Chapter 1

Experimental Spinal Cord Injury Models in Rodents: Anatomical Correlations and Assessment of Motor Recovery

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Additional information is available at the end of the chapter

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Abstract

Human traumatic spinal cord injury (SCI) causes disruption of descending motor and ascending sensory tracts, which leads to severe disturbances in motor functions. To date, no standard therapy for the regeneration of severed spinal cord axons in humans exists. Experimental SCI in rodents is essential for the development of new treatment strategies and for understanding the underlying mechanisms leading to motor recovery. Here, we provide an overview of the main rodent models and techniques available for the investigation of neuronal regeneration and motor recovery after experimental SCI.

Keywords: spinal cord injury, regeneration, plasticity, rodent, motor recovery

1. Introduction

The challenge of spinal cord injury (SCI) research is to find the right model for testing new treatment strategies. Although rodents differ from humans in many aspects, the research on primates is prohibited in many countries, and there are very strict regulations on experimenting with nonhuman primates [1]. Therefore, rodent models are the first choice for testing the effectiveness and mechanisms of new potential treatments for SCI. Rodents, especially mice, provide the additional advantage of transgenic technologies (knock out and knock in) that can be helpful in SCI research. In this chapter, an extensive description is provided on the currently available rodent SCI models, methods of treatment application, histological analysis of regenerating axons, and functional analysis of motor recovery.

2. Anatomy of the longitudinal axon tracts in rodents and humans

In order to understand the impact of SCI, it is important to have some basic knowledge about the long axon tracts that are interrupted by the lesion. Descending tracts control various motor functions. Sensory information from ascending tracts is also essential for posture, balance, and coordination of movements. Here, the main projections from the brain to the spinal cord and vice versa are summarized.

2.1. Descending motor tracts

The descending tracts in the spinal cord (**Figure 1**, left-hand side) run from the brain and brainstem to the spinal cord and are all involved in motor control [2].

2.1.1. Corticospinal tract (CST)

The corticospinal tract (CST) is variable between species. The motor cortex in rodents, generally referred to as the sensorimotor cortex (a rostrocaudal gradient of motor and sensory areas), is not as well defined as it is in humans, who have separate areas for sensory and motor cortex. The CST is responsible for the control of fine movements of distal musculature (e.g., fingers). Pyramidal neurons in layer V of the motor area give rise to the corticospinal axons that run via the internal capsule to the brainstem pyramids where they cross. It then depends on the species which path the majority of CST axons follow. In primates, almost all crossed fibers run in the lateral CST, located in the dorsolateral part of the lateral column. In rodents, most fibers are located in the dorsal CST (dCST), running in the ventral part of the dorsal columns. In some species, a ventral CST (vCST) is also observed. The CST axons terminate mainly in lamina 3–6 of the grey matter. In humans, up to 20% of CST axons terminate directly on motoneurons in lamina 9. CST terminals are glutamatergic.

2.1.2. Rubrospinal tract (RST)

The rubrospinal tract (RST) plays a role in general locomotion and in some species controls more skilled motor tasks together with the CST. It arises from the caudal magnocellular part of the red nucleus and crosses in the ventral tegmental decussation. The RST descends in the dorsal part of the spinal cord lateral column. The axons terminate in laminae 5 and 6 (sometimes 7) in the cervical and lumbosacral enlargements corresponding to the limbs. In rats, direct termination on lamina 9 motoneurons has been reported. The RST is prominent in rodents, whereas in animals with a large lateral CST (e.g., primates and humans), the RST is smaller. RST axons use glutamate as neurotransmitter.

2.1.3. Reticulospinal tracts (ReST)

The reticular formation in the brainstem plays a role in the preparation of movements and postural control. Reticulospinal tracts run medially and laterally in the ventral part of the spinal cord white matter. Whereas the medial reticulospinal tract (ReST) remains uncrossed, part of the lateral ReST fibers cross to the contralateral side. The ReST does not form a clear bundle

but intermingles with fibers from other tracts, for example, the vestibulospinal and spinothalamic tracts. The axons terminate in laminae 5–9 and can be either glutamatergic or GABAergic [3].

2.1.4. Vestibulospinal tracts (VeST)

The medial and lateral vestibulospinal tracts (VeSTs) are responsible for the initiation of limb and trunk extensor activity, which is important for posture. The lateral VeST arises from the lateral vestibular nucleus and does not cross, whereas the medial VeST originates from both the medial and the spinal vestibular nuclei and partially crosses to the contralateral side. Both run in the ventral white matter and terminate in laminae 7–8, providing glutamatergic input [3].

2.1.5. Raphespinal and coeruleospinal tracts

The Raphe nuclei give rise to the raphespinal projections, which together with the coeruleospinal projections (from the locus coeruleus) modulate (among others) motor functions. The raphespinal projections include a non-serotonergic component that runs in the dorsolateral funiculus and is involved in gating pain, as well as a serotonergic component that runs in the ventrolateral white matter, terminating in the intermediate grey and on motoneurons in the ventral horn. The noradrenergic coeruleospinal fibers run without crossing in the ventral funiculus and project throughout the grey matter.

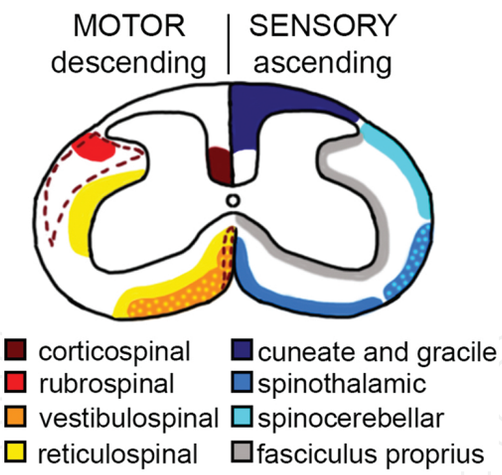

Figure 1. Spinal cord anatomy: schematic representation of the main ascending sensory tracts (right) and descending motor tracts (left) in a transverse section of the rodent spinal cord. Dotted areas represent locations where tracts are intermingled. Dashed lines indicate the location of the corticospinal tract in humans.

2.2. Ascending sensory tracts

The ascending tracts in the spinal cord (**Figure 1**, right-hand side) convey sensory information from the periphery to central nervous system (CNS) areas involved in walking, posture, and information processing about noxious stimuli [4].

2.2.1. Gracile and cuneate tracts

These two large ascending pathways contain axons from the dorsal root ganglia (DRGs) and provide sensory information from the limbs and trunk. In rodents, an additional dorsal column nucleus contains afferent axons from the tail. The tracts synapse in the gracile and cuneate nuclei located in the medulla oblongata. The second-order axons then cross the midline and run through the medial lemniscus to the thalamus. A subpopulation of DRG neurons synapses locally on dorsal horn neurons, whose axons also project to the gracile and cuneate nuclei. This is called the post-synaptic dorsal column pathway, whereas those DRG axons that do not synapse locally constitute the direct dorsal column pathway.

2.2.2. Spinothalamic, spinoreticular, and spinovestibular tracts

Several sensory tracts run in the ventrolateral funiculus of the spinal cord. These include the spinothalamic tract that conveys nociceptive, thermal, crude touch, and pressure information from the DRGs to the thalamus. The spinoreticular tract provides pain information to brainstem nuclei of the reticular formation. The spinovestibular tract is important for bringing proprioceptive signals to the vestibular nuclei. Several other tracts are present in the ventrolateral funiculus, such as the spinomesencephalic, spinoparabrachial, spinohypothalamic, and spinocervical tracts, each providing information to specific brain regions, that is, mesencephalon, parabrachial nuclei, hypothalamus, and lateral cervical nucleus in the upper cervical cord, respectively.

2.2.3. Spinocerebellar tracts

Projection axons from the spinal cord to the cerebellum are located in the dorsolateral and ventrolateral funiculi (**Figure 1** right-hand side). They carry proprioceptive information from the muscles and tendons to the cerebellum, so that adjustments of posture and coordination of movements can take place.

2.3. The propriospinal system

The spinal cord's "own" projection system refers to neurons that are located in the spinal cord, whose axons interconnect various spinal cord levels [5]. This so-called propriospinal system constitutes a large part of the white matter. It comprises interneurons that are connected to either other interneurons or directly to motoneurons. With respect to locomotor control, short-axon propriospinal neurons are also called premotoneurons, because they modulate corticospinal and sensory input to motoneuron pools controlling fore- and hindlimb activity. The long-axon propriospinal neurons form connections between the cervical and lumbosacral enlargements and are responsible for coordination of fore- and hindlimbs. These axons run in the fasciculus proprius (**Figure 1**, right-hand side).

The propriospinal neurons also modulate input to the lumbar central pattern generator (CPG), a local system involved in reflexive stepping in total absence of supraspinal input [5]. Serotonin from brainstem neurons has been shown to play a major role in CPG activation [6, 7].

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3. Rodent spinal cord injury models

The choice of SCI models is important in view of comparability with human SCI, but practical issues should also be considered. Although human lesions are usually compressions (but some may be sharp wounds as well or a mixture of both), from the experimental point of view it might be important to have a more "clean" and reproducible cut. Treatment strategies that fail to cause regeneration through a spinal cord transection lesion will probably have equally small effects after contusion lesions. On the contrary, treatments that induce regeneration in a transection model should then be tested and optimized in a contusion model. Partial injury models are useful for the investigation of the locomotor recovery over time, since not only regeneration but also sprouting from spared axon tracts can occur (see Section 5). Models of complete transection are used to study regeneration without the possibility of plasticity processes bypassing the lesion. While the complete transection of the spinal cord is a very reproducible injury, disadvantages of this lesion model are the poor degree of regenerative

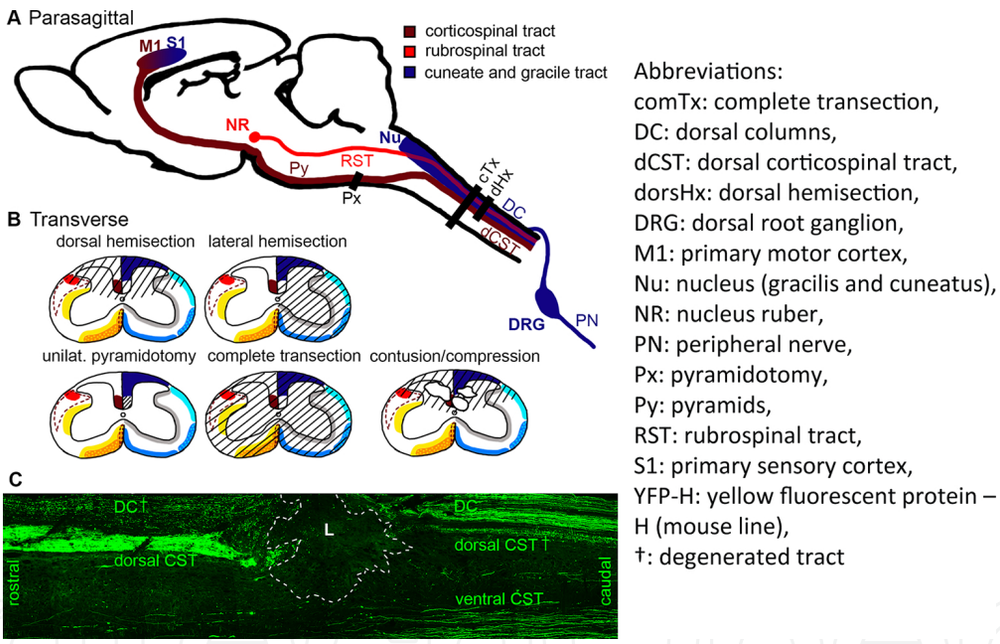

Figure 2 Spinal cord lesions in rodent models of SCI: (A) schematic representation of a parasagittal section through the brain and spinal cord (modified from Paxinos & Watson, The Rat Brain in Stereotaxic Coordinates, 6th Edition). Tracts with clear localizations are indicated. It should be noted that these do not run in the same spinal cord section, with the RST (red) running more laterally than the CST (brown) and the cuneate and gracile tracts (blue). The dorsal hemisection, complete transection, and the pyramidotomy lesions are represented as black bars. (B) Schematic drawings of transverse sections through the spinal cord with bilateral motor tracts depicted left and bilateral sensory tracts depicted right (for exact description of the tracts see **Figure 1**). Dashed areas represent the extent of tissue damage produced by the different injury paradigms. Note that motor and sensory tracts run in both spinal cord hemispheres but are depicted separately for better understanding. (C) Histological parasagittal section through the spinal cord of YFP-H mice (The Jackson Laboratory) 7 days after a dorsal hemisection (compilation of 2 sagittal sections). Descending CST axons are intact rostrally and have degenerated caudally from the lesion site (dashed white line). Ventral CST fibers are not lesioned (plane of section causes apparent lack rostrally). Ascending dorsal column axons are intact caudally and have degenerated rostrally.

growth of the severed axons and the general inadequacy for most motor tests. In this section, the technical principles of each model in rats and mice are described.

3.1. Dorsal hemisection (Hx)

Spinal cord transection lesions are generally applied using scissors, scalpel blades, or fine retractable wire knives. The advantage of wire knives (McHugh Millieux) is that a SCI can be performed with high precision, because they can be attached to a stereotactic frame. The dorsal Hx (Figure 2A–C) is the most used SCI paradigm for the investigation of the regeneration of CST and, depending on the extent of lateral lesion, it also includes the RST. It is mostly performed at thoracic level 8 (T8) and involves the laminectomy at T8-9, opening of the dura mater and subsequent lesioning of the spinal cord [8, 9]. For mice, microdissection spring scissors (Fine Science Tools) are used to hemisect the spinal cord. Since this procedure is inherently variable, the experimenter needs to test various depths to determine the desired extension of the lesion. A more controlled technique for dorsal Hx in mice was described by Hill et al. (2009) who used a so-called Vibraknife (LISA-Vibraknife; Louisville, KY) [10, 11]. Dorsal hemisection lesions are usually applied at thoracic spinal cord levels and result in the formation of a dense inhibitory scar [12, 13]. Depending on the severity of the lesion, the animals spontaneously recover a certain degree of walking that can be further ameliorated by regeneration promoting treatments.

3.2. Lateral Hx

For lateral Hx, the lateral half of the spinal cord is transected in mostly the same technical procedure as the dorsal Hx, with the difference that the tracts on one side are left intact (Figure 2B). These lesions provide the advantage of an internal control situation [14], which is also reflected in the behavioral testing, where paw preferences are often scored (see Section 7.5.). Lateral Hx experiments are usually performed at cervical levels, allowing the analysis of both fore- and hindlimb recovery. Mostly, a lesion at cervical level C5 is produced, but some groups have specialized on the analysis of breathing musculature after a lesion at C2 [15].

3.3. Complete transection (Tx)

For a complete transection (Figure 2B), small scissors are generally used to transect the spinal cord after having cut the meninges. Alternatively, the dura mater is opened just enough to allow the insertion of a spinal cord hook (Fine Science Tools) between dura and spinal cord. The hook is then used to lift the cord in order to completely cut the spinal cord. The dura mater can be closed with fine sutures (10-O) after the procedure. The complete Tx model is useful to investigate the effect of treatments on the axonal regeneration, and on (limited) recovery of locomotor function. After a complete SCI in rats, there is usually the development of fluid-filled cavities, whereas in mice this is generally not the case [16].

3.4. Contusion and compression injury

Contusion injuries are the most widely used lesion type in SCI research, since the majority of human SCI involves a contusion or compression pathology. Several commercially available systems can be used to inflict standardized graded contusion injuries. These include the NYU MASCIS impactor (New York University Multicenter Animal SCI Study) [17], the OSU impactor (Ohio State University electromagnetic SCI device) [18, 19], the IH impactor (Infinite Horizon) [20], and the spinal cord compression device (Kopf Instruments). In general, a controlled pressure is exerted on the spinal cord after laminectomy by either dropping or placing a weight onto the cord, controlling the force and/or velocity [21]. Depending on the species (in rats more than in mice), contusion injury leads to cyst formation (Figure 2B), a feature also displayed by human SCI patients [19, 21]. Thoracic contusions are usually performed to induce dorsal bilateral lesions, whereas contusions at the cervical level are performed unilaterally [20].

Compression/decompression models are frequently used to investigate the occlusion of the central canal, another common symptom of SCI in human patients. To perform an experimental compression, injury clips, balloons, or forceps are widely used [21]. Vascular clips and calibrated forceps can be used to create graded and reproducible injuries. The clip compression model and the contusion injury model show some resemblances as they both inflict the injury via pressure to the outer surface of the spinal cord. These models can be fine-tuned so that injuries of varying degrees can be created. They lead to the formation of fluid-filled cysts which are surrounded by spared tissue. The remaining tissue continuity and axon sparing makes them also a suitable model for locomotor functional tests. For the same reason, however, SCI contusion and compression models are not as well suited as transection models to investigate the neuronal and axonal regeneration.

3.5. Pyramidotomy

An exclusive CST-only lesion can be achieved by pyramidotomy, a transection at the height of the pyramids [22] (Figure 2A–B). The injury of the CST by pyramidotomy does not greatly affect locomotion in rodents. Rats and mice use the CST primarily for fine finger movements, which is greatly relevant for human patients. For the study of motor recovery, specific forepaw tests are used (see Section 7.5.). Since this lesion is usually performed unilaterally, the intact side serves as an internal control and is also used for studying plasticity-related regeneration mechanisms [23].

3.6. New SCI models

Scientists are continuously looking for models that resemble the human injuries more closely. For example, two recent studies focused on lumbosacral SCI, a type of injury affecting an estimated one-third of patients [24, 25]. A model combining SCI and traumatic brain injury was recently introduced, because a proportion of SCI patients additionally suffer from head injuries, for example, due to traffic or diving accidents [26]. Finally, a recent publication on a

closed-body SCI by applying a high-pressure air blast in mice provides a model resembling human traumatic SCI [27].

4. The application of treatments

After the choice of the appropriate lesion model for therapy development, the next decision in experimental SCI is the technique to use to apply a treatment. The application method determines the timing, frequency, and duration of the treatment. This section provides technical details of current methods for applying treatments in the various SCI models.

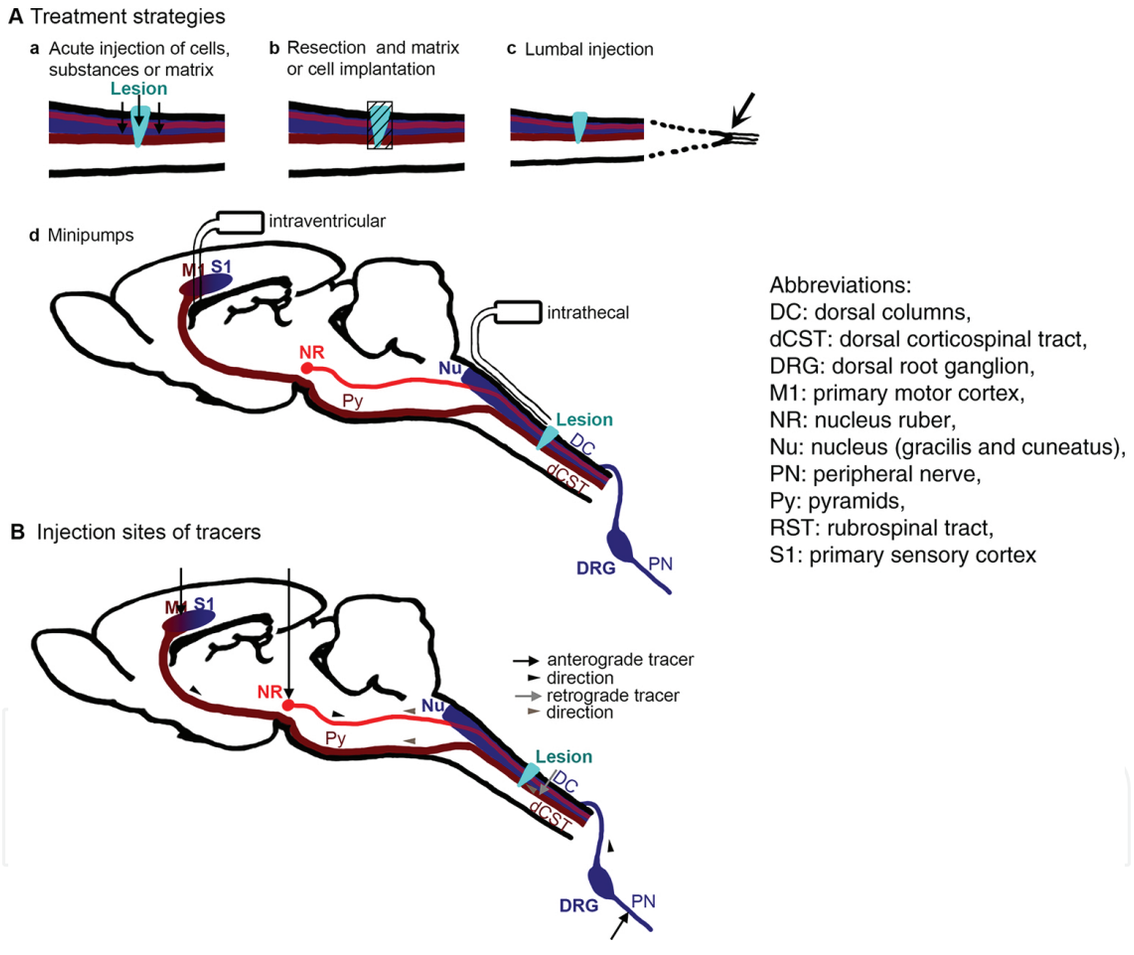

Figure 3 Strategies for the application of treatments and tracers exemplified in a schematic drawing of a dorsal Hx lesion: (A) treatments can be applied by (a) injection into the lesion site and/or adjacent tissue, (b) resection of the chronic lesion scar and subsequent matrix or cell implantation, (c) intrathecal lumbar injection, and (d) infusion over prolonged periods with minipumps and catheters either intraventricularly, intrathecally, or epidurally. The epidural catheter can be guided not only from rostral direction through the cisterna magna but also from the caudal side by performing an additional laminectomy [9, 28]. (B) Anterograde tracers are injected into the motor cortex and nucleus ruber in order to label CST and RST, respectively, and in the peripheral nerve to label the dorsal column axons. Injection of a retrograde tracer caudally to the lesion site is applied in order to visualize the cell bodies corresponding to regenerated axons or local interneuron circuits.

4.1. Injection into the spinal cord parenchyma

The simplest method for acutely applying a therapy is the (single) injection of a substance at the time of surgery. In some models, such as the dorsal Hx, the lesion site is open, so the treatment might potentially diffuse too quickly out of the area. Therefore, treatments are often injected in the intact tissue immediately adjacent to the lesion (Figure 3Aa). The injection volume should not be too high (<1 μl), and the injection should be performed slowly so that additional damage to the tissue is avoided. Controlled injection is achieved either by using a pump (e.g., the Pump 11 Elite Nanomite, Harvard Sachs Elektronik) or by introducing a delay of several minutes between injection and retraction of the needle. The injection method is most suitable for single acute treatments, because any additional doses will require additional surgery.

4.2. Lumbar injection into the CSF

A therapeutic can be applied to the CSF by lumbar intrathecal injection (Figure 3Ac), described in detail for mice by Lu et al, 2013. Shortly, the animal is subjected to a brief inhalation narcosis and kept in half-sleep by keeping its head in a dark environment. The L5 vertebra can be localized between the iliac crests of the hip bones. A 30-gauge needle is used to puncture the skin and enter the spine between the L5 and L6 spinous process. When the dura mater is punctured, a reflective flick of the tail is induced and up to 5 μl liquid can be applied [29]. This method is useful for renewing treatments multiple times after the initial injection.

4.3. Intrathecal application via minipumps

For continuous long-term application of liquid therapeutics, the use of minipumps is a standard delivery method (Figure 3Ad). Pumps can be implanted subcutaneously and attached to a catheter for intrathecal delivery. Minipumps either release the liquid via osmosis (Alzet®) or they use a programmable microprocessor (iPrecio®). They are commercially available in different sizes and with varying pumping rates and time periods. The subcutaneously placed minipumps can be removed after the required delivery period. The minipumps are connected to a catheter which can be inserted in the brain for intraventricular infusion [30], or the catheter can be guided through the epidural space underneath the vertebrae toward the lesion site [19, 28]. It is important to consider that the catheter by itself can produce a compression of the spinal cord. This is especially problematic in mice, because of their size, although special mouse catheters are available commercially (Alzet®).

4.4. Cellular transplantation strategies

Cell therapy is generally the focus in neurodegenerative diseases such as Alzheimer's or Parkinson's disease where the common goal is to replace degenerated neurons. In contrast, SCI is characterized by damage of the neuronal processes, whereas the corresponding cell bodies are located in various areas of the brain, brainstem, and DRGs, thus complicating cell replacement. Moreover, the projection neurons are thought to undergo atrophy in contrast to dying [31]. Therefore, cellular therapeutic approaches for experimental SCI concentrate on the

spinal cord, where local cells are affected by the primary and secondary injury events. The therapies on the one hand aim to replace glial cells or local neurons. Peripheral nerve grafts or Schwann cell cables have been used to bridge the lesion [32, 33]. Transplanted oligodendrocyte precursor cells or Schwann cells have been shown to remyelinate axons, whereas olfactory ensheathing or mucosal cells may provide axon guidance and trophic support [34–36]. Cell therapies using embryonic stem cells, neural stem/progenitor cells, or induced pluripotent stem (iPS) cells mostly aim to provide local pools of neurons that might serve as relay stations, analogue to propriospinal neurons [37–40]. Stem cells might also differentiate into glial cells that can remyelinate axons. On the other hand, stem cells can bridge the lesion gap and promote regeneration by the secretion of trophic factors, the support of angiogenic events, or the inhibition of glutamate toxicity. These effects have been reported for mesenchymal stem cells, bone marrow mesenchymal stromal cells, or unrestricted somatic stem cells from umbilical cord blood [41–43].

The transplantation of (stem) cells is usually performed by injection of cell suspensions into the spinal cord parenchyma (Figure 3Aa). This can be performed acutely by injecting cells into the intact tissue adjacent to the lesion. Alternatively, the lesion is allowed to form over a certain time period (usually 7 days, also called subacute), and a new surgery is performed to inject the cells directly into the lesion site. Factors to consider are cell survival, migration, differentiation into neural/glial cell types, axon outgrowth, and synaptic contacts in the case of neuronal transplants and secretion of regeneration-supportive factors in the case of nonneural transplants.

4.5. Implantation of matrices

Although many studies have proven the beneficial effects of autologous or heterologous cellular grafts in acute and chronic SCI models in animals [44, 45], the use of cell transplantation in human patients often remains a controversial issue [46, 47]. The search for artificial biomaterials for the implantation into the injured spinal cord has been prompted due to the limited access to autologous donor material and immunological problems associated with allograft rejection.

Cavities or cysts that often form after SCI are a major obstacle impeding axonal regeneration. Therefore, the reconnection across the trauma cavity by means of scaffolds or matrices is a major focus in SCI research. In order to provide a favorable growth substrate for regenerating axons, a bridging material should provide and combine several structural, physicochemical, and molecular properties [48]. Materials should ideally be easily modifiable, serve as a scaffold for matrix molecules and/or cellular transplants, and further be immunologically inert and absorbable [49]. Positive results with acellular matrices have been obtained in numerous studies [45, 49–55]. Important advances have recently been reported in the development of biosynthetic conduits for spinal cord repair. Biosynthetic conduits equipped with ECM molecules and different cell lines, and supplemented with neurotrophic growth factors, have been shown to yield encouraging results in the treatment of experimental SCI [51].

In chronic SCI, cavity formation has occurred and a lesion scar has formed, which presents a stable physical and molecular barrier to axonal regeneration. Cavities and sites of scar resection

can be treated with bridging or scaffolding materials. Interesting effects were achieved with a polyethylene glycol (PEG) treatment in a chronic SCI paradigm [56]. PEG was used to fill the cavity that was created by resection of the 5-week-old lesion scar in spinal cord-injured rats (Figure 3Ab). After 8 months, long-distance axonal regeneration through and beyond the graft was observed. The PEG matrix was repopulated by blood vessels, astrocytes, and Schwann cells, the latter remyelinating bundles of regenerating axons. These histological parameters were accompanied by long-lasting functional motor improvement. This study suggests that the chronically lesioned tracts are still able to regenerate when provided with the right extracellular environment [56].

4.6. Implantation of a mechanical microconnector system

Complete transections result in a gap between the two spinal cord stumps. Recently, a novel and unique connector device was described [57]. The purpose of this mechanical microconnector system is to reconnect severed spinal cord tissue stumps in the submillimeter range. The microconnector consists of two elliptical discs lined with numerous honeycombed holes. After implantation into the injured rat spinal cord, the device is connected to a vacuum pump, and the tissue stumps are brought into close apposition via the application of negative pressure. The connector discs have a rough surface, allowing the adherence of the spinal cord tissue. Additional features of the mechanical microconnector system are an internal canal system and an inlet tube, which can be connected either to a syringe or to an osmotic minipump to achieve application of therapeutics into the lesion area. Even the implantation of the device alone was sufficient for axon regeneration and led to a significant improvement of locomotor function following complete transection of the thoracic spinal cord [57].

4.7. Electrical stimulation and neuroprothesis

Electric field stimulation has been shown to promote enhanced and/or oriented neurite outgrowth, thereby offering potential additional treatment strategies after PNS but also CNS injury [58–60]. For SCI treatment, epidural stimulation has been used to create electric fields to restore motor functions [61, 62]. Electrical current is applied at varying frequencies and intensities to the areas of the lumbosacral spinal cord, activating the CPG. The CPG can initiate stepping function even without any input from the brain. The lab of Grégoire Courtine developed a neuroprosthetic that achieves a high-fidelity control of leg kinematics. A closed-loop system, using muscle activity and other kinematic parameters in real-time to feed back into the system, allowed neuromodulation during walking [63]. Another study used neuroprosthetic intervention in the form of a Neurochip 2 recurrent brain-computer interface in a cervical hemisection model. The neurochip delivered electrical stimulation and measured in parallel the electromyographic (EMG) activity of the muscles, thus adjusting the stimulation according to the muscle activity. Animals that received this so-called targeted, activity-dependent stimulation displayed increased skilled forepaw reaching as compared to animals receiving non-targeted stimulations or physical training [64].

Although it has no direct effect on the regeneration of axons after SCI, epidural stimulation is a very promising approach already used for the rehabilitation of SCI patients with promising results [65].

4.8. Exercise and training

The first studies suggesting that exercise might stimulate motor recovery were performed using environment enrichment [66, 67]. During the last decades, several investigators developed new experimental settings to perform motor training of animals. For example, forced walking on treadmills, training either bipedal or quadrupedal stepping, has been shown to improve locomotor recovery after SCI [68–70]. The combination of treadmill training with epidural stimulation and the administration of serotonergic and dopaminergic agonists seemed to be especially effective in restoring motor activity. Extensive plasticity of corticospinal, brainstem, and intraspinal connectivity was shown to underlie the observed functional recovery [61].

Recently, Starkey et al. (2015) developed a new type of cage with enriched environment over three floors with different types of training possibilities (e.g., grasping tasks, ladder walking, and climbing). This so-called "natural habitat cage" was combined with a new three-dimensional animal tracking system to allow high-impact, self-motivated training. Interestingly, differences were observed between the animals' overall activity and preference for certain tasks. Healthy as well as SCI animals trained in these cages performed better in experimental tests for fine motor control of fore- and hindlimb [71]. For forelimb training, a robotic rehabilitation system was recently developed, in which the animal has to pull a bar to receive food. This setup could also be used to measure forelimb strength [72].

4.9. Other types of treatments

Systemic treatments (intravenous, intraperitoneal, subcutaneous) are not discussed above, although they are clinically relevant. For treatments outside the spinal column, it should in general be known whether the applied therapeutic can cross the blood-brain barrier. A much higher concentration must be applied peripherally to achieve an effective concentration centrally. Since human SCI almost invariably involves surgery, the possibility of local treatment is given.

4.10. Combination treatments

It has become more and more apparent that combination therapies will be necessary to successfully treat SCI. The above described matrices, cell transplantations, electrical stimulation, and training paradigms all offer possibilities of combination with trophic factors, pharmacological treatments, agonists or antagonists of neurotransmitters, anti-inhibitory treatments, and so forth. It seems likely that holistic treatments combining several regeneration mechanisms will be clinically more successful to target the multitude of SCI systems.

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5. Possible treatment effects on neurons

5.1. Regeneration versus sprouting

For researchers, the "holy grail" is the regeneration of the injured fibers through the lesion scar and the subsequent reinnervation of their targets. After an initial retraction phase, the axons of the above-described tracts usually start regrowing toward the lesion site. Treatments can increase the regenerative growth of various tracts through and beyond the lesion site [73]. Although this process could be called "sprouting," it is important to distinguish between regenerative sprouting of the severed tract with the goal of regrowth toward the original connections and plastic sprouting, with the goal to find alternatives routes (**Figure 4**). Functional recovery of locomotion can also be achieved through plasticity of intact fibers that may form contralateral sprouts or make new synapses with local propriospinal neurons (**Figure 4**). In the last decade, the propriospinal system became a major focus for SCI. It can serve as a detour for bypassing the scar. Injured descending axons have been shown to sprout and rewire to propriospinal neurons, whose axons are located in the spared tissue and project into the lower denervated spinal cord [69, 74]. Also, the propriospinal interneurons can sprout to innervate new targets below the lesion. In addition, these neurons might regenerate better than the projection neurons, because of the shorter distance of the axon stump to the cell body. They have been shown to upregulate growth-associated proteins and have a high intrinsic capacity for plasticity [75]. In partial injury models, such as dorsal or lateral transection or contusion,

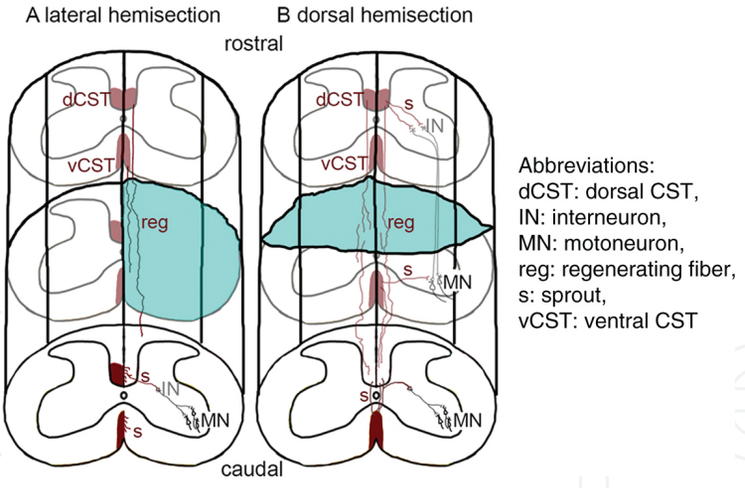

Figure 4 Axonal mechanisms leading to motor recovery exemplified for the dCST in two lesion models: (A) in the case of lateral Hx lesions, the contralateral intact dCST axons caudal to the lesion can sprout (s) and synapse with propriospinal interneurons (IN) connected to motoneurons (not necessarily at the same rostrocaudal level). The vCST on the intact side can also sprout to the ipsilateral side. Regeneration of the CST through the lesion is depicted by irregular lines since regenerating axons generally display a meandering and less straight course than the original tracts. (B) In the dorsal Hx model axons of the dCST can regenerate either through the lesion (irregular lines) or to form sprouts to make local connections with propriospinal interneurons whose axons run ventrally below the lesion and are connected to caudal motoneurons. The intact vCST can sprout and extend to the degenerated dCST tract or form new connections with interneurons that contact local motoneurons. Abbreviations: dCST: dorsal CST, IN: interneuron, MN: motoneuron, reg: regenerating fiber, s: sprout, vCST: ventral CST.

both regeneration from injured tracts and sprouting from spared tracts can be studied. It may be of importance to note that sprouting can be undirected, so that aberrant neuronal circuits may be formed [76]. Treatments can enhance sprouting and direct the sprouts to establish functional circuits.

5.2. Neuroprotection

After the primary insult, secondary damage due to, among others, inflammation, oxidative stress, and blood-brain barrier dysfunction causes the death of neurons (and glia) in the tissue surrounding the lesion [77]. The loss of local motor neurons leads to more extensive motor deficits in addition to the impairments caused by the injury to the descending motor tracts. The loss of spinal interneurons may disrupt intraspinal connections between motor centers. Therefore, a neuroprotective action of a treatment might, first, reduce functional impairments and, second, increase the possibility of local plasticity via interneurons (see Section 5.1.). For the analysis of neuroprotection, quantification of (moto-) neurons is performed at various distances rostrally and caudally from the lesion center [78]. A treatment could also lead to the protection of the brain and brainstem projection neurons from death or atrophy [8, 31, 79]. Quantification of the lesion size and spared white matter in standardized lesion models might also provide information about the protective effects of a treatment strategy.

6. Tracing and/or immunohistochemical (IHC) staining of motor and sensory tracts

The next step in SCI research is the histological analysis of regenerated axons. Short-term studies (up to several weeks after the injury) give information about early injury events, whereas long-term studies (up to several months or even years after the injury) are useful to investigate long-term effects and behavioral outcomes with a treatment compared to a control. In order to visualize regenerating axons from the specific spinal cord tracts, these can be marked via axonal tracing (Figure 3B) or detected by immunohistochemistry (IHC). In this section, the main techniques used in experimental SCI in rodents are summarized.

6.1. Tracing methods

6.1.1. Conventional tracing

Axonal tracing is an important tool for the investigation of regeneration after SCI (Figure 3B), holding the advantage that specific axonal populations are precisely marked. Conventional tracers label axons and neurons via the axonal transport [80]. Neuronal tracers can label the axons anterogradely (toward the axon terminal) which is the preferred method for analyzing their sprouting and regeneration. Retrogradely transported tracers (towards the cell body) are injected at the distal side of a lesion, in order to quantify the number of neurons with regenerated (distal) fibers and to visualize propriospinal neurons (see Section 6.4.).

Preferred application methods are pressure injection (liquid tracers), iontophoretic injection of electrically charged tracer molecules, or the insertion of dye crystals (carbocyanine dyes) [80]. The tracer can be detected via confocal microscopy using either its own fluorescence or IHC. The ideal survival time after the tracing depends on the tracer used, the distance between the site of tracer application and the area of interest, and the rate of its transport in the axons. A drawback of conventional tracing techniques is that in most cases not all axons of a neuronal population take up and transport the tracer substance. Many classical retrograde tracers are only, or more efficiently, taken up by injured axons and axon terminals, whereas the rate of the uptake by uninjured axons of passage is rather small. This can lead to nonspecific results [81].

Some examples for widely used monosynaptic neuronal tracers are the enzyme horseradish peroxidase, biotinylated dextran amine (BDA), and Fluoro Gold™. Examples of nonviral polysynaptic tracers are bacterial toxins, such as cholera toxin B. The drawback of nonviral polysynaptic tracers is, however, the dilution of signal after each synaptic step [82]. For the purpose of multisynaptic tracing, viral tracings are more suitable.

6.1.2. Viral tracings

When transneuronal tracing is desired, viral tracings are the method of choice. Transneuronal tracing is useful for the investigation of multisynaptic pathways and circuits [82]. The virus, which expresses a reporter gene in order to achieve the tracing, can replicate in the neurons and then infect other neurons which are connected via synapses. The virus replication further amplifies the signal, thereby avoiding the problem of signal dilution [8]. Very importantly, viral vector systems are very effective tools for gene therapeutic approaches. Frequently used viral systems used for axon tracing are adeno-associated viral vectors [83], lentiviral vectors [84], rabies virus [82], and herpes simplex virus [85]. The combination of viral tracings and gene therapy further offers the possibility to deliver a vector into specific areas.

A very elegant approach to investigate axonal pathways and their regeneration is the combination of viral tracing with optical tissue clearing and light sheet laser scanning microscopy [86–88].

6.2. Anterograde tracing of defined tracts

6.2.1. Motor cortex—CST tracing

In SCI research, the CST is the most established model tract for the investigation of regeneration and the associated locomotor functional outcome. Its origin in the sensorimotor cortex and its course through the pyramidal decussations and, in rodents, the dorsal center part of the spinal cord allow a very precise labeling and localization of the tract. By using a stereotactic frame, precise injections of the tracer of choice are applied into the sensorimotor cortex [8, 89]. In general, tracing is performed 2 (mice) to 3 (rats) weeks before sacrifice of the animals for histological analysis. In the case of BDA, tissue sections need to be stained with streptavidin coupled to a fluorescent marker. Fluorescently labeled BDA is available, but the signal is

usually still enhanced by post-staining. Analysis is performed by confocal microscopy, counting regenerating axon profiles in and beyond the lesion site.

6.2.2. Nucleus ruber—RST tracing

The nucleus ruber can be traced in the same way as the CST; however, it is much smaller in size and therefore easier to miss [73].

6.2.3. Ascending sensory tracts: CTB tracing or CGRP staining

Cholera toxin β (CTB) is a tracer that is transported anterogradely, retrogradely and, as a recent study suggested, even transneuronally [90]. This tracer is used frequently to label the ascending sensory tracts in the dorsal column of the spinal cord. For this purpose, CTB is injected into the sciatic nerve that is crushed to achieve maximum uptake of the tracer [90]. This allows the specific analysis of the regeneration of ascending axons corresponding to the hindlimbs. In contrast, IHC staining for the marker calcitonin gene-related peptide CGRP allows the detection of axon profiles entering the spinal cord at all spinal segments. This, however, compromises the analysis of CGRP axons beyond the lesion, since axons from intact spinal levels above the lesion will also stain positively.

6.3. Raphespinal and coeruleospinal tracts

Because of their neurotransmitters serotonin (5-HT) and noradrenaline (NA), whose key synthesizing enzyme is tyrosine hydroxylase (TH), the tracts descending from the Raphe nuclei and locus coeruleus can be investigated by IHC using 5-HT- and TH-specific antibodies [73]. Since their fibers run both ventrally and dorsally, care should be taken to analyze only areas that are relevant to the localization of the lesion, for example, only the dorsal funiculus in case of a dorsal hemisection, with respect to regeneration. The possibility of sprouting from ventral axons cannot be ruled out, and some types of interneurons also express 5-HT.

6.4. Retrograde tracing

A very valuable tool for tracing regenerating neurons is retrograde tracing. When a tracer injected distally from the lesion site marks neurons proximally from the lesion site, these neurons have regenerated their fibers (provided the tracer is precisely located to the lesioned and not the spared region, and tracer diffusion can be ruled out). It can also answer the question whether axons from the intact side sprouted to the lesioned side. This technique was applied to show which brainstem nuclei were projecting into the distal cord [14] and to trace propriospinal interneuron networks [70]. If a retrograde tracer is applied to the spinal cord proximal to the lesion site, it can also be used to quantify the neurons "associated" with the lesion, for studying cell death or atrophy of neuronal populations [8].

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7. Assessment of motor function

In order to assess motor recovery after experimental SCI and putative regenerative treatments, several functional tests are available. The choice of the tests depends not only on the lesion model but also on the costs, because some tests require specific commercial systems.

7.1. Basso, Beattie, and Bresnahan (BBB) locomotor score and subscore (rat) and Basso Mouse Scale (BMS, mouse)

The BBB open-field test, developed by Basso, Beattie, and Bresnahan [91], is an established test for the evaluation of hindlimb locomotor function of SCI rats. It is suitable for thoracic SCI models where it has become the first choice test to evaluate locomotor function [92]. The BBB score is based on the classification of hindlimb locomotor function using a scale which ranges from 0 (no spontaneous movement of the hindlimbs) to 21 (normal movement, coordinated walking pattern). For the evaluation procedure, the rats are placed in a defined open field where they are observed and evaluated by two trained observers. The animal's movements in the open field are scored over 4 minutes according to the criteria of the BBB locomotor rating scale [91]. The evaluation of coordination, an important parameter of the intermediate and late phases of the BBB, is not always clear without any doubt. This entails ratings in the medium-range scale intervals often leading to an artificial plateau. Therefore, and because usually not all aspects of locomotion are influenced by a treatment, the determination of a BBB subscore can be helpful to improve the sensitivity of the test [93]. Furthermore, additional automatic gait analysis helps to avoid potential subjective evaluation of coordination [94]. An advantage of the BBB locomotor rating scale is that preoperative training—which is a general requirement for many locomotor behavioral tests—is not necessary. However, as is generally the case for behavioral tests, preoperative handling of the experimental animals and their familiarization with the test surroundings are useful. Additionally, adaptations of the original BBB locomotor scale have been described also for severe thoracic injuries such as complete spinal cord transection [56, 95]. Since such severe lesions result in maximum BBB scores of 8–10, the spreading of the low and intermediate BBB values (BBB 1–10) allows a distinct evaluation of less prominent locomotor behavioral improvements.

The small size and rapid speed of mice caused investigators to develop a mouse-specific scale, the BMS [96]. The procedure of the animal walking in an open field is basically the same as described above, but parameters like coordination, paw position, and trunk instability are evaluated in a slightly different way than for rats. Similarly, for unilateral cervical SCI new locomotor rating scales have been developed, such as the forelimb locomotor assessment scale (FLAS) [97] or the forelimb locomotor scale (FLS) [98].

7.2. Horizontal ladder rung test and Gridwalk

The horizontal ladder walking test is used for the evaluation of fine motor control, coordination, and foot placing accuracy, all of which require certain degrees of sensory feedback. Therefore, this test is particularly useful for the investigation of locomotion after a thoracic CST injury. Video analyses of the runs allow the assessment of multiple parameters [99]. The

mistakes the animals make during walking are evaluated and classified into predefined categories. The test apparatus consists of metal rungs (3 mm in diameter) placed between Plexiglas walls in predefined intervals (1–5 cm for rats). The spacing patterns should be regularly alternated to make sure that the animals' locomotor function and not their cognitive functions are evaluated. Care should be taken to provide gaps between the rungs that are neither too narrow (mistakes being made by an animal might not be observable) nor too large (the animal cannot walk across without fear of falling, or without having to jump between rungs). During pretraining, the animals learn to cross the horizontal ladder without interruption. Post-injury runs are recorded with a (high-speed) video camera from an angle slightly below the rod plane. This ensures the possibility to detect precise movements of all four paws and their digits. For evaluation, the predefined foot placing mistakes are counted. For mice, the procedure is similar, with smaller spacing (approximately 15 mm). For both species, several parameters of skilled walking can be observed, including correct placement, slight and deep slip, total miss, (partial) replacement, and correction [22, 99]. Alternative to the ladder test, the Gridwalk test makes use of grids to asses skilled walking.

7.3. Automated gait analysis methods

7.3.1. CatWalk™

The CatWalk™ system for automated quantitative gait analysis in SCI rats was developed by Hamers et al (2001). Classically, gait analysis in the form of footprint analyses has been (and still is by some groups) performed by painting the animal's paws with ink and letting it run on paper (or, an elegant variation, with developer and photographic paper) [100]. Static measures such as the distance between paws and toes could be measured, but no spatiotemporal resolution was achieved. The Catwalk™ system consists of a glass plate through which fluorescent light is internally reflected. When a mouse or rat places its paw on the glass, the light is deflected from the glass and the paw print lights up. The intensity is related to the pressure or weight support, which provides additional information about the functionality of the paw. A high-speed camera placed below the glass plate records all the runs (originally, a mirror projected the light toward the camera, but the commercial version (Noldus) images directly). A narrow walkway corridor on top of the glass plate ensures that the animals walk in a straight line. After a few days of habituation training, the animals walk steadily through the corridor. Recording is performed in the dark, but the commercial setup has a lid with red light above the walkway, so that the outline of the animal is visualized. After analysis, main parameters of interest are the stride length (step size), the base of support (distance between left and right paws), the walking speed, the duration of the swing and stand phase, the regularity index as a measure of coordination, and the intensity of the prints. Many more parameters can be studied, the choice of which can be based on the animal model [101, 102]. The CatWalk™ system has been used in the following SCI models: thoracic CST Tx, RST Tx, and dorsal Hx [8, 103]; thoracic contusion [101, 102]; pyramidotomy models [22]; and lateral cervical spinal cord contusion [104, 105], and in recent studies assessing the effects of training and gene therapy [106, 107]. The CatWalk™ can furthermore be combined with the horizontal

ladder test by placing the ladder above the glass plate, so that footslips light up because the animal touches the glass plate [108].

7.3.2. Automated gait analysis using treadmill

The CatWalk™ is semi-automated, because the animals must voluntarily walk across the walkway and need pretraining. Again, scientists are striving to improve the existing systems (Neckel, 2015). New fully automated gait analysis platforms have been developed, including the DigiGait™ (Mouse Specifics, Inc.) [109–111] and the TreadScan™ (Clever Sys Incorporated) systems [112]. These two systems use transparent treadmills allowing the animals' gait analysis at constant speed, including the possibility to measure at different speeds.

7.3.3. MotoRater and kinematic analysis

The growing number of SCI models is accompanied by the need to modify the test systems. Recently, a new method for profiling locomotor recovery was developed in the lab of Martin Schwab [113]. This setup, now commercially available as the so-called MotoRater (TSE Systems), makes use of mirrors to image the mouse or rat that is walking in a Plexiglas basin from three sides (left, right, and below). The animals are tattooed on anatomical landmarks such as ilias crest, trochanter major of the hip, condylus lateralis of the knee, malleolus lateralis of the ankle, and the tip of the fifth toe. This way the walking is precisely monitored as stick diagrams and followed in time. As with the CatWalk™, the kinetics of even-ground walking patterns are analyzed. In contrast to the Catwalk, the researchers included new levels of difficulty in this system. A horizontal ladder is introduced to monitor precise paw placement and forelimb-hindlimb coordination. Alternatively, the basin is filled with water, either at levels where animals are wading (3 cm for rats, 1 cm for mice) or at levels where the animals have to swim. Wading brings the advantage that the water provides weight support. Furthermore, the animal's strength can be measured, because of the desire of the animal to raise its body as much as possible out of the water. In the original article, three types of SCI were compared (dorsal Hx, ventral Hx, and lateral Hx). For each lesion model, various aspects of the test revealed to be suitable in different ways. For example, skilled walking and overground locomotion are most suitable for the evaluation of thoracic dorsal Hx. For thoracic ventral Hx, wading was described to be the better test and for cervical lateral Hx, the authors observed improvement of hindlimb movements during wading and swimming. Due to the forepaw impairment, cervical Hx animals can hardly perform the ladder test and are poor at normal even-ground locomotion. Further studies of the same group made use of the MotoRater to assess the contribution of the brain stem nuclei to locomotor recovery [14] and the effects of training on motor skills after SCI [71].

Another kinematic gait analysis system makes use of reflective markers at essentially the same hallmarks as the MotoRater system (iliac crest, hip, knee, ankle, metatarsophalangeal joint, and toe). A motion capture system (SIMI Reality Motion Systems) is used to analyze gait parameters combined with electromyogram recording (EMG) [69, 70].

7.4. Sensory testing

Although less relevant than motor recovery, the recovery of sensory functions has a potential impact on locomotion. Furthermore, lesioned animals can develop neuropathic pain [114] which may be attenuated or, worse, aggravated by a treatment. Sensory tests performed after SCI include mechanical and nociceptive tests.

Sensorimotor reflexes can be tested by light touch to the paw, causing contact placing of the paw. Proprioceptive placing is elicited by stretching a tendon or joint [21]. Von Frey filaments are used to assess the animal's sensitivity to sub-threshold mechanical stimuli. For this purpose, filaments of increasing thickness are applied to the foot sole, exerting a defined force. This is normally not painful to the animal, so that only animals that suffer from mechanical allodynia (pain reaction from a normally non-painful stimulus) withdraw their paw from the filament. The minimum force eliciting a pain response is scored as paw withdrawal threshold [115]. Electronic versions of this test are available commercially (e.g., IITC Life Science, Ugo Basile). For the assessment of cutaneous hyperalgesia (increased pain from a pain-provoking stimulus), a hot plate or a commercial Plantar Test setup (e.g., Hargreaves Apparatus, Ugo Basil) [116] is used. The paw of interest is placed on a source of radiant heat or, in the case of the Planar Test, an infrared beam is precisely aimed at the central part of the animal's sole. The paw withdrawal time is recorded. Each paw is tested three times since the animal can also withdraw the paw spontaneously. Compared to the traditional hot plate test setup, the Plantar Test has the advantage of an automated, and therefore, accurate end-point detection [116].

For the majority of the sensory tests, the animal has to be able to move (withdraw) the paw. They can, therefore, generally not be performed with severely and completely spinal cord-injured animals that often lack the ability to perform limb movements below the level of the injury. For severely injured animals, the tail-flick test, a modification of the plantar hot plate test where the base of the tail is heated, can be applied [92].

7.5. Forelimb tests

For cervical hemisection lesions and for pyramidotomy, specific tests to analyze forelimb motor recovery have been developed [21]. Since these lesions are usually one-sided, the healthy side serves as an internal control. First, new locomotor rating scales (alternatives for the BBB) have been developed, such as the forelimb locomotor assessment scale (FLAS) [97] or the forelimb locomotor scale (FLS) [98]. Second, broad tests for paw preference are applied, such as the cylinder test, where the choice of the weight-bearing forelimb is monitored [22], and the grooming test, where the preferred paw for grooming is scored. Popular tests assessing dexterity include pasta eating or the Irvine, Beatti, Bresneham (IBB) forelimb rating scale, where the forelimb function is assessed, while the rat is eating a round-shaped cereal [117]. Furthermore, tests for the assessment of fine finger movements include the single pellet-grasping test or the staircase test [21, 71]. In these skilled forepaw tests, mice or rats have to reach for and grasp sugar pellets through a slit in a Plexiglas wall or from wells in a staircase setup (Lafayette Instruments (rat), Campden Instruments (mouse)). Video analyses of the sessions allow the assessment of multiple parameters. Some groups use the horizontal ladder as well to score forepaw locomotion, but the animals are usually poor at performing this test.

To further quantify grip a commercial grip strength meter is available (TSE Systems, Ugo Basile, Columbus Instruments), or the ability of the animal to keep its balance and hold on stably to an inclined plane (or cage grid) is measured.

7.6. Important considerations for functional testing

Several studies indicate that the choice of the motor tests should be based on the type of injury and the degree of impairment [113, 118]. For thoracic dorsal Hx, the horizontal ladder test and CatWalk™ gait analysis systems are suitable since even-ground walking and skilled walking are impaired, but display recovery over time. In the case of ventral Hx, the wading and swimming paradigms in the MotoRater provide more useful information on impairment and recovery. Cervical lateral Hx animals also perform better during wading and swimming. With regard to swimming, an assessment tool was developed in Sweden, where parameters like fore- and hindlimb usage, hindlimb alternation and position, trunk instability, body angle, and tail movements are precisely scored [119].

Care should, however, always be taken with the evaluation of the results. Animals can develop compensation strategies to perform a task in a different way than before the injury [118]. For example, animals primarily use their hindlimbs for swimming, but after a thoracic injury, they utilize their forelimbs. Therefore, distance or speed may recover, but the actual functional recovery of the hindlimbs might be still impaired. Another example is the grasping of food pellets that animals with forelimb impairment cannot do. Some animals tend to successfully develop a scooping strategy to retrieve pellets [118]. Investigators should be aware of this and monitor the strategies the animals use. The use of video equipment to accompany a test is therefore advisable.

The strain of the animals (and even the substrain produced by different suppliers) also plays an important role. Some animal strains perform better than others in tests which require the acquisition of certain skills [118, 120]. For example, in the staircase-skilled forepaw reaching test, Lister-hooded and Long-Evans rats perform much better than Lewis rats and Fischer rats [121, 122]. Housing is also of importance, since the amount of motor activity in the cage can provide training effects. This might mask a treatment effect, because the spontaneous recovery due to training may be too prominent. A popular cage enrichment in the form of sunflower seeds might compromise skilled grasping tests [118]. On the contrary, if the chosen test is too difficult for the animals in view of their impairment, recovery of function might be missed too. Other variables like circadian rhythms and stress can introduce variability. Therefore, it is vital to habituate the animals to the experimenters, to perform pre-injury recordings of the basal performance of the animals in the tests and to perform testing always at the same time of day under the same circumstances.

8. Discussion and conclusions

This chapter provides an overview of the main rodent models, experimental treatment strategies, histological analysis methods, and motor tests that are available for the investigation

of neuronal regeneration and locomotor function after experimental SCI. The choice of the appropriate model depends on the research question and on the type of human injury which the investigation is based on. There is ongoing controversy regarding the comparability of experimental blunt versus sharp lesions to the clinical situation of human patients. Contusion/compression injuries are very suitable for studying human traumatic SCI. These types of injury maintain tissue continuity even in the most severe cases, which is also observed in the vast majority of human spinal cord traumata. However, spared tissue bridges might compromise the analysis of treatment effects in experimental SCI. Moreover, blunt force spinal cord traumata are often accompanied by sharp lesions like maceration, laceration, or transection, for example by bone splinters. Therefore, sharp transections are also valid models, not least because they are easier to control and reproduce.

SCI experiments in rodents are essential for the development of new treatment strategies. They aim to extensively test treatment effects on multiple nerve tracts, to elucidate their mechanisms of action and, using multiple motor and sensory tests, to shed light on their ability to restore function. It is highly important to know whether a treatment is effective via neuroprotection, spared axon sprouting, or axon regeneration, since this will influence the choice of treatment that suits the patient best. Patients with incomplete lesions may benefit from plasticity-inducing treatments, whereas patients suffering from complete injuries require therapeutic strategies that induce regeneration. Patients with contusion lesions or complete injuries might further benefit from matrix or stem cell implantation to fill up cavities. When a treatment strategy displays promising effects in rodent SCI models, the next step will be to test it in a model system that is more close to human patients. In primates, the CST projects mainly dorsolaterally and originates from both left and right motor cortex, because a number of CST axons decussate along the spinal cord midline. These axons are capable of forming detour circuits reconnecting the motor cortex with denervated spinal cord areas in monkeys with lateral cervical Hx [123–125]. Due to the comparability with the anatomy of humans, the nonhuman primate cervical Hx model has been proposed to be a suitable model to test the recovery of forelimb skills after SCI [126].

Rodent research provided numerous important insights into the SCI field. To name a few, the regeneration and/or sprouting responses of tracts involved in locomotion, the involvement of the propriospinal system, the CPG circuits, and the ability to stimulate these without supraspinal input all contributed to a better understanding of human spinal cord pathophysiology. Numerous treatments have been tested and have provided even more insights into how the various systems can be manipulated. However, to date, despite many years of extensive research, there are no clinical standard therapies for SCI which significantly increase the regenerative response to such a degree that they achieve strong (locomotor) improvements in human patients. This reflects the complexity of SCI. Although many treatments did not reach the clinic, they have been of enormous value to understanding the mechanisms of regeneration leading to functional motor recovery.

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