gupta, D., Carmel, J.B., 2017. Paired motor cortex and cervical epidural electrical stimulation timed to converge in the spinal cord promotes lasting increases in motor responses. J. Physiol. 595, 6953–6968. https://doi.org/10.1113/JP274663

Misonou, H., Mohapatra, D.P., Park, E.W., Leung, V., Zhen, D., Misonou, K., Anderson, A.E., Trimmer, J.S., 2004. Regulation of ion channel localization and phosphorylation by neuronal activity. Nat. Neurosci. 7, 711–718. https://doi.org/10.1038/nn1260

Mitew, S., Gobius, I., Fenlon, L.R., McDougall, S.J., Hawkes, D., Xing, Y.L., Bujalka, H.,

Gundlach, A.L., Richards, L.J., Kilpatrick, T.J., Merson, T.D., Emery, B., 2018. Pharmacogenetic stimulation of neuronal activity increases myelination in an axon-specific manner. Nat. Commun. 9, 1–16. https://doi.org/10.1038/s41467-017-02719-2

Miyasaka, Y., Yamamoto, N., 2021. Neuronal Activity Patterns Regulate Brain-Derived Neurotrophic Factor Expression in Cortical Cells via Neuronal Circuits. Front. Neurosci. 15. https://doi.org/10.3389/fnins.2021.699583

Mo, M., Zhou, M., Wang, L., Qi, L., Zhou, K., Liu, L.F., Chen, Z., Zu, X.B., 2015. CCL21/CCR7 enhances the proliferation, migration, and invasion of human bladder cancer T24 cells. PLoS One 10, 1–12. https://doi.org/10.1371/journal.pone.0119506

Molteni, R., Zheng, J.Q., Ying, Z., Gómez-Pinilla, F., Twiss, J.L., 2004. Voluntary exercise increases axonal regeneration from sensory neurons. Proc. Natl. Acad. Sci. U. S. A. 101, 8473–8478. https://doi.org/10.1073/pnas.0401443101

Monneau, Y., Arenzana-Seisdedos, F., Lortat-Jacob, H., 2016. The sweet spot: how GAGs help chemokines guide migrating cells. J. Leukoc. Biol. 99, 935–953. https://doi.org/10.1189/jlb.3mr0915-440r

Monnier, P.P., Sierra, A., Schwab, J.M., Henke-Fahle, S., Mueller, B.K., 2003. The Rho/ROCK pathway mediates neurite growth-inhibitory activity associated with the chondroitin sulfate proteoglycans of the CNS glial scar. Mol. Cell. Neurosci. 22, 319–330. https://doi.org/10.1016/S1044-7431(02)00035-0

Moore, D.L., Blackmore, M.G., Hu, Y., Kaestner, K.H., Bixby, J.L., Lemmon, V.P., Goldberg, J.L., 2009. KLF family members regulate intrinsic axon regeneration ability. Science (80-.). 326, 298–301. https://doi.org/10.1126/science.1175737

Murray, P.J., Allen, J.E., Biswas, S.K., Fisher, E.A., Gilroy, D.W., Goerdt, S., Gordon, S., Hamilton, J.A., Ivashkiv, L.B., Lawrence, T., Locati, M., Mantovani, A., Martinez, F.O., Mege, J.L., Mosser, D.M., Natoli, G., Saeij, J.P., Schultze, J.L., Shirey, K.A., Sica, A., Suttles, J., Udalova, I., vanGinderachter, J.A., Vogel, S.N., Wynn, T.A., 2014. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. Immunity. https://doi.org/10.1016/j.immuni.2014.06.008

Nadeau, S., Filali, M., Zhang, J., Kerr, B.J., Rivest, S., Soulet, D., Iwakura, Y., Vaccari, J.P. de R., Keane, R.W., Lacroix, S., 2011. Functional recovery after peripheral nerve injury is dependent on the pro-inflammatory cytokines IL-1β and TNF: Implications for neuropathic pain. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2840-11.2011

Nakanishi, O., Suetsugu, S., Yamazaki, D., Takenawa, T., 2007. Effect of WAVE2 phosphorylation on activation of the Arp2/3 complex. J. Biochem. 141, 319–325. https://doi.org/10.1093/jb/mvm034

Nascimento, A.I., Mar, F.M., Sousa, M.M., 2018. The intriguing nature of dorsal root ganglion neurons: Linking structure with polarity and function. Prog. Neurobiol. 168, 86–103. https://doi.org/10.1016/j.pneurobio.2018.05.002

Nash, B., Thomson, C.E., Linington, C., Arthur, A.T., McClure, J.D., Mcbride, M.W., Barnett, S.C., 2011. Functional duality of astrocytes in myelination. J. Neurosci. 31, 13028–13038. https://doi.org/10.1523/JNEUROSCI.1449-11.2011

Nesic, O., Perez-Polo, R., Xu, G.Y., McAdoo, D., High, K.W., Hulsebosch, C., High, K.W., Hulsebosch, C., 2001. IL-1 receptor antagonist prevents apoptosis and caspase-3 activation after spinal cord injury. J. Neurotrauma. https://doi.org/10.1089/089771501750451857

Nguyen, P.T., Dorman, L.C., Pan, S., Vainchtein, I.D., Han, R.T., Nakao-Inoue, H., Taloma, S.E., Barron, J.J., Molofsky, A.B., Kheirbek, M.A., Molofsky, A. V., 2020. Microglial Remodeling of the Extracellular Matrix Promotes Synapse Plasticity. Cell 182, 388-

403.e15. https://doi.org/10.1016/j.cell.2020.05.050

Niederöst, B., Oertle, T., Fritsche, J., McKinney, R.A., Bandtlow, C.E., 2002. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. J. Neurosci. 22, 10368–10376. https://doi.org/10.1523/jneurosci.22-23-10368.2002

Niemi, J.P., DeFrancesco-Lisowitz, A., Cregg, J.M., Howarth, M., Zigmond, R.E., 2016. Overexpression of the monocyte chemokine CCL2 in dorsal root ganglion neurons causes a conditioning-like increase in neurite outgrowth and does so via a STAT3 dependent mechanism. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2015.09.018

Nocera, G., Jacob, C., 2020. Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. Cell. Mol. Life Sci. 77, 3977–3989. https://doi.org/10.1007/s00018-020-03516-9

Norenberg, M.D., Smith, J., Marcillo, A., 2004. The pathology of human spinal cord injury: defining the problems. J. Neurotrauma 21, 429–440. https://doi.org/10.1089/089771504323004575

Nossent, A.Y., Bastiaansen, A.J.N.M., Peters, E.A.B., de Vries, M.R., Aref, Z., Welten, S.M.J., de Jager, S.C.A., van der Pouw Kraan, T.C.T.M., Quax, P.H.A., 2017. CCR7-CCL19/CCL21 axis is essential for effective arteriogenesis in a murine model of hindlimb ischemia. J. Am. Heart Assoc. 6, 1–17. https://doi.org/10.1161/JAHA.116.005281

Novak, M.L., Koh, T.J., 2013. Phenotypic transitions of macrophages orchestrate tissue repair. Am. J. Pathol. https://doi.org/10.1016/j.ajpath.2013.06.034

O'Shea, T.M., Burda, J.E., Sofroniew, M. V., 2017. Cell biology of spinal cord injury and repair. J. Clin. Invest. 127, 3259–3270. https://doi.org/10.1172/JCI90608

Ogunshola, O.O., Antic, A., Donoghue, M.J., Fan, S.Y., Kim, H., Stewart, W.B., Madri, J.A., Ment, L.R., 2002. Paracrine and autocrine functions of neuronal vascular endothelial growth factor (VEGF) in the central nervous system. J. Biol. Chem. 277, 11410–11415. https://doi.org/10.1074/jbc.M111085200

Oh, S.K., Choi, K.H., Yoo, J.Y., Kim, D.Y., Kim, S.J., Jeon, S.R., 2016. A Phase III Clinical Trial Showing Limited Efficacy of Autologous Mesenchymal Stem Cell Therapy for Spinal Cord Injury. Neurosurgery 78, 436–447. https://doi.org/10.1227/NEU.0000000000001056

Okada, S., Hara, M., Kobayakawa, K., Matsumoto, Y., Nakashima, Y., 2018. Astrocyte reactivity and astrogliosis after spinal cord injury. Neurosci. Res. 126, 39–43. https://doi.org/10.1016/j.neures.2017.10.004

Okada, S., Nakamura, M., Katoh, H., Miyao, T., Shimazaki, T., Ishii, K., Yamane, J., Yoshimura, A., Iwamoto, Y., Toyama, Y., Okano, H., 2006. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat. Med. 12, 829–834. https://doi.org/10.1038/nm1425

Opatz, J., Küry, P., Schiwy, N., Järve, A., Estrada, V., Brazda, N., Bosse, F., Müller, H.W., 2009. SDF-1 stimulates neurite growth on inhibitory CNS myelin. Mol. Cell. Neurosci. https://doi.org/10.1016/j.mcn.2008.11.002

Ortiz, F.C., Habermacher, C., Graciarena, M., Houry, P.Y., Nishiyama, A., Oumesmar, B.N., Angulo, M.C., 2019. Neuronal activity in vivo enhances functional myelin repair. JCI Insight 4, 1–15. https://doi.org/10.1172/jci.insight.123434

Oudega, M., Perez, M.A., 2012. Corticospinal reorganization after spinal cord injury. J. Physiol. 590, 3647–3663. https://doi.org/10.1113/jphysiol.2012.233189

Oyinbo, C.A., 2011. Secondary injury mechanisms in traumatic spinal cord injury a nugget of this multiply cascade. Acta Neurobiol. Exp. (Wars). 71, 281–299.

Palmisano, I., Danzi, M.C., Hutson, T.H., Zhou, L., McLachlan, E., Serger, E., Shkura, K., Srivastava, P.K., Hervera, A., Neill, N.O., Liu, T., Dhrif, H., Wang, Z., Kubat, M., Wuchty, S., Merkenschlager, M., Levi, L., Elliott, E., Bixby, J.L., Lemmon, V.P., Di Giovanni, S., 2019. Epigenomic signatures underpin the axonal regenerative ability of dorsal root ganglia sensory neurons. Nat. Neurosci. 22, 1913–1924. https://doi.org/10.1038/s41593-019-0490-4

Palomer, E., Carretero, J., Benvegnù, S., Dotti, C.G., Martin, M.G., 2016. Neuronal activity controls Bdnf expression via Polycomb de-repression and CREB/CBP/JMJD3 activation in mature neurons. Nat. Commun. 7. https://doi.org/10.1038/ncomms11081

Pandey, V., Martinez, A.K.F., Bastea, L.I., Döppler, H.R., Eisenhauer, J., Le, T., Edenfield, B., Storz, P., 2021. Cxcl10/cxcr3 signaling contributes to an inflammatory microenvironment and its blockade enhances progression of murine pancreatic precancerous lesions. Elife 10, 1–24. https://doi.org/10.7554/eLife.60646

Park, J.B., Yiu, G., Kaneko, S., Wang, J., Chang, J., He, Z., 2005. A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. Neuron 45, 345–351. https://doi.org/10.1016/j.neuron.2004.12.040

Park, K.K., Liu, K., Hu, Y., Smith, P.D., Wang, C., Cai, B., Xu, B., Connolly, L., Kramvis, I., Sahin, M., He, Z., 2008. Promoting Axon Regeneration in the Adult CNS by Modulation of the PTEN/mTOR Pathway. Sci. (American Assoc. Adv. Sci. 322, 963–966.

Park, S., Koppes, R.A., Froriep, U.P., Jia, X., Achyuta, A.K.H., McLaughlin, B.L., Anikeeva, P., 2015. Optogenetic control of nerve growth. Sci. Rep. 5, 1–9. https://doi.org/10.1038/srep09669

Pecze, L., Blum, W., Schwaller, B., 2013. Mechanism of capsaicin receptor TRPV1-mediated toxicity in pain-sensing neurons focusing on the effects of Na+/Ca2+ fluxes and the Ca2+-binding protein calretinin. Biochim. Biophys. Acta - Mol. Cell Res. 1833, 1680–1691. https://doi.org/10.1016/j.bbamcr.2012.08.018

Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R., Fainzilber, M., 2005. Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. Neuron 45, 715–726. https://doi.org/10.1016/j.neuron.2005.01.023

Pinto-Costa, R., Sousa, S.C., Leite, S.C., Nogueira-Rodrigues, J., da Silva, T.F., Machado, D., Marques, J., Costa, A.C., Liz, M.A., Bartolini, F., Brites, P., Costell, M., Fässler, R., Sousa, M.M., 2020. Profilin 1 delivery tunes cytoskeletal dynamics toward CNS axon regeneration. J. Clin. Invest. 130, 2024–2040. https://doi.org/10.1172/JCI125771

Piotrowska, A., Rojewska, E., Pawlik, K., Kreiner, G., Ciechanowska, A., Makuch, W., Zychowska, M., Mika, J., 2018. Pharmacological blockade of CXCR3 by (±)-NBI-74330 reduces neuropathic pain and enhances opioid effectiveness - Evidence from in vivo and in vitro studies. Biochim. Biophys. Acta - Mol. Basis Dis. 1864, 3418–3437. https://doi.org/10.1016/j.bbadis.2018.07.032

Poeta, V.M., Massara, M., Capucetti, A., Bonecchi, R., 2019. Chemokines and chemokine receptors: New targets for cancer immunotherapy. Front. Immunol. 10, 1–10. https://doi.org/10.3389/fimmu.2019.00379

Pollard, T.D., 2016. Actin and actin-binding proteins. Cold Spring Harb. Perspect. Biol. 8, 1–17. https://doi.org/10.1101/cshperspect.a018226

Poniatowski, Ł.A., Wojdasiewicz, P., Krawczyk, M., Szukiewicz, D., Gasik, R., Kubaszewski, Ł., Kurkowska-Jastrzębska, I., 2017. Analysis of the Role of CX3CL1 (Fractalkine) and Its

Receptor CX3CR1 in Traumatic Brain and Spinal Cord Injury: Insight into Recent Advances in Actions of Neurochemokine Agents. Mol. Neurobiol. https://doi.org/10.1007/s12035-016-9787-4

Popovich, P.G., Wei, P., Stokes, B.T., 1997. Cellular inflammatory response after spinal cord injury in Sprague- Dawley and Lewis rats. J. Comp. Neurol. https://doi.org/10.1002/(SICI)1096-9861(19970120)377:3<443::AID-CNE10>3.0.CO;2-S

Pöyhönen, S., Er, S., Domanskyi, A., Airavaara, M., 2019. Effects of neurotrophic factors in glial cells in the central nervous system: Expression and properties in neurodegeneration and injury. Front. Physiol. 10, 1–20. https://doi.org/10.3389/fphys.2019.00486

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. https://doi.org/10.1242/jcs.01694

Purves, D., Augustine, G.J., Fitzpatrick, D., Hall, W.C., LaMantia, A.-S., McNamara, J.O., Williams, S.M., 2004. Neuroscience, 3rd ed. Sinauer Associates.

Puttagunta, R., Tedeschi, A., Sória, M.G., Hervera, A., Lindner, R., Rathore, K.I., Gaub, P., Joshi, Y., Nguyen, T., Schmandke, A., Laskowski, C.J., Boutillier, A.L., Bradke, F., Di Giovanni, S., 2014. PCAF-dependent epigenetic changes promote axonal regeneration in the central nervous system. Nat. Commun. 5. https://doi.org/10.1038/ncomms4527

Qian, C., Zhou, F.Q., 2020. Updates and challenges of axon regeneration in the mammalian central nervous system. J. Mol. Cell Biol. 12, 798–806. https://doi.org/10.1093/jmcb/mjaa026

Qiu, J., Cai, D., Filbin, M.T., 2000. Glial inhibition of nerve regeneration in the mature mammalian CNS. Glia 29, 166–174.

Rabiller, L., Labit, E., Guissard, C., Gilardi, S., Guiard, B.P., Moulédous, L., Silva, M., Mithieux, G., Pénicaud, L., Lorsignol, A., Casteilla, L., Dromard, C., 2021. Pain sensing neurons promote tissue regeneration in adult mice. npj Regen. Med. 6, 1–9. https://doi.org/10.1038/s41536-021-00175-7

Rappert, A., Biber, K., Nolte, C., Lipp, M., Schubel, A., Lu, B., Gerard, N.P., Gerard, C., Boddeke, H.W.G.M., 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. https://doi.org/10.4049/jimmunol.168.7.3221

Richardson, P.M., Issa, V.M.K., 1984. Peripheral injury enhances central regeneration of primary sensory neurones. Nat. 309, 791–793. https://doi.org/10.1038/309791a0

Riol-Blanco, L., Sánchez-Sánchez, N., Torres, A., Tejedor, A., Narumiya, S., Corbí, A.L., Sánchez-Mateos, P., Rodríguez-Fernández, J.L., 2005. The Chemokine Receptor CCR7 Activates in Dendritic Cells Two Signaling Modules That Independently Regulate Chemotaxis and Migratory Speed. J. Immunol. 174, 4070–4080. https://doi.org/10.4049/jimmunol.174.7.4070

Rishal, I., Fainzilber, M., 2014. Axon-soma communication in neuronal injury. Nat. Rev. Neurosci. 15, 32–42. https://doi.org/10.1038/nrn3609

Rizeq, B., Malki, M.I., 2020. The Role of CCL21/CCR7 Chemokine Axis in Breast Cancer Progression. Cancers (Basel). 12, 1036-. https://doi.org/10.3390/cancers12041036

Rodríguez-Fernández, J.L., Criado-García, O., 2020. The Chemokine Receptor CCR7 Uses Distinct Signaling Modules With Biased Functionality to Regulate Dendritic Cells. Front. Immunol. 11, 1–10. https://doi.org/10.3389/fimmu.2020.00528

Romer, S.H., Deardorff, A.S., Fyffe, R.E.W., 2016. Activity-dependent redistribution of Kv2.1 ion channels on rat spinal motoneurons. Physiol. Rep. 4, 1–11. https://doi.org/10.14814/phy2.13039

Rosenzweig, E.S., Courtine, G., Jindrich, D.L., Brock, J.H., Ferguson, A.R., Strand, S.C., Nout, Y.S., Roy, R.R., Miller, D.M., Beattie, M.S., Havton, L.A., Bresnahan, J.C., Edgerton, V.R., Tuszynski, M.H., 2010. Extensive spontaneous plasticity of corticospinal projections after primate spinal cord injury. Nat. Neurosci. 13, 1505–1512. https://doi.org/10.1038/nn.2691

Rosito, M., Deflorio, C., Limatola, C., Trettel, F., 2012. CXCL16 orchestrates adenosine A 3 receptor and MCP-1/CCL2 activity to protect neurons from excitotoxic cell death in the CNS. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.4046-11.2012

Rosito, M., Lauro, C., Chece, G., Porzia, A., Monaco, L., Mainiero, F., Catalano, M., Limatola, C., Trettel, F., 2014. Trasmembrane chemokines CX3CL1 and CXCL16 drive interplay between neurons, microglia and astrocytes to counteract pMCAO and excitotoxic neuronal death. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2014.00193

Rost, B.R., Schneider-warme, F., Schmitz, D., Hegemann, P., 2017. Primer Optogenetic Tools for Subcellular Applications in Neuroscience. Neuron 96, 572–603. https://doi.org/10.1016/j.neuron.2017.09.047

Rotshenker, S., 2011. Wallerian degeneration: The innate-immune response to traumatic nerve injury. J. Neuroinflammation 8. https://doi.org/10.1186/1742-2094-8-109

Ruschel, J., Bradke, F., 2018. Systemic administration of epothilone D improves functional recovery of walking after rat spinal cord contusion injury. Exp. Neurol. 306, 243–249. https://doi.org/10.1016/j.expneurol.2017.12.001

Ruschel, J., Hellal, F., Flynn, K.C., Dupraz, S., Elliott, D.A., Tedeschi, A., Bates, M., Sliwinski, C., Brook, G., Dobrindt, K., Peitz, M., Brüstle, O., Norenberg, M.D., Blesch, A., Weidner, N., Bunge, M.B., Bixby, J.L., Bradke, F., 2015. Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. Sci. (American Assoc. Adv. Sci. 348, 347–352. https://doi.org/10.1126/science.aaa2958

Sala-Jarque, J., Mesquida-Veny, F., Badiola-Mateos, M., Samitier, J., Hervera, A., del Río, J.A., 2020. Neuromuscular Activity Induces Paracrine Signaling and Triggers Axonal Regrowth after Injury in Microfluidic Lab-On-Chip Devices. Cells. https://doi.org/10.3390/cells9020302

Saliani, A., Perraud, B., Duval, T., Stikov, N., Rossignol, S., Cohen-Adad, J., 2017. Axon and myelin morphology in animal and human spinal cord. Front. Neuroanat. 11, 1–19. https://doi.org/10.3389/fnana.2017.00129

Sami, A., Selzer, M.E., Li, S., 2020. Advances in the Signaling Pathways Downstream of Glial-Scar Axon Growth Inhibitors. Front. Cell. Neurosci. 14, 1–12. https://doi.org/10.3389/fncel.2020.00174

San Ruiz-Miguel, J.E., Letourneau, P.C., 2014. The role of Arp2/3 in growth cone actin dynamics and guidance is substrate dependent. J. Neurosci. 34, 5895–5908. https://doi.org/10.1523/JNEUROSCI.0672-14.2014

Sánchez-Sánchez, N., Riol-Blanco, L., De La Rosa, G., Puig-Kröger, A., García-Bordas, J., Martín, D., Longo, N., Cuadrado, A., Cabañas, C., Corbí, A.L., Sánchez-Mateos, P., Rodríguez-Fernández, J.L., 2004. Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells. Blood 104, 619–625. https://doi.org/10.1182/blood-2003-11-3943

Sánchez-Ventura, J., Giménez-Llort, L., Penas, C., Udina, E., 2021. Voluntary wheel running preserves lumbar perineuronal nets, enhances motor functions and prevents hyperreflexia

after spinal cord injury. Exp. Neurol. 336. https://doi.org/10.1016/j.expneurol.2020.113533

Sandner, B., Puttagunta, R., Motsch, M., Bradke, F., Ruschel, J., Blesch, A., Weidner, N., 2018. Systemic epothilone D improves hindlimb function after spinal cord contusion injury in rats. Exp. Neurol. 306, 250–259. https://doi.org/10.1016/j.expneurol.2018.01.018

Sandrow-Feinberg, H.R., Izzi, J., Shumsky, J.S., Zhukareva, V., Houle, J.D., 2009. Forced exercise as a rehabilitation strategy after unilateral cervical spinal cord contusion injury. J. Neurotrauma 26, 721–731. https://doi.org/10.1089/neu.2008.0750

Sasaki, M., Abe, R., Fujita, Y., Ando, S., Inokuma, D., Shimizu, H., 2008. Mesenchymal Stem Cells Are Recruited into Wounded Skin and Contribute to Wound Repair by Transdifferentiation into Multiple Skin Cell Type. J. Immunol. 180, 2581–2587. https://doi.org/10.4049/jimmunol.180.4.2581

Satake, K., Matsuyama, Y., Kamiya, M., Kawakami, H., Iwata, H., Adachi, K., Kiuchi, K., 2000. Nitric oxide via macrophage iNOS induces apoptosis following traumatic spinal cord injury. Mol. Brain Res. https://doi.org/10.1016/S0169-328X(00)00253-9

Saxena, A., Wagatsuma, A., Noro, Y., Kuji, T., Asaka-Oba, A., Watahiki, A., Gurnot, C., Fagiolini, M., Hensch, T.K., Carninci, P., 2012. Trehalose-enhanced isolation of neuronal sub-types from adult mouse brain. Biotechniques 52, 381–385. https://doi.org/10.2144/0000113878

Scandella, E., Men, Y., Legler, D.F., Gillessen, S., Prikler, L., Ludewig, B., Groettrup, M., 2004. CCL19/CCL21-triggered signal transduction and migration of dendritic cells requires prostaglandin E2. Blood 103, 1595–1601. https://doi.org/10.1182/blood-2003-05-1643

Schlau, M., Terheyden-Keighley, D., Theis, V., Mannherz, H.G., Theiss, C., 2018. VEGF triggers the activation of cofilin and the Arp2/3 complex within the growth cone. Int. J. Mol. Sci. 19. https://doi.org/10.3390/ijms19020384

Schumann, K., Lämmermann, T., Bruckner, M., Legler, D.F., Polleux, J., Spatz, J.P., Schuler, G., Förster, R., Lutz, M.B., Sorokin, L., Sixt, M., 2010. Immobilized Chemokine Fields and Soluble Chemokine Gradients Cooperatively Shape Migration Patterns of Dendritic Cells. Immunity 32, 703–713. https://doi.org/10.1016/j.immuni.2010.04.017

Schwaiger, F.W., Hager, Gerhard, Schmitt, A.B., Horvat, A., Hager, Gundel, Streif, R., Spitzer, C., Gamal, S., Breuer, S., Brook, G.A., Nacimiento, W., Kreutzberg, G.W., 2000. Peripheral but not central axotomy induces changes in Janus kinases (JAK) and signal transducers and activators of transcription (STAT). Eur. J. Neurosci. 12, 1165–1176. https://doi.org/10.1046/j.1460-9568.2000.00005.x

Segarra-Mondejar, M., Casellas-Díaz, S., Ramiro-Pareta, M., Müller-Sánchez, C., Martorell-Riera, A., Hermelo, I., Reina, M., Aragonés, J., Martínez-Estrada, O.M., Soriano, F.X., 2018. Synaptic activity-induced glycolysis facilitates membrane lipid provision and neurite outgrowth. EMBO J. 37, 1–16. https://doi.org/10.15252/embj.201797368

Senger, J.L., Chan, K.M., Macandili, H., Chan, A.W.M., Verge, V.M.K., Jones, K.E., Webber, C.A., 2019. Conditioning electrical stimulation promotes functional nerve regeneration. Exp. Neurol. 315, 60–71. https://doi.org/10.1016/j.expneurol.2019.02.001

Senger, J.L.B., Verge, V.M.K., Macandili, H.S.J., Olson, J.L., Chan, K.M., Webber, C.A., 2018. Electrical stimulation as a conditioning strategy for promoting and accelerating peripheral nerve regeneration. Exp. Neurol. 302, 75–84. https://doi.org/10.1016/j.expneurol.2017.12.013

Sengottuvel, V., Leibinger, M., Pfreimer, M., Andreadaki, A., Fischer, D., 2011. Taxol facilitates axon regeneration in the mature CNS. J. Neurosci. 31, 2688–2699. https://doi.org/10.1523/JNEUROSCI.4885-10.2011

Shannon, L.A., Calloway, P.A., Welch, T.P., Vines, C.M., 2010. CCR7/CCL21 migration on fibronectin is mediated by phospholipase Cγ1 and ERK1/2 in primary T lymphocytes. J. Biol. Chem. 285, 38781–38787. https://doi.org/10.1074/jbc.M110.152173

Shannon, L.A., McBurney, T.M., Wells, M.A., Roth, M.E., Calloway, P.A., Bill, C.A., Islam, S., Vines, C.M., 2012. CCR7/CCL19 controls expression of EDG-1 in T cells. J. Biol. Chem. 287, 11656–11664. https://doi.org/10.1074/jbc.M111.310045

Sharma, K., Selzer, M.E., Li, S., 2012. Scar-mediated inhibition and CSPG receptors in the CNS. Exp. Neurol. 237, 370–378. https://doi.org/10.1016/j.expneurol.2012.07.009

Shen, Y., Tenney, A.P., Busch, S.A., Horn, K.P., Fernando, X., Liu, K., He, Z., Silver, J., Flanagan, J.G., 2009. PTPsigma-receptor for CSPGs. Science (80-.). 326, 592–596. https://doi.org/10.1126/science.1178310.PTP

Sheridan, G.K., Murphy, K.J., 2013. Neuron-glia crosstalk in health and disease: Fractalkine and CX3CR1 take centre stage. Open Biol. https://doi.org/10.1098/rsob.130181

Shim, S., Ming, G.L., 2010. Roles of channels and receptors in the growth cone during PNS axonal regeneration. Exp. Neurol. 223, 38–44. https://doi.org/10.1016/j.expneurol.2009.10.001

Shin, J.E., Cho, Y., Beirowski, B., Milbrandt, J., Cavalli, V., DiAntonio, A., 2012. Dual Leucine Zipper Kinase Is Required for Retrograde Injury Signaling and Axonal Regeneration. Neuron 74, 1015–1022. https://doi.org/10.1016/j.neuron.2012.04.028

Shinozaki, M., Iwanami, A., Fujiyoshi, K., Tashiro, S., Kitamura, K., Shibata, S., Fujita, H., Nakamura, M., Okano, H., 2016. Combined treatment with chondroitinase ABC and treadmill rehabilitation for chronic severe spinal cord injury in adult rats. Neurosci. Res. 113, 37–47. https://doi.org/10.1016/j.neures.2016.07.005

Siegle, J.H., López, A.C., Patel, Y.A., Abramov, K., Ohayon, S., Voigts, J., 2017. Open Ephys: An open-source, plugin-based platform for multichannel electrophysiology. J. Neural Eng. 14. https://doi.org/10.1088/1741-2552/aa5eea

Silver, J., 2016. The glial scar is more than just astrocytes. Exp. Neurol. 286, 147–149. https://doi.org/10.1016/j.expneurol.2016.06.018

Sindrilaru, A., Scharffetter-Kochanek, K., 2013. Disclosure of the Culprits: Macrophages—Versatile Regulators of Wound Healing. Adv. Wound Care. https://doi.org/10.1089/wound.2012.0407

Sivasankaran, R., Pei, J., Wang, K.C., Zhang, Y.P., Shields, C.B., Xu, X.M., He, Z., 2004. PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. Nat. Neurosci. 7, 261–268. https://doi.org/10.1038/nn1193

Smith, P.D., Sun, F., Park, K.K., Cai, B., Wang, C., Kuwako, K., Martinez-Carrasco, I., Connolly, L., He, Z., 2009. SOCS3 Deletion Promotes Optic Nerve Regeneration In Vivo. Neuron. https://doi.org/10.1016/j.neuron.2009.11.021

Sofroniew, M. V., 2020. Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity. Trends Immunol. 41, 758–770. https://doi.org/10.1016/j.it.2020.07.004

Song, G., Yang, R., Zhang, Q., Chen, L., Huang, D., Zeng, J., Yang, C., Zhang, T., 2019. TGF-β Secretion by M2 Macrophages Induces Glial Scar Formation by Activating Astrocytes In Vitro. J. Mol. Neurosci. https://doi.org/10.1007/s12031-019-01361-5

Song, W., Cho, Y., Watt, D., Cavalli, V., 2015. Tubulin-tyrosine ligase (TTL)-mediated increase in tyrosinated α-tubulin in injured axons is required for retrograde injury signaling and axon regeneration. J. Biol. Chem. 290, 14765–14775. https://doi.org/10.1074/jbc.M114.622753

Song, Y., Brady, S.T., 2015. Post-translational modifications of tubulin: Pathways to functional diversity of microtubules. Trends Cell Biol. 25, 125–136. https://doi.org/10.1016/j.tcb.2014.10.004

Sonn, I., Nakamura, M., Renault-Mihara, F., Okano, H., 2019. Polarization of Reactive Astrocytes in Response to Spinal Cord Injury is Enhanced by M2 Macrophage–Mediated Activation of Wnt/β-Catenin Pathway. Mol. Neurobiol. https://doi.org/10.1007/s12035-019-01851-y

Spira, M.E., Oren, R., Dormann, A., Gitler, D., 2003. Critical calpain-dependent ultrastructural alterations underlie the transformation of an axonal segment into a growth cone after axotomy of cultured Aplysia neurons. J. Comp. Neurol. 457, 293–312. https://doi.org/10.1002/cne.10569

Starkey, M.L., Davies, M., Yip, P.K., Carter, L.M., Wong, D.J.N., McMahon, S.B., Bradbury, E.J., 2009. Expression of the regeneration-associated protein SPRR1A in primary sensory neurons and spinal cord of the adult mouse following peripheral and central injury. J. Comp. Neurol. 513, 51–68. https://doi.org/10.1002/cne.21944

Stern, S., Hilton, B.J., Burnside, E.R., Dupraz, S., Handley, E.E., Gonyer, J.M., Brakebusch, C., Bradke, F., 2021. RhoA drives actin compaction to restrict axon regeneration and astrocyte reactivity after CNS injury. Neuron 109, 3436-3455.e9. https://doi.org/10.1016/j.neuron.2021.08.014

Sternson, S.M., Roth, B.L., 2014. Chemogenetic tools to interrogate brain functions. Annu. Rev. Neurosci. 37, 387–407. https://doi.org/10.1146/annurev-neuro-071013-014048

Steward, O., Willenberg, R., 2017. Rodent spinal cord injury models for studies of axon regeneration. Exp. Neurol. 287, 374–383. https://doi.org/10.1016/j.expneurol.2016.06.029

Stoll, G., Griffin, J.W., Li, C.Y., Trapp, B.D., 1989. Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. J. Neurocytol. 18, 671–683. https://doi.org/10.1007/BF01187086

Su, Y., Shin, J., Zhong, C., Wang, S., Roychowdhury, P., Lim, J., Kim, D., Ming, G.L., Song, H., 2017. Neuronal activity modifies the chromatin accessibility landscape in the adult brain. Nat. Neurosci. 20, 476–483. https://doi.org/10.1038/nn.4494

Sun, S., Chen, D., Lin, F., Chen, M., Yu, H., Hou, L., Li, C., 2016. Role of interleukin-4, the chemokine CCL3 and its receptor CCR5 in neuropathic pain. Mol. Immunol. https://doi.org/10.1016/j.molimm.2016.08.006

Svitkina, T., 2018. The actin cytoskeleton and actin-based motility. Cold Spring Harb. Perspect. Biol. 10, 1–21. https://doi.org/10.1101/cshperspect.a018267

Szepesi, Z., Manouchehrian, O., Bachiller, S., Deierborg, T., 2018. Bidirectional Microglia–Neuron Communication in Health and Disease. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2018.00323

Takahashi, K., Rochford, C.D.P., Neumann, H., 2005. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J. Exp. Med. https://doi.org/10.1084/jem.20041611

Tanimura, S., Takeda, K., 2017. ERK signalling as a regulator of cell motility. J. Biochem. 162, 145–154. https://doi.org/10.1093/jb/mvx048

Taylor, A.M., Blurton-Jones, M., Rhee, S.W., Cribbs, D.H., Cotman, C.W., Jeon, N.L., 2005. A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nat. Methods 2, 599–605. https://doi.org/10.1038/nmeth777

Tedeschi, A., Dupraz, S., Curcio, M., Laskowski, C.J., Schaffran, B., Flynn, K.C., Santos, T.E., Stern, S., Hilton, B.J., Larson, M.J.E., Gurniak, C.B., Witke, W., Bradke, F., 2019. ADF/Cofilin-Mediated Actin Turnover Promotes Axon Regeneration in the Adult CNS. Neuron 103, 1073-1085.e6. https://doi.org/10.1016/j.neuron.2019.07.007

Tedeschi, A., Dupraz, S., Laskowski, C.J., Xue, J., Ulas, T., Beyer, M., Schultze, J.L., Bradke, F., 2016. The Calcium Channel Subunit Alpha2delta2 Suppresses Axon Regeneration in the Adult CNS. Neuron 92, 419–434. https://doi.org/10.1016/j.neuron.2016.09.026

Tennant, K.A., Taylor, S.L., White, E.R., Brown, C.E., 2017. Optogenetic rewiring of thalamocortical circuits to restore function in the stroke injured brain. Nat. Commun. 8, 1–14. https://doi.org/10.1038/ncomms15879

Tian, L., Ma, L., Kaarela, T., Li, Z., 2012. Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. J. Neuroinflammation. https://doi.org/10.1186/1742-2094-9-155

Tomlinson, J.E., Žygelyte, E., Grenier, J.K., Edwards, M.G., Cheetham, J., 2018. Temporal changes in macrophage phenotype after peripheral nerve injury. J. Neuroinflammation. https://doi.org/10.1186/s12974-018-1219-0

Tong, J., Li, L., Ballermann, B., Wang, Z., 2013. Phosphorylation of Rac1 T108 by Extracellular Signal-Regulated Kinase in Response to Epidermal Growth Factor: a Novel Mechanism To Regulate Rac1 Function. Mol. Cell. Biol. 33, 4538–4551. https://doi.org/10.1128/mcb.00822-13

Tonra, J.R., Curtis, R., Wong, V., Cliffer, K.D., Park, J.S., Timmes, A., Nguyen, T., Lindsay, R.M., Acheson, A., DiStefano, P.S., 1998. Axotomy Upregulates the Anterograde Transport and Expression of Brain-Derived Neurotrophic Factor by Sensory Neurons. J. Neurosci. 18, 4374–4383. https://doi.org/10.1523/JNEUROSCI.18-11-04374.1998

Torres-Bacete, J., Delgado-Martín, C., Gómez-Moreira, C., Simizu, S., Rodríguez-Fernández, J.L., 2015. The Mammalian Sterile 20–like 1 Kinase Controls Selective CCR7-Dependent Functions in Human Dendritic Cells. J. Immunol. 195, 973–981. https://doi.org/10.4049/jimmunol.1401966

Tremblay, M.Ě., Lowery, R.L., Majewska, A.K., 2010. Microglial interactions with synapses are modulated by visual experience. PLoS Biol. 8. https://doi.org/10.1371/journal.pbio.1000527

Trettel, F., Di Castro, M.A., Limatola, C., 2019. Chemokines: Key Molecules that Orchestrate Communication among Neurons, Microglia and Astrocytes to Preserve Brain Function. Neuroscience. https://doi.org/10.1016/j.neuroscience.2019.07.035

Tsuda, M., Inoue, K., Salter, M.W., 2005. Neuropathic pain and spinal microglia: A big problem from molecules in “small” glia. Trends Neurosci. https://doi.org/10.1016/j.tins.2004.12.002

Turtle, J.D., Strain, M.M., Reynolds, J.A., Huang, Y.J., Lee, K.H., Henwood, M.K., Garraway, S.M., Grau, J.W., 2018. Pain input after spinal cord injury (SCI) undermines long-term recovery and engages signal pathways that promote cell death. Front. Syst. Neurosci. 12, 1–14. https://doi.org/10.3389/fnsys.2018.00027

Tyssowski, K.M., DeStefino, N.R., Cho, J.H., Dunn, C.J., Poston, R.G., Carty, C.E., Jones, R.D., Chang, S.M., Romeo, P., Wurzelmann, M.K., Ward, J.M., Andermann, M.L., Saha, R.N., Dudek, S.M., Gray, J.M., 2018. Different Neuronal Activity Patterns Induce Different Gene Expression Programs. Neuron 98, 530-546.e11. https://doi.org/10.1016/j.neuron.2018.04.001

Udina, E., Furey, M., Busch, S., Silver, J., Gordon, T., Fouad, K., 2008. Electrical stimulation of intact peripheral sensory axons in rats promotes outgrowth of their central projections. Exp.

Neurol. 210, 238–247. https://doi.org/10.1016/j.expneurol.2007.11.007

Ulvmar, M.H., Hub, E., Rot, A., 2011. Atypical chemokine receptors. Exp. Cell Res. 317, 556–568. https://doi.org/10.1016/j.yexcr.2011.01.012

Ulvmar, M.H., Werth, K., Braun, A., Kelay, P., Hub, E., Eller, K., Chan, L., Lucas, B., Novitzky-Basso, I., Nakamura, K., Rülicke, T., Nibbs, R.J.B., Worbs, T., Förster, R., Rot, A., 2014. The atypical chemokine receptor CCRL1 shapes functional CCL21 gradients in lymph nodes. Nat. Immunol. 15, 623–630. https://doi.org/10.1038/ni.2889

Umpierre, A.D., Bystrom, L.L., Ying, Y., Liu, Y.U., Worrell, G., Wu, L.J., 2020. Microglial calcium signaling is attuned to neuronal activity in awake mice. Elife 9, 1–24. https://doi.org/10.7554/eLife.56502

Van Den Brand, R., Heutschi, J., Barraud, Q., DiGiovanna, J., Bartholdi, K., Huerlimann, M., Friedli, L., Vollenweider, I., Moraud, E.M., Duis, S., Dominici, N., Micera, S., Musienko, P., Courtine, G., 2012. Restoring voluntary control of locomotion after paralyzing spinal cord injury. Science (80-.). 336, 1182–1185. https://doi.org/10.1126/science.1217416

Van Raemdonck, K., Umar, S., Palasiewicz, K., Volkov, S., Volin, M. V., Arami, S., Chang, H.J., Zanotti, B., Sweiss, N., Shahrara, S., 2020. CCL21/CCR7 signaling in macrophages promotes joint inflammation and Th17-mediated osteoclast formation in rheumatoid arthritis. Cell. Mol. Life Sci. 77, 1387–1399. https://doi.org/10.1007/s00018-019-03235-w

Venkatesh, I., Mehra, V., Wang, Z., Califf, B., Blackmore, M.G., 2018. Developmental Chromatin Restriction of Pro-Growth Gene Networks Acts as an Epigenetic Barrier to Axon Regeneration in Cortical Neurons. Dev. Neurobiol. 78, 960–977. https://doi.org/10.1002/dneu.22605

Venkatesh, I., Simpson, M.T., Coley, D.M., Blackmore, M.G., 2016. Epigenetic profiling reveals a developmental decrease in promoter accessibility during cortical maturation in vivo. Neuroepigenetics 8, 19–26. https://doi.org/10.1016/j.nepig.2016.10.002

Wagner, F.B., Mignardot, J.B., Le Goff-Mignardot, C.G., Demesmaeker, R., Komi, S., Capogrosso, M., Rowald, A., Seáñez, I., Caban, M., Pirondini, E., Vat, M., McCracken, L.A., Heimgartner, R., Fodor, I., Watrin, A., Seguin, P., Paoles, E., Van Den Keybus, K., Eberle, G., Schurch, B., Pralong, E., Becce, F., Prior, J., Buse, N., Buschman, R., Neufeld, E., Kuster, N., Carda, S., von Zitzewitz, J., Delattre, V., Denison, T., Lambert, H., Minassian, K., Bloch, J., Courtine, G., 2018. Targeted neurotechnology restores walking in humans with spinal cord injury. Nature 563, 65–93. https://doi.org/10.1038/s41586-018-0649-2

Wahane, S., Halawani, D., Zhou, X., Zou, H., 2019. Epigenetic regulation of axon regeneration and glial activation in injury responses. Front. Genet. 10, 1–20. https://doi.org/10.3389/fgene.2019.00640

Wahl, A.S., Büchler, U., Brändli, A., Brattoli, B., Musall, S., Kasper, H., Ineichen, B. V., Helmchen, F., Ommer, B., Schwab, M.E., 2017. Optogenetically stimulating intact rat corticospinal tract post-stroke restores motor control through regionalized functional circuit formation. Nat. Commun. 8. https://doi.org/10.1038/s41467-017-01090-6

Wang, D., Ichiyama, R.M., Zhao, R., Andrews, M.R., Fawcett, J.W., 2011. Chondroitinase combined with rehabilitation promotes recovery of forelimb function in rats with chronic spinal cord injury. J. Neurosci. 31, 9332–9344. https://doi.org/10.1523/JNEUROSCI.0983-11.2011

Wang, H., Liu, N.K., Zhang, Y.P., Deng, L., Lu, Q.B., Shields, C.B., Walker, M.J., Li, J., Xu, X.M., 2015. Treadmill training induced lumbar motoneuron dendritic plasticity and behavior recovery in adult rats after a thoracic contusive spinal cord injury. Exp. Neurol.

271, 368–378. https://doi.org/10.1016/j.expneurol.2015.07.004

Wang, K.C., Kim, J.A., Sivasankaran, R., Segal, R., He, Z., 2002. p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature 420, 74–78. https://doi.org/10.1038/nature01176

Wang, Qi, Zhang, S., Liu, T., Wang, H., Liu, K., Wang, Qiujun, Zeng, W., 2018. Sarm1/Myd88-5 Regulates Neuronal Intrinsic Immune Response to Traumatic Axonal Injuries. Cell Rep. https://doi.org/10.1016/j.celrep.2018.03.071

Wang, Y., Cheng, X., He, Q., Zheng, Y., Kim, D.H., Whittemore, S.R., Cao, Q.L., 2011. Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult oligodendrocyte precursor cells by increasing the expression of bone morphogenetic proteins. J. Neurosci. 31, 6053–6058. https://doi.org/10.1523/JNEUROSCI.5524-09.2011

Wang, Y., Huang, J., Li, Y., Yang, G.-Y., 2012. Roles of Chemokine CXCL12 and its Receptors in Ischemic Stroke. Curr. Drug Targets. https://doi.org/10.2174/138945012799201603

Wanner, I.B., Anderson, M.A., Song, B., Levine, J., Fernandez, A., Gray-Thompson, Z., Ao, Y., Sofroniew, M. V., 2013. Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. J. Neurosci. 33, 12870–12886. https://doi.org/10.1523/JNEUROSCI.2121-13.2013

Ward, P.J., Clanton, S.L., English, A.W., 2018. Optogenetically enhanced axon regeneration: motor versus sensory neuron-specific stimulation. Eur. J. Neurosci. 47, 294–304. https://doi.org/10.1111/ejn.13836

Ward, P.J., Jones, L.N., Mulligan, A., Goolsby, W., Wilhelm, J.C., English, A.W., 2016. Optically-induced neuronal activity is sufficient to promote functional motor axon regeneration in vivo. PLoS One 11, 1–16. https://doi.org/10.1371/journal.pone.0154243

Weering, H.R.J. va., Jong, A.P.H. d., Haas, A.H. d., Biber, K.P.H., Boddeke, H.W.G.M., 2010. CCL21-induced calcium transients and proliferation in primary mouse astrocytes: CXCR3-dependent and independent responses. Brain. Behav. Immun. 24, 768–775. https://doi.org/10.1016/j.bbi.2009.04.007

Wei, J.J., Kim, H.S., Spencer, C.A., Brennan-Crispi, D., Zheng, Y., Johnson, N.M., Rosenbach, M., Miller, C., Leung, D.H., Cotsarelis, G., Leung, T.H., 2020. Activation of TRPA1 nociceptor promotes systemic adult mammalian skin regeneration. Sci. Immunol. 5, 1–9. https://doi.org/10.1126/SCIIMMUNOL.ABA5683

Welniarz, Q., Dusart, I., Roze, E., 2017. The corticospinal tract: Evolution, development, and human disorders: Corticospinal Tract Human Disorders. Dev. Neurobiol. (Hoboken, N.J.) 77, 810–829. https://doi.org/10.1002/dneu.22455

Weng, Y.L., An, R., Cassin, J., Joseph, J., Mi, R., Wang, C., Zhong, C., Jin, S.G., Pfeifer, G.P., Bellacosa, A., Dong, X., Hoke, A., He, Z., Song, H., Ming, G. li, 2017. An Intrinsic Epigenetic Barrier for Functional Axon Regeneration. Neuron 94, 337-346.e6. https://doi.org/10.1016/j.neuron.2017.03.034

Weng, Y.L., Wang, Xu, An, R., Cassin, J., Vissers, C., Liu, Yuanyuan, Liu, Yajing, Xu, T., Wang, Xinyuan, Wong, S.Z.H., Joseph, J., Dore, L.C., Dong, Q., Zheng, W., Jin, P., Wu, H., Shen, B., Zhuang, X., He, C., Liu, K., Song, H., Ming, G. li, 2018. Epitranscriptomic m6A Regulation of Axon Regeneration in the Adult Mammalian Nervous System. Neuron 97, 313-325.e6. https://doi.org/10.1016/j.neuron.2017.12.036

Wenger, N., Moraud, E.M., Gandar, J., Musienko, P., Capogrosso, M., Baud, L., Le Goff, C.G., Barraud, Q., Pavlova, N., Dominici, N., Minev, I.R., Asboth, L., Hirsch, A., Duis, S., Kreider, J., Mortera, A., Haverbeck, O., Kraus, S., Schmitz, F., DiGiovanna, J., Van Den

Brand, R., Bloch, J., Detemple, P., Lacour, S.P., Bézard, E., Micera, S., Courtine, G., 2016. Spatiotemporal neuromodulation therapies engaging muscle synergies improve motor control after spinal cord injury. Nat. Med. 22, 138–145. https://doi.org/10.1038/nm.4025

Wenger, N., Moraud, E.M., Raspopovic, S., Bonizzato, M., DiGiovanna, J., Musienko, P., Morari, M., Micera, S., Courtine, G., 2014. Closed-loop neuromodulation of spinal sensorimotor circuits controls refined locomotion after complete spinal cord injury. Sci. Transl. Med. 6. https://doi.org/10.1126/scitranslmed.3008325

West, A.E., Greenberg, M.E., 2011. Neuronal activity-regulated gene transcription in synapse development and cognitive function. Cold Spring Harb. Perspect. Biol. 3, 1–21. https://doi.org/10.1101/cshperspect.a005744

Williams, J.L., Holman, D.W., Klein, R.S., 2014. Chemokines in the balance: Maintenance of homeostasis and protection at CNS barriers. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2014.00154

Woller, S.A., Eddinger, K.A., Corr, M, Yaksh, T L, Corr, Maripat, Yaksh, Tony L, 2017. Woller S.A, Eddinger K.A, Corr M, Yaksh T.L, An overview of pathways encoding nociception. Clin Exp Rheumatol 2017; 35 (suppl. 107) S40-S46.

Wong, Y.H., Lee, C.M., Xie, W., Cui, B., Poo, M.M., 2015. Activity-dependent BDNF release via endocytic pathways is regulated by synaptotagmin-6 and complexin. Proc. Natl. Acad. Sci. U. S. A. 112, E4475–E4484. https://doi.org/10.1073/pnas.1511830112

Woodham, P., Anderson, P.N., Nadim, W., Turmaine, M., 1989. Satellite cells surrounding axotomised rat dorsal root ganglion cells increase expression of a GFAP-like protein. Neurosci. Lett. 98, 8–12. https://doi.org/10.1016/0304-3940(89)90364-9

Wu, D., Jin, Y., Shapiro, T.M., Hinduja, A., Baas, P.W., Tom, V.J., 2020. Chronic neuronal activation increases dynamic microtubules to enhance functional axon regeneration after dorsal root crush injury. Nat. Commun. 11, 1–16. https://doi.org/10.1038/s41467-020-19914-3

Wu, D., Murashov, A.K., 2013. MicroRNA-431 regulates axon regeneration in mature sensory neurons by targeting the Wnt antagonist Kremen1. Front. Mol. Neurosci. 6, 1–13. https://doi.org/10.3389/fnmol.2013.00035

Wu, J.C., Huang, W.C., Chen, Y.C., Tu, T.H., Tsai, Y.A., Huang, S.F., Huang, H.C., Cheng, H., 2011. Acidic fibroblast growth factor for repair of human spinal cord injury: A clinical trial - Clinical article. J. Neurosurg. Spine 15, 216–227. https://doi.org/10.3171/2011.4.SPINE10404

Wu, J.C., Huang, W.C., Tsai, Y.A., Chen, Y.C., Cheng, H., 2008. Nerve repair using acidic fibroblast growth factor in human cervical spinal cord injury: A preliminary Phase I clinical study. J. Neurosurg. Spine 8, 208–214. https://doi.org/10.3171/SPI/2008/8/3/208

Wu, S.C., Rau, C.S., Lu, T.H., Wu, C.J., Wu, Y.C., Tzeng, S.L., Chen, Y.C., Hsieh, C.H., 2013. Knockout of TLR4 and TLR2 impair the nerve regeneration by delayed demyelination but not remyelination. J. Biomed. Sci. 20, 1. https://doi.org/10.1186/1423-0127-20-62

Wu, X.B., Cao, D.L., Zhang, X., Jiang, B.C., Zhao, L.X., Qian, B., Gao, Y.J., 2016. CXCL13/CXCR5 enhances sodium channel Nav1.8 current density via p38 MAP kinase in primary sensory neurons following inflammatory pain. Sci. Rep. https://doi.org/10.1038/srep34836

Wynn, T.A., Vannella, K.M., 2016. Macrophages in Tissue Repair, Regeneration, and Fibrosis. Immunity 44, 450–462. https://doi.org/10.1016/j.immuni.2016.02.015

Xiong, Y., Huang, F., Li, X., Chen, Z., Feng, D., Jiang, H., Chen, W., Zhang, X., 2017.

CCL21/CCR7 interaction promotes cellular migration and invasion via modulation of the MEK/ERK1/2 signaling pathway and correlates with lymphatic metastatic spread and poor prognosis in urinary bladder cancer. Int. J. Oncol. 51, 75–90. https://doi.org/10.3892/ijo.2017.4003

Xue, W., Fan, Z., Li, L., Lu, J., Zhai, Y., Zhao, J., 2019. The chemokine system and its role in obesity. J. Cell. Physiol. 234, 3336–3346. https://doi.org/10.1002/jcp.27293

Yang, B., Zhang, F., Cheng, F., Ying, L., Wang, C., Shi, K., Wang, J., Xia, K., Gong, Z., Huang, X., Yu, C., Li, F., Liang, C., Chen, Q., 2020. Strategies and prospects of effective neural circuits reconstruction after spinal cord injury. Cell Death Dis. 11. https://doi.org/10.1038/s41419-020-2620-z

Yang, L., Blumbergs, P.C., Jones, N.R., Manavis, J., Sarvestani, G.T., Ghabriel, M.N., 2004. Early Expression and Cellular Localization of Proinflammatory Cytokines Interleukin-1β, Interleukin-6, and Tumor Necrosis Factor-α in Human Traumatic Spinal Cord Injury. Spine (Phila. Pa. 1976). https://doi.org/10.1097/00007632-200405010-00004

Yang, P., Wen, H., Ou, S., Cui, J., Fan, D., 2012. IL-6 promotes regeneration and functional recovery after cortical spinal tract injury by reactivating intrinsic growth program of neurons and enhancing synapse formation. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2012.03.019

Yap, E.L., Greenberg, M.E., 2018. Activity-Regulated Transcription: Bridging the Gap between Neural Activity and Behavior. Neuron 100, 330–348. https://doi.org/10.1016/j.neuron.2018.10.013

Yin, Y., Cui, Q., Li, Y., Irwin, N., Fischer, D., Harvey, A.R., Benowitz, L.I., 2003. Macrophage-derived factors stimulate optic nerve regeneration. J. Neurosci. https://doi.org/10.1523/jneurosci.23-06-02284.2003

Yin, Y., Henzl, M.T., Lorber, B., Nakazawa, T., Thomas, T.T., Jiang, F., Langer, R., Benowitz, L.I., 2006. Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. Nat. Neurosci. https://doi.org/10.1038/nn1701

Ying, Z., Roy, R.R., Edgerton, V.R., Gómez-Pinilla, F., 2005. Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. Exp. Neurol. 193, 411–419. https://doi.org/10.1016/j.expneurol.2005.01.015

Yoshizaki, S., Tamaru, T., Hara, M., Kijima, K., Tanaka, M., Konno, D. jiro, Matsumoto, Y., Nakashima, Y., Okada, S., 2021. Microglial inflammation after chronic spinal cord injury is enhanced by reactive astrocytes via the fibronectin/β1 integrin pathway. J. Neuroinflammation 18, 1–15. https://doi.org/10.1186/s12974-020-02059-x

Zareen, N., Dodson, S., Armada, K., Awad, R., Sultana, N., Hara, E., Alexander, H., Martin, J.H., 2018. Stimulation-dependent remodeling of the corticospinal tract requires reactivation of growth-promoting developmental signaling pathways. Exp. Neurol. 307, 133–144. https://doi.org/10.1016/j.expneurol.2018.05.004

Zhang, Q., Cao, D.L., Zhang, Z.J., Jiang, B.C., Gao, Y.J., 2016. Chemokine CXCL13 mediates orofacial neuropathic pain via CXCR5/ERK pathway in the trigeminal ganglion of mice. J. Neuroinflammation. https://doi.org/10.1186/s12974-016-0652-1

Zhao, P., Waxman, S.G., Hains, B.C., 2007. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine-cysteine chemokine ligand 21. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2209-07.2007

Zhou, B., Yu, P., Lin, M.Y., Sun, T., Chen, Y., Sheng, Z.H., 2016. Facilitation of axon regeneration by enhancing mitochondrial transport and rescuing energy deficits. J. Cell Biol. 214, 103–119. https://doi.org/10.1083/jcb.201605101

Zhou, S., Shen, D., Wang, Y., Gong, L., Tang, X., Yu, B., Gu, X., Ding, F., 2012. microRNA-222 Targeting PTEN Promotes Neurite Outgrowth from Adult Dorsal Root Ganglion Neurons following Sciatic Nerve Transection. PLoS One 7, 1–11. https://doi.org/10.1371/journal.pone.0044768

Zhou, Y.-Q., Liu, D.-Q., Chen, S.-P., Sun, J., Zhou, X.-R., Xing, C., Ye, D.-W., Tian, Y.-K., 2019. The Role of CXCR3 in Neurological Diseases. Curr. Neuropharmacol. 17, 142–150. https://doi.org/10.2174/1570159x15666171109161140

Zhou, Z., Hong, E.J., Cohen, S., Zhao, W. ning, Ho, H. yi H., Schmidt, L., Chen, W.G., Lin, Y., Savner, E., Griffith, E.C., Hu, L., Steen, J.A.J., Weitz, C.J., Greenberg, M.E., 2006. Brain-Specific Phosphorylation of MeCP2 Regulates Activity-Dependent Bdnf Transcription, Dendritic Growth, and Spine Maturation. Neuron 52, 255–269. https://doi.org/10.1016/j.neuron.2006.09.037

Zhu, Y., Lyapichev, K., Lee, D.H., Motti, D., Ferraro, N.M., Zhang, Y., Yahn, S., Soderblom, C., Zha, J., Bethea, J.R., Spiller, K.L., Lemmon, V.P., Lee, J.K., 2017. Macrophage transcriptional profile identifies lipid catabolic pathways that can be therapeutically targeted after spinal cord injury. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2751-16.2017

Zigmond, R.E., 2012. gp130 cytokines are positive signals triggering changes in gene expression and axon outgrowth in peripheral neurons following injury. Front. Mol. Neurosci. https://doi.org/10.3389/fnmol.2011.00062

Zigmond, R.E., Echevarria, F.D., 2019. Macrophage biology in the peripheral nervous system after injury. Prog. Neurobiol. 173, 102–121. https://doi.org/10.1016/j.pneurobio.2018.12.001

Zlotnik, A., Yoshie, O., 2012. The Chemokine Superfamily Revisited. Immunity 36, 705–716. https://doi.org/10.1016/j.immuni.2012.05.008

Zlotnik, A., Yoshie, O., 2000. Chemokines: A new classification system and their role in immunity. J. Cult. Herit. 1, 121–127. https://doi.org/10.1016/s1074-7613(00)80165-x

Zörner, B., Schwab, M.E., 2010. Anti-Nogo on the go: From animal models to a clinical trial. Ann. N. Y. Acad. Sci. 1198, 22–34. https://doi.org/10.1111/j.1749-6632.2010.05566.x

Zychowska, M., Rojewska, E., Piotrowska, A., Kreiner, G., Nalepa, I., Mika, J., 2017. Spinal CCL1/CCR8 signaling interplay as a potential therapeutic target – Evidence from a mouse diabetic neuropathy model. Int. Immunopharmacol. https://doi.org/10.1016/j.intimp.2017.09.021

Annex

Some of the results from chapter 1 in this thesis have been preprinted in bioRxiv:

Genetic control of neuronal activity enhances axonal growth only on permissive substrates

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