Stem cell therapy and Coordination dynamics therapy to improve Spinal cord injury

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Summary

During competition a motocross athlete suffered a clinically complete spinal cord injury (SCI) at the Thoracic 11/12 levels according to MRIs (magnetic resonance imaging). Six weeks after the accident the subject began intensive Coordination Dynamics Therapy (CDT) at an up-to-date therapy centre. After 6 months of therapy, when further improvements were only marginal, the patient opted for haematopoietic stem cell therapy in addition to ongoing CDT. During two years of stem cell therapy, including 4 sessions of stem cell application, and ongoing coordination dynamics therapy, improvement remained marginal – no more than what would have been achieved with continuing only CDT. It is concluded that this haematopoietic stem cell therapy did not have any beneficial effect on the repair of the spinal cord in this patient. Differences in the regeneration capacity between commonly used laboratory animals and human are addressed. On the basis of a frog model for regeneration, cell communication, and neural control, it is discussed why complete SCI in human are difficult to improve and why for stem cell therapies more proper human knowledge is needed to induce structural repair and direct it to the injured sites of the neuronal networks. Further research is needed to improve and justify the clinical application of stem cell therapy. A thoughtful combination of stem cell therapy and CDT may have a chance of structural repair even in complete SCI. However, objective measures are needed to quantify improvement in MRI (anatomic measure), EMG (measuring of motor programs by sEMG, electrophysiologic measure), and measurements of coordination dynamics (kinesiologic measure).

Key-words: Stem cell therapy – Coordination dynamics therapy – Human – Spinal cord injury – Regeneration – Coordination dynamics – Cell communication – Regeneration

Introduction

Coordination dynamics therapy (CDT) is a movement and learning therapy which can partly repair the injured, malfunctioning, or degenerating human CNS (25-31, 33, 34, 36). In severe injuries, especially spinal cord injury (SCI), substantial structural repair is needed in addition to functional repair. The assumption in CDT is that structural repair can be induced when training at limits (37). All repair mechanisms should be activated then including local release of target-derived growth factors that would attract regenerating fibres, or facilitated growth of regenerating fibres along active spared tract fibres (62). The weak point in CDT may still be limited structural repair in severe SCI.

When administering stem cell therapy, it is believed that structural repair can be achieved with the building of new nerve cells and new connections. The building of new nerve cells and connections is directed to the injured site using accompanying movement therapy (8).

A previous publication reported a case of a patient who suffered a complete SCI at the Th11/12 levels (according to MRI diagnostics) during a motocross competition and who underwent optimal

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coordination dynamics therapy for 6 months (37). During the therapy period objective diagnostics were performed including measurements of coordination dynamics, surface EMG (electromyography), and MRI (6 months after the accident). Most of the improvements achieved during these 6 months of intensive CDT were probably due to a functional repair. Achievement of significant further progress seemed to be possible only through substantial structural repair with many new connections growing or sprouting across the injury site and becoming functional. This patient with an anatomical complete SCI (according to MRI and function) was therefore an ideal case to assess if stem cell therapy can achieve meaningful structural repair.

Below it will be reported that the haematopoietic stem cell therapy, administered in a well-known Russian clinic, did not achieve any substantial progress in this patient within two years of therapy. The reasons why this stem cell therapy may not have worked and why the combination of this stem cell therapy and CDT did not achieve substantial progress are discussed.

On the basis of an animal model it is demonstrated that the research of neural control, cell communication, and cell proliferation has to include, in addition to morphology, also functional measurements on the cellular level.

Methods

Coordination Dynamics Therapy

Schalow Coordination Dynamics Therapy (CDT) is a neuronal network learning therapy, which includes the training of automatisms (creeping, crawling, walking, and running; to stimulate endogenous stem cell reserves to replace lost tissue upon training at limits), old-learned movements, rhythmic dynamic stereotyped movements (to functionally repair neural assemblies (spinal oscillators)) and device imposed movements to repair the impaired phase and frequency coordination of CNS self-organization. Some of the administered movements are shown in Fig. 2. For further details of the therapy method, see Refs. 37 (more practical, previous publication) and 35 (more theoretical). For understanding the repair of the integrative functions of the human CNS, the System Theory of Pattern Formation (applied to the injured and malfunctioning human CNS) is used.

System Theory of Pattern Formation

By cooperative and competitive interplay, the many billions of neurons of the human CNS generate dynamics in self-organization (by phase and frequency coordination) which cannot be explained by the properties of single neurons and neuron assemblies alone. A dynamic system theory of self-organization and pattern formation of the human CNS was derived from the concepts of synergetic. Collective variables or order parameters capture the collective coordinated activity of the neurons of the CNS. The specific equations of motion (the dynamics) of these collective variables generate the time course of organizational states.

The collective variables can be designated by the vector **X** and the coordination pattern dynamics can be formulated abstractly by equations of motion (59):

$$d\mathbf{X}/dt = \mathbf{F}_{intr}(\mathbf{X}) + \Sigma c_{inf}\mathbf{F}_{inf}(\mathbf{X},t) \qquad (2)$$

where $\mathbf{F}_{intr}$ designates the intrinsic dynamics. The summation is done over different types of so-called behavioural information, $\mathbf{F}_{inf}$, such as environmental, memorized, or intended behavioural information. The relative strength of different influences is parameterized by c_{inf}. For a mathematical solution to equation (2) for the special movement 'jumping on the springboard' in the Haken-Kelso-Bunz model, see Ref. 34. With the behavioural information $\mathbf{F}_{inf}(\mathbf{X},t)$ (treadmill walking, jumping on springboard, …) we can change the intrinsic dynamics $\mathbf{F}_{intr}(\mathbf{X})$ of the neuronal network organization of the human CNS.

In a complex system like the CNS, patterns can be generated by the system seeking cooperative stability. The system has the tendency to slip into the collective states to which it is attracted. Commonly attractive states and attractors are pictured as a ball in a potential well or, more generally, in an attractor layout. When an infant crawls, its arms and legs are strongly attracted to the pace and trot gait patterns (attractor states). Changes in CNS functioning are characterized as continuous stabilization and destabilization over time of preferred attractor states.

The equations of motion of the collective variables (2) have important clinical implications for treatment.

1. Behavioural requirements $\mathbf{F}_{inf}$ (like intention, support, and instruction) affect the whole coordination dynamics, including stability, rather than only certain coordination pattern itself.
2. Intrinsic coordination tendencies captured by the intrinsic dynamics systematically influence the performed pattern because the degree to which intrinsic tendencies conflict or agree with the required patterns determines the variability of the performed coordination pattern.
3. Reduction in stability, when intrinsic and informational requirements conflict, may lead to loss of stability and abrupt change as behavioural information is changed smoothly.
4. The intrinsic dynamics $\mathbf{F}_{intr}$ include vegetative and higher mental functions, which indicate that via exercising coordinated movements with support and instructions ($\mathbf{F}_{inf}$), urinary bladder function and intelligence can partly be repaired following CNS injury.

The novel step in this formulation (formula 2) is that not only do the patterns of trained movements improve but also other, non-trained functions (like vegetative and higher mental functions) of the CNS may improve (learning transfer). Further, we have a theory-based tool at hand to increase the stability of physiologic network states (for example movements) and to decrease the stability of pathologic neuronal network states (different kinds of spasticity). This means that we have a theoretical tool at hand to understand how we can repair the injured or malfunctioning human CNS on the macroscopic level by learning.

The dynamics of CNS organization is partly reflected in the temporal stability of coordination patterns, which can be assessed through a process of pattern change. When a subject exercises on the special CDT and recording device (Fig. 2A, left), the device imposes all continuously changing coordinations between arm and leg movements between pace and trot gait. The mean differential stability (arrhythmicity of exercising) per minute of exercising is referred to as coordination dynamics value, and is used to quantify CNS functioning. Since the quality of CNS functioning can be quantified by a single value, progress in CNS functioning can be evaluated in percentages and can be used to judge improvements in CNS functioning during therapy-induced learning in patients (Fig. 1) or during development (32, 33).

In addition to stability, symmetries structure the state space of a multicomponent system with multiple patterns. If symmetry is impaired in a patient with a CNS injury, the CNS organization switches more easily from a movement state into a spastic state or into another movement state (for further details, see Ref. 35).

Stem cell therapy

A 100-year-old dogma was that a mature adult's brain remains stable like a computer with fixed memory and processing power. One can lose brain nerve cells but one cannot gain new ones. If we locate certain functions to certain parts of the brain and/or nerve cells, then this dogma seems understandable. How could we remember anything and how can we have a certain character if the CNS were capable of structural changes? But if CNS functions are generated by a self-organization of neuronal networks, which can be changed by learning, then single cells, network parts, and brain parts are not that important any more with respect to movement, vegetative, and higher mental functions. The stability of important CNS functions (pattern formation) would not be in conflict with the building of new nerve cells and network changes in the adult human CNS.

In the last years it was found that new nerve cells can be built in the adult CNS (7, 8, 16, 49, 50). The building of new neurons is called neurogenesis. Multipotent neural stem cells divide periodically in the brain, giving rise to other stem cells and the progeny that can grow up to be either neurons or support cells (glial cells = astrocytes and oligodendrocytes). But to mature, these neural precursors must migrate away from the influence of the multipotent stem cells. Similar to the development of the brain, only those progeny cells survive which form active connections with meaningful functions; the others die.

Whether the young cells that persist become neurons or glial cells depends on where in the brain they end up and what type of activity is occurring in that brain region at that time. It takes more than one month in animals from the moment when a new neuron is formed from a stem cell until it becomes fully

functional and able to send and receive information. Thus, neurogenesis is a process, not an event, and the one that is tightly controlled (8). In the Discussion it will be analyzed which activity might be necessary to make use of the process of building new nerve cells.

New neurons seem to arise only in the fluid-filled ventricles in the forebrain and in the hippocampus. The progeny cells travel from the ventricles to the olfactorius bulbs and the hippocampus where new nerve cells seem to be needed and make functional contacts there. The hippocampus may need many new neurons to increase the brain's capacity to process and store novel information. There have been reports that new neurons were also found outside the hippocampus and olfactory bulb. In the human spinal cord during development, distinct and dynamic populations of neural precursors have been found, which are developmentally regulated (45).

For therapies two types of stem cells are used: adult neural stem cells and embryonic (and may be foetal and neonatal) stem cells. The adult neural stem cells are rare and left over from early embryonic development. Neurobiologists and physicians make patients believe that there is enough knowledge available on the molecular and cell communication mechanisms (and growth factors) of the human CNS so that neurogenesis can be controlled and the environmental stimuli at the injury site regulated, so that the neurogenesis can be directed to the injured or malfunctioning site of the CNS where it is needed and used for repair.

The haematopoietic stem-cell therapy (51) used in the patient described in this article belongs to the treatment with adult stem cells. The principle procedure is the following. To stimulate haematopoietic stem-cell production, the patient receives subcutaneous injections of granulocytic colony-stimulating factor (Neupogen® or Filgrastim). Then the patient is hooked up to a blood separator to isolate the stem cells and afterwards the stem cells are concentrated and re-injected (5.3-million) by a transfusion intrathecally into the subarachnoid space (i.e. into the spinal fluid, across the protective blood-brain barrier) through a L3-L4 lumbar puncture. The stem-cell procedure is accompanied by a daily intensive physical rehabilitation of 2 hours. Following the injection of the stem cells, the patients suffer from pain for one or two days (inflammation?).

According to the author's knowledge the physical rehabilitation, which the patient obtained in the clinic, was mainly muscle training. Therefore the cellular environment at the injury site of the spinal cord, and sensory-motor apparatus functionally related to the injury site, were stimulated only little to direct a possible neurogenesis soon after the application to the functional needed injury site (see below).

Repair strategies used in stem cell therapy and CDT

Nerve fibre growing strategy (animal)

An implicit assumption of much animal SCI research, at least until recently, has been that the major goal is to induce damaged axons to grow, to reconnect to appropriate targets and thereby restore functions as is possible in the peripheral nervous system (PNS). The major focus of animal research has therefore been to overcome the failure of axons to regenerate. The three main aims of such research are: to initiate and maintain axonal growth and elongation; to direct regenerating axons to reconnect with their target neurons; and to reconstitute original circuitry (or the equivalent), which will ultimately lead to functional restoration. Only the first aim has been extensively studied and convincingly demonstrated (60) in animals. Such repair strategy can be designated as 'nerve fibre growing strategy'. In the Discussion differences in the capacity for reparative regeneration are compared between animal and human in the PNS and CNS.

Cell replacement strategy (animal and human?)

In SCI not only the white matter with the tracts is damaged, but also the grey matter with the spinal neuronal networks. Especially in severe cervical spinal cord new motoneurons and new premotor neuronal networks are needed to reconstitute original circuitry at the damaged site. The axons of the newly built motoneurons have to grow down to the muscles and re-innervate peripheral muscles to restore functions of arm, hand and fingers and sensory fibres may have to grow along Bügners bands in nerve roots (PNS) into the spinal cord (CNS) to re-innervate spinal circuitry and tract neurons. Stem cell research is making believe that original circuitry (or the equivalent) can be reconstituted and chal-

lenges the view that neural stem cells achieve their therapeutic efficacy exclusively by a cell-replacement mechanism. Neural stem cells may also promote CNS repair through their intrinsic neuroprotective ability, which is mainly exerted by undifferentiated stem cells releasing, at the site of tissue damage, a milieu of neuroprotective molecules, temporally and spatially orchestrated by environmental needs. Therapeutic cell plasticity can be viewed as the capacity of somatic stem cells to adapt their fate and function to specific environmental needs, which arise as a result of pathological conditions (54). Since no improvement was achieved with the administered haematopoietic stem cell therapy, the results do not support the cell replacement strategy in human.

Recapitulating developmental grows and guidance repair strategy (animal and human)

Most crucial developmental guidance cues (for structural repair) need to be characterized and perhaps re-expressed at the proper location to guide regenerating axons (62). To trigger the injured spinal cord (and it functional surrounding) to re-express endogenous neurotrophins at appropriate levels and locations developmental important automatisms may have to be exercised in humans. Ongoing pre- and postsynaptic activity in the target area is not sufficient in guiding the axons and dendrites that extend from adult-born neurons. Such automatism are creeping, crawling, up-righting, walking, running and training balance. Autonomic automatisms like swallowing, breathing, continence (urinary bladder and bowel) and blood circulation also have to be trained. Plasticity of both intact and recreated circuits needs to be engaged. A child, which has not crawled sufficiently before walking, will have coordination problems for the rest of the life, unless CDT is administered. Through pattern learning neuronal networks become progressively more organized.

Phase and frequency repair strategy

The human CNS is organizing itself by time and space-coordinated firing of neurons. This coordinated firing is materialized by relative phase and frequency coordination on the single neuron level, on the ensemble level and on the movement level (64). The coordinated firing between up to billions of neurons in the CNS becomes impaired following injury, malformation or degeneration. An improvement of this impaired relative phase and frequency coordination will partially repair CNS functioning. Such improvement of phase and frequency coordination is achieved upon exercising very exact coordinated movements between arms and legs on special CDT devices (Fig. 2A, left). The highly coordinated movement induced afferent input entrains the neuronal networks to improve its self-organization.

Neuronal network learning repair strategy (human)

Since a total regeneration in humans remains far off, if not impossible, a feasible goal, which will improve outcomes regardless of regeneration, is to optimize of what is left. A major focus of human SCI research has therefore been the functional reorganization of the injured CNS. What ever the injury, malfunction or degeneration is, the organization of the integrative functions of the human CNS can be changed in the way that physiologic functions are re-learned (see system theory of pattern formation). The problem to be solved is how to teach the human CNS to use the endogenous repair and reorganization mechanisms. Knowledge of human CNS functioning is needed for the network repair by learning. Significant recovery from incomplete SCI can be achieved through encouraging sprouting and guidance from spared tracts, as well as maximizing plasticity of spared and regenerated neuronal networks by exercising integrative patterns to recruit all spared tract fibres. But a functional reorganization is limited if a SCI is complete (according to function and anatomy, quantified by MRI) and the distal spinal cord is disconnected from supraspinal centres as in the patient of this report. Encouraging sprouting and guidance from spared tracts, as well as maximizing plasticity of spared circuits, is not possible any more. Encouraging growing of somatic nerve fibres through autonomic connections outside the spinal cord for relearning was unsuccessful in this patient within 2.5 years. The administered stem cell therapy was unsuccessful to induce the missing structural repair. A challenge for the researchers using the replacement strategy (see above) would be to demonstrate some structural repair in chronic complete SCI in human.

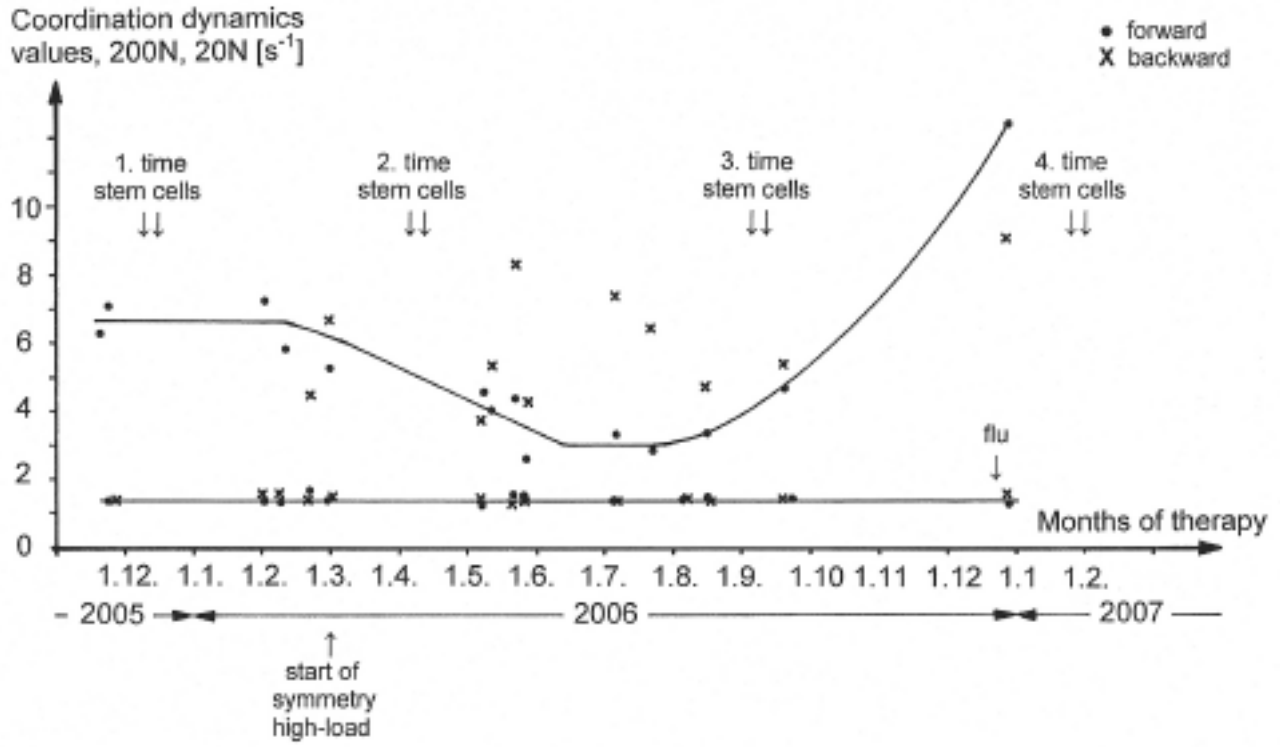

Fig. 1. – Coordination dynamics values for 20 (lower trace) and 200N (upper trace) in dependence on the therapy duration. Notice that the periods of stem cell therapy (injections) did not change the coordination dynamics values significantly.

Neuro-feedback and bio-feedback repair strategies (human)

Neuronal network learning can be enhanced through neuro-feedback and bio-feedback. In addition to the movement induced afferent input from receptors of skin, muscles and tendons, additional movement-coordinated auditory-visual input to the activated neuronal networks can further enhance the entrainment of neuronal networks for repair. The visualisation of the task (seeing the arm and leg movements, bio-feedback) seems to be more efficient for movement performance improvement than abstract feed-back (watching EMG or coordination dynamics traces, neuro-feedback).

Results

It was shown in a previous publication that a patient with a complete spinal cord injury (SCI) at the Th11/12 levels improved strongly within the first 3 months of coordination dynamics therapy (CDT) (37), as quantified by the values of coordination dynamics for 200N (Fig. 1 of Ref. 37). Clinically, movements, including crawling, improved initially as well. Yet thereafter improvement was only minimal. It was concluded that initial improvement was achieved due to functional repair/functional reorganization, and further recovery would have been possible only if there had occurred substantial structural repair. In CDT it is argued that structural repair will occur after more than 6 months of intensive therapy. However, the more severe is the injury, the more difficult it may be to achieve structural repair during CDT, even if the patient is training at capacity limits with more than 30 hours of training per week, which is what this particular patient was doing (see Discussion).

The changes brought about in the functioning of the CNS after 2 years of CDT and stem cell therapy are partly summarized in Fig. 1 via plotting the values of coordination dynamics for 20N (low load, lower trace) and 200N (high load, upper trace). This figure serves as the continuation of Fig. 1 of the previous publication (37) with the subsequent results of ongoing therapy. The values of coordination dynamics values for 20N do not reveal significant changes for the subsequent two years of therapy. For this low

load the patient could easily and smoothly turn by mainly using his healthy arms. But for a load of 200N, even this top-fit patient would need the legs for optimal exercising in the sitting position on this special CDT device. Therefore, the 200N values of coordination dynamics should show changes due to a structural (and functional) repair of the spinal cord. As can be seen in Fig. 1, the 200N values of coordination dynamics did not change markedly with ongoing therapy. If there would have been structural repair, transient significant increase of the values should have occurred because of the enlargement of the neuronal networks proceeding from the brain to the disconnected caudal spinal cord across the injury site via repaired networks.

After the first session of stem cell therapy, the 200N values of coordination dynamics did not change (Fig. 1). When starting symmetry high-load testing and exercising, coordination dynamics for 200N improved (values reduced), which means that the functioning of the CNS improved. The second, third, and fourth stem cell injections had no strong effect on coordination dynamics, i.e. on the functioning of the CNS, either. The slight increase in the values of coordination dynamics at the end of 2006 were due to the exhaustion caused by flu, but not to an enlargement of the neuronal networks in the spinal cord achieved by regeneration (Fig. 1), as the high-load (coordination dynamics) hysteresis curve indicated exhaustion (not shown). After the fourth stem cell injection at the end of February 2007, the values of coordination dynamics (till the end of 2007) remained similar to those at the end of 2006 (not included in Fig. 1).

The patient himself felt no improvement in leg function in response to additional stem cell therapy. The small improvement that occurred during the two years of therapy was achieved with the movement therapy itself.

In conclusion, the 4 sessions of stem cell therapy within the two years had no beneficial effect on the recovery of leg function in this patient with a complete SCI at the Th11/12 levels. It even seems that, instead, pure, but optimal CDT could have achieved more improvement.

Discussion

It will be discussed why pure coordination dynamics therapy (CDT) was not very successful so far in this patient and why the combined stem cell therapy and CDT were also not very successfully. The argument that the regenerative capacity may differ from patient to patient is right, but cannot explain this only little progress.

Structural spinal cord repair by CDT (naturally induced stem cell therapy)

The injured or malfunctioning human CNS can partly be repaired functionally and structurally by CDT (25-34, 36). The functional repair has been proven to be successful in more than 100 patients, but the induction of substantial structural repair still remains a problem. It was reasoned so far, that when pushing the patient to or over his/her limits (previous publication, 37), all 'intrinsic' self-repair mechanisms are activated (the known and the unknown ones) including neurogenesis. When trying to induce neurogenesis at the injury site naturally, the following strategies are used.

For the discussion a patient with a SCI at C5/6 levels is used. It is assumed that according to the MRI, approximately 5% of the spinal cord has survived during injury. At least after improving the trunk stability, the patient is able to exercise very exact coordinated movements across the SCI site and to activate (and over-activate) the spared tract fibres at the injury site and also the functionally connected neuronal network parts. When training on the special CDT device for arm and leg turning movements (Fig. 2A, left), most coordinated afferent and efferent inputs are crossing the injury site at spinal cord segments C5/6 between the cervical intumescence (where the stereotyped arm movements are mainly located) and the lumbosacral intumescence (where the stereotyped leg movements are mainly located) in the rostral-caudal and caudal-rostral direction, because arms and legs are both partly activated volitionally. Because of the exactness of the coordination between arm and leg movements (and the movement induced afferent inputs) and that neurons work as coincidence (and coordination) detectors (30), the threshold of action potential generation at the axon hillock is reached with much less afferent input to the neurons (30) than in the case of less exact coordination between arm and leg movements. The communica-

Fig. 2. – Coordinated arm and leg movements (imposed by devices) performed during coordination dynamics therapy for activating the injured spinal cord in the rostral and the caudal direction substantially. A. Special coordination dynamics therapy device (left side) for turning movements in the lying position; the patient of this report with complete spinal cord injury (SCI) at Th11/12 levels is exercising on it. On the right side an airwalker (trot gait in poor coordination; a patient with incomplete SCI). B. Neurowalker for exercising in trot gait coordination in the upright position; a patient with severe cervical SCI C5/6; self-made device to achieve exact coordination. C. Free treadmill walking in the backward direction; a patient with severe C5/6 SCI. D. Free treadmill walking in the forward direction of a patient with a severe C5/6 SCI. The legs are supported by the therapist (the author on the left side). The patient is partly activating the leg movements via the arm movements. The coordination between arm and leg movements is extremely exact, especially for running. E. Device for exercising exact pace gait coordination; partly self-made.

tion between the neurons involved in the generation of the coordinated arm and leg movements over the injury site is strongly enhanced. More spared tract fibres and synapses are activated in the case of most coordinated movements than in those movements which are less coordinated.

Since probably different kinds of afferent and efferent tract fibres survived the injury, different kinds of highly coordinated arm and leg movements have to be trained (and over-trained) to activate the different kinds of spared tract fibres (in the white matter) and neurons (in the grey matter) to fully recruit the residual connectivity across the injury site. Therefore a functional reorganization of the neuronal networks should probably precede the structural repair, because only those fibres can be activated at the limits which the patient is able to recruit for activation. Also, this actually means that both functional and structural repair have to work together (as a team).

Highly coordinated arm and leg movements, imposed by devices, are administered when the patient is exercising on the special CDT therapy devices for turning (Fig. 2A, left), exercising alternating arm and leg movements in the trot gait coordination on a self-made neurowalker (Fig. 2B), and exercising coordinated arm and leg movements in the pace gait coordination on a partly self-made instrument (Fig. 2E). Highly coordinated arm and leg movements, which are not directly imposed by a device, are the free walking and running on a treadmill in the forward (Fig. 2D) and the backward direction (Fig. 2C). The legs of the patient are supported by therapists and the patient moves arms dynamically coordinated with legs (52). The patient is inducing walking and running with the arms naturally. The therapists have to feel the movements in the legs of the patient, adapt to, support and enhance them. The exact timing of arm and leg movement is given by the patient. The interpersonal coordination between the patient and the therapists has to be extremely good to achieve such upright natural highly coordinated movements. The training of assisted crawling in the pace and trot gait coordination also activates coordinated arm and leg movements with their driving neuronal networks across the injury site, but the coordination is mostly not as exact as for walking and running on the treadmill.

Successful activation as a stimulus for repair

The successful coordinated communication across the injury site (that means in the rostral and the caudal direction) is probably important for inducing structural repair. The feedback of the functional success across the injury site and the overloading of the spared neuronal networks at the injury site may be the stimulus for neurogenesis. In Hebbs learning (10) only those synapses which are successfully activated are increasing in strength. This means that the feedback of successful action is of importance. In general, the feedback of successful self-organization of physiologic network patterns may also be of importance for structural repair.

Factors influencing the building of new nerve cells and connections

The successful activation (at the patient's limits) of the spared neuronal networks at the injury site may induce the building of new neuronal networks working functionally in coordination with the spared ones. But what factors (or combination of factors) are activating the building of new networks for repair? What concentrations of growth factors are needed? What are the distances of action of growth or proliferation factors to induce cell communication?

Even though there is little known on the regenerative capacity of the human CNS and the substances and factors influencing it successfully, still some reasoning's may be useful to understand and enhance structural repair.

Stimulus strength for structural repair (spared tract fibres and cells at the injury site)

In coordination dynamics therapy, the primary stimulus for structural repair will come from the working at the limits of the neurons (somas, dendrites, axons, synapses) involved in bridging functionally and structurally the injured site caudally and rostrally. If more neurons and tract fibres are spared at the injury site and can be activated during therapy, then the summed stimulus for repair will be bigger. In consequence, to induce substantial structural repair, it is of importance to recruit as many tract fibres and neurons at the injury site as possible (through activation of different movement patterns)

and the activation is probably more efficient if performed simultaneously (coordinated integrative activation over the injury site).

This also means that a structural and functional repair is more difficult to achieve in more severe injuries. Therefore studies are needed to compare the outcome to the extent of the injury (quantified by MRI (anatomy)) upon an up-to-date therapy.

Communication distance for the proliferation of neurons

If the distance between the spared host cells and the precise area vacated by lost neurons at the injury site and the administered neural stem/precursor cells were of importance for the proliferation of neurons and their integration with the spared networks, then one would have to add neural stem/precursor cells as close as possible to the injury site or one would have to inject the cells into the injured site with the risk of damage of spared networks.

If a close contact of cell membranes were even necessary to stimulate the proliferation of cells, then the very close environment of spared host neurons would be of importance also to allow a touch communication between cells.

How close the communication between neural stem/precursor cells and host cells may have to be and how complex the communication, for changing cell functions, between cells can be, it will be discussed with respect to an animal model. Such comparable deep knowledge may be necessary to understand the neurogenesis in human with respect to repair because the human spinal cord does not regenerate spontaneously. If we want to establish a repair mechanism in human, which is not innate (the regeneration of the spinal cord), extreme deep knowledge is needed.

Frog model for studying neural control and communication between two different kinds of motoneurons and two different kinds of muscle cells – Demonstration of the complexity of cell communication during development and repair

Cell communication distances

In frog (rana temporaria) there are two types of motoneurons and two types of muscle fibres. Large motoneurons with thick fast conducting axons (comparable to α_1-motoneurons in man (24)) innervate the twitch muscle fibres (comparable to fast fatigue muscle fibres in human) and small motoneurons with thin slowly conducting axons (comparable to α_3-motoneurons in human (24)) innervate slow muscle fibres (comparable to slow (red) muscle fibres in human) selectively (13, 14, 17-19). In this frog model, which many researchers have used, I will concentrate on the innervation of the slow muscle fibres during the metamorphosis from the tadpole to the small frog (19) and during repair (14) to draw some conclusions about distances and complexity of cell function changes during cell communication. The cell communication mechanisms during metamophpsis from the tadpole to the frog (19) and during re-innervation following denervation in the adult frog (14) are very similar. When in frog tadpoles the legs start to move (forelimbs cannot be seen at that stage), the thick fast conducting and fast growing axons of the large motoneurons innervate their own twitch muscle fibres, but also the slow muscle fibres. When later the thin slowly conducting and slowly growing axons of the small motoneurons reach the (pyriformis) muscle, they identify their slow muscle fibres by a close contact, make functional contact with them and push the motor endplates of the large motoneurons away (Fig. 3a-f). The competition of the two different kinds of α-motoneurons for forming a stable functioning motor endplate on the slow muscle fibre membrane takes place by a close contact (Fig. 3a-f). The contact distance between the large motoneuron endplate with the slow muscle fibre is 0.1μm (Fig. 3b) and the contact distance between the 'en grappe' endplate of the small motoneuron with the slow muscle fibre is also 0.1μm (Fig. 3a, c). The motoneurons are separated from the slow muscle fibre membrane by a basal membrane (Fig. 3b, c). The contact between the two motoneurons during the competitive communication is closer. The distance between the two cell membranes is less than 0.05μm (Fig. 3a, b, c).

In conclusion, in this frog model the distance of the cooperative and competitive interplay between the two nerve cells and the muscle cell is in the range of 0.1μm or less.

If in man the communication distance between cells for proliferation or withdrawal were also less than 0.1μm, then it would be difficult to induce neurogenesis at the injury site. The MRI of our patient

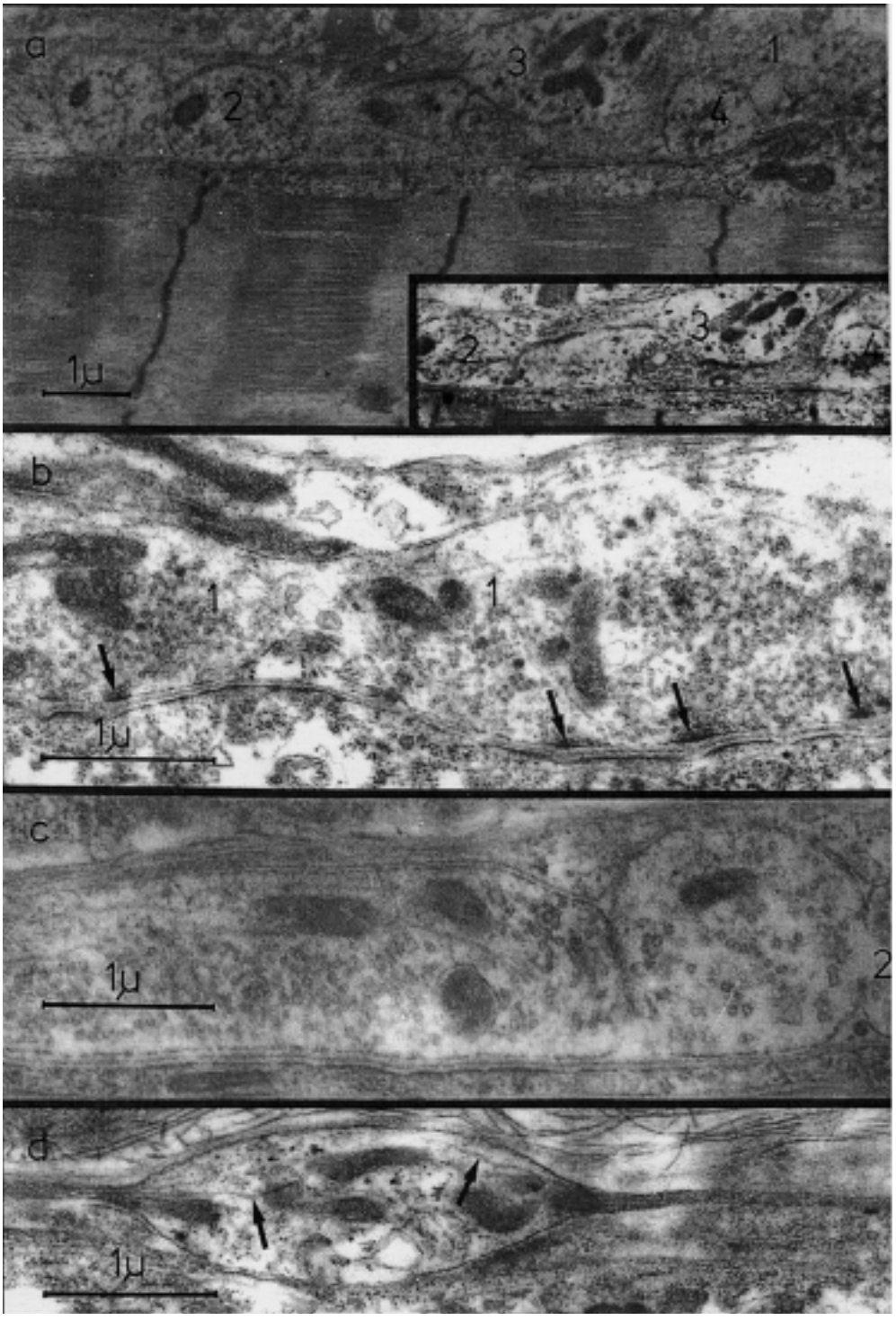

Fig. 3a-d. – Electron microscopy (EM) of a slow muscle fibre with synapses of a fast (marked with '1') and a slowly conducting axon (marked with '2-4'); pyriformis muscle of a frog at the end of the metamorphosis from the tadpole to the small frog (tail length 0.5 mm). By serial sections, including the sections 'a' through 'd', a picture of a part of the slow muscle fibre with the two synapses could be drawn ('e', 'f'). a. Filaments of the slow muscle fibre show no M-line. Endplates of the fast and slow axons are strongly intermingled; both have contacts with the slow muscle fibre membrane. b. Nerve ending only from the fast axon; active zones are marked with arrows; no synaptic folding opposite to the active zones. c. Synapse profile of the slow axon; no active zones and no synaptic folding. d. Axon enlargement with marked microtubules.

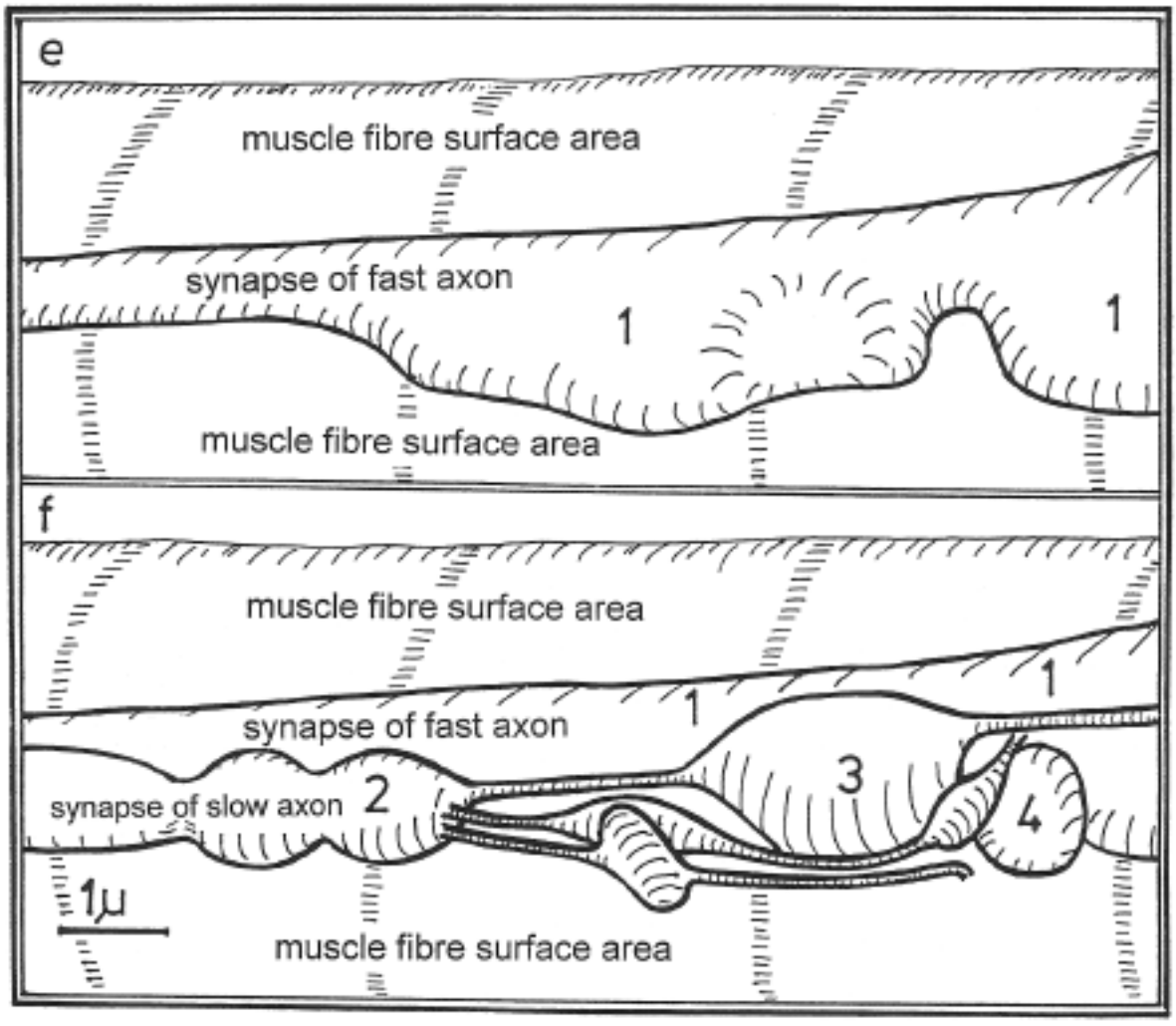

Fig. 3e-f – Three-dimensional reconstruction of the synapses of the fast and slowly conducting axons attached to the slow muscle fibre; obtained from EM serial sections, including the ones from Fig. 3a-d; in 'e' only the synapse of the fast axon is shown; in 'f' the synapses of both the slow and the fast axon are shown.

looked very unhealthy at the injury site (Fig. 4 of Ref. 37). Probably the honeycomb structure represents the scar tissue which may have contained a few healthy spared tract fibres. It seems difficult to see how the transplanted neural stem/precursor cells could get sufficiently close contact with the spared host neurons at the injury site to proliferate to certain neurons or glial cells.

The rate of neurogenesis may depend on the number of spared (activated) tract fibres, host neurons and synapses at the injury site

If the stimulus for the proliferation of new nerve cells were substances, released from the spared tract neurons at the injury site, working over longer distances to attract neural stem/precursor cells for proliferation, then the concentrations and gradients of attractor substances would be of importance. The concentration of naturally released stimulation factors may depend on the number of spared tract neurons which can be activated during therapy (see above). In such a situation incomplete spinal cord injuries would have a much better chance for regeneration with the help of neurogenesis, because many more host neurons are spared at the injury site to secrete target-derived growth or proliferation factors or stimulate the secretion from other cells.

Addition of growth factors (assumed to be known and working in man) may induce uncontrolled growth of cells, axons, and dendrites, because

there is no specific information in the CNS neuronal networks, 'telling' the nerve cells where to proliferate for achieving specific physiologic functions. How can specific chemoattractive gradients build up to lead to selective and specific homing of transplanted cells? If the movement therapy (accompanying the stem cell therapy) is supposed to supply that specific information, then the movement therapy must be thoughtful. A simple muscle power training or positioning of the limbs would not activate spared networks at the injury site substantially and specifically. The mechanisms of specific information transfer from certain movements to induce homing of transplanted cells at the injury site (the movement-repair interface) should be studied functionally and structurally.

Cell identification complexity during functional and structural changes

In stem cell therapy it is assumed that multipotent neural stem cells divide to become neural precursors, which migrate to the injured CNS site and proliferate there to become different kinds of neurons and glial cells. To follow up the processes of neurogenesis and cell changes, different markers are needed for the identification. The complexity and difficulty of such cell identification, communication, functional changes and neural control (functionally and structurally) will be demonstrated again in this frog model.

The slow muscle fibres can be distinguished with the electron microscope from the twitch muscle fibre by the missing M-line (Fig. 3a). This identification criterion is independent whether the slow muscle fibre is innervated by the slow thin (small motoneuron) or the fast conducting thick (large motoneuron) axon. The thin axons form the motor endplates of 'en grappe' shape type (19) on the slow muscle fibre with no dense zones (Fig. 3a, c). The thick fast conducting axons (normally innervating twitch muscle fibres) form extended motor endplates on the slow muscle fibre with dense zones but with no synaptic folding opposite to the dense zones (Fig. 3b) as on twitch muscle fibres (not shown). Even though the contractile filaments look the same whether the slow muscle fibre is innervated by slow or fast axons in the electron microscope, the contractile properties are different. If the slow muscle fibre is innervated by its own slowly conducting thin axon, the slow muscle fibre can hold the contraction for a long time (14, 19) and the calcium, released from the sarcoplasmatic reticulum, is enhanced for a long time (14). If the slow muscle fibre is innervated by the 'wrong' fast conducting axon (normally innervating twitch muscle fibres), the slow muscle fibre contracts only shortly (14, 19) and the increased calcium concentration is also only transiently (14). The two kinds of α-motoneurons control the contractile properties and the excitation-contraction coupling of the slow muscle fibre differently, even though the structure of the slow muscle fibre, judged by the electron microscope, is the same. The slowly conducting motoneurons make the slow muscle fibre 'slow' and the fast conducting motoneurons partly 'fast'. If the slow muscle fibre is innervated by its own slowly conducting thin axon, the muscle fibre membrane does not generate an action potential (lack or block of Na^+-channels) following depolarization. Naturally the slow muscle fibre is depolarized electrotonically (slowly) by the endplate potentials of 'en grappe' type motor endplates of one or two small motoneurons. The depolarization spreads passively (electrotonically) along the muscle fibre. The membrane properties for hyperpolarization (for identification) and depolarization have both a slow time course. If, however, the slow muscle fibre is innervated by a thick fast conducting axon, then the slow muscle fibre membrane can generate action potentials (sodium channels are present and active), which are conducted actively along the slow muscle fibre membrane. The membrane properties of the slow muscle fibre are partly fast for depolarization and slow for hyperpolarization. Functionally the slow muscle fibre can be identified safely by the slow time course during hyperpolarizing current pulses, when impaling micro-electrodes, independent whether the slow muscle fibre is innervated by fast or slow axons. Therefore, the two different neurons are controlling the membrane properties of the slow muscle fibre only partly. If the slow muscle fibre is partly only denervated, then the neurons control even only parts of the membrane (18). For the neural control of the slow muscle fibre by the two different neurons, a functioning synapse is not necessary. Before a functioning synapse can be identified electrically, membrane and contractile properties have changed already (14, 19).

What are the consequences for the research in animals and humans? First, it is quite difficult to identify a slow muscle fibre reliably during the changes of its properties. Putting, for example, marker molecules into active sodium channels would not be sufficient for the identification of the muscle fibre type, because slow muscle fibres can also have active sodium channels. Second, the neural control of cells can be quite complex, since only certain properties are under neural control. Third, highly qualified research is necessary to understand safely what processes are really taking place in and at the cells. Morphology and electrophysiology (for measuring function) are needed.

Similarity between development and repair

This model of the interaction of 3 cells (4 cells, when including twitch muscle fibres) is of special interest for the repair, because the neural control of the slow muscle fibre is very similar during development (19) and during repair (re-innervation) (14) following denervation. Therefore for repair we may learn from the development (32, 33).

Trophic substance for cell communication

It is important to note that without a fully functioning synapse, the motoneuron can change membrane and contractile properties of muscle fibres in the frog model. This is achieved presumably through a trophic substance (38), which may be stored in dense core vesicles and is released from the vesicles, when they come in contact with the membrane of the nerve ending. The released substance diffuses from the nerve ending to the muscle cell. It is not known how the neuron is getting the feedback from the muscle fibre. The transport to the soma would be no problem because of the retrograde axonal transport in microtubules (Fig. 3d) in addition to the anterograde transport. It is important that we may have specific trophic substances for communication in addition to the communication via chemical synapses.

Specificity in communication

Normally the thin axons of small α-motoneurons innervate the slow muscle fibres and the thick axons of large α-motoneurons the twitch muscle fibres specifically. Therefore there exists specificity between the 4 cells, but when one motoneuron type is missing (small α-motoneuron), the other one is taking over (large ··-motoneuron). Of primary importance for the muscle cell seems to be the innervation by a motoneuron. Of secondary importance is the innervation by the right motoneuron. This means that the specificity is only relative. It is unclear what the specificity of cell communication in the CNS of humans is like.

Difference between frog and human

For sure, there will be big differences between the nervous system of frog and human and we can expect a higher complexity in the CNS than in the PNS. If we have already such a complexity between motoneurons and muscle fibres in the frog, then we can expect a tremendous complexity during repair in the human CNS.

Also, the motor learning for the repair of the injured CNS during CDT is a very complex process and is not deeply understood. But this mechanism is, for sure, working in human and the capacity for learning is largest in human.

Differences in the capacity for reparative regeneration between animal and human in the PNS and CNS

To improve techniques in the repair of the human CNS, reliable human data are needed. Exploration from one species to another one is hazardous, and more so, as the phylogenetic separation increases (2, 12, 42). The assumptions made from commonly used laboratory animals have general applicability, but ultimately must be tested in man, if possible (12).

The capacity for reparative regeneration is smaller, the higher an animal is on the phylogenetic scale (2, 15). Rats and dogs can have their nerves severed and not reanastomosed, yet their regenerative efforts are so strong that nerve continuity and motor and sensory return will occur (2, 3). For a gap of 8 mm after nerve transaction more than 50% of the nerve fibres could be counted in the distal stump of the rat sciatic nerve (11). In the dog, nerve fibres can cross a gap of 4 to 5 cm (9). In human, a cut peripheral nerve has to be adapted (best motor and sensory fascicles adapted separately to avoid mismatch), because nerve fibres cannot cross a gap (for references see Ref. 23). If

such differences in the capacity for reparative regeneration (PNS) between animal and human were similar in the CNS, then regeneration experiments in rat spinal cords would be only of limited consequence for human.

In the rat, the number of regenerating axons following spinal cord injury is always small and the distance of regrowing is modest (60). Regenerating axons are most numerous in the few millimetres caudal to the lesion, and longer regenerating axons become progressively rare. The longest single regrown axons were 10 to 15 mm long (60). Therefore it seems that the degree of regeneration in the rat spinal cord is same or smaller than in the PNS.

It has been reported that the transplantation of both embryonic stem cells and embryonic stem cell-derived neural (neuronal or glial) progenitors (54) is able to efficiently promote CNS regeneration in preclinical models (rat) of acute spinal cord injury (53, 55). The failure of the stem cell therapy in the patient of this article with a complete spinal cord injury (according to MRI and function), in comparison to the success in rat, may indicate that there is also a large difference in the capacity for reparative regeneration between rat and human in the CNS. But in the patient of this report, adult stem cells (low capacity for reparative regeneration) were used and in the rat embryonic stem cells (high capacity for regeneration, but risk of teratocarcinoma formation) were transplanted. Even if the formation of teratocarcinoma could be avoided, when using embryonic stem cells, probably still a functional reorganization of the human CNS by an efficient movement therapy is needed, since during development the healthy human CNS also needs the training of movements (running, jumping, training balance) to improve its functioning (32, 33). CDT is a specific movement therapy which can improve the development of the pathologically functioning CNS (cerebral palsy (31)) and can improve the functioning of the injured human CNS in traumatic brain injury (26, 34), hypoxic brain injury (33), stroke (25) and spinal cord injury (27, 28).

To sum up, to draw conclusions from the regeneration capacity of the rat brain or tissue culture to the human PNS or CNS is only justified, if the differences in the capacity of regeneration are taken into consideration.

Difference in the increase of motor units

There are also differences in the increase of motor units after partial denervation of muscles between rat and man. The rat can increase the motor unit by collateral spouting of nerve fibres in the muscle by, perhaps, about 400%, but the monkey only by about 50 to 100% (5). This means that the monkey (and most likely also the human) needs nearly the whole number of motor fibres to keep useful functions (6). Rats, on the other hand, may need only a small number of motoneurons for running apart from coordination (and balance).

Difference in the relearning of function between animals and man

Sperry transposed the nerve supply of flexor and extensor muscles in the rat (40) and in the monkey (41): the monkey relearned the task, the rat did not. Monkeys also differ from dogs (43) and rats (41) in the physiotherapy they need. In Sperry's experiment on monkeys, their learning to flex or extend the elbow in one situation did not necessarily become generalized to other performances. This indicates that the neuronal readjustment was not localized solely in the spinal centres, but involved reorganization at the supraspinal level (44). Surprisingly few trials were required for poliomyelitis patients to use transposed tendons successfully (48). The visualisation of the task seemed to be the prime aid to the patients.

Differences in the complexity of regeneration

Lesion-induced reorganization of the CNS following dorsal rhizotomies has been demonstrated in animals (4, for further literature see Refs. 20-23). There is regeneration of the spinal cord in goldfish following a lesion (1), but the regeneration is only a partial one (1) and the swimming function is comparably simple in comparison with the functions of arms, hands and legs in human. Upon CDT, the trunk stability and trunk movements recover first in the patients with severe cervical SCI.

Speed versus degree of regeneration

It was shown that the regeneration in rat peripheral nerves can be improved by 20% by the application of nucleotides (46, 47). The increase of regeneration was quantified by morphometry (nerve fibre diameter distributions) and electrophysiology methods (single nerve fibre conduction velocity distributions), but after much longer periods of time (not published), the control rats became as good as the experimental rats. This means that long-term (long-time) studies are needed to differentiate between the capacity of regeneration and the speed of regeneration.

Spontaneous recovery may mimic repair

It has been reported that autologous bone marrow transplantation in patients with subacute spinal cord injury improved motor and/or sensory function 3-4 weeks (therapeutic time window) following injury (56), but the spinal shock phase, following SCI, lasts in human already 3 to 4 weeks following the injury. Therefore the success of the stem cell transplants may be more caused by the recovery from the spinal shock in incomplete SCI (most injuries are incomplete) than by the transplantation. Further, in incomplete SCI there is spontaneous recovery from the injury up to one year after the accident. In the first 6 months following the injury may be 80% of the spontaneous recovery is done and after 12 months may be 95% of the spontaneous recovery is completed. Actually the conventional SCI centres with their inefficient repair treatments benefit from this spontaneous recovery and often make believe that the improvement of the patient is due to their treatment. The success of the autologous bone marrow transplantation may further be explained by the spontaneous recovery of the SCI and not by the transplantation. To show that a treatment method can improve SCI, the treatment should be administered to patients with chronic injuries (27, 28). After a few years, for sure, the spinal shock and spontaneous recovery phases are over.

Importance to repair vegetative functions

The repair of the continence is most important for SCI patients. Before World War II most patients died of urinary bladder infections. Guttmann (57) succeeded in keeping paraplegics alive by introducing intermittent catheterization. The continence problem has not been touched in the so-called preclinical and clinical studies. Human functions, as for example, the continence, cannot be mimicked easily by an animal model. The rat is moving on 4 limbs. The weight of the intestine is therefore not so much on the pelvic floor as in human (no stress-continence). If half of the spinal cord is only injured in rat experiments, the rat can probably keep the continence without or little repair.

The patient of this report learned to use the bladder reflex for emptying the urinary bladder. Urinary bladder infections do not occur any more. The patient did not feel any improvement of urinary bladder functions due to the 4 sessions of stem cell therapy. He can manage the continence and he is independent in every day life due to the movement therapy.

System theory of pattern formation can explain learning transfer, stability, and symmetry; central pattern generators cannot.

Central pattern generators (CPGs) (63) are commonly used to understand the coordinated locomotor function in connection with SCI (60, 62). To understand the integrative functions of the human CNS, the system theory of pattern formation (35) or CPGs can be used; now it will be discussed that CPGs cannot explain learning transfer, stability, and symmetry but the system theory of pattern formation can.

For explanation, the neuronal network patterns walking, jumping on springboard, urinary bladder continence and extensor spasticity (pathologic pattern derived from the antigravity system) are taken. If a patient with an incomplete SCI trains assisted walking (Fig. 2) then the CPG (circuitry or network) for walking improves. If he trains jumping on springboard, the CPG for jumping improves. To improve urinary bladder continence is already difficult to understand in the framework of CPGs. The reduction of extensor spasticity in relation to movements cannot be understood in the framework of CPGs.

With the system theory of pattern formation (see Method) the improvement of the neuronal network

patterns walking, jumping, and continence and the reduction of extensor spasticity can be understood. By training the movement patterns walking and jumping on the springboard, not only the trained movements improve themselves, but also other untrained patterns improve like the urinary bladder function and the extensor spasticity. In an attractor layout of the collective states the attractors for walking and jumping get deeper (higher stability), the pathologic attractor extensor spasticity gets more shallow (lower stability) and the complex attractor continence changes to become more physiologic. There is learning transfer from the walking and jumping patterns to the continence and spasticity patterns. Also, the learning transfer from a movement to its symmetry counterpart is of importance (35). Backward walking (Fig. 2C) improves forward walking. If, for example, a stroke patient has not been walking physiologically for 5 years, his pathologic forward walking became an old-learned movement and is difficult to change by learning. However, through exercising backward walking (which is new to the patient) he can improve his forward walking by learning transfer.

Olfactory bulb transplant

In complete SCI olfactory bulb transplant operations are also offered. A piece of the olfactory bulb is taken from the nose and used as an interponate between the rostral and caudal spinal cord. Assuming that the olfactory bulb has a high regenerative capacity in human and can induce growing connectivity over the injury site, several objections still remain against such transplantation. First, only very few SCIs are really complete. In the patient of this report probably still a few tract fibres were spared, even though the MRI showed a complete SCI. During transplantation the patient would loose some spared tract fibres and may be some regenerated fibres. An operation would only be justified if the patient is surely getting more new tract fibres than he is loosing in the operation procedure.

In microsurgery of peripheral nerve injury there are standards to achieve optimal results as to reduce mismatch (sensory and motor nerve fascicles have to be adapted) and to gain sufficient fibre numbers (20-23). During an operation of the spinal cord one cannot see the ascending and descending tracts in the white matter for a separate reconstruction. Also, one cannot cut very much out of the spinal cord to see the healthy tissue very well because then further functions would be lost. Such an operation seems to be very risky already from the point of view of the technical details of the operation.

The patient of this report will probably not try out the olfactory bulb transplantation, because his friend with a SCI did not get any functions back within 2.5 years after the operation.

Building of new nervous tissue and new motoneurons in the spinal cord

For the repair of a cervical spinal cord injury not only the rostral and the caudal white matter spinal cord parts have to be reconnected for information transmission but also the grey matter at the injured site has to be repaired by building of new cells for getting new motoneurons for muscle function from the damaged or destroyed spinal cord segments. Especially in cervical spinal cord injury C5/6, functional improvements of patients depend also strongly on the building of new motoneurons at the damaged site and the regeneration of their axons along Bügners bands in nerve roots and peripheral nerves to the muscles. The process of re-innervation may need in human more than two years (growing speed of axons ≈ 1-2 mm/day plus time for the building of synapses) following the building of new motoneurons. Knowledge of the regeneration in the CNS and PNS is needed to understand the time scales for repair.

Movements to direct the neurogenesis during stem cell therapy

Especially in the repair of cervical SCI, sufficient arm and leg power is a big problem. Simple muscle power training methods for directing the neurogenesis during stem cell therapy will bring only little improvement in ambulation. First, a motor unit (the number of the muscle fibre which a motoneuron can innervate (supply)) can only be increased by 50% (see above). Second, the hypertrophy of muscle fibres is limited. Third, during the building of new nerve

cells in the spinal cord grey matter, the building of the different kinds of motoneurons has to be induced. For the proliferation of precursors to α_1-motoneurons (innervating fast fatigue muscle fibres) to generate power, for example, dynamic movements like jumping on the springboard would be needed. During the movement therapy to direct the neurogenesis to the injured networks, one also has to think what kinds of neurons or neuronal networks (fast, slow,...) one wants to stimulate for building. Since this will be a very difficult task, different kinds of integrative arm and leg movements (Fig. 2) have to be trained with low speed, high speed, little and high power. The integrative automatisms are especially helpful, because one can reach muscle groups (and their innervating neuronal networks), which one cannot reach with simple volitional muscle training movements.

In rat, for example, the repair conditions are quite different. Nowadays only half of the spinal cord is cut. Otherwise one would have to empty the urinary bladder manually. First, a patient with 50% of the spinal cord spared can probably relearn walking and running and can probably become continent (which has to be proven). Therefore, this animal model for repair is far away from human reality with respect to a severe spinal cord injury with may be 5% of the spinal cord only spared. Second, in rat a motor unit can be increased by up to 400% (see above). Frankly speaking, the rat can walk and run with a few motoneurons (without body-weight support) apart from the balance and the limb coordination; but a human cannot. It has been reported that as little as 10% of spinal white matter tracts were sufficient after subtotal spinal cord transection to permit spontaneous walking without external support in adult cats (61). The running of the rat in a running wheel is actually a good movement, because this running is an integrative automatism. Therefore, it could well be that the improvement of movements in rat following the cutting of 50% of the spinal cord and application of nerve grows factors and/or stem cells is mainly caused by a functional reorganization with only little structural repair. The staining or marking of neurons or regenerating fibres may not be sufficient to demonstrate successful regeneration (see above). In addition to morphology, the functioning of single neurons has to be measured. Even the comparison between experimental and control rats is not sufficient, because it could well be that in the experimental rats just the speed of regeneration is enhanced and not the capacity of regeneration (see above).

In-vitro experiments

Patients are made to believe that stem cell therapy is working by showing them growing nerve fibres in in-vitro experiments. In culture experiments one can perform experiments on the living tissue under certain controlled conditions which are in-vivo not possible. But the culture conditions are far from human reality.

In the frog model of above (Fig. 3) one can partly substitute for the neurotrophic control what thin axons are exerting onto slow muscle fibres (control on the incorporation of Na-channels and generation of action potentials) by performing potassium contractures with the slow muscle fibre for several days in culture medium (39). Depolarization of the slow muscle fibre membrane is achieved by increasing the potassium concentration outside the muscle fibres. Such neural control is, for example, not possible to perform in human (in-vivo), because at least the heart would stop working.

Conclusion

On the basis of human data and a frog model for regeneration, cell communication and neural control, it seems that the repair of the human nervous system, including the building of new nerve cells (self-renewal, proliferation, differentiation, migration), dendrites and axons and their functional integration in the existing networks, is an extremely complex and specific process of functional and structural repair in the human nervous system. The substitution of cells close to the injured spinal cord during an operation or the injection of cells may be a too simple strategy for the repair of the human CNS. Knowledge of the different repair mechanisms and how they are induced in human and their time scales is necessary for proper therapy. Recently stem cell-mediated repair after SCI has been nicely and extensively reviewed (54, 58, 60, 62). The emphasis was on animal research apart from conventional human

anatomy. Differences in the capacity of regeneration between animal and human are missing. The necessity to measure also the functioning of new nerve cells was not emphasized. For the integration of new nerve cells and for functional reorganization the theory of central pattern generators was used, even though the plasticity of central pattern generators cannot explain learning transfer, stability of movement patterns and spasticity reduction. The System Theory of Pattern Formation, applied to the injured or malfunctioning human CNS, and the self-organization of neuronal networks (35) by phase and frequency coordination (64), which can explain human CNS self-organization, reorganization and the integration of new nerve cells, was not reviewed. Stem cell therapies seem to be still in the animal experimental stage and have not reached the stages of human research (this article) and human applicability (54). It is actually a pity that a potentially qualified method (stem cell therapy), which could contribute substantially to the structural repair of the injured human CNS, may be wasted just because the necessary proper human research is not supported and performed.

Ethical issues

Many patients with a severe C5/6 SCI do not try to improve their functional state following the injury because they believe in the argument of conventional rehabilitation centres that nothing can be done (brain-wash number one = 'choose your wheelchair for the rest of your life'). Some of the patients, who escaped this first brain-wash, fall prey to the second brain wash, namely the existence of miracle pills or cells. Patients emphasize that they do not want to be misled with hope to success.

As long as universities are not organizing the necessary human neurophysiology and clinical research properly, the situation may not change.

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