Transplanting neural progenitor cells to restore connectivity after spinal cord injury

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Abstract

Spinal cord injury remains a scientific and therapeutic challenge with great cost to individuals and society. The goal of research in this field is to find a means of restoring lost function. Recently we have seen considerable progress in understanding the injury process and the capacity of CNS neurons to regenerate, as well as innovations in stem cell biology. This presents an opportunity to develop effective transplantation strategies to provide new neural cells to promote the formation of new neuronal networks and functional connectivity. Past and ongoing clinical studies have demonstrated the safety of cell therapy, and preclinical research has used models of spinal cord injury to better elucidate the underlying mechanisms through which donor cells interact with the host and thus increase long-term efficacy. While a variety of cell therapies have been explored, we focus here on the use of neural progenitor cells obtained or derived from different sources to promote connectivity in sensory, motor and autonomic systems.

Traumatic spinal cord injury (SCI) occurs in an instant yet sets off a cascade of molecular and cellular events that evolve over days to months following the initial trauma[1] (FIG. 1). Depending on the spinal level at which the injury occurs and the severity of the injury, motor, sensory and autonomic functions are disrupted, dramatically impacting quality of life and incurring major costs for effective management. Potential therapeutic approaches must therefore address diverse and multifaceted pathophysiological processes, including the haemorrhage, oxidative stress, inflammatory signalling and immune cell infiltration that are unleashed in the acute phase of injury[2–6]. At a later stage, major therapeutic goals include reversing demyelination, combating chronic neuroinflammation, neutralizing local growth-limiting factors, promoting regeneration or sprouting of injured axons and restoring lost neural circuitry and connectivity[7–12]. These are challenging tasks due to the complexity of injury progression, which varies temporally and spatially. Thus, despite the

significant progress that has been achieved over decades of research in understanding SCI pathophysiology, there remain no effective therapies in the clinic and it is unclear how a single treatment will satisfactorily address these diverse challenges.

In the past decade, the field of neural cell transplantation has made exciting progress, with advances in cellular engineering, better understanding of neural development and network formation, growing appreciation of neural plasticity and how it can be therapeutically harnessed and the application of relevant combinatorial approaches. Cell transplantation therapies are highly promising due to their ability to provide multiple benefits at the molecular, cellular and circuit levels[13]. Many different cell transplantation strategies are currently being investigated for use in SCI. These include the transplantation of non-neuronal cell types, such as Schwann cells, olfactory ensheathing cells, oligodendrocyte progenitor cells and mesenchymal stem cells[13]. These strategies are focused on providing neuroprotective benefits, promoting remyelination and modulating the immune response in the injured spinal cord. However, this Review is focused on the transplantation of neural progenitor cells (NPCs), which provide the neural building blocks of the new glial cells (astrocytes and oligodendrocytes) and neurons that are necessary to build neural networks and promote connectivity (and thus functional recovery)[14]. As we describe herein, recent advances in NPC transplantation research have revealed the remarkable ability of engrafted neurons to synaptically integrate into the injured nervous system, highlighting the potential for reconstruction of complete neural circuits that can support recovery of complex neurological functions.

Oligodendrocyte progenitor cells

Cells that can differentiate into oligodendrocytes and produce myelin. They are also known as oligodendrocyte precursor cells, often described as Ng2 cells (chondroitin sulfate proteoglycan neuron/glia antigen 2) or polydendrocytes and were previously known as oligodendrocyte type 2 astrocyte (O-2A) progenitor cells.

Neural progenitor cells (NPCs).

Neural cells with less proliferative potential than neural stem cells. NPCs give rise to glial and neuronal cell types that are present in the CNS in the developing embryo, neonate and adult rodent. embryonic NPCs include neuronal-restricted precursors and glial-restricted precursors.

The new neurons generated following NPC transplantation have been shown in animal models to anatomically and functionally integrate with host neural circuits and to support the establishment of novel neuronal relays across the site of injury[15–25]. NPC-derived glial cells[26–32] not only support the graft-derived neurons but also confer additional therapeutic benefits. These include supporting the regeneration, extension and remyelination of injured host axons that are necessary for the relay[17,33–49], providing neuroprotection for both host and graft neurons[50–52] and attenuating glial scar formation[33,42,53]. Thus, NPC transplantation can be considered a potentially powerful combinatorial therapy.

> ### Neuronal relays
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> At their simplest, three synaptically connected neurons; in the case of transplantation after spinal cord injury, these are the injured neuron, the transplant-derived neuron and the target neuron.

> ### Glial scar
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> The fibroglial cell layer surrounding the core of a lesion after spinal cord injury, composed of chondroitin sulfate proteoglycans and fibrous connective tissue.

In this Review, we describe recent advances in the preparation and characterization of NPCs from embryonic tissue and pluripotent cells and the application of transplantation strategies to improve connectivity in sensory, motor and autonomic systems, as well as the incorporation of new ideas about neural plasticity and the use of scaffolds to promote the formation of neuronal connections. For further information on the role of other neural cells (such as astrocytes and oligodendrocyte progenitor cells) and the elucidation of the mechanisms associated with their therapeutic potential, we refer the reader to a number of recent reviews[13,54–60].

Brief history of neural cell therapy

The idea of transplanting neural tissues as a strategy for replacement and repair following injury and in degenerative diseases originated in the late nineteenth century and early twentieth century[61,62]. In 1890, one of the earliest attempts to use this approach[63] transplanted brain tissue from adult cats into adult dogs; the results were encouraging but survival was limited. This was followed by experiments that transplanted neonate rat cortical tissues into the cortex of littermates, which increased survival[64]. At the same time, it was recognized that the limitations of regeneration in the adult mammalian CNS are, in part, due to a lack of neurotrophic support. Thus, implants of peripheral nerve were used to support the growth of severed nerves[65]. Many decades later, this work was followed up in studies that transplanted peripheral nervous system 'bridges' into the injured CNS, underscoring the potential for regeneration in a growth-permissive environment[66]. Another important advance was the first successful human kidney transplant, using as a donor the recipient's identical twin[67], which opened the door to autologous grafting and later allografting using immunosuppression. The 1970s and 1980s saw a rapid rise in the number of transplantation studies that demonstrated how fetal neural tissues can be used effectively to repair the CNS in neurological disorders and CNS injuries (reviewed in reF.[68]).

> ### Autologous grafting
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> Transplantation of cells or tissue derived from the individual's own body, including autografts (transplants of tissue from one point to another in the same individual's body, such as a skin or nerve graft) as well as grafts of reprogrammed autologous cells (for example, induced pluripotent stem cells).

> ## Allografting
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> Transplantation of tissue or cells from a genetically non-identical member of the same species. When cells from a different species are transplanted, they are xenografts.

During this period, two notable preclinical studies were conducted in models of Parkinson disease. These studies demonstrated as a proof of concept that transplantation of fetal brain tissue enriched with dopaminergic neurons could restore dopaminergic inputs to the denervated striatum and partially reverse functional deficits[69,70]. Later work demonstrated that transplantation of fetal brain tissue (taken from the ventral mesencephalon, which is enriched in dopaminergic neurons) resulted in local dopamine production and long-term functional improvements that were associated with transplant survival and integration[71]. This led to clinical trials[72,73] which concluded that dopamine neuron grafts can survive, integrate, reinnervate the striatum and promote functional recovery in some patients. There were, however, a number of challenges, including the limited availability of embryonic tissue, variable results, immunosuppression required for allografts and persistent ethical issues[74,75].

Similar advances were made in SCI, beginning with a study that reported that donor spinal cells may be able to promote axonal repair by bridging the injury and/or forming new neuronal relays[76]. Subsequent studies showed that transplants obtained from the appropriate tissue (such as the developing spinal cord) taken from animals of the appropriate age (for example, embryonic day 14 (E14)) could be transplanted into various models of SCI in rats, where they not only survived but also integrated with surrounding host tissue and developed identifiable neural morphology[53,77]. In these studies, the donor fetal spinal cord (FSC) showed a significant degree of organotypic differentiation, by forming regions with the cytological and neurotransmitter characteristics of the adult spinal cord[78]. Immunocytochemical and neural tracing experiments showed that host afferent axons expressing calcitonin gene-related peptide regenerated into the transplants, indicating that the FSC tissue encouraged regeneration of adult axotomized neurons[35]. Retrograde tracers showed that axons from the transplants extended into the host spinal cord as far as 5 mm from the host–graft interface[38]. From the findings taken together, the authors of these studies concluded that "intraspinal grafts of fetal spinal cord tissue can establish a short-range intersegmental circuitry in the injured, adult spinal cord" and "may contribute to the formation of a functional relay between separated segments"[38].

> ## Fetal spinal cord
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> (FSC). Tissue or cells originating from animals at the fetal stage or embryonic stage of development. This cell population has been extensively characterized and widely applied to studies of animal spinal cord injury.

A key limitation faced by those conducting the pioneering FSC transplantation experiments was the difficulty of identifying the grafted cells within the host tissue. Later, with the availability of labelled neural tissue obtained from transgenic rats expressing alkaline

phosphatase (and later green fluorescent protein), the long-term integration of fetal E14 spinal cord tissue transplants was confirmed, together with the projection of axons over several spinal segments[29]. It is remarkable that even without the use of the transgenic animals and advanced molecular tools that most studies rely on today, the early studies were able to demonstrate that fetal transplantation met the fundamental requirements of effective connectivity: the generation and survival of new neurons, the growth of axons into and out of the transplant and a modest level of functional recovery[79].

Moving to NPCs

In the 1990s progress was made in defining the population of neural progenitor and neural stem cells (NSCs) that are present in the developing mammalian spinal cord. This was accompanied by the development of techniques for isolating and culturing these cells[80]. These studies discovered that at early stages of development the spinal cord contains mostly multipotent neuroepithelial cells, which mature into lineage-restricted progenitors, including neuronal-restricted precursors (NRPs)[81], and glial-restricted precursors (GRPs)[82]. Both NRPs and GRPs can be isolated directly from the E13.5 rat spinal cord or can be generated from multipotent neuroepithelial cells[83]. Neuroepithelial cells transplanted into the adult spinal cord showed poor survival, but transplants of NRPs and GRPs showed robust and long-term survival, expressing markers of mature neurons, astrocytes and oligodendrocytes, as well as synaptic markers[27]. These studies indicated that progenitor cells may provide a promising cellular replacement candidate for neural cells, including neurons. It is important to note that, during development, NRPs generate the specific progenitors of ventral and dorsal lineage neurons via coordinated spatial and temporal regulation of gene expression, underscoring the complexity of the challenge of rebuilding damaged spinal circuits[84,85]. A comparison between the properties of FSC and NRP and/or GRP transplants, both derived from E14 spinal cord, showed that FSC cells are able to project longer axons[29]. This reflects the complex composition of fetal tissue, which contains non-neural cells and extracellular matrix molecules in addition to neurons. Indeed, following transplantation of dissociated FSC tissue, in which the enzyme trypsin is used to degrade extracellular matrix components, graft survival and axon growth required the addition of a cocktail of growth and matrix factors[17]. Another difference between the two transplant sources is that the culturing of the NRPs or GRPs results in an alteration in the composition of the donor neuronal phenotype in comparison with that of the cells present in FSC tissue[22], including a downregulation of several ventral transcription factors that are developmentally expressed in motor neurons as well as some spinal interneurons. The implications of these biases in composition for spinal cord transplantation and/or repair and the possibility of selecting or engineering selective populations (such as motor neurons or specific excitatory or inhibitory interneurons) is discussed later in this Review.

Neural stem cells

(NSCs). Multipotent neural cells with high proliferative potential that can generate both neurons and glial cells, such as the neuroepithelial cells present in the developing and adult spinal cord of rodents.

> **Extracellular matrix**
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> The non-cellular component that provides physical and chemical scaffolding for cells and signalling for tissue differentiation and homeostasis. in the context of spinal cord injury, it refers to the molecular components of the scar, such as chondroitin sulfate proteoglycans.

There have been parallel advances in identifying and characterizing NSCs and NPCs in the adult mammalian CNS. Pioneering work reported the presence of multipotent NSCs in the adult rat brain and spinal cord[86,87]. NPCs prepared from the adult CNS have since been extensively used for transplantation experiments in animal models of SCI[60,88], and their self-renewal capacity presents advantages. In recent years, however, the emphasis in cell transplantation studies has shifted to NPCs derived from pluripotent embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells). These NPCs can be obtained with differentiation protocols that can generate either neuronal progenitors[89] or more distinct populations of cells, such as interneurons[90] (BOX 1). The clear advantages of iPS cells for autologous grafting made them candidates for clinical trials[91]; however, the advantages of pluripotent cells come with concerns about tumour formation that may mean that additional purification steps are required to minimize the potential risk.

Improving connectivity with NPCs

In addition to the selection of particular types of cell transplants for the specific task of restoring connectivity, it is also important to target distinct neural systems in which such a strategy may be most effective. These may include those responsible for locomotion and sensory functions, those responsible for life-threatening deficits (impaired breathing, autonomic dysreflexia) and those that remain a priority to injured individuals (bladder, upper extremity function). While our Review is focused on improving connectivity with NPC transplants, it is important to note that cell transplantation strategies may have a number of additional aims — including remyelination, immunomodulation, stimulation of endogenous stem cells and attenuation of the scarring process — that may contribute to restoration of function in these systems (reviewed in REFS[13,60,92]). There are also other non-transplant strategies that may enhance intrinsic growth potential, such as modulating the expression of PTEN and SOCS3 (REFS[93,94]), or targeting extrinsic factors that limit growth (such as inhibitory molecules associated with scarring and myelin debris) to allow bridging and a generate more effective donor–host interface (reviewed in REF.[95]). Many studies have also focused on a variety of pharmacological strategies to modulate the inflammatory response and provide neuroprotection at the early stages of SCI to reduce the long-term damage of the secondary injury (reviewed in REFS[96,97]). More recently there has been promising and exciting progress in promoting neural plasticity and improved function through the use of neural interfacing such as epidural stimulation together with activity-based training[98].

Transplantation of NPCs in sensory systems.

Sensory neurons of the dorsal root ganglia project both to the periphery and along the spinal cord to the brainstem. Animal models in which this spinal cord pathway (known

as the dorsal column) is lesioned allow regeneration and connectivity after SCI and the recovery of sensory function to be studied[99]. In these models, transplants of NRPs together with GRPs have been examined for their capacity to form a functional relay between injured dorsal column sensory axons and their targets in the dorsal column nuclei (DCN) in the brainstem[16] (FIG. 2). NRPs appeared to be good candidates for this role as it was known that they can generate neurons[27,100], survive long term after transplantation and form synaptic connections with host neurons when grafted into the adult spinal cord[29,30]. Furthermore, it was shown that brain-derived neurotrophic factor (BDNF) can promote directional axon growth from NPCs transplanted into the injured spinal cord, providing a means to guide connectivity with appropriate targets[101]. The target for the first of these studies was the sensory axons of rat ascending dorsal column neurons, which comprise a tight bundle of fibres within the spinal cord dorsal columns. The injury model used in the study generated a complete unilateral injury of the dorsal columns, severing the tract at cervical spinal segment C1, and was followed by acute transplantation of E13.5 rat spinal cord-derived NRPs and/or GRPs[16]. A week later, a lentivirus expressing BDNF was injected into the DCN to generate a trophic gradient for directional axon growth. Tracing analysis showed that the host sensory axons regenerated into the transplant and made synaptic connections that were verified by immuno-electron microscopy. Graft-derived neurons extended axons into the target DCN and made synaptic connections. Functional analysis demonstrated that activation of regenerated dorsal column axons through stimulation of the sciatic nerve induced FOS expression, indicating neural activity, in graft-derived neurons. The same stimulation also evoked responses in the DCN with a delay consistent with transmission through a neuronal relay[16].

This strategy of relay formation became the basis for subsequent transplantation studies that aimed to address and resolve distinct issues, including long-term graft survival, the generation of new neurons, the growth of host axons into the graft and graft axons out of the graft, and the generation of functional synapses within the transplant and with putative targets (reviewed in REF.[54]). These studies emphasized the advantage of using immature neurons for transplantation, owing to their enhanced capacity to grow and overcome the inhibitory environment of the injury[102], and also highlighted the essential role of donor astrocytes in generating permissive conditions for neuronal survival, differentiation, axon growth and synapse formation[103]. It has been shown that this relay strategy can be applied with NPCs derived from ES cells, iPS cells or cells derived from sources enriched in an appropriate population of cells (such as glutamatergic or GABAergic neurons)[54,59,104,105]. Selectively transplanting subpopulations of cells with particular neuronal phenotypes may also allow more directed treatments: for example, GABAergic neurons have been transplanted to treat neuropathic pain in models of SCI because they can mitigate the loss of presynaptic inhibition onto the dorsal horn neurons that are involved in gating sensory information[106]. These studies also illustrated the multilevel analysis of transplant success that is required at the anatomical, physiological and eventually network levels. However, it is important to realize the limitations of the relay approach and the need for additional modifications designed to increase transplant survival, increase regeneration of host neurons and maximize functional recovery without maladaptive plasticity, as well as the need to test this strategy in chronic injury.

Neuropathic pain

Pain resulting from injury to the somatosensory nervous system. Neuropathic pain resulting from spinal cord injury typically manifests itself as sharp, shooting or burning sensations experienced in the absence of noxious stimulation or exaggerated pain responses on noxious stimulation.

Maladaptive plasticity

Spontaneous reorganization of spared neural circuits in such a way that it produces undesired neurological outcomes such as pain or spasticity.

Transplantation of NPCs in motor systems.

Restoration of voluntary motor function has long been a central therapeutic goal of NPC transplantation after SCI. Studies have used diverse lesion models, cell sources, anatomical assessments and behavioural tests (reviewed in REFS[89,107–113]), producing a wide variety of results that have sometimes included negative data[114] and a failure to replicate previous findings[115]. These studies have also highlighted the many mechanisms by which cell transplants can promote the recovery of functional connectivity and provided important lessons to be considered when potential therapeutics are being advanced from the preclinical stage to the translational stage.

One of the first studies to report enhanced motor functional recovery in SCI used FSC tissue transplanted 10 days after a contusion lesion in adult rats[116]. Spontaneous locomotor activity (that is, open-field locomotion) and motivated locomotor performance (gait analysis during locomotion to reach a food reward) were assessed. Despite an absence of significant improvements in some assessments of generalized locomotor performance, such as inclined plane and grid walking, rats with transplants exhibited improvement in specific aspects of gait (the base of support and stride length of the hindlimbs) during motivated locomotion[116]. This showed that detailed quantitative assessments of isolated aspects of motor function may be required to reveal subtle and/or targeted effects of treatment. Indeed, locomotion is a complex behaviour that requires integration of descending motor commands, sensory feedback, alternating excitation and inhibition of motor units and intersegmental coordination of motor outputs[117]. This study did not assess connectivity between the graft and the host, so mechanisms supporting this functional improvement remain unclear. More broadly, it is still unclear how graft–host neural relays might support the coordinated integration of the multiple neural pathways involved in motor behaviour. Progress in this area will require application of concepts learned from neuroanatomical and physiological studies of the intact and injured nervous system[118–120].

Contusion lesion

Spinal cord injury produced by a blunt force impact, typically resulting in incomplete neurological deficits with partial function remaining below the level of injury. This lesion

model has been widely used in experimental studies due to its anatomical similarities to most human spinal cord injury.

Along these lines, there has been significant progress recently related to the corticospinal tract (CST), a supraspinal descending pathway implicated in skilled motor function. In a recent study focused on targeted restoration of forelimb motor function[42], dissociated NPCs were transplanted into the site of a rat spinal cord dorsal column lesion that axotomized the descending axons of the CST. Following graft maturation, large numbers of CST axons were found to have grown into the NPC grafts. These axons established functional synapses onto graft-derived neurons, which in turn projected axons into the caudal host spinal cord. Performance on a skilled forelimb reaching task showed that rats that received NPC transplants performed significantly better than controls from 5 weeks after transplantation. This indicated that corticospinal axons can regenerate into the NPC transplant (exhibiting a preference for transplants with a caudalized (spinal cord) identity) and form relays through a combination of monosynaptic and polysynaptic projections[42]. In separate studies, other host motor system axons have been shown to project into transplants after SCI, including reticulospinal, rubrospinal and serotonergic axons[17,21,45]. In one of these studies, graft-derived synapse formation onto neurons in the caudal host spinal cord, as well as the presence of complete graft-mediated electrophysiological relays, was reported[17]. In a recent study, it was observed that some of the axons emerging from donor NPC transplants were myelinated by host oligodendrocytes, generating myelin sheaths similar to those of axons in the intact spinal cord[121]. This observation that graft-derived axons can be recognized and myelinated by host systems suggests that conduction of impulses through newly formed graft–host relays might be improved. Despite the focus on supraspinal tracts in these studies, it is important to consider that spinal interneurons are also strong candidates for mediating functional relays, as has been demonstrated in studies in which supraspinal control of stepping has been recovered through indirect propriospinal relay connections[118].

Recovery of complex motor behaviours is an immensely challenging goal of SCI research. At present, it is plausible that NPC transplantation may promote recovery of only selected, less complex aspects of motor control, rather than complete restoration of walking or hand function. Indeed, to date all studies have reported only partial recovery at best. Therefore, it is critical that preclinical efficacy studies are designed to use not only rigorous behavioural assessments but also rigorous statistical analyses designed to detect subtle but biologically meaningful effects of treatment. This is demonstrated by the findings of a recent study of human NSC transplantation into cervical SCI in non-human primates (rhesus monkeys)[122]. In this study, subjects were evaluated in an open-field task that sampled more 25 features of forelimb motor function. Although the authors reported that transplantation did not significantly improve any of the individual features of motor performance, principal component analysis revealed significant improvements in "an overall measure of motor function that combines all measures as compared to monkeys without surviving grafts"[122]. This type of multivariate statistical approach may be a particularly powerful method to reveal the effects of treatment in non-human primate models, which risk being underpowered for single variable statistics. Finally, motor behavioural outcomes in animal studies must be interpreted with regard to potential impact on quality of life. For a

large fraction of the human population with SCI, a small degree of hand function may have great implications for quality of life and independence.

Principal component analysis

An approach that uses an orthogonal transformation to convert observations that may be correlated into a set of uncorrelated variables referred to as principal components.

Transplantation of NPCs in respiratory networks.

Impaired breathing remains a leading cause of morbidity and death after SCI[123]. Accordingly, a range of strategies for repairing injured respiratory networks have been explored. A focus has been the phrenic motor network, which controls the diaphragm (FIG. 3). From a preclinical perspective, the phrenic network is a relatively simple neural network of readily identifiable supraspinal and spinal neuronal components which controls function of a single muscle performing a simple task and requires no training. This has enabled rigorous assessment of the reparative potential of cell therapies in this system[15,18,20,22,23,124]. As in most other systems, the goals of cell therapy for respiratory networks have included providing functionally relevant new neurons and/or a growth-permissive substrate to serve as a bridge to growing host axons. Building on prior work with glial progenitors in other SCI models[125], recent work demonstrated in mice that transplanted GRPs promote growth of injured bulbospinal respiratory axons through the site of injury[126], improving phrenic motor recovery.

NPC transplants have also been used within the phrenic motor network to provide a new population of neurons that can contribute to the formation of novel neuronal networks that relay information across the injury site (FIG. 3). Among the first of these studies were two that used FSC transplants to repair injured phrenic motor circuitry following a lateral hemisection at the C2 spinal level in adult rats[15,18]). In one of these studies, transneuronal tracing techniques revealed that by 4 weeks after transplantation donor cells synaptically integrated with the denervated phrenic network[15]. A subsequent study showed that the donor cells also received synaptic input from host neurons both rostral and caudal to the injury[18]. Electrophysiological recording revealed examples of spontaneous donor neuron activity that was in phase with inspiratory and expiratory phases of breathing, and was responsive to altered respiratory drive[18]. Combining transplantation with respiratory training[127] increased this patterned respiratory activity within donor cells. Finally, it has been shown that host phrenic motor neuron output is significantly improved following transplantation[128]. This capacity for anatomical and functional improvement was also demonstrated following a lateral hemisection in adult rats at the C4 spinal level, in the heart of the phrenic motor neuron pool, and where the majority of human SCIs occur[129].

While these hemisection-type injuries offer a reproducible proof-of-principle preclinical injury model, the neuropathological consequences of human SCI more closely resemble contusion injury or compression injury. A growing number of studies are now assessing the efficacy of neural transplantation in such models. Mechanically dissociated FSC tissue transplanted into the lesion site 1 week after a C3/4 contusion injury (subacutely) was

recently shown to result in synaptic integration of donor neurons — including cholinergic interneurons — with the injured phrenic motor network and spontaneous respiratory activity[23]. Serotonergic host axons and putative boutons were also seen in close proximity to these donor neurons, providing further evidence of host–donor innervation. This result was replicated by the transplantation of lineage-restricted NPCs[22], which were derived from the developing rodent spinal cord. The latter study also showed that enriching donor progenitors for a specific subset of spinal interneurons — CHX10-expressing, excitatory V2a neurons — further enhanced recovery. However, the extent of donor–host integration as well as the extent of functional improvement was variable. Thus, despite the functional benefits seen with FSC transplantation in these animal models, some caveats remain that need to be explored further[14]. The results of these recent experiments suggest not only that care should be taken in selecting the appropriate donor cell phenotype but also that transplantation without additional interventions to promote integration may not be enough to release their full therapeutic potential.

> **Compression injury**
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> A condition that puts pressure on the spinal cord, which can be achieved in animal models of spinal cord injury using calibrated clips or by placing a specific weight in the epidural space. (A mixed contusion–compression spinal cord injury model can also be generated by delivering an initial blunt impact followed by sustained pressure.)

Transplantation of NPCs in autonomic systems.

SCI at high spinal levels can affect autonomic function. For example, the loss of supraspinal control over sympathetic flow originating from sites caudal to the injury results in cardiovascular dysfunction (FIG. 4). Specifically, the altered tonic activity of sympathetic preganglionic neurons (SPNs) as a result of the loss of descending modulatory input causes abnormal haemodynamics at rest and orthostatic hypotension[130,131]. Autonomic dysreflexia, a serious cardiovascular disorder characterized by dangerous episodic hypertension, occurs due to bursting of massive sympathetic discharges in response to sensory or visceral stimuli below the level of injury[132]. Autonomic dysreflexia is another leading cause of morbidity and death in individuals with SCI and its treatment is considered to be high priority for improving quality of life[133]. Currently, treatments for this disorder (which is managed mostly by antihypertensive medications) have transient effects without addressing the primary sympathetic dysregulation. There is therefore an opportunity for experimental transplantation strategies using NPCs to restore the regulation of SPNs and ultimately improve long-term cardiovascular function. Autonomic dysreflexia has been modelled in multiple studies by transection of the adult rat spinal cord at spinal level T4 coupled with noxious colorectal distension[134–136]. Transplantation of E14 rat brainstem-derived NSCs in this injury model promoted recovery of basal cardiovascular parameters and alleviated autonomic dysreflexia[136] (FIG. 4). Anatomical analysis showed survival and differentiation of the graft into catecholaminergic and serotonergic neurons, the projection of host supraspinal medullar neurons into the graft and long-distance axon growth and topographical innervation of caudal SPNs by the graft-derived neurons. Taken together, the results indicated the formation of functional relays to restore supraspinal regulation of

denervated SPNs[136]. It is possible that this approach could be further refined through the transplantation of serotonergic neuron-enriched fetal raphe cells or serotonergic neurons prepared by specific neuronal differentiation of ES cells or iPS cells.

Another functional deficit after SCI that is associated with the autonomic nervous system is the loss of voluntary micturition control, which is also rated as an important therapeutic goal for quality of life in people with SCI[133]. Importantly, bladder dysfunction is a cause of urinary tract infection, which is a major cause of death in people with SCI and remains a concern given the increase in antibiotic-resistant bacteria[137]. Lower urinary tract function is controlled by a combination of supraspinal, spinal and peripheral neurons[138]. Following SCI, interruption of afferent neuronal pathways initially produces bladder areflexia. Over time, new spinal neuronal circuits are established that enable the emergence of a spontaneous bladder reflex to facilitate involuntary urine voiding[139]. However, there is a frequent occurrence of bladder hyperactivity and detrusor–sphincter dyssynergia causing inefficient voiding[140].

One of the first studies to test the efficacy of NPC transplantation on restoration of bladder function used a midthoracic contusion injury in rats followed by a subacute grafting of E14-derived NRPs and/or GRPs[141]. Rats with transplants exhibited an accelerated recovery from bladder areflexia, with a decrease in the high micturition pressure and amelioration of dyssynergia between the bladder and the urethral sphincter[141]. As dyssynergia is associated with the loss of brainstem projections into the lumbosacral spinal cord, it appears likely that the transplant reduced the secondary injury, providing protection to these modulatory systems. The fewer episodes of detrusor hyperreflexia suggested an attenuation of the hyperactive bladder reflexes because of diminished sprouting from bladder afferents. This is therefore an example of NPC transplants modulating the host environment and providing local protection and axonal sparing for descending pathways, rather than bridging connectivity. Indeed, transplantation of human GRPs in an animal model of a similar spinal cord contusion also showed improvement of bladder function, which may again reflect local beneficial effects of the transplant with respect to neuroprotection and axon sparing[125]. A recent study showed that transplanted NPCs derived from human ES cells differentiated to resemble GABAergic inhibitory medial ganglionic eminence neurons, received synaptic inputs and improved bladder function[142]. As GABA is crucial to coordinated bladder function, these improvements were hypothesized to be due to the influence of human ES cell-derived medial ganglionic eminence-like neurons on the function of the inhibitory interneurons that modulate parasympathetic preganglionic neurons and the motor neurons that innervate the detrusor muscle and external urethral sphincter, respectively.

An example of improved bladder function that did result from transplant-mediated improvement of connectivity was provided by studies that used peripheral nerve autografts, acidic fibroblast growth factor and chondroitinase ABC to treat a complete T8 spinal cord transection in adult rats and mice, showing restoration of supraspinal control of bladder function[143]. Urodynamic analysis revealed that the treatment was associated with regeneration of serotonergic neurons across the lesion and into the distal portion of the spinal cord.

> ### Chondroitinase ABC
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> A bacterial enzyme that degrades polysaccharide chains on chondroitin sulfate proteoglycans. This enzyme has been used as a potential therapeutic treatment for spinal cord injury due to its degradation of axon growth-inhibiting chondroitin sulfate proteoglycans that are present in the extracellular matrix of the injured spinal cord.

Cell therapy in clinical trials

The data described so far demonstrate the potential of NPC transplants to improve connectivity through a combination of host regeneration and formation of neuronal relays. However, as noted already, there are a number of additional mechanisms through which NPC transplants have been shown to provide therapeutic benefit in preclinical models, including remyelination, immunomodulation, neuroprotection and stimulation of axon growth[60].

Several efforts have been made to translate neural cell therapies into the setting of human injuries as described in this section and Supplementary Table 1, which includes all the registered clinical trials evaluating NSC transplantation in SCI regardless of their therapeutic targets. Building on the early work with FSC tissue, a small-scale clinical study was initiated to test the feasibility of transplanting FSC-derived cells into people with post-traumatic syringomyelia[144–147]. This suggested that spinal tissue could be safely transplanted[147]. The next evolution of this work was a trial[148] in which a mixture of human NSCs and NPCs derived from the fetal telencephalon was transplanted into patients with cervical SCI and which confirmed the safety of NPC transplantation. More recently, key clinical trials were conducted with oligodendrocyte progenitor cells[149,150], olfactory ensheathing cells[151], autologous Schwann cells[152,153] and human NSCs (HuCNS-SC)[154].

In 2009, the biopharmaceutical company Geron gained FDA approval to take human ES cell-derived oligodendrocyte progenitor cells into phase 1/2 clinical trials in people with subacute thoracic SCI[150]. This was a turning point not only for treatment of SCI but also for the stem cell field more generally as the cells were the first pluripotent stem cell-derived cells to be approved for clinical trials. After five people had been enrolled, the trial ended in 2011; however, in 2014, Asterias Biotherapeutics received FDA approval to continue and expand the trial, now including individuals with cervical-level injuries and incorporating an increased dose of cells for increased efficacy[150] and approval to begin a phase 2 trial[155]. Another trial — the StemCells 'Pathway' trial (phase 1/2) — was a single-blind, randomized controlled trial that was initiated in 2011 and ended in 2016 after 17 people had been treated[156]. Despite reports of some functional gain[154], the company lacked the financial support to continue the trial. In 2014, Neuralstem (now Seneca BioPharma) initiated an open-label phase 1 trial on the use of human spinal cord-derived NSCs in people with chronic thoracic or cervical SCI[157]. Eight patients have been enrolled to date and recruitment continues[158].

These clinical trials led to the engineering and development of hardware specifically for transplanting cells into the human spinal cord[159,160]. It has also become clear that it is important to evaluate cells derived from the same sources in preclinical studies and subsequent clinical studies to reduce variability in outcomes: the donor cells used in the StemCells trial were a different, clinical-grade cell line than the research-grade cell line used in preclinical studies; however, only the latter cell line showed positive effects in animals[114]. As new strategies for derivation of donor cells are developed, it is likely that more rapid progression to clinical trials will be possible. For example, with the rapid advances in iPS cell technology and preclinical testing of the efficacy[161–163], there are now plans to test human iPS cells in people with SCI[91].

Despite these advances, hurdles for translation persist, and neural cell transplantation has yet to become an approved therapy. Because of the relatively small population of people with SCI, the cost–benefit ratio for developing and testing treatments can be prohibitively high and the competition for 'qualified' patients at the subacute stage of injury can be fierce. However, the significant socio-economic burden and the growing incidence and prevalence of SCI should stimulate continued innovation and the push for translation. With consistent improvements in communication between academic scientists, clinical professionals, industry representatives, funding agencies, governments, people with SCI and advocates, there is hope that, as a unified network, greater support can be attained to drive clinical translation forward and overcome these hurdles.

Challenges

The advances in understanding the complex process of SCI together with the exciting progress in NSC biology have positioned NPC transplants as a promising therapeutic tool and as a focus of research to elucidate strategies for cell replacement in the CNS. Nevertheless, there are still many challenges to be faced at the cell biological and neuroscience systems levels.

Improve graft survival.

The challenge of graft survival was identified in the early studies using fetal transplants: these showed an initial loss of graft cells followed by pro-liferation of the NPC population, which eventually integrated with the host tissue[29]. Initial strategies to improve the survival and efficacy of transplants included a delay between injury and transplantation and the addition of neurotrophins[164]. Later, when FSC tissue treated with trypsin was used, these measures were supplemented by a cocktail of growth factors and a fibrin matrix[165]. The challenges of NPC survival in the injury environment[166] have further been addressed by inclusion of GRP-derived astrocytes to generate a permissive microenvironment[29]; however, this strategy was ineffective in a severe injury such as a complete transection[31]. Other strategies that have been used to promote survival of grafted NPCs include chondroitinase treatment[167], genetic modifications[168] and cell preconditioning[169]. A promising direction to address both survival and efficacy has been the use of biomaterials, as discussed in recent reviews[60,170] and BOX 2.

Improve host regeneration.

There is good evidence that transplants of NPCs initiate robust long-distance donor axon growth in the host[17,29]. Research is therefore now focused on the ability of host descending and ascending axons, which have variable capacity to regenerate and/or sprout[45,171], to grow into the transplant and overcome the inhibitory environment of the injury. A growing understanding of the intrinsic mechanisms of CNS axon growth and regeneration[172,173] has allowed molecular, pharmacological and rehabilitative strategies to target and improve plasticity of distinct axon populations, including the CST[174]. Similarly, a growing understanding of the composition of the molecules and the cells in the injury environment[175,176] has allowed strategies to neutralize or modulate their effects[177]. In particular, we have gained a better understanding of the various cellular and molecular components of the lesion environment[95], challenging long-held assumptions about the reasons underlying regeneration failure[178]. Combined intrinsic and extrinsic growth-promoting manipulations have been successfully used to promote extensive regeneration of propriospinal axons after SCI; this suggests that combining intrinsic or extrinsic manipulations with NPC transplantation might also support greater regeneration compared with transplantation alone[179]. Following SCI and NPC transplantation, the process of axonal growth and regeneration has to be carefully defined and assessed with respect to the CST, the serotonergic system and a variety of brainstem tracts (as discussed in REF.[108]). While host regeneration and improved axon growth are essential to building a functional relay and direct connectivity, it is also necessary to pay attention to the age-related decline in regeneration capacity[180] and the process of remyelination (BOX 3). It is also important to minimize maladaptive plasticity associated with spasticity or pain[181] and to be aware of situations in which regeneration can suppress function or interfere with functional recovery[182,183]. Finally, it is important to consider the mechanistic differences between host axon regeneration into grafts and synaptogenesis with graft neurons (relay formation) versus long-distance host axon regeneration through a graft to extend into caudal regions of host spinal cord. Although non-neural cell grafts and biomaterial scaffolds have historically been used to promote long-distance regeneration through sites of SCI[184,185], neural grafts can also act as permissive scaffolds. For example, in preliminary work, the expression of the transcription factor KLF6 in corticospinal neurons was shown to promote long-distance growth through NPC transplants, allowing synaptogenesis with caudal spinal cord neurons[186].

Establish and maintain functional synaptic connections.

To provide compelling evidence of connectivity through functional synapses, a multilevel approach is required. Immunohistochemistry is often used to show expression of presynaptic and postsynaptic proteins. Electron microscopy provides ultrastructural evidence for the formation of the postsynaptic density and can be combined with immuno-electron microscopy to identify the transplant-derived cell through the expression of reporters. However, synapse assembly and maturation are complex multistep processes that ultimately result in stable and functional synapses capable of transporting synaptic vesicles and expressing proper receptors and transporter proteins[187]. An indirect method to assess the presence of stable and mature synapses is to measure the transneuronal spread of neurotropic viruses[188,189]. However, the most direct evidence of functional connectivity

is generated by electrophysiological methods to show the transmission of signals through the putative relay, which can provide information on the formation and strength of monosynaptic and multisynaptic connections[16,17,25,42]. Improved knowledge of synaptic plasticity during development and adulthood suggests new strategies for transforming the initial connection mediated by axon regeneration and relay formation into stable functional synaptic connections[175]. Thus, targeted efforts may be needed to promote synaptic maturation and facilitate stability and function in an activity-dependent manner so as to train new circuits to generate meaningful function.

Identify the best neurons for improving connectivity.

There are a variety of spinal interneurons with different physiological and functional properties, which are often identified by unique developmental transcription factor expression profiles[190,191]. These cells modulate motor neuron activity, relay information along spinal cord segments and to the contralateral spinal cord and play an important role in neuroplasticity following SCI[118,192]. However, the spinal cord-derived NPCs used in most transplantation studies contain diverse populations of interneuron progenitors whose composition may change during culturing and expansion relative to the original fetal tissue[22]. It is now possible to direct the acquisition of specific phenotypic fates, either through directed differentiation of pluripotent cells[193–196] or by isolating NPCs from appropriate regions of the developing nervous system[15,42,44,136]. Building on the knowledge of interneuron diversity and evidence for plasticity within spinal networks, several studies have begun to explore which of these neurons contribute to plasticity and might show therapeutic advantages. In the respiratory network, excitatory premotor V2a spinal interneurons have been shown to contribute to anatomical phrenic plasticity after cervical SCI[197] and transplants enriched with these cells improve functional recovery[22]. In parallel, transplantation of inhibitory (GABAergic) interneurons has been shown to attenuate pain-associated behaviours following SCI[142,198–200]. Aside from transplantation of interneurons, there is growing interest in spinal cord motor neuron replacement strategies (reviewed in REF.[58]). However, survival of transplanted postmitotic motor neurons remains poor, and promoting differentiation of NPCs into motor neurons is difficult in the harsh lesion environment[58].

With advances in cellular engineering, and a better understanding of the neuronal and glial components that contribute to plasticity, future work can tailor cell therapies to progenitors that can be most beneficial for survival, specificity of connectivity, network function and recovery. Matching of neuronal phenotypes in the relay is important to ensure the fidelity of the transmitted signal, including the timing and pattern of the activity, particularly for skilled voluntary motor abilities and discriminative touch[54]. It is likely that tailored cell therapies will eventually be combined with other treatments such as gene therapy, neural stimulation or activity-based therapies that can better direct growth and strengthen synaptic connectivity for lasting functional recovery.

Guidance of transplanted cells to appropriate targets.

Early studies were focused on host axonal growth into the graft and non-specific synaptic formation[101]. More recent studies have explored the use of transplants with regional

specificity. For example, homologous reconstitution of the lesioned adult spinal cord with caudalized NSCs or primary spinal cord-derived NPCs supports robust regeneration of corticospinal axons, which form functional excitatory synapses with the neural replacement graft[42]. Similarly, it was demonstrated that sensory axons regenerate into appropriate organotypic domains of neural progenitor grafts (dorsal horn-like domains) and that these domains are avoided by regenerating corticospinal axons[44]. This indicates that injured adult axons retain the ability to recognize appropriate and inappropriate targets, which is conducive to restoration of circuitry. On the distal side of the relay, robust growth of axons from NPC transplants into the host spinal cord has been identified to originate from neurons located in caudal regions of the transplant[201]. Thus, in the absence of specific directional guidance, these neurons are most likely to extend axons caudally[201], which raises the possibility that they might make maladaptive connections and highlights the need for directional and guided growth. Indeed, one study showed that intraspinal NSC grafts caused aberrant sprouting, resulting in allodynia[181]. To address this issue, some studies have used a neurotrophic gradient to promote and guide graft-derived axons towards the putative target[16]. The use of NPCs, which are composed of both neuronal and glial progenitors, may also allow guided glial progenitor cell migration from the injury and/or transplant site, creating a permissive environment for axon growth[202]. However, it is also possible that transplants with regional specificity may retain their ability to project towards the appropriate targets and establish area-specific circuits (as has been shown in brain transplants[203–205]).

Integrating with adaptive plasticity to restore mapping and function.

Assuming that connectivity can be restored sufficiently to provide a relay that forms stable synapses, two major issues remain. The first is the requirement to form faithful maps and the other is the need to retrain the new circuits to support meaningful function. In the example of relay formation in the sensory system following the axotomy of the dorsal column tract, there is good anatomical and physiological evidence for restored somatotopic connectivity with the DCN[16]. However, even if a sufficient number of axons reach the putative target, it may be realistically impossible to restore the original specificity of the spatial connectivity generated during development[206]. This is likely to degrade the quality of the sensory information from the skin unless regenerating sensory axons can retain the ability to recognize appropriate postsynaptic partners. Furthermore, SCI initiates a reorganization process within the brain that can involve substantial cortical remapping as a result of sensory or motor deficits[207–210]. NPC transplants that promote connectivity must therefore restore some of the 'lost' cortical mapping. Given that cortical reorganization is a dynamic process, it is hoped that it can be refined and reshaped by retraining the new circuits during activity. Inducing neuronal activity could also be used to promote the remyelination and functionality of the axons[211].

Spontaneous plasticity within the adult nervous system following injury suggests that transplantation strategies may also rely on the ability of grafts to support functional integration with spared and reorganized host circuits. For example, plasticity in the propriospinal system allows recovery of some locomotor function after injury[212], and synaptic integration of NPC grafts with spared host propriospinal neurons was shown to

be necessary for improved locomotor outcomes[19]. Likewise, injury to the corticospinal system induces sprouting of spared as well as injured CST axons[210]. With use of a selective chemogenetic silencing approach, it was demonstrated that a small number of spared (dorsolateral) corticospinal axons can mediate spontaneous functional recovery of skilled locomotion after axotomy of the dorsal CST[213]. In light of the findings that NPC grafts support recovery of CST-mediated motor function following axotomy of dorsal and dorsolateral CST fibres[42], it is possible that graft integration with spared as well as regenerating fibres may be a potent therapeutic target for relay formation in transplantation studies.

Avoiding maladaptive connectivity.

Plasticity within the injured nervous system can be adaptive, but growth and reorganization of other pathways can lead to maladaptive outcomes. For example, plasticity within nociceptive systems early after SCI has been shown to contribute to long-term functional deficits such as hypersensitivity of nociceptive signalling and inhibition of locomotor recovery[214–216]. A question of high clinical relevance, therefore, is whether NPC grafts pose the risk of enhancing maladaptive plasticity in the injured spinal cord. One concern is that NPC grafts can promote sprouting of nociceptive afferent fibres into both the host spinal cord and the graft tissue following transplantation[44,171,217]. In one study, transplanted cells failed to promote locomotor improvement, and instead caused thermal hyperalgesia and mechanical allodynia of the forepaws that was associated with sprouting of calcitonin gene-related peptide-expressing fibres into the spinal cord dorsal horn rostral to the site of injury[218]. Notably, the grafted cells differentiated primarily into astrocytes, suggesting that if they are transplanted into an inappropriate injury or at the wrong time after injury, graft-derived astroglia may inadvertently promote pain-associated outcomes. Similarly, another study showed that naive primary FSC cells differentiated mostly to astrocytes after transplantation and produced allodynia[181]. In this study, primary FSC cells transduced with the neurogenic transcription factor neurogenin 2 gave rise to grafts containing significantly greater numbers of neurons and fewer astrocytes. Neurogenin 2-expressing grafts reduced sprouting of nociceptive fibres, attenuated allodynia and promoted enhanced motor recovery[181]. Together, these findings suggest a potential role of transplanted astrocytes in the development of pain-like states through plasticity of nociceptive systems, potentially occurring through the secretion of astrocyte-derived growth factors that promote sprouting of nociceptive axons[217,218]. However, not all neural grafts have been shown to have maladaptive effects, and graft-derived astrocytes are also known to have critical roles in producing a permissive microenvironment conducive to graft survival and host regeneration[16,41] and attenuating sensory dysfunction[219] as well as spasticity[219,220]. This highlights a critical need to gain more mechanistic understanding of how specific graft components and distinct types of astrocytes influence the growth of host systems.

Efficacy in large-animal models.

Although most experimental SCI studies have used rodents, large-animal models are key in the translation to human trials[221] because they are able to better match body size, neuroanatomy, immunology and complexity of neurological functions[222]. Moreover, differences in spinal cord lesion size and anatomy pose a special consideration in the

'scaling up' of cell transplantation therapies. Whereas the rodent spinal cord lesion site spans a few millimetres, human injuries are typically centimetres in length, requiring longer-distance axon growth for neural relay formation. A recent study reported the successful transplantation of allogeneic iPS cells into a porcine SCI model[223] and demonstrated long-term survival of grafts in 2–3-cm-long lesion sites. The transplanted iPS cells differentiated into glial cells and neurons and produced a modest improvement in motor function. Non-human-primate SCI models offer a further advantage to examine functional efficacy due to their high neuroanatomical and functional similarity to humans. Important studies have used marmoset models of SCI[224–226] and demonstrated that transplantation of allogeneic ES cell-derived NPCs resulted in remyelination of host axons by graft-derived oligodendrocytes, synaptic connectivity between host and graft-derived neurons and recovery of both locomotion and forelimb function[226]. Marmoset spinal cord anatomy, however, is much smaller than that of humans, underscoring the importance of recent studies showing functional efficacy with human FSC-derived NPCs transplanted into sites of cervical spinal cord hemisection in macaque primates spanning approximately 5 mm (REF.[122]). These grafts extended hundreds of thousands of axons rostrally and caudally from the lesion site, formed synapses with host neurons, supported regeneration of host corticospinal axons and promoted recovery of forelimb motor function. While these findings lend hope to the potential clinical efficacy of transplanted NPCs, large-animal and primate studies are costly and time-intensive and are likely to be used mostly for verification of data obtained with rodents.

Future perspectives

A haunting issue, which is rarely addressed, is that preclinical experiments are designed to use precise injury models with precise matching of animals and protocols between studies to obtain reproducible data. By contrast, the clinical reality is that there is considerable variability in injury location and severity, the time of treatment after injury and the types of treatment provided at early stages. While the study of variability between males and females, now mandated by the NIH[227], is being addressed preclinically, getting robust data across all of the sources of variability found in the clinic remains a challenge. Part of the solution may be to demonstrate the efficacy of any potential therapeutic approach in a range of injuries and animal models. One potential approach may be to evaluate transplantation efficacy in canines with naturally occurring canine SCI. This large-animal model offers unique parallels to the human SCI population with diverse genetic backgrounds, heterogeneous location and severity of SCI, and anatomical similarity to the human contusion–compression lesion[228]. The high prevalence of naturally occurring SCI in dogs also allows the long-term evaluation of clinically relevant outcomes in large cohorts, using surgical and imaging techniques identical to those used in the clinic[228]. Furthermore, there is an urgent need to align the injury assessment criteria for therapeutic efficacy in animal behaviour tests with the neurological examinations following SCI that will be acceptable across clinical trials. There has been promising progress in improved clinical evaluation over the American Spinal Injury Association impairment scale[229,230] and innovative assessment related to quality of life[231].

The limitations of the rodent model and the need for additional and appropriate animal models to address both the gap in the size between rodents and humans and differences in the neuroanatomy (particularly for the CST) has been recognized with significant advances in porcine and primate work, which need to continue. Another encouraging development is the emphasis of patient priorities for therapies with growing research using NPC transplants to address autonomic function, bladder control and respiration. This is likely to expand to consider the consequences of SCI on multiple organs of the body.

As most clinical translation involves allogeneic transplants, patients will have to be immunosuppressed, resulting in increased risks of infection, malignancy and other side effects. Allografts can be tested in animals only with cells from their own species, and testing human cell transplants therefore presents the conundrum of a xenograft model, a gap that might be minimized with use of primates. Use of iPS cells allows autologous grafting, but the complexity of the procedures, their cost and the need for screening of genetic stability are still major obstacles. Nevertheless, clinical trials with pluripotent cells are in the pipeline, and creative solutions include banking of iPS cell lines that are HLA-matched to large sections of the population and therefore will be less likely to require immunosuppression[91].

Concern has been raised about the migration of transplanted cells into other areas of the CNS forming ectopic aggregates that can induce abnormal activities[232]. It is possible that these concerns might be mitigated by the use of scaffolds to minimize migration and provide a stable local matrix as demonstrated in primate transplantation[122]. Similarly, the development of pluripotent cell lines and differentiation protocols may provide better control and greater efficiency in deriving cells with specific phenotypes but presents risks of tumour formation.

Although many NPC transplants are designed for cell replacement and restoration of connectivity in SCI, it is recognized that functional improvement may be the result of other mechanisms, such as neuromodulation, neuroprotection, synaptic reorganization, improved angiogenesis or remyelination (or a combination of these factors), which need to be considered in the design and interpretation of clinical trials[92]. The advantages of multifunctional NPC transplants pose a challenge to our mechanistic understanding, but as our understanding improves, we will be able to use more effectively advances in engineering to direct donor cells to treat specific systems in a targeted fashion.

Combination therapy may still be required with NPC transplants. This might include, for example, exercise or activity protocols to strengthen synaptic connections and promote plasticity, drug therapy at the acute phase to reduce secondary damage and the inclusion of scaffolds.

Despite representing the majority of patients, chronic SCI is still understudied and remains more of a challenge than the acute/subacute injury. Nevertheless, clinical trials — including those initiated by Neuralstem, NeuroRegen and Novagenesis — are moving towards treatments for chronic SCI[233–236] (see Supplementary Table 1).

Several other practical difficulties face cell therapy, including the complex and expensive production of cells, their cryopreservation, delivery protocols and the relatively small population of eligible patients. The FDA and other regulatory bodies have until recently had limited experience with cell therapy; however, the growing number of approved clinical trials with strong evidence of safety and the recent guidelines from the International Campaign for Cures of Spinal Cord Injury Paralysis[237] are paving the way for a more clearly defined process for future therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Box 1 |

From pluripotent cells to neural progenitor cells

The history of pluripotent cell research began with the discovery of embryonal carcinoma cells derived from teratocarcinomas in the 1950s[240]. These were subsequently shown to be pluripotent and capable of continuous expansion, leading to the generation of cell lines[241]. The next significant step was the establishment in culture of pluripotential cells derived from mouse blastocysts and grown as embryonic stem cells (ES cells)[242,243]. These cells became instrumental for the study of cell differentiation and lineage analysis and enabled the production of transgenic animals[244,245] and gene targeting by homologous recombination[246]. The intense interest in ES cells resulted in the discovery of differentiation protocols for a variety of somatic cells, including neurons[104]. It was not until 1996 that the first human ES cell lines were established[247], as well embryonic germ lines derived from primordial human germ cells[248]. The discovery of a xeno-free system, which abolished the need for animal products to grow ES cells, cleared the way for future application in the clinic[249].

The next breakthrough occurred in 2006 with the remarkable demonstration of the generation of induced pluripotent stem cells (iPS cells) from skin fibroblasts using four transcription factors: POU5F1, SOX2, MYC and KlF4 (REF.[250]). This direct cell reprogramming opened the field of regenerative medicine to unprecedented opportunities for cell replacement and repair, including the potential for autologous transplantation using patient-derived cells. In the past decade, rapid progress has improved iPS cell technology by enabling the derivation of iPS cells from a variety of cells (including human cells), modifying the reprogramming process to avoid the use of the oncogene *MYC* or vectors with biosafety concern and using the cells for disease modelling[251]. As the epigenetic background of cells affects their genetic profile and differentiation potential, researchers using iPS cell technology can face a dilemma: whether to use cells derived from less invasive procedures (such as CD34-expressing cells from the blood) or to use cells of a common ectodermal germ layer origin, such as keratinocytes (derived from skin biopsy), which have higher efficiency in producing neural progenitor cells[252]. It is also possible to generate neural progenitor cells directly from somatic cells without reprogramming into pluripotent cells using a combination of a small number of factors[253]. This strategy presents opportunities for autologous grafting without immunosuppression and with lower risk of tumorigenicity; however, it remains challenging to obtain highly efficient and consistent results from reprogramming.

There have also been continued efforts to obtain efficient and effective protocols for the differentiation of pluripotent cells into neural stem cells[254]. Protocols have recently moved from the preparation of a population of unspecified neural progenitors to the production of cells with specific phenotypes, such as dopaminergic neurons[255], cortical neurons[256], motor neurons[257] and spinal interneurons[90], for potential cell replacement in the CNS. There has also been progress in obtaining cells of glial lineages from pluripotent cells[150].

In the transition from animal models to clinical trials, additional attention must be paid to safety and scaling. Safety concerns are focused on the preparation of clinical-grade cells and the elimination of pluripotent cells capable of producing teratomas, while the scaling issue requires the creation of government-approved cell banks containing sufficient cells for transplantation procedures. In spinal cord injury (SCI), for example, tens of millions of cells are required per patient. Finally, business models remain a challenge for SCI therapeutics, especially for biotechnology companies that need outside funding. Additional perspectives on the role of iPS cell transplants in SCI can be found in recent reviews[59,60].

Box 2 |

Scaffolds to the rescue

The intersection between material engineering and neuroscience has produced a rich and productive area of research that leverages the ingenuity of engineering design to address the complexities of CNS injury and therapeutics. For the design of appropriate scaffolds to support transplantation of neural progenitor cells, engineers have considered parameters such biocompatibility, biodegradability, permeability, biomechanical properties, the possible addition of extracellular proteins, the controlled release of growth factors and surface topography (such as alignment of the scaffolds for directional axon growth)[60,170]. Important studies have been performed using natural polymers as scaffolds — including collagen, laminin, fibrin, hyaluronic acid, alginate, chitosan and self-assembled peptides used as hydrogels — which in general are biocompatible and biodegradable, with low immunogenicity[170]. These materials also have beneficial biological activities which can be matched with the transplanted cells for improved survival and differentiation[60]. By contrast, synthetic biomaterials such as polyglycolic acid, polylactic acid and poly(lactic-glycolic acid) as well as synthetically fabricated scaffolds such as polycaprolactone and nanotubes allow consistent production and control over a wide range of physical properties but often present challenges with respect to compatibility and potential toxicity[184,185].

Hydrogels have often been used as a supportive matrix for the delivery of cells into the injury cavity to improve transplant survival[258] and can be modified with molecules (such as platelet-derived growth factor to promote oligodendrocyte differentiation[259]) or constructed with polymers that allow local delivery of cellular transplants into the injured spinal cord. Injectable scaffolds are viscous liquid-forming space-filling hydrogels[260] that can be designed to form channels that guide axon growth[261] and can be prepared with aligned endothelial cells that provide both vascular structures and directional axon growth[262]. Recent technology allows the printing of 3D scaffolds and the generation of microstructure to promote and guide axon growth[25]. Furthermore, 3D cell cultures have been used for the fabrication of tissue-like constructs that can foster human neural stem cell maturation and regeneration in the injured spinal cord[263].

Box 3 |

The remyelination enigma

Demyelination following spinal cord injury (SCI) has been documented in animal models[264] and humans[265] as a result of loss of oligodendrocytes and degeneration events at the secondary stages of injury. Importantly, this loss can occur directly as a result of injury even without axonal compromise (primary demyelination) or indirectly as a result of axonal degeneration (secondary demyelination). effective remyelination may fail because of deficiency in the host progenitor cells and their recruitment, or incompetence of differentiation and maturation[266]. As a result, injured host neurons or newly growing axons may remain dysfunctional. What remains contentious is whether chronic demyelination makes a significant contribution to the deficits observed following SCI and is therefore an important therapeutic target[267–272]. Reaching a definite conclusion about the importance of the remyelination process to the success of neural progenitor cell (NPC) transplantation therapies is complicated by the variations between the transplantation studies with respect to the model of injury and its level and the type of functional analysis as well as by the other potential benefits of NPC transplantation besides myelination.

When examining the remyelination process following SCI, one has to consider the endogenous glial progenitors expressing neural/glial antigen 2 (NG2), which proliferate following injury and differentiate into remyelinating cells[273]. This response is lasts for months after SCI[274]. To enhance remyelination through NPC transplantation, preclinical studies have been focused on the use of oligodendrocyte progenitor cells (OPCs), which can be isolated from the CNS or derived from embryonic stem cells and induced pluripotent stem cells. One important study[269] examined transplantation of human embryonic stem cell-derived OPCs into a thoracic contusion in rats. The transplanted cells survived, redistributed themselves over short distances and differentiated into oligodendrocytes. Rats that received OPCs 7 days after injury exhibited enhanced remyelination and improved locomotor ability. By contrast, when OPCs were transplanted 10 months after injury, there was no enhanced remyelination or locomotor recovery. However, it is important to note that the role of remyelination in promoting functional improvements was not directly tested in these studies. A second study was conducted to support the clinical use of OPC therapy for cervical injuries[150], testing OPCs in a nude rat model of cervical SCI. The OPCs were found to significantly improve locomotor performance when administered directly into the cervical spinal cord 1 week after injury, and the functional improvement was associated with reduced cavitation and increased sparing of myelinated axons within the injury site. The study also showed that OPC migration is limited to the spinal cord and brainstem and did not cause any adverse clinical observations.
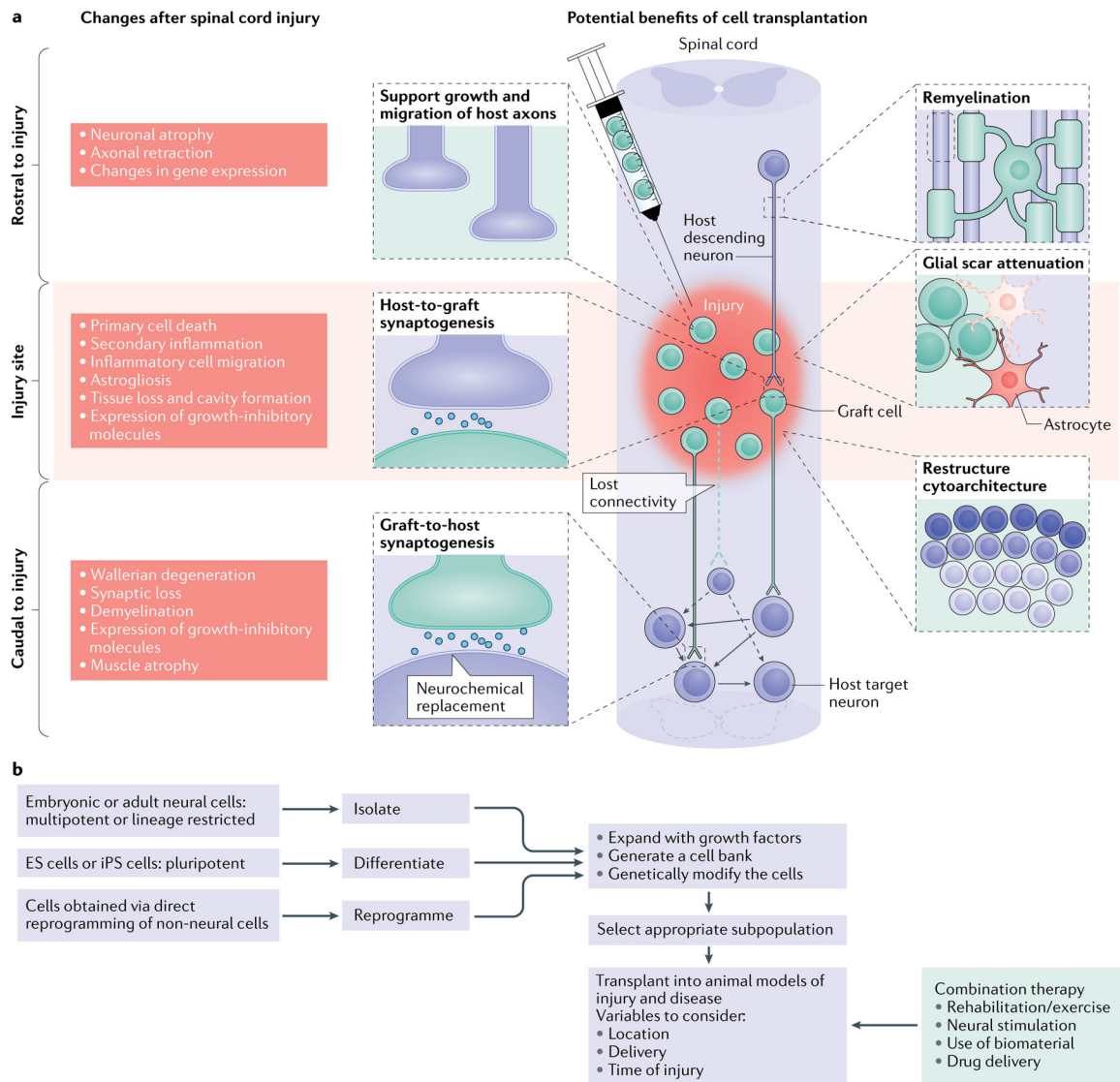
Fig. 1 |. Spinal cord injury: pathophysiological events and potential therapeutic targets.
a | The left side of the schematic illustrates the complex changes that occur after spinal cord injury (SCI), which differ temporally and spatially. In a descending tract, such as that illustrated here, these changes include events rostral to the injury (including axon degeneration and changes in gene expression), at the level of the injury (encompassing acute tissue damage and cell death as well as chronic secondary injury and inflammation) and caudal to the injury (including both neural events such as demyelination and non-neural events such as muscle atrophy). Similar changes occur in ascending tracts; however, in this case the location of the events in relation to the injury will be reversed. The injured spinal cord schematic illustrates potential therapeutic targets for cell transplantation, including remyelination, support of host axon growth, glial scar attenuation, synaptogenesis and the restructuring of spinal cord cytoarchitecture. **b** | The flow chart depicts the decisions that must be made when a cell transplantation strategy for SCI is being developed and the processing steps involved. It shows choices of cells for transplantation in SCI,

the process of their preparation, modification and selection and the parameters of their delivery alone and as part of a combination therapy. For cell choices, a wide range of neural progenitor cells can be obtained from embryonic and adult tissue, from pluripotent cells (embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells)) and from direct reprogramming of non-neural cells. These cells can be expanded with growth factors to generate cell banks and/or can be genetically modified (to overexpress growth factors, for example). It is then possible to select a subpopulation of the resulting neurons for transplantation. The transplantation process needs to consider variables such as the location of the transplant (that is, transplantation directly into the injury site, intrathecally or systemically), the delivery method (that is, injection as a cell suspension or as part of a hydrogel scaffold) and the timing (for example, subacute transplantation versus transplantation after a 2-week delay after injury). A number of different types of combination therapy can also be initiated at different times and act synergistically with the transplant. In particular, attention should be paid to rehabilitation strategies, various neural stimulation modalities, the use of biomaterials and drug delivery.
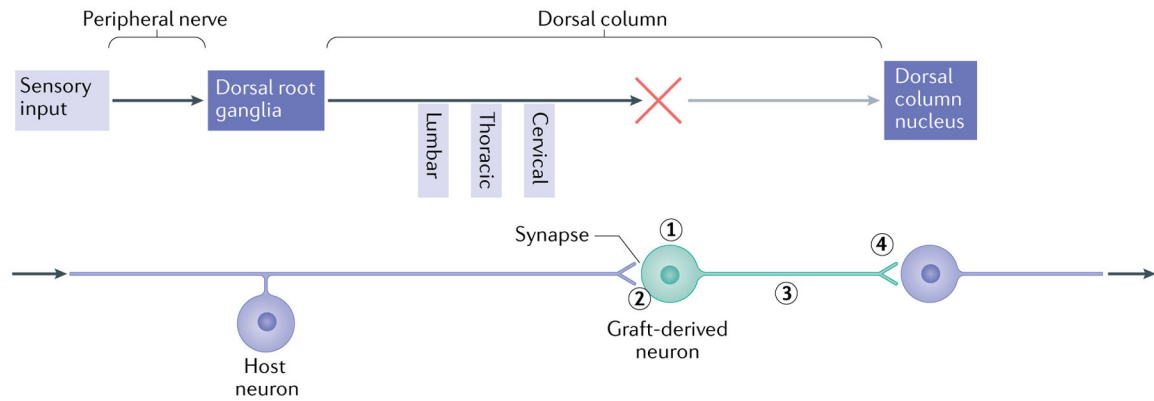
Fig. 2 |. Forming a relay using neural progenitor cells.
The schematic illustrates the elements required to use neural progenitor cells (NPCs) to form a relay following spinal cord injury, using the example of the sensory system in the presence of a cervical lesion of the dorsal column and transplants of neuronal-restricted precursors (NRPs) and/or glial-restricted precursors (GRPs). The upper part illustrates the elements of the ascending sensory pathway, disrupted by a cervical injury that interrupts the connectivity of sensory axons (dorsal column) with the dorsal column nucleus (DCN) in the brainstem. The lower part shows the steps required to restore the connectively using NPC transplants that form a relay. First, the transplant must survive and generate neurons with the appropriate phenotype (excitatory, for example) (**1**). The use of a mixture of NRPs and GRPs has been found to be effective, as the GRP-derived astrocytes generate a permissive environment for survival and differentiation of neurons[16]. Second, the host axons must grow into the graft and form synaptic connections (**2**). It appears that the presence of astrocytes in the graft attracts the sensory neurons[40], but other strategies include the induction of the growth potential of host neurons through the repression of genes such as *PTEN* and *SOCS3* (REF.[238]). Finally, the axons of transplanted neurons must undergo directional extension to the target (along a neurotrophic gradient to the DCN[101]) (**3**) and form synaptic connections (**4**). To verify the formation of a functional relay, analysis needs to be performed at different levels. Structural analysis includes the tracing of axon growth from the host and the transplant and obtaining evidence of synaptic structure by electron microscopy. Physiological analysis may involve the stimulation of axons followed by assessment of the expression of FOS in downstream neurons as well as measures of signal transmission through the transplant. Functional analysis will include behaviour tests indicative of restored connectivity of the specific tracts.
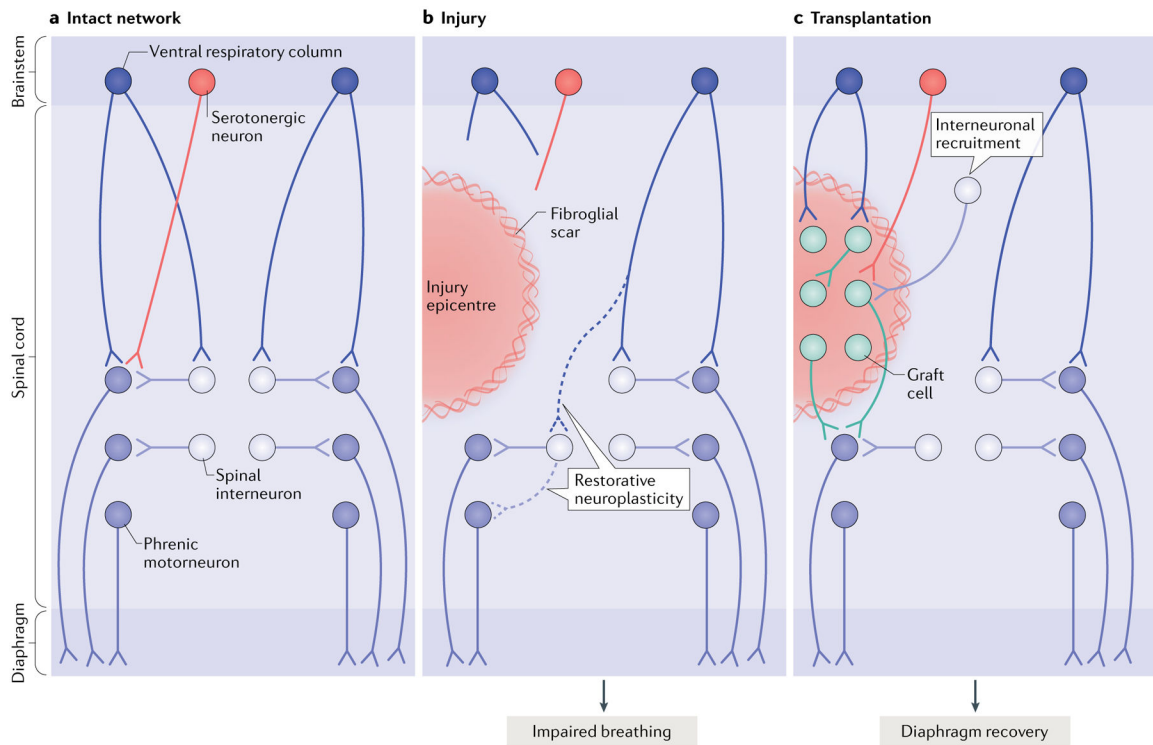
a Intact network **b** Injury **c** Transplantation

Brainstem

Ventral respiratory column

Serotonergic neuron

Spinal cord

Fibroglial scar

Injury epicentre

Spinal interneuron

Restorative neuroplasticity

Phrenic motorneuron

Interneuronal recruitment

Graft cell

Diaphragm

Impaired breathing

Diaphragm recovery

Fig. 3 |. Restoring connectivity in the respiratory system.
The diagram depicts the intact (part **a**), injured (part **b**) and transplant-treated (part **c**) spinal phrenic motor circuit within the cervical spinal cord. Respiration is driven by brainstem neurons in the ventral respiratory column that directly — or indirectly via spinal interneurons — innervate the phrenic motor neuron pool (distributed from cervical level C3 to cervical level C6). Phrenic motor neuron activity is also modulated by serotonergic pathways and populations of spinal interneurons as breathing conditions change. Phrenic motor neurons on each side of the spinal cord innervate half of the diaphragm on each side of the body via phrenic nerves. Injury (part **b**) can compromise descending projections, as well as phrenic spinal interneurons and motor neurons. Spared spinal neurons caudal to the injury are therefore denervated. While this is devastating, some limited recovery of diaphragm activity can occur ipsilateral to the injury via restorative neuroplasticity (dashed lines), and spared monosynaptic and polysynaptic pathways from the contralateral spinal cord (via brainstem and spinal interneurons, respectively) can facilitate plasticity in these lateralized spinal injuries. However, the extent of recovery is minimal and deficits persist. A number of cell therapies have been used to promote repair and plasticity within injured respiratory pathways. Neural progenitor cell transplants are perhaps the most often used, as they can modify glial scarring at the lesion border and provide the building blocks for tissue repair. Transplantation of neural progenitor cells into the injured phrenic network in animal models (typically directly into the lesion site as shown in part **c**) has resulted in extensive synaptic integration between donor neurons themselves, between host spinal and brainstem neurons and donor neurons and between donor and spinal phrenic neurons. This synaptic integration also coincides with enhanced plasticity of existing and newly formed pathways, and improved respiratory activity[15,18,22,23]. Without any other intervention, transplantation

of cells alone is likely to lead to the formation of a vast range of new connections, which are likely to differ between treated recipients[22,23], and to the recruitment of novel interneuron populations to establish novel neural networks. Research is under way to develop strategies that control this integration and connectivity. Other models of injury affecting the phrenic network and additional mechanisms of recovery are discussed elsewhere[239].
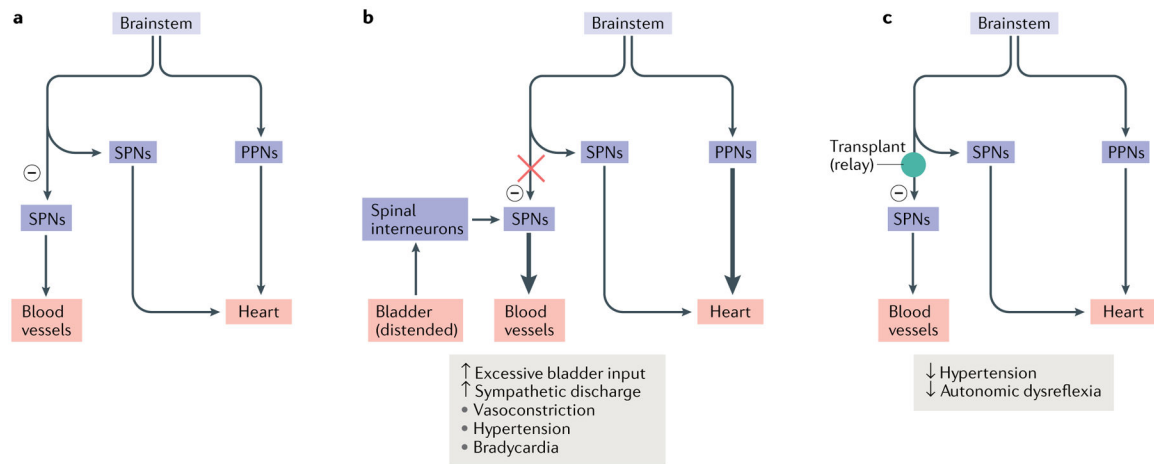
Fig. 4 |. Restoring connectivity in autonomic systems.
The majority of the vasculature is controlled by sympathetic activity, while the heart is regulated by both the sympathetic system and the parasympathetic system. Sympathetic preganglionic neurons (SPNs) in the spinal cord project to the periphery and synapse onto sympathetic postganglionic neurons. The latter extend axon terminals into the blood vessel and heart. **a** | In normal conditions, sympathetic excitation induces vasoconstriction and thus increases blood pressure. Subsequently, baroreceptor-mediated parasympathetic excitation decreases the heart rate. In addition, supraspinal vasomotor pathways provide inhibitory regulation (indicated by a minus sign) to suppress the sympathetic activity to blood vessels, leading to recovery of normal blood pressure. **b** | After spinal cord injury, spinal SPNs lose this descending inhibitory modulation. When excessive sensory or visceral stimulation below the level of injury (for example, bladder distension) activates SPNs via interneurons, the massive discharge of SPNs causes vasoconstriction and increases blood pressure. This causes baroreceptor-mediated bradycardia to occur. However, the absence of supraspinal inhibitory signals to caudal SPNs means that blood pressure remains high. The resulting simultaneous hypertension and bradycardia is known as autonomic dysreflexia. **c** | Transplantation of early-stage neurons into the lesion of the spinal cord reconstitutes supraspinal vasomotor pathways. Grafted cells relay supraspinal inhibitory signals across the lesion to target neurons in the caudal portion of the spinal cord, which can restore sympathetic regulation of cardiovascular function after spinal cord injury. PPNs, parasympathetic preganglionic neurons.