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Repair strategies for traumatic spinal cord injury, with special emphasis on novel biomaterial-based approaches

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ABSTRACT

As a part of the central nervous system (CNS), the adult mammalian spinal cord displays only very poor ability for self-repair in response to traumatic lesions, which mostly lead to more or less severe, life-long disability. While even adult CNS neurons have a certain plastic potential, their intrinsic regenerative capacity highly varies among different neuronal populations and in the end, regeneration is almost completely inhibited due to extrinsic factors such as glial scar and cystic cavity formation, excessive and persistent inflammation, presence of various inhibitory molecules, and absence of trophic support and of a growth-supportive extracellular matrix structure. In recent years, a number of experimental animal models have been developed to overcome these obstacles. Since all those studies based on a single approach have yielded only relatively modest functional recovery, it is now consensus that different therapeutic approaches will have to be combined to synergistically overcome the multiple barriers to CNS regeneration, especially in humans. In this review, we particularly emphasize the hope raised by the development of novel, implantable biomaterials that should favor the reconstruction of the damaged nervous tissue, and ultimately allow for functional recovery of sensori-motor functions. Since human SCI pathology depends on the vertebral level and the severity of the traumatic impact, and since the timing of application of the different therapeutic approaches appears very important, we argue that every case will necessitate individual evaluation, and specific adaptation of therapeutic strategies.

1- Introduction

Human traumatic spinal cord injury (SCI) can lead to life-long disability with loss of sensation and voluntary motor functions (paralysis). Neurological outcomes depend on the level of the spinal segment affected (resulting in either paraplegia, or tetraplegia in case of cervical trauma), and the severity of the local damage (partial, or complete destruction of the underlying nervous tissue). Very often, the case of SCI patients is further aggravated by diverse complications, such as spasticity and loss of autonomous urinary function, and/or associated with development of various respiratory, digestive, musculo-skeletal or circulatory disorders, as well as infectious diseases (septicemia, pneumonia) [1]. Both individual, and societal costs for this condition are extremely high. SCI pathology is particularly frequent among young adults between 18 and 35 years, and touches more men than women (four out of five in the U.S.: traffic accidents, sports, ...). Incidence of SCI is estimated at around 54 new cases per year per million population in the U.S., and while the average life expectancy of SCI patients has greatly improved during the last decades, cases may become more severe due to an ageing population. First-year post-trauma care (hospitalization and re-education) costs are by far the most important (about 1 Mio. \$ for a patient with complete tetraplegia), but summed up annual costs during a normal life time can be much higher, even without considering indirect costs such as losses in productivity and wages*.

2- SCI pathology on the molecular and cellular level

While peripheral nervous system (PNS) can adapt to traumatic lesions by regenerating axons and, depending on the lesion severity, restore synaptic connectivity, adult mammalian CNS (brain and spinal cord) displays only a poor capacity for self-repair in response to traumatic lesions. Major differences in the endogenous repair mechanisms of PNS vs. CNS concern the nature of their respective neurons and glial cells, and particularly their local extracellular environment, as well as the specific inflammatory processes provoked by a lesion.

Thus, although adult neurons of the mammalian CNS can initially survive a traumatic lesion, regrowth of their axons through the lesion site ultimately fails due to a cascade of cellular and molecular events, starting within minutes after the initial impact and continuing for weeks and even years, leaving the affected neural tissue in a permanently altered, regeneration-inhibited state [for refs. see 2, 3]. In the last two

decades, several factors have been identified that contribute to the complexity of the SCI pathophysiology. The injury-associated vascular damage inevitably leads to hypoxia, hemorrhage and edema, thereby accelerating necrosis of the damaged neural tissue. The associated breakdown of the blood-spinal cord barrier (BSBC) favors infiltration of blood-derived monocytes/macrophages that, together with activated microglia (the immune resident cells), will spread inflammation, further exacerbated by formation of free radicals and glutamate excitotoxicity [4]. This detrimental environment will cause secondary damage through neurodegenerative lesions that spread caudally and rostrally from the initial impact, aggravating the neurological deficits [2, 5, 6, and refs. therein]. The cavities that develop because of necrosis of the neural tissue become walled off by the astrocytic scar, creating a barrier to axon growth that is both mechanical, and chemical due to expression of inhibitory molecules such as chondroitin sulfate proteoglycans (CSPG) as well as certain semaphorins and matrix-metalloproteinases. Axon regrowth is further inhibited by degradation products of CNS myelin, namely MAG (myelin associated glycoprotein), Omgp (oligodendrocyte myelin glycoprotein), and Nogo, and hampered by the low levels of neurotrophic factors present in adult CNS [for review, 6].

3- Therapeutic approaches: what has been tried so far?

Few, if any, effective therapies exist for the thousands of patients suffering from spinal cord trauma. The only treatment for human CNS injury approved thus far, methylprednisolone administration, only marginally improves clinical outcomes, despite promising preclinical results. Nevertheless, evidence from studies over the past three decades indicates that mammalian CNS maintains, to a previously unexpected level, a capacity for neural tissue remodeling and axonal plasticity (i.e., sprouting). Indeed, depending on the level of their intrinsic capacity to activate a regeneration-associated gene (RAG) expression program, damaged axons are able to regenerate to some degree and within a certain time window [7, 8]. Unraveling of the mechanisms underlying such remodeling has provided the basis for the development of experimental strategies to treat SCI using animal models, particularly in rodents. Such strategies include neutralization of myelin-derived inhibitors (e.g., anti-Nogo-antibodies [9]), or downstream inhibition of the related intracellular signaling pathways [Rho-GTPase signaling; [10]], and degradation of inhibitory components of the glial scar (CSPG

degradation by Chondroitinase-ABC enzyme [3, 11, 12; for review, 6]. Axon growth through the traumatic lesion site can also be stimulated by administration of neurotrophic factors, or by manipulation of pro-regenerative or inhibitory neuronal signaling pathways (mTOR/PTEN [13]). Regeneration of neural tissue is further enhanced by transplantation strategies employing genetically modified fibroblasts, Schwann cell bridges, olfactory ensheathing cells, or neuronal progenitors and stem cells (see above reviews, and [14]).

Furthermore, some progress has been reached by active rehabilitation programs, shown to be effective and beneficial at least for patients with incomplete SCI scoring C-D on the ASIA (American Spinal Injury Association) scale, and even for a small number with cervical injury [15-17]. Certainly the most promising breakthrough concerning rehabilitation is the recent introduction of epidural electrical stimulation (EES), a technique intended to reactivate and reinforce spared, but so far "subfunctional" descending (supraspinal) neural connections ("subfunctional" since these sparse residual connections are initially not robust enough to eventually drive muscle function). Thus, epidural implantation of an anode-cathode electrode array just below the lesion site coupled to a pulse generator, has allowed complete motor paraplegic patients to acquire standing and stepping capacities when associated with task-specific training. To this end, the technique has been more and more refined, from an initially more diffuse stimulation towards "multi-modal rehabilitation" sessions, in which the combination of individual electrodes delivering pulses of variable frequency and amplitude is fine-tuned for particular tasks and notably, for bilateral function. Electromyogram analysis helps determining the best-suited stimulation pattern for eliciting a response from specific muscles. After a certain number of training sessions with gravity assistance, some patients are finally capable of voluntary leg movements without the need for concomitant EES [18, 19]. Progress through EES training was reported to be particularly rapid when lumbosacral proprioceptive circuits were stimulated with a temporal and spatial selectivity in correspondence with the intended movement, thereby allowing even weak residual supraspinal commands to act on the appropriate spinal motor neuron pools. After several months of spatio-temporally coordinated EES-assisted training, patients that had been paralyzed for several years thus regained the capacity for voluntary control of muscle function even in the absence of EES [20].

4- Regeneration-inhibiting obstacles to overcome

Unfortunately, despite the significant progress in recent years, translation of experimental therapeutic strategies to clinical practice is still limited. More preclinical research is needed in view of the diverse, important caveats that need to be overcome or be better controlled, as outlined below:

- Adult axon growth through the lesion (regeneration or sprouting of axons), the real contribution of which to functional recovery is largely unknown. Thus, the number of fibers sprouting or regenerating through the lesion site is always small, and the distance of outgrowth is modest (e.g., for corticospinal axons that would need to grow for long distances), even more so in the context of human spinal cord anatomy [21, 22].

- Incomplete restoration of the blood-spinal cord barrier (BSCB), and poor revascularization at the lesion site: this impairs self-repair, as well as grafting interventions due to poor oxygen and nutrient supply for transplanted cells [6, 23].

- Demyelination and remyelination: Oxidative stress, excitotoxicity, low oxygen supply, and the persisting inflammatory process lead to the death of oligodendrocytes. Although oligodendrocyte precursor cells (OPCs) are recruited to the injury site [24], the subsequent spontaneous remyelination will usually remain abnormal or incomplete (for review, [25]). Schwann cells migrating into the lesion site, or arising from resident OPCs [26], also participate in the remyelination process, but their survival is rapidly compromised [27, 28]. Since denuded axons are particularly vulnerable, much effort has been devoted to boost their remyelination, either by transplanting OPCs that may be generated from human embryonic stem cells [29], or by inducing recruitment and differentiation of resident OPCs *via* trophic factors, hormones, or by targeting potential inhibitory factors in the environment such as LINGO-1, Sema-3A, and CSPGs (for review, [30]). However, although these approaches did seem to favor functional recovery to a certain degree, most are not exclusively acting on myelination, and recent work has put a serious doubt on the actual contribution of oligodendrocyte-driven remyelination to spontaneous locomotor recovery [31].

- Retrograde degeneration of axons ("axon die-back"): Axons damaged by the initial impact that are no longer connected to their target will eventually undergo a retrograde degeneration process, thereby liberating more toxic myelin degradation products that in turn, aggravate the spreading of inflammation beyond the primary lesion site.

- Scarring is recognized as a major obstacle to CNS repair, as it both physically and chemically impedes axon regrowth. However, it should be noted that astrogliosis is also beneficial for neuronal repair, since reactive astrocytes at the lesion site are essential for neural protection and regulation of the injury-induced inflammatory response. Therefore, efforts to avoid completely scar formation through reduced expression of genes selectively expressed by reactive astrocytes, or by eliminating proliferating astrocytes themselves, appear not suitable as therapeutic approach for SCI (for review, [32]). On the contrary, there is direct evidence that scar-forming astrocytes are indispensable for enhancing axon regeneration from adult neurons [33], see discussion in [2].

- Immune response and inflammation: inflammation, involving multiple cell types and effector molecules, can have both negative (secondary damage), and positive effects [for review, 34]. Wound healing generally involves an ordered immune response that relies on time-dependent activation of phenotypically and functionally distinct macrophage subsets. Thus, during the first days in normal wound healing outside the CNS, "classically activated" inflammatory macrophages (M1) assure the clearance of cell debris and ECM material. Thereafter, diverse subtypes of "alternatively activated", anti-inflammatory macrophages (M2) take over that will contribute to tissue reconstitution, then remodeling (also termed "resolution") via release of anti-inflammatory cytokines, stimulation of proliferation of fibroblasts and endothelial cells (angiogenesis), and production of ECM [35, 36]. This sequence, however, is deregulated after SCI. The immune response to SCI initially implies a release of proinflammatory cytokines (mostly by activated tissue-resident microglia), reactive oxygen species (ROS) and nitric oxide (NO_x), thereby attracting blood-borne M1 macrophages that will release even more pro-inflammatory factors (pro-inflammatory feedback loop). While at first beneficial due to clearance of debris, this inflammatory reaction will later accelerate glial scar formation, neural cell death, and axon retraction and demyelination [34, 35, 37-39]. M2 macrophages are initially present in the lesion, but disappear rapidly (around 1 week post-lesion in the rat), whereas pro-inflammatory M1 macrophages persist indefinitely [2, 35, 37]. Today, this impaired immune response cascade appears as one of the major obstacles to SCI repair, and may even compromise some repair strategies such as cell therapy. Precise, time-dependent regulation of the necessary balance between inflammatory and anti-inflammatory macrophages may thus have enormous therapeutic

potential [40].

Today, it is consensus among researchers in the SCI field that maximal restoration will most likely be achieved with a combination of therapies overcoming the multiple hindrances, and synergistically improving functional recovery. Accordingly, several such combined therapies were shown to boost axon regeneration and tissue restoration after SCI, e.g. associating ChABC treatment with peripheral nerve autograft [41, 42], or ChABC or Nogo-A antibody treatments with locomotor training [43, 44]. Strategies employing a complex cocktail of trophic factors together with implantation of stem cell-derived neurons or embryonic neurons embedded in a fibrin polymer bridge were also explored [45, and refs. therein].

5- Novel biomaterial-based approaches

New hope for SCI treatment appears to arise not least thanks to recent progress in the field of bio-inspired new materials that has already seen the development of promising tools for medical applications in wound healing and regeneration of various tissues. For SCI treatment, biomaterials in the form of implantable hydrogels appears particularly interesting, and evidence is accumulating that future combinatorial approaches will need to integrate such "bio-scaffolds" as substitute for lost neural extracellular matrix (ECM). Various biomaterials have been designed, both natural and synthetic, differing in their physical state (solutions, hydrogels, solids with controlled porosity), in situ biodegradability, and specific functionalization (charged surface favoring cell attachment, capacity for drug delivery, etc.). Ideally, any such material should have properties specifically adapted to the spinal cord environment, i.e. allow for cell migration and attachment, and axon growth, improve revascularization, and be well integrated such as to not generate a frontier at the host tissue-scaffold interface [for review, see 46-48]. Essential parameters of the polymer considered so far are stiffness, permeability, swelling, and degradation. In preclinical tests, diverse hydrogels have shown significant therapeutic potential for CNS lesion repair, in particular for SCI, but generally also have some specific disadvantages [49].

Among the synthetic polymers, the methacrylate-derived pHEMA (poly-(2-hydroxyethyl) methacrylate) is one of the hydrogels exhibiting mechanical properties similar to those of nerve tissue, and has been used mainly in SCI [50]. It provides good cell adhesion and neural development when combined with ECM molecules, such as

laminin or collagen. Neurogel in particular, a cross-linked pHEMA hydrogel, has led to the development of a revascularized bridge that promotes spinal cord restoration in rats and cats, and also reduces glial scarring [51]. Other hydrogels of similar composition, such as collagen-impregnated poly (glyceryl methacrylate) (pGMA), have been tested in brain lesions. However, pHEMA hydrogel synthesis implies the use of a toxic monomer, of which only the polymerized form can be used.

Polyethylene glycol (PEG) is another quite attractive synthetic hydrogel for SCI repair, as it is hydrophilic, non-toxic, biodegradable and bioabsorbable. Injectable in liquid form, PEG can be cross-linked by photopolymerization. Its use in experimental SCI has yielded encouraging results, including a chronic lesion paradigm, although the latter only after surgical resection of the glial scar prior to PEG injection [52, 53]. PEG-based hydrogels can further be used to generate implantable microspheres for drug release (e.g. of neurotrophic factors) into the spinal lesion site [54].

Natural carbohydrate-based polymers, notably of the glycosaminoglycan (GAG) type, are likely to form physical hydrogels, the degradation of which can be modulated by their microstructure and crosslinking degree. Various ECM components, such as collagen and hyaluronic acid (HA), as well as plasma-derived polymers, such as fibrin, were also employed as spinal cord scaffolds, in addition to agarose, alginate, and chitosan [55-57].

Natural polymers (e. g. GAGs, or polysaccharides, i. e. polymers of sugar moieties) can generally be considered biocompatible with the host tissue, and are non-toxic with a modular resorption rate, properties rendering them attractive for nerve tissue engineering. Agarose was the first biomaterial used as hydrogel, but proved unsuitable for tissue repair because it is not biodegradable, and its gelation temperature lies below 37°C. Alginate hydrogel scaffolds are also not biodegradable, although they can be colonized by cells, and may stimulate axonal growth. Collagen, the most common ECM component in mammals, is widely used as a scaffold to promote axon regeneration in PNS, but is not sufficient by itself in CNS. Fibrin, commonly used as scaffold and particularly for cell therapy [ex., 56], degrades too rapidly, and when contaminated with blood-borne pathogens activates astrocytes and resident inflammatory cells (microglia). Matrigel™ (BD Biosciences) is the trade name for a gelatinous protein mixture obtained from mouse sarcoma, liquid below 4°C and gelling rapidly above this temperature. Generally used as a three-dimensional substrate for cell culture studies, the use of

Matrigel™ in clinical therapy is limited because its exact composition is unknown. Hyaluronan (hyaluronic acid or hyaluronate), a hydrogel forming polysaccharide (GAG), is widely used in cosmetology and regenerative medicine. Tested in different models of PNS lesions, hyaluronan hydrogels are capable of being invaded by nerve fibers, and stimulate axon outgrowth. However, hyaluronan is not very effective in the case of traumatic CNS injury because of its rapid degradation, especially in an inflammatory environment.

Chitosan, derived from chitin by deacetylation, refers to a family of linear copolysaccharides of N-acetyl-D-glucosamine and D-glucosamine residues, and is structurally and functionally similar to GAGs (heparan sulfates, or other oligosaccharides such as hyaluronic acid). Several investigators have used chitosan-based biomaterials (usually combined with other natural or synthetic compounds), together with other strategies to improve axon regrowth. In most of these studies the biomaterial was designed as a structure containing macro- or microchannels for seeding cells into their inner lumen (*i.e.* neural stem and progenitor cells [NSPCs], or Schwann cells), and/or for trophic factor delivery [58-60]. However, the effect of the biomaterial alone was either not reported, or shown to have no significant effect on axon regeneration. Thus, these studies did generally not analyze the effect of the biomaterial used alone on scar formation (glial reaction), inflammation, and vascularization, with the exception of a report by [58], who found that chitosan is principally compatible with SC tissue, and does not provoke an inflammatory reaction. Chitosan-based materials and derivatives are indeed receiving increased attention in tissue engineering because of their unique and appealing biological properties, such as biocompatibility, biodegradability, and non-toxicity that make them suitable in the biomedical field. They have been extensively evaluated for regeneration of epithelial and soft tissues. Their beneficial characteristics include preservation of cellular phenotypes, binding and activity enhancement of bioactive factors, and synthesis and deposition of tissue-specific extracellular matrix [61, 62]. Moreover, other properties such as analgesic, antitumor, hemostatic, anticholesterolemic, anti-microbial, permeation enhancing, and antioxidant effects have also been documented [for review, 63, 64]. Accordingly, our recent study employing chitosan as fragmented physical hydrogel suspension (Chitosan-FPHS) showed that its implantation in acute rat spinal bilateral dorsal hemisection promoted the reconstitution of spinal tissue with functional vasculature. It did so not least by

modulating the inflammatory response, favoring inflammation resolution through macrophage polarization towards the M2 phenotype. Chitosan-FPHS also diminished fibrous glial scarring, and the border between lesion site and intact tissue was rendered permissive for regrowth of numerous axons into, and for some even beyond the lesion site. Growing axons were myelinated or ensheathed by endogenous Schwann cells that migrated into the lesion site and whose survival was prolonged. This structural remodeling was associated with significant, long-lasting recovery of locomotor function [2]. Thus, implantation of Chitosan-FPHS into an acute SCI lesion site seems a very promising strategy, and our perspective for a follow-up of the present study is to test its relevance in a chronic lesion. Determination of the suitable time window for chitosan hydrogel implantation is also important when considering a combination with other strategies, such as cell therapy or drug delivery. Furthermore, for a proof of concept, this strategy should be tested in an experimental model more relevant to humans, i.e. a contusion lesion.

At this point, it should be mentioned that the use of novel biomaterials for therapeutic approaches in the field of SCI is still in its infancy, and particular efforts should be made to carefully evaluate the effect of any biomaterial at first alone, before passing on to combinatorial strategies, and employing them in preclinical and clinical tests. Indeed, analyzing the effect of a combinatorial treatment is not possible without a thorough understanding of the mechanisms of action and the outcome of each treatment individually. Even then, this does not preclude the possibility that therapies, which when used alone do show a beneficial effect, will not always work synergistically when combined [see for ex., 65-67]. Nevertheless, since the introduction of bioengineering in SCI therapeutic approaches it has become evident that implanting a biomaterial will be important as support structure ("bio-scaffold") for the reconstitution of injured tissue, once potential safety issues can be ruled out, and the feasibility has been proven both for acute, and chronic spinal cord injury. We have good reason to believe that biomaterial formulations that will finally make it into clinical trials, will be able to prevent the irreversibility of a complete lesion, in the acute phase by creating a pro-regenerative environment preventing necrotic cavity formation, in cases of chronic lesions by potentiating the outcome of current approaches like re-education, or electrical stimulation.

6- Concluding remarks

Today, patients with complete SCI remain paralyzed for the rest of their life, with no possible relief in the foreseeable future. Therefore, there is enormous societal expectation for a therapeutic approach to come, and the slightest hope for improving the condition of patients is the focus of a lot of attention and compassion. For this reason, one has to carefully manage news about progress in the field in order not to raise false hope.

Regeneration of the CNS is a major challenge for modern tissue engineering in fundamental and clinical research, and research on SCI in particular is of high priority in North America and in Europe (EU recom. 1560-2002). SCI offers tremendous challenges for fundamental and clinical scientists, as it requires in-depth knowledge of the cellular and molecular mechanisms triggered by the injury, of axon growth in general, as well as of the anatomical and functional organization of the spinal cord and connected CNS structures. As outlined above, therapeutic strategies may, in addition to providing a biomaterial-based scaffold replacing lost ECM, target the balance between pro- and anti-inflammatory factors, stabilize affected neurons and axons to prevent excessive neurodegeneration, degrade regeneration-inhibitory molecules, and use cell therapy to provide "bridges" of neural cells allowing to be contacted by regrowing or sprouting axons [68].

Only few clinical trials (phase-1/2a) with FDA approval for chronic SCI are currently under way [69]. Thus, small biotech companies are exploring safety, tolerance, and efficacy of transplanting human neural stem cells (StemCells Inc., and Neuralstem Inc.) or oligodendrocyte progenitors (Asterias Biotherapeutics), and implantation of scaffolds based on a synthetic, resorbable (degradable) biomaterial (polylysine-coated PLGA: InVivo Therapeutics). These companies had demonstrated the efficacy of the proposed treatment in pre-clinical studies, using the BBB locomotor rating scale (open field test, [70]), which provides a baseline for data comparison. Recently, the FDA also approved a phase-1 clinical trial to evaluate transplantation of human autologous Schwann cells, conducted by the Miami Project [71].

The main challenge remains therapeutic efficacy, which is still rather limited for the diverse strategies developed so far. Indeed, the consequences of traumatic SCI are so complex and diverse that a combination of several treatments with complementary mechanisms of action is required, necessitating the establishment of multidisciplinary

collaborations between neuro-surgeons and researchers in multiple fields. Moreover, because of the lesion variability from one case to another (level, volume, severity; acute or chronic), it may not be feasible to devise just one "standard" approach. Rather, several strategies should be developed and evaluated in different lesion paradigms, thus giving the clinician in charge the possibility to use the best-suited combination of treatments according to the case.

Disclosure of interest

The authors declare that they do not have any competing interests.

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Fig. legend - Chitosan-FPHS implantation leads to reduced astrocytic scar formation, robust axon regrowth, and improved locomotor function:

(A) Preparation of fragmented physical hydrogel suspension (FPHS) from chitosan: Briefly, chitosan powder is dissolved in acetic acid, a gel is formed from the solution by neutralization in ammonia vapor, and after intense washing in H₂O the gel is fragmented into ~20 µm particles by blending with an Ultra-Turrax apparatus. (B) Untreated (left) vs. chitosan-FPHS-filled (right) bilateral spinal cord dorsal hemisection, 3 weeks after the lesion. Note that with chitosan-FPHS treatment, the astrocytic scar (GFAP-labeling, green) is reduced, numerous axons (neurofilament [NF] labeling, red) are growing through the lesion site, and no lesion cavity is forming. The hydrogel is invaded by numerous cells reconstructing a nervous tissue (nuclei stained with Dapi). (C) Chitosan-FPHS-treated lesioned animals exhibit stronger, and more rapid locomotor function recovery, as demonstrated here using the BBB (Basso, Beattie, Bresnahan) locomotor scale (based on an "open field" test where animals freely moving in an enclosure are filmed, and the quality of their hindlimb movements is evaluated by specially trained, but "naïve" observers with respect to the applied treatment). Only animals with a BBB of "0" the day after the lesion were selected for further evaluation; a BBB of "10" corresponds to full weight support by the hindlimbs. The difference between treated and untreated animals is significant from 3-4 weeks onwards. (D) Gait analyses *via* the GaitLab system (the animal is filmed from underneath while moving through a 1m long corridor; all four paws are marked in different colors by the evaluation software), a first analysis being performed the day before the injury, the following once a week when animals have regained weight support, *i.e.* at around 6 weeks after the lesion for the majority of animals (BBB score 9/10). Right after the lesion, the hindpaws (turned upside down) are simply pulled behind the animal that advances only with its forelimbs; 10 weeks later, the same animal has regained weight support, and coordination of fore- and hindlimb movements.

