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Towards a Definition of Recovery of Function

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ABSTRACT

In this review we consider recovery of function after spinal cord injury, and, in particular, recovery improved following intraspinal cellular transplants. Some recovery occurs spontaneously and this can be especially dramatic in neonates, supporting the notion that developing and adult spinal cord respond differently to injury. Recovery can be improved in both neonates and adults by appropriate cellular transplants into the injury site. We describe several functional tests used in animals with spinal lesions and transplants. We compare the effects of transplants of fetal tissue and genetically modified fibroblasts into neonatal and adult injury sites on recovery of motor and sensorimotor function. Fetal tissue transplants support greater recovery and elicit more regeneration in neonates than in adults. Transplants of fibroblasts modified to produce neurotrophic factors however support both recovery and axonal growth even in adults. The contribution of the transplant to recovery is shown by the loss of function that follows a second lesion just rostral to the original lesion/transplant site. The effect of the re-lesion indicates that the recovery is mediated by the presence of the transplant but the way in which transplants act to promote recovery may include a number of mechanisms, including regeneration and sprouting, neuroprotection, and modifications of organization of spared CNS structures.

Key words: locomotion; neonatal vs. adult; neurotrophic factors kinematics; spinal cord; transplants

INTRODUCTION

SPINAL INJURY destroys some cells and axotomizes others. The cells are not replaced, the axons don't regenerate, and the functions dependent on these pathways are lost. If we can rescue injured neurons or replace them, and induce damaged neurons to regenerate their axons, the growing axons will encounter a hostile environment, with cysts and scars, inhibitory proteins and an active immune response. If they were to regenerate despite these barriers, the absence of appropriate guidance molecules might make it difficult for the growing axons to find appropriate targets. We now know, however, that there are ways to deliver trophic and anti-apoptotic factors that can protect neurons from cell death and atrophy and that can stimulate the expression of the genes required for an injured neuron to regenerate its axon. We can use transplants or other implanted channels to provide bridges through which the axons can regenerate. We can use enzymes or vaccines or antibodies to modify the environment in ways that will counter the inhibitory effects. We still don't know whether guidance molecules will be expressed but there are suggestions that patterned activity that occurs during physical training can modify central

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circuits. Thus, the seemingly insurmountable problems encountered in trying to repair the injured spinal cord are being reduced to practical issues.

THE RELATION BETWEEN REPAIR AND RECOVERY

The essential issue is to identify the extent to which repair, assessed morphologically, is associated with recovery of useful function. We know empirically that certain interventions can improve recovery but we do not know the mechanisms underlying the improvement and therefore how further improvement might be achieved. Three approaches that have been studied include the transplantation of cells or tissues or bioactive molecules into the injured spinal cord, pharmacotherapy to replace missing transmitters, and rehabilitative regimes that train the spinal cord. Each of these interventions is likely to be mechanistically independent and thus combinations could act synergistically to promote repair and recovery (Murray, 2001).

DO TRANSPLANTS IMPROVE RECOVERY?

We will review here our experiments using transplants to repair injuries and restore function after spinal injury in neonatal and adult rats. Recovery, with or without transplants, is greater when the injury is made to neonates than when the same injury is made to an adult (Bregman et al., 1993; Diener and Bregman, 1998). The mechanisms of recovery after spinal injury may be quite different in animals injured as neonates and as adults and it is important to identify the features of recovery that occur after injury and after transplantation in both neonates and adults. Understanding the basis of the better recovery in neonates may suggest strategies for intervention that will improve function in the adult.

Developing and Adult Spinal Cord Respond Differently to Injury

Neonatal neurons are more likely to die as a result of axotomy but there is more recovery of function than one would see after a similar lesion in an adult, a phenomenon known as "the infant lesion effect." The infant lesion effect may reflect the contribution of late developing pathways that continue to grow into the spinal cord postnatally in the absence of myelin-based inhibitors and in the presence of abundant growth promoting molecules. Regeneration of cut axons or sprouting by intact axons is also more likely to occur in the more permissive environment of the developing CNS. The surviving supraspinal systems may be considerably reorganized as a result of a neonatal injury. Novel pathways can therefore develop after a neonatal lesion, permitting novel strategies that will enable the animals to regain a better functional outcome. In adult injuries the neurons are more resistant to retrograde degeneration, and therefore more neurons survive injury, but there are likely to be greater persisting deficits. This is at least partly because of the presence of growth inhibitors in the adult spinal cord which can contribute to the decreased plasticity in the adult CNS and perhaps also to a decreased ability to develop novel strategies to perform functions.

What Kind of Transplant to Use?

Different kinds of transplants have been shown to prevent cell death, to modify cortical representation of the body, to replace lost cells, to promote regeneration, to reduce scar formation, to neutralize inhibitors, to provide a bridge for growing axons and to remyelinate axons. Initial experiments used fetal tissue as a source of transplant tissue (Reier et al., 1992; Howland et al., 1994; Miya et al., 1997; Giszter et al., 1998; Deiner and Bregman, 1998) but the development of efficient gene transfer methods has enabled the preparation of designer cells that can provide specific molecules. Still, we remain in search of the ideal cells for transplantation because we do not yet know which features are essential (Fischer and Liu, 2001; Takami et al., 2002; Chen et al., 2002).

Transplantation into Neonates

A mid-thoracic transection in a neonatal rat severs ascending and descending axons and these axons do not regenerate through the resulting cavity. Many of the axotomized neurons either shrink or die but the caudal spinal cord, although partially denervated, remains largely intact. In particular, the pattern generators in lumbar spinal cord are spared but they are missing their descending input. The transection, however, is likely to induce sprouting from dorsal roots, providing increased afferent input and there will be additional reorganization within the lumbar spinal cord in response to the partial deafferentation. Thus, the lumbar spinal cord will be anatomically and functionally dissimilar to lumbar cord in a normal animal. Transplantation of cells into the lesion site should further modify the anatomical and functional organization of the partially denervated spinal cord.

In our experiments, we trained groups of neonatally spinalized and transplanted rats to walk on a treadmill, beginning at about 3–4 weeks of age, and then assessed the extent of weight-supported locomotion and the kinematics of this locomotion to identify the deficit associ-

ated with lesion and the improvement associated with the transplant. Adult rats consistently show 100% weight supported steps on a treadmill but after a midthoracic transection they become permanently paraplegic (0% weight-supported steps). In contrast, about 20% of rats that received a midthoracic transection as neonates develop some weight supported stepping. The remainder develop little or no useful hindlimb function. It is particularly informative to study those spinalized rats that develop locomotor function to gain an understanding of how an isolated lumbar spinal cord can contribute to locomotor function. A transplant of fetal tissue or of fibroblasts genetically modified to produce trophic factors increases the percentage of rats that develop weight-supported locomotion to 50–60% suggesting that the presence of a transplant increases the likelihood of functional recovery (Miya et al., 1997; unpublished results). There is always considerable variability in the recovery—some transplant recipients never develop hindlimb support, some show intermittent support and some develop fairly constant weight-supported locomotion. This variability may reflect the variability in descending systems induced to regenerate (Hase et al., 2001).

A second transection rostral to the initial lesion has little effect on animals transected as adults; that is, they remain paraplegic. In animals that develop weight-supported locomotion after neonatal spinalization, weight-supported stepping is initially decreased but then recovers to levels similar to what they had achieved prior to the second lesion. Thus, persisting modifications in the lumbar spinal cord circuitry must account for a basal level of development of the ability to locomote in a subset of these animals. In neonatally transplanted animals, there is also an initial loss of function followed by a recovery of weight supported locomotion but the recovery did not reach the levels achieved prior to the second lesion. The presence of the transplant thus appears to add additional components that further improve the recovery or the development of locomotion (Miya et al., 1997). Presumably the further improvement represents an interaction between transplant-mediated modifications, including reorganization of existing pathways, axonal growth and neuroprotection, and modifications in spinal pathways that occurred as a result of neonatal denervation.

Understanding the capabilities and the basis of recovery following neonatal injury is a critical issue. Several factors are important considerations, including whether the injured animal is revealing the extent of its functional capabilities and how the functional capabilities that are observed are achieved. To assess the functional limitations of recovery, increasingly more complex tasks must be used. Tasks more complex than treadmill locomotion reveal additional deficits in both spinalized and transplanted rats. In addition, more careful analysis of locomotion reveals both deficits and some of the mechanisms (e.g., novel strategies) that are used to accomplish the locomotion. These studies show that neonatally injured animals, with or without transplants, develop weight supported locomotion that differs in several ways from that used by intact animals.

When analyzing locomotion it is important to consider the biomechanical task that must be solved. The weight supporting and locomoting animal must maintain its center of mass progressing forward with bounded lateral motion. This involves selecting and controlling the gait pattern, organizing the kinematics of each limb's swing phase and selecting and controlling the ground reaction forces applied during each stance phase. In the intact rat these processes form a graceful progression with efficient use of energy storage mechanisms and muscular effort. Mechanisms to support part of these processes are located in the spinal cord as pattern generators and primitives (Kiehn et al., 1997; Giszter et al., 2000). Recent studies (Edgerton et al., 2001; Lacquaniti et al., 1999) suggest that more detailed kinematic aspects of this coordinated progression, perhaps kinematic plans, are embedded in the spinal cord circuitry. In addition, as these

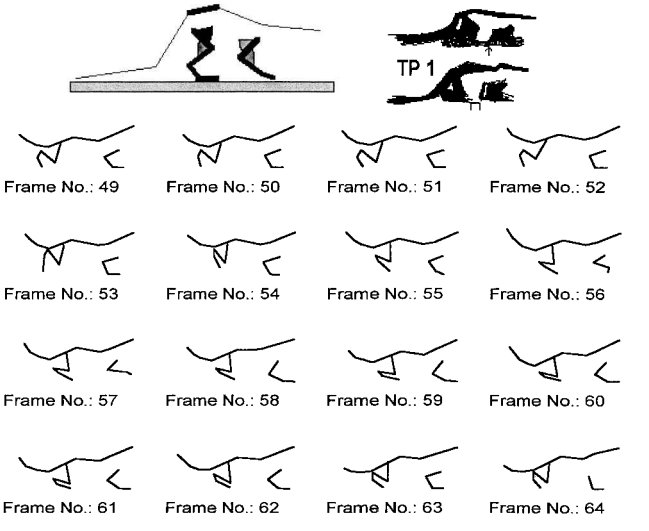

FIG. 1. Diagram on upper left shows a stick figure constructed from digitized images of a rat. The individual joint angles were calculated in MATLAB to create Figures 3 and 4. The stick figures on the upper right enable kinematic analysis of a control rat (N1) and a transplant recipient (TP1). Note hunched posture and shorter hindlimb stride length in TP1 compared to N1. The lower portion of the figure shows a series of stick figures from adjacent frames (collected at 60 Hz) during a step cycle in another transplant recipient (TP2). Gait, kinematics, and simultaneous electromyography can be synchronized using video records in this way.

authors also consider, the kinetic aspects of control must also be mastered for function. We have examined neonatal rats in a number of ways to try to understand how kinetic and kinematic factors interact in the recovery of weight supporting locomotion observed in neonatal injured rats.

Kinematic analysis shows a hunched posture and a shorter hindlimb stride length in injured rats (Miya et al., 1997; Kim et al., 1999). Thus gait is altered (Fig. 1). The intralimb organization of movement in the hindlimb is also different (Figs. 2, 3). In intact animals, the hip and knee flex in a phase-lagged process. In the hindlimb the hip moves and then the knee moves, and similarly in the forelimb the shoulder flexes before the elbow. In neonatally lesioned animals that achieve weight support, the hip and knee movements in the hindlimb are in phase, flexing at the same time. Interlimb and even the forelimb's intralimb coordination are different (Figs. 3, 4). Although a coordination pattern similar to a normal rat's pattern can be achieved, this generally occurs during intervals of *absence* of weight support and rarely, if ever, during weight-bearing locomotion (see TP12, Fig. 3). Thus kinematic analyses tell us that when weight-bearing locomotion is achieved the stride length and coordinations are modified, but there is a kinetic demand or postural impairment such that to achieve good weight bearing the animal has to adopt a severely disrupted or modified gait and intralimb joint coordination.

We can also study locomotion by measuring ground reaction forces and the movement of the center of pressure as an animal walks across a platform that is equipped with force plates. The notion to which our kinematic analyses and kinetic considerations (Raibert, 1996) leads us is as follows: good weight bearing locomotion of neonatal injured rats is akin to a person pulling a rickshaw or pushing a wheelbarrow. In the rats' case the rickshaw has legs. This model of biomechanical function makes specific strong predictions regarding the control of locomotion and begins to explain the kinematic disruptions during weight bearing that were remarked on above. A prediction is that trunk muscles, since they act

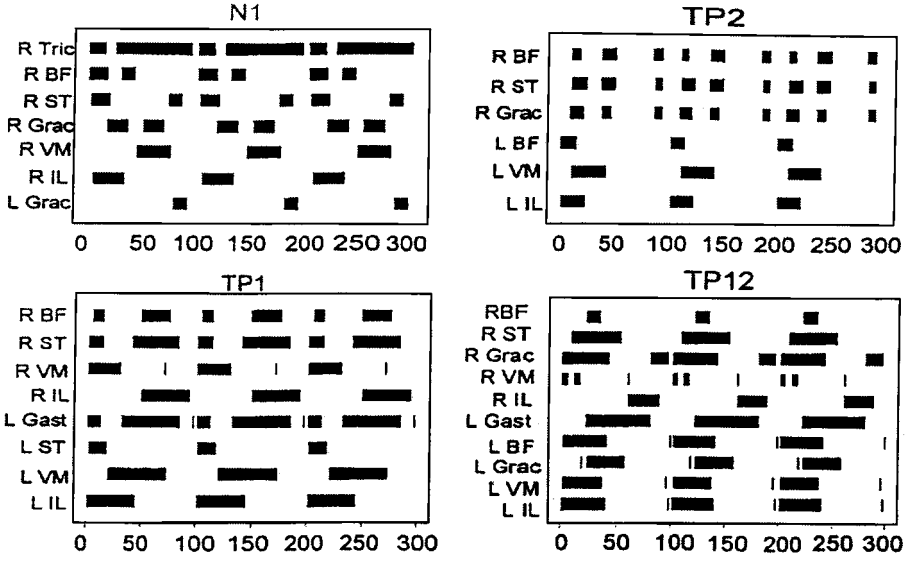

FIG. 2. Gait and underlying EMG pattern: EMG patterns in a normal rat (N1) and three rats with neonatal transections and transplants. Two rats, TP1 and TP2, showed good weight support (>80% steps weight supported), while TP12 had only intermittent weight support. In the weight-supported locomotion of transplant rats, the gait pattern differs considerably from normal, consistent with an effort to use a gait with greater static stability. We show here the EMG patterning associated with these gaits. When the average EMG had reached 30% of its peak normalized value, it was classified as firing. A horizontal bar was plotted to show when a particular muscle was firing. Three identical average cycles were plotted repeatedly for each animal to aid in understanding the rhythmicity and phasing. Notice that in the absence of weight-supported locomotion in TP12, the left illiopsoas (L IL) and left vastus medialis (L VM) were coactivated, but in the normal rat (N1) they fired as antagonists, as was expected. The intermittent weight support in TP12 may have been caused partly by this intralimb muscle phasing deficit. TP12, however, showed the proper antagonistic relationship between the right iliopsoas (R IL) and the right vastus medialis (R VM). Variable interlimb phasing of muscles was characteristic of all transplant recipients. Simple motor patterns, or gait or limb kinematics that were highly consistent among the transplant rats were generally not observed during weight supported locomotion.

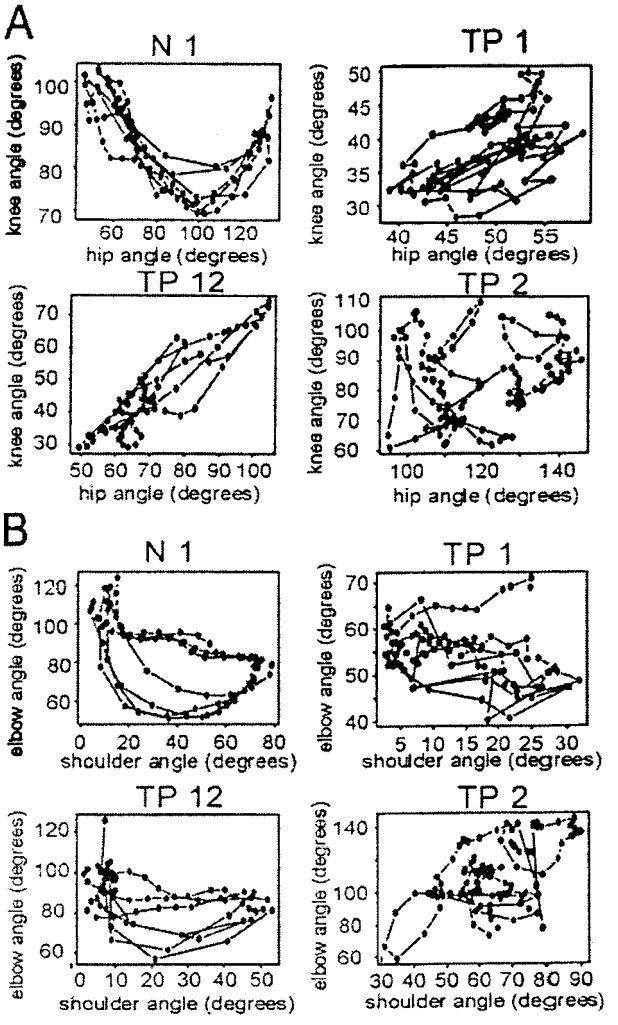

FIG. 3. Intralimb joint angle phase plots. **(A)** Knee to hip relationship. The coupling (phase relationships) of the hip and knee angles differed among normal rats and transplant rats. The cup-shaped plot of hip and knee angles of normal rat N1 was not observed in transplant rats. TP2, the animal with the best weight support, had no clearly distinguishable pattern in either its forelimb or hindlimb phasing (see B below). An animal with moderate weight support (TP1), also differed significantly from the normal pattern, but had a tendency to an in-phase joint coupling (trajectories tend to cluster around a line). TP12 showed clear in-phase joint use during a bout of locomotion with no weight support. The hip and knee angle relationship had collapsed into a straight line. **(B)** Elbow to shoulder relationship. The normal animal showed an elliptical pattern of coordination of elbow and shoulder use. TP12, who did not display weight support during this testing period, had a coupling between elbow and shoulder similar to the normal animal's pattern. In contrast, TP1 and TP2 showed less orderly joint coupling.

as the "hands" on the rickshaw must play a critical role. This is borne out in physiological analysis of corticospinal mechanisms (Giszter et al., 1998). A second prediction is that the forelimbs and hindlimbs may be used in opposition much of the time in order to control and balance the load of the weight supported haunches and this may account for the hunched posture. A third prediction is that the spinal stepping systems may be mostly autonomous in these rats and consequently to achieve stance without stepping requires isolating the hindlimbs from perturbations that could initiate stepping.

We have tested these ideas with ground reaction force measurements, center of pressure measurements and with stance testing. A normal rat supports itself during locomotion with a diagonal two-legged support. The center of pressure lies on this diagonal and balance is a dynamic process, in which the center of pressure advances during forward progression in a relatively linear fashion between dwell points in keeping with analyses of legged locomotion (Raibert, 1986; Full and Koditschek, 1999). The operated animals, in contrast, use a gait in which three legs are usually in contact with the ground to provide a static base of support. In this case, the center of pressure can vary within this triangular area as the animal progresses. As a result the animal tends to walk in a zigzag fashion across the platform with the body rolling and yawing (Miya et al., 1997). The trunk is likely to play a central role in limiting yaw and roll and indeed voluntary trunk control seems to associate closely with the degree of function achieved (Giszter et al., 1998). This meandering gait requires more energy but both the transected and transplanted animals that develop weight support have been able to use their impaired motor functions to achieve a goal, which is to walk across this platform, using an inefficient but stable mechanism for forward progression. An important question regarding the energetic inefficiency of this gait is at what point an animal may voluntarily abandon weight-supported locomotion, despite the experimenter's inducements, not because it cannot achieve weight support, but because it is not consistent with the animal's internal measures and goals specified by evolution.

TRANSPLANTS INTO SPINAL INJURY SITES IN ADULTS

A transection injury produces severe and permanent deficits in the adult. This is a model that may be useful in identifying interventions that can dramatically improve function by encouraging long distance regeneration, but may be less helpful in identifying more subtle improvements. In order to study transplant-mediated recovery in the adult in which improvement may be more modest, we have chosen a less extensive surgical model, a subtotal hemisection that ablates the lateral funiculus. This lesion destroys the rubrospinal tract, the dorsal spinocere-

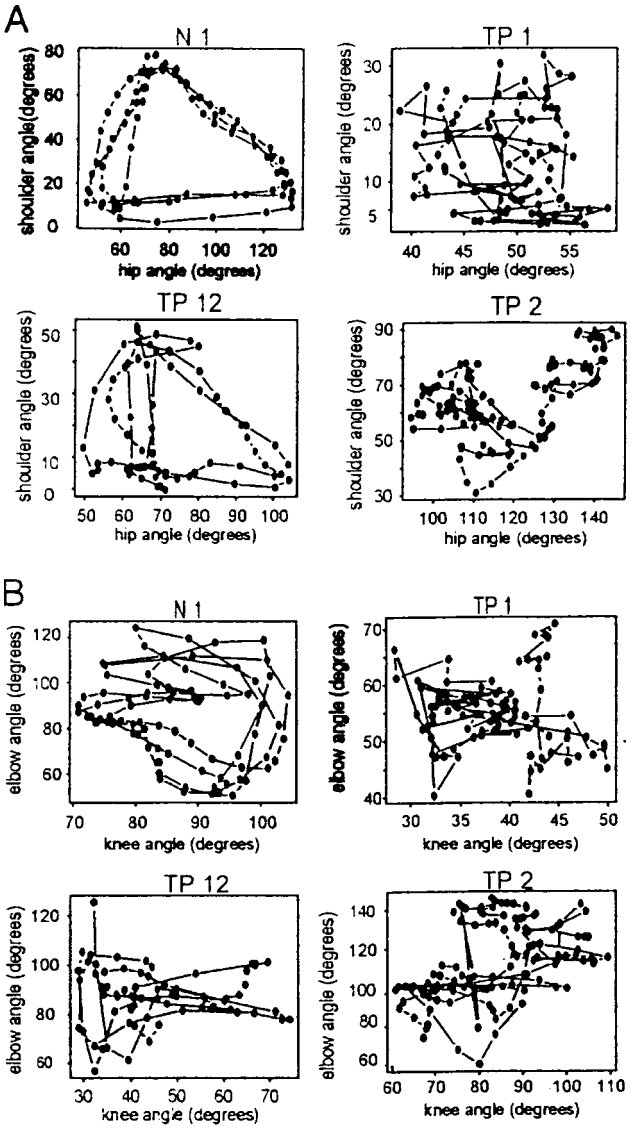

FIG. 4. Interlimb joint angle phase plots. **(A)** Shoulder hip relationship. Both the normal rat and TP12 displayed coupling patterns in the proximal joints. TP12 displayed a coupling similar to the normal rat's pattern in the proximal joints (hip and shoulder). This is particularly notable, because the TP12 displayed no weight support in this test period, during which good coordination was demonstrated. TP2 had a repeating pattern in the coordination of the proximal joints, but it differed from the normal and TP12. TP1 had no distinguishable pattern. This suggests that coupling mechanisms might be obscured by weight support and associated compensations in TP1 and TP2. **(B)** Elbow knee pattern. For the more distal joints (knee and elbow shown) none of the transplant rats showed a clear pattern. Normal animals had a repeatable cycle.

bellar tract, the spinothalamic tract, Lissauer's tract and local propriospinal pathways; the lesion spares some of the ipsilateral gray matter, and the dorsal columns and corticospinal tracts and the ventral funiculus on both sides. Adult rats show considerable spontaneous recovery of motor functions from the initial deficits but the recovery is incomplete. We did not use fetal spinal cord as a source of transplant tissue because in previous experiments we found that in the adult, unlike the neonate, fetal tissue does not elicit significant regeneration. Instead, we transplanted fibroblasts genetically modified to produce the neurotrophic factor BDNF (Fb-BDNF). Transplants of genetically modified fibroblasts have been shown to elicit robust axonal growth, both regenerative and sprouting, in incomplete lesion paradigms (Grill et al., 1997; Liu et al., 1999).

We compared recovery on a series of tests in rats that received a transplant of Fb-BDNF into the lateral funiculus lesion site with an operated control rats that received a transplant of unmodified fibroblasts (Fb-UM) or a gelfoam implant (Kim et al., 2001). Both experimental and operated control rats showed good recovery of locomotion as assessed by the BBB open field locomotion (Basso et al., 1995) and locomotion on a narrow beam test. These tests were therefore not very sensitive to the injury and thus not sensitive to the effects of the therapeutic intervention. Tests that were more sensitive included tests of spontaneous forelimb usage (the cylinder test), walking on a rope which is a more challenging locomotor task than walking on a narrow beam, swimming which is locomotion without weight support, and removal of an adhesive patch placed on the forepaw, which is a sensorimotor task.

The Cylinder Test

The cylinder test is particularly useful since it depends on the fact that a rat placed in a new environment (a transparent cylinder) will spontaneously explore that environment. The rats explore by rearing, using their hindlimbs and supporting themselves with their forelimbs placed on the walls of the cylinder. An intact rat will support itself with right forelimb or left forelimb or both forelimbs with an equal likelihood of each. An animal with a lateral funiculus lesion will only very rarely use the affected (ipsilateral) forelimb. In contrast, an animal with an Fb-BDNF transplant will use both limbs at least some of the time. A second lesion just rostral to the transplant eliminates spontaneous usage of this limb in those animals with an Fb-BDNF transplant but does not modify the function (or lack of function) in the operated control animals. Thus recovery of the spontaneous use of the affected forelimb can be attributed to the presence of the transplant of BDNF-secreting fibroblasts. We can obtain more information about how the animal moves when the cylinder is equipped with force plates on the base and walls. This instrumentation enables us to measure the forces applied by the animal while rearing and exploring

with each limb and to assess the center of pressure motions or "stabilogram" during stance, during rearing and during exploration. In examining such data, it is important to recognize that a normal rat may be able to use dynamic balance controls that an injured rat may not use. The consequence of this can be that simpler measures, such as stabilogram variance, may not vary monotonically with severity of injury or disability. For example, a severely injured rat may have high variance during rearing because its static balance control is poor, while a normal rat may have high variance during rearing because it is using dynamic balancing mechanisms that do not require low variance. These measures therefore need precise baseline data to be used effectively.

The cylinder test shows that Fb-BDNF transplants can increase the use of the affected limb and that this can be abolished by a second lateral funiculus lesion just rostral to the transplant/lesion site. Postural deficits remain in both operated control and Fb-BDNF transplant recipients. As we improve our therapeutic intervention we can hope to identify additional functional improvements using more precise measurements such as those obtained with force plates.

The Rope Walking Test

Animals with a lateral funiculus lesion perform well on a narrow beam test and also show few deficits on the BBB test. A more complex test is walking on a rope, a task that challenges both forelimb-hindlimb coordination and balance (Kim et al., 2001). Normal rats have little trouble walking with good coordination and balance on a stout rope. Operated control animals, in contrast, make many errors in foot placement and posture, and often slip or fall from the rope. Fb-BDNF recipients, although deficient compared to normal rats, can nevertheless cross the rope with weight supporting steps and few falls Thus the transplant appears to improve the ability to locomote under these more challenging conditions.

We used the second lesion model to evaluate more directly the role of the transplant. In this test, a relesion had little effect on the operated control rats but produced a dramatic loss of function in the Fb-BDNF recipients, such that they were now significantly more deficient than the operated control rats. Thus the transplant seems to have contributed to the recovery of locomotion on a rope-crossing test and furthermore the neural circuitry may have been permanently modified so that the spinal cord has become dependent on the transplant.

The Patch Removal Test

We used a sensory test, the patch removal test, to identify modifications in sensory processes (Kim et al., 2001). When an adhesive patch is placed on the forelimb of an intact rat, the rat responds immediately by pulling the patch off with its teeth. We measured the latency to remove the patch by the animals in our experimental groups. All rats, injured or intact, quickly removed the patch from the left (unaffected) limb. Both experimental and operated control rats showed a much greater latency to removal of the patch from the affected limb but the BDNF-Fb recipients showed a significantly shorter latency than the operated controls. In some cases, the operated control rats did not even attempt to remove the patch, suggesting a more severe sensory impairment. After the relesion all of the animals showed almost complete inability to respond to the presence of the patch or remove it. With the patch removal test, we can see that transplant recipients respond more quickly to the presence of the patch, which suggests an improvement in perception of the stimulus. The re-lesion significantly impairs function only this group.

WHAT DOES THE SECOND LESION (RE-LESION) TELL US?

The results of a second lesion, the re-lesion, indicate whether the presence of the transplant contributes to the recovery; it is however not simple to identify the mechanisms by which the transplant exerts its effect. First, the second lesion will inevitably cut some axons that had been spared and it thus enlarges the lesion. Nevertheless, operated control animals rarely show additional deficits when the second lesion is placed close to the initial injury so we can assume that loss of function in transplanted animals is due to the loss of the effect of the transplant rather than to the enlargement of the lesion. The intent of the second lesion is to damage those axons that have grown into at least the rostral part of the transplant. The second lesion damages these axons but it will also damage axons spared by the initial lesion but induced to sprout into the rostral part of the transplant. The second lesion, however, would spare axons that have regenerated along a novel trajectory or axons that have sprouted as a result of the transplant but have either not entered the transplant or have not entered the rostral portion of the transplant. Thus, there are several possible outcomes to a relesion experiment. One is that recovery is abolished, as in the cylinder test. This result would suggest that the recovered function is due primarily to the presence of the transplant. Another outcome is a more severe deficit after the second lesion, as in the case of the rope-crossing test. This result suggests that the spinal cord has become dependant in some way on the circuitry provided by the transplant. The second lesion has actually inter-

fered with a compensatory response based on regeneration, sprouting, or neuroprotection. A third possibility is that there is partial sparing of the recovered function, as in the weight-supported locomotion that neonatally spinalized animals developed. The retransection in this case only abolished part of the weight-supported locomotion ability and the remaining weight supported locomotion capacity is presumably due to the modifications that occurred as a result of the transection and the developing lumbar spinal cord.

MECHANISMS BY WHICH TRANSPLANTS CAN MODIFY CIRCUITRY

We know that transplants of fetal tissue or of fibroblasts genetically modified to produce trophic factors can modify the circuitry of the lesioned spinal cord. Spinal injury can produce retrograde degeneration of some axotomized neurons. There is some controversy over whether the cells that can no longer be recognized have in fact died or whether they have atrophied below the level of recognition but there is no controversy over the retrograde degenerative changes in a significant numbers of cells in certain nuclei, for example, Red Nucleus, Clarke's nucleus, after a lateral funiculus lesion (Himes and Tessler, 2001; Kwon et al., 2002; Liu et al., 2002; Novikova et al., 2002). This degeneration is apoptotic and axotomized neurons that would normally atrophy or die as a result of this injury can be rescued by interventions that include supplying trophic factors. This neuroprotection is equally effective in rescuing neurons axotomized either in neonates or adults.

Axonal growth, either through regeneration or sprouting, is another potential contributor to repair and recovery. In neonates, in addition to the possibility of regenerating and sprouting axons, there are also late developing axons that don't grow into the spinal cord until after the lesion has been made. Finally surviving supraspinal systems, including the corticospinal system, may undergo substantial reorganization in response to neonatal lesions and this may play a key role in recovery of functions such as weight support (Giszter et al., 1998; Fouad et al., 2001).

SUMMARY

It seems clear that cellular transplantation into spinal cord lesion sites can ameliorate some motor and sensory deficits arising from the injury, but we don't at present have a good understanding of how transplants work. We know that some injured neurons are rescued but we don't know whether the rescued neurons are functional. We know that some cut axons regenerate and that some spared axons sprout but we don't know whether the regenerating and sprouting axons are functional. We therefore don't know which elements of the novel circuitry contribute to improved function. Behavioral tests can identify some levels of recovery but they may not adequately assess functional changes arising from modifications in local circuitry and there is therefore the danger that potentially useful interventions may be inadequately assessed functionally. Behavioral tests can identify directions for the potentially more powerful physiological tests which may be required for the next generation of experiments designed to test mechanisms of recovery.

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