Olfactory ensheathing cell transplants have been shown to exert neuroprotective and prorregenerative effects in animal models of SCI. Its capacity to promote functional neural repair, however, remains unclear. The authors studied axonal growth and locomotor recovery after C-7 contusion injury and OEC transplantation in adult rats.

Methods. Twenty-four male Wistar rats underwent a mild C-7 contusion injury that completely disrupted the dorsal corticospinal tract (DCST). In 14 rats OECs were transplanted into the lesion, and 10 were used as controls. At 3 months post-contusion, the kinematics of locomotion were assessed, and the CST was traced by injecting dextran tetramethylrhodamine bilaterally into the cerebral cortex. The animals were killed 2 weeks after tracer injection, and their spinal cords were studied immunohistochemically.

Although the survival of transplanted cells varied, they were present in all cases. The authors observed neither OEC migration nor DCST axon regeneration in any of the cell transplant–treated rats. Corticospinal axons ended in retraction bulbs at the proximal edge of the lesion or, exceptionally, a few micrometers inside the transplant. The results of neurofilament immunohistochemical analysis provided evidence of neurites from systems other than the DCST growing into the transplant, but in some cases these neurites formed loops of pathological appearance. Contusion injury of C-7 caused chronic locomotor deficits that did not improve after OEC transplants.

Conclusions. The findings in this study indicate that OEC transplants alone are not sufficient for neural repair and functional recovery after SCI. In addition, OECs can induce abnormal axonal growth, making further studies necessary before considering their clinical use.

KEY WORDS • cervical spine • contusion injury • olfactory ensheathing cell • transplantation • corticospinal tract • rat
Olfactory ensheathing cell transplants after SCI

eration when transplanted in thoracic segments 1 week after contusion injury. These authors used anterograde tracers to study several neural systems, including the cortico-, reticulo-, and vestibulospinal tracts, and did not find axons growing through the transplant in any case, although some neuroprotective effect was noted.

Several facts encouraged us to assess the effects of OEC transplants in contused cervical spinal cords. First, approximately two thirds of human traumatic SCI occurs at cervical segments, about half of which exhibit the pathological features of contusion. Second, the distance of the injury site from the neuronal cell body appears to be an important determinant of axonal regeneration, because neurons from brainstem nuclei show a greater regenerative response after induction of cervical than after thoracic spinal cord lesions. Third, we have recently shown that mild C-7 contusion injury produces permanent forelimb deficits in elbow extension and body support during locomotion. Segmental neuronal death and axonal destruction were associated with these deficits. Thus, the C-7 contusion model might be particularly useful for verifying the effects of mixed protective/preregenerative treatments, which could improve locomotion in the absence of long axonal regeneration. In the present study, no evidence of functional repair was observed 3 months after mild contusion injury and OEC transplantation.

Materials and Methods

Culture and Purification of OECs

Olfactory ensheathing cells were prepared as previously described. Briefly, young adult (7–8-week-old) male Wistar rats, reared at the Cajal Institute, were used. The olfactory nerve and glomerular layers of the olfactory bulb were dissected, digested with trypsin and deoxyribonuclease, preplated for 6 hours at 37°C in uncoated flasks to remove microglia and macrophages, and finally plated on flasks coated with poly-L-lysine. Primary cultures were grown in a mixture of DMEM-F12 (1:1) supplemented with penicillin/streptomycin and 10% fetal bovine serum. The medium was replaced 48 hours later by a mixture of DMEM-F12 and conditioned medium (1:1) obtained from purified OEC cultures. Approximately 10 days were needed to produce confluent cultures and proceeding to negative cell purification. The cells were detached with trypsin–EDTA acid and recovered by centrifugation. Suspensions of OECs were incubated with a mixture of Dynabeads (Dynal, Oslo, Norway) M-450, coated with goat anti-mouse immunoglobulin G and M-280 (streptavidin coupled) to which anti-rat Thy 1 and biotinylated Isocut B4 were previously linked. The tube was placed in a magnetic cell separator and the cells remaining in suspension were withdrawn by pipette and plated. This secondary culture was allowed to recover for 2 days, before being labeled with Fast Blue. Fluorescent cell labeling was performed by adding Fast Blue (5 μg/ml [Sigma-Aldrich, Chemical Co., St. Louis, MO]) to the culture medium for 12 hours, after which the cells were washed, detached with trypsin–EDTA, suspended in DMEM at approxi-
mately 10,000 cells/ml and kept at 4°C until transplanta-
tion (-1 hour). Six separate cultures of the same cells were plated onto circular coverslips and processed for p75 NGF receptor (AB-1554; Chemicon), neurofilament (N-0142; Sigma-Aldrich Chemical Co.), or GFAP (Z-0334; Dako) immunohistochemical analysis. Stained sections were observed using a microscope equipped for fluorescence and photographed at a resolution of 2776 × 2048 pixels, with a digital image system (DP50; Olympus, Hamburg, Germany). Measurement of the extent of the lesion was performed on transverse and parasagittal sections after processing for van Gieson staining.

Assessment of Locomotion

Kinematics of locomotion were measured on video recordings (50 frames per second) captured while the rats walked spontaneously on a flat and transparent (120-cm-long, 14-cm-wide) surface toward a dark box. The camera was placed orthogonal to the line of movement and only bidimensional analysis, of the right side of the animal, was performed. The locomotion analysis was conducted 3 months after contusion injury, in subgroups of animals with C-7 contusion (seven rats), C-7 contusion and OEC transplantation (nine rats; transplant paradigm 1), and normal animals (nine rats). Points corresponding to hip, knee, ankle, metatarsus, shoulder, elbow, and wrist were marked on the shaved skin, and the movement of the points was automatically digitized using motion analysis software (WINanalyze: Mikromak GmbH, Münster, Germany) and processed to obtain the data of interest. The duration of stance (contact) and swing phases for the four limbs were determined with the help of a mirror placed at 45° below the walking surface. At least six gait cycles were randomly selected for analysis and the follow-
ing variables were measured and mean values computed: 1) duration of the stance and swing phases for the four limbs; 2) forward

J. Neurosurg: Spine / Volume 3 / October, 2005
formation of cysts as previously described. When values were chosen at a probability value less than 0.05.

Statistical Analysis

One-way analysis of variance and the Student-Newman-Keuls posttest were used to compare kinematics data from normal, C-7–injured, and C-7–injured/OEC-transplanted animals. Values are presented as the means ± 1 standard error of the mean. Significance values were chosen at a probability value less than 0.05.

Results

The weight-drop technique is frequently used in experimental SCI because it reproduces the main pathological features of human spinal cord contusion. We used this technique to injure the rat C-7 segment. Identifying and measuring each segment before histological processing confirmed that the lesions always involved C-7, although a small variation was observed in some cases when the distal portion of C-6 was damaged while that of C-7 was spared. The damage extended longitudinally 2.93 ± 0.49 mm (range 2.3–3.6 mm), causing the formation of cysts as previously described. When observed in the transverse plane, the damage consisted of a central cavity that completely interrupted the DCST, whereas to various extents the dorsal columns and peripheral white matter were spared (Fig. 1). The contusion did not break the meninges, thus preventing the invasion of fibroblasts into the lesion. When the animals were killed (3 months postinjury), the cysts were essentially acellular, although thin cellular trabeculae were observed in some cases. We have recently shown that this lesion, in addition to damaging the DCST, the dorsal columns, and the central gray matter, causes the death of approximately 40% of triceps brachii motor neurons.

Transplanted Cell Identification, Survival, and Migration

Evaluation of spinal cord sections in the nine animals in which Fast Blue–labeled OECs were transplanted in five points across the lesion zone (see Materials and Methods) showed intense blue fluorescence, located in OEC nuclei, 3 months after transplantation (Figs. 2–4). No OEC migration was observed. Transplanted cells remained at the lesion-implant site, and processes from the host astrocytes grew into the transplant (Figs. 2C and 4D). A disorganized mass of fluorescent cells, contiguous with the host tissue and completely filling the cyst, was found in four of nine animals (Fig. 2A). In another three cases, the mass of fluorescent cells filled approximately 1 mm2 of the lesion zone and was surrounded by cell-free cysts (Figs. 2B and 3A). In the remaining two transplant-treated animals poor OEC survival was observed, with the cells forming trabeculae inside the cysts (Fig. 4). Fast Blue was found mainly in oval nuclei closely resembling those of OECs (Figs. 2C and 3D); however, the label was also transferred to some host cells, particularly macrophages and ependymal cells, apparently in amounts inversely proportional to transplant survival. Immunostaining for GFAP and p75 NGFR permitted a better assessment of the cell types exhibiting Fast Blue fluorescence. Long GFAP-positive processes were found inside the transplant, but they were never costained with Fast Blue, indicating that they belonged to host astrocytes. Interestingly, these long astrocyte processes intermingled with OECs and neurites (Figs. 2C and 4D). We found in every transplanted animal numerous Fast Blue–positive, p75 NGFR positive cells (see for example Fig. 4E and F). Olfactory ensheathing cells are known to express p75 NGFR, a property used for their identification within two weeks of transplantation; however, Schwann cells also express p75 NGFR, and it is well known that they invade the lesion zone in rodent and human SCI. Indeed, we identified p75 NGFR–positive cells in the tissue surrounding the lesion of nontransplanted controls, the cysts of which were essentially acellular except for thin trabeculae. Thus, the Fast Blue/p75 NGFR–positive cells observed in the lesion could be either transplanted OECs or host Schwann cells. Finally, many Fast Blue–positive cells were negative for p75 NGFR and GFAP, and their oval nuclei were clearly different from macrophages. It is likely that these cells were mostly transplanted OECs.

Effects of Transplants on Nerve Fiber Growth

The DCST was completely interrupted in all injured animals, and OEC transplants did not promote its regeneration (Figs. 2–4), even when a mass of cells filled the injured zone. Dorsal CST axons ended as retraction bulbs at the proximal edge of the lesion, or they occasionally penetrated a few micrometers into the lesion in both transplanted and control animals. Numerous neurofilament-positive processes of undetermined origin were observed, however, within the transplants (Figs. 2E, 3C and E, and 4C). They appeared to arise from axons spared from lesion and from spinal neurons. These processes followed the random orientation of transplanted cells, aligning longitudinally in the spinal cord in some cases (Figs. 2E and 4C) and forming aberrant loops in others (Fig. 3C and E). In five rats OECs were implanted into the contusion zone as well as 0.5 mm rostral and 0.5 mm caudal to it, with the aim of promoting OEC–axon contact and regeneration; however, Fast Blue–labeled cells remained confined to the injection site, and corticospinal axons never grew across the lesion, ending at its proximal edge as occurred in specimens in transplant paradigm 1.

Effect of OEC Transplants on Locomotor Recovery

Rat locomotion after mild contusion injury of C-7 has been recently characterized. Although the animals were able to feed themselves immediately after recovering from anesthesia and did not require special postinjury care, they exhibited evident forelimb impairment as well as trunk instability. They stood well on their hindlimbs from the 1st postoperative days, but locomotion was abnormal because of a lack of body support provided by the forelimbs. By 2 weeks postoperatively, all rats recovered limb locomotion in all four limbs, and impairments were not apparent to an untrained person; however, specific chronic locomotor deficits persisted, particularly reduced elbow extension and deficient forelimb-related body support. The animals recovered stability by increasing the stance phases of both fore- and hindlimbs, at the cost of reducing forward velocity. There was no obvious loss of locomotor hindlimb function, at least at low walking velocities on a flat surface.
Olfactory ensheathing cell transplants did not improve the impairments observed after C-7 contusion. In comparison with normal animals, both contusion- and contusion/OEC transplanted animals exhibited reduced elbow extension (normal $144 \pm 5.4^\circ$; contused $118 \pm 5.4^\circ$; and contused/transplanted $116 \pm 1.8^\circ$; $p < 0.0001$). During the first evaluations of injured animals, the forelimb was not capable of supporting the body, collapsing, instead, at the beginning of the stance phase. The animals compensated for this impairment by increasing the duration of the stance phase of both the hindlimbs and the forelimbs (mean for the four limbs: normal $0.26 \pm 0.02$ seconds; contused $0.46 \pm 0.04$ seconds; and contused/transplanted $0.41 \pm 0.04$ seconds; $p < 0.0001$). As a result of this compensation, the percentage of time that more than two limbs were supporting the body during the walking cycle was increased, maintaining stability but reducing forward velocity (normal $0.37 \pm 0.02$; contused $0.18 \pm 0.02$; and contused/transplanted $0.21 \pm 0.01$ m/second; $p < 0.0001$). It is important to mention that these adaptations in the walking cycle may not be definitive. Our preliminary observations indicate that elbow extension deficit persists in C-7–injured animals retrained to walk, whereas stance phase duration and spontaneous forward velocity can reach almost normal values. The animals compensate for forelimb dysfunction by changing the temporal and geometrical organization of the walking cycle and transferring more body weight to the hindlimbs (JE Collazos-Castro, unpublished observations). For a better understanding of these adaptations, a combination of kinematics, kinetic, and electrophysiological studies is necessary.

**Discussion**

We explored the use of OEC transplants for repairing C-7 contusion injuries. No regeneration of the DCST was observed. Collaterals from other axons grew into the transplanted animals, following the random orientation of the cells and sometimes forming loops of pathological appearance. Furthermore, OEC transplants did not improve locomotor deficits. These observations stand in contrast to those reported in previous studies in which regeneration was observed after cervical DCST electrolytic lesioning and OEC transplantation, and yet they are in agreement with recent studies in which no regeneration was found after thoracic spinal cord contusion or transection.

The first requirement for successful lesion repair is survival of the transplant. In the present study, a mass of cells inside the cysts was observed only in transplant-treated animals; however, it was not clear which of the observed cells belonged to the host and which were transplanted cells. The use of techniques for specific cell identification is necessary to estimate the proportion of surviving transplanted cells. Transplanted OECs had been accurately followed in some studies, but never for longer than 3 weeks, and the number of surviving cells has never been estimated. We labeled OECs with Fast Blue obtaining an intense longlasting fluorescence. Unfortunately, after OECs were transplanted the marker spread to some host cells, particularly macrophages, ependymal cells and probably Schwann cells. Immunohistochemical analysis showed that a portion of Fast Blue–labeled cells were also positive for p75 NGFR. These cells may be either transplanted OECs or Schwann cells that invaded the transplanted and were labeled by Fast Blue transfer. Fast Blue/
p75–labeled cells intermingled with axons and were found associated with long astrocyte processes growing into the transplant, a behavior similar to that of astrocytes after Schwann cell transplantation into the brain. Numerous neurites grew into the transplant site, but no regeneration of the CST was observed. Qualitatively similar effects.
have been demonstrated after Schwann cells or OECs were transplanted in rats with spinal cord contusion or transection. Although OECs and Schwann cells are considered different cell types, their molecular phenotypes share several similarities. Properties such as the ability to migrate far from the implant site were thought to differentiate OECs from Schwann cells and to facilitate axonal regeneration in spinal cord–transected rats; however, in neither the present study nor in others involving better cell identification techniques have OECs been observed migrating away from the transplant site, and the previous observations of CST regeneration have not been confirmed. What seems clear is that the growth of some axonal processes, particularly serotonergic and sensory axons, is favored by OEC transplants; however, it must be recalled that serotonergic and sensory axons are those that grow spontaneously after SCI. Furthermore, it must also be remembered that the sprouts observed invading the transplants may be terminal sprouts from nondamaged axons ending near the lesion or collaterals from uninjured axons ending in unrelated targets and that their sprouting into the lesion area may not necessarily be advantageous for functional recovery. Indeed, spontaneous Schwann cell invasion and axonal sprouting from

Olfactory ensheathing cell transplants after SCI

FIG. 3. Parasagittal sections of the spinal cord of a transplant-treated animal 3 months postinjury. A: Low-power photomicrograph showing the DCST (red) and the transplant (blue-violet). The outer borders of the spinal cord have been outlined in yellow and those of cyst cavities in white. The transplant divided the damaged area into two smaller cysts. Magnifications of the squares are shown in B and C, processed for neurofilament immunohistochemistry. B: Dorsal CST traced with DTMR (red). Most axotomized axons persisted near the lesion zone at 3 months postinjury. C: Although DCST did not regenerate, numerous neurites from other systems grew on the transplanted cells, as evidenced by neurofilament staining (green). This zone is shown magnified in D and E. D: Transplanted cells forming loops into the lesion zone. E: Neurites follow the random orientation of OECs. Scale bars = 0.5 mm (A) and 0.2 mm (B–E).
FIG. 4. Parasagittal sections obtained in the spinal cord of one animal with poor transplant survival 3 months postinjury. A: Low-power photomicrograph showing the DCST and the spinal cord immunostained for neurofilament. Most of the lesion zone was occupied by cysts, divided by trabeculae in which Fast Blue–positive cells, GFAP-positive processes, and neurites were found. Magnifications of the squares are shown in B and C. B: Dorsal CST axons ended on the cyst wall. C: Trabeculae containing numerous neurites aligned with the longitudinal axes of the spinal cord. D: A serial section adjacent to C submitted to GFAP immunohistochemical analysis; the large arrow signals long GFAP-positive processes oriented longitudinally, whereas the small arrow indicates macrophages loaded with DTMR. E: Fast Blue–marked cells inside the trabeculae. F: Enlargement of the square shown in E, showing numerous cells positive for p75 NGFR. Scale bars = 1 mm (A), 0.2 mm (B–E), and 0.05 mm (F).
Olfactory ensheathing cell transplants after SCI

sensory and spinal neurons into the lesion is well known after SCI in mammals, humans included, and is not associated with functional benefits. In this context it is worth noting that OECs do not associate with axons after they are implanted into the injured spinal cord, but they do enhance Schwann cell migration into the lesion, the latter cells associated with myelinated and unmyelinated axons. Our results are compatible with the hypothesis that Schwann cells could be responsible for the increased axonal sprouting observed after OEC transplantation in models of SCI.

It is unlikely that Fast Blue labeling had caused the failure of OECs to promote DCST regrowth because the marker did not affect OEC viability in vitro, and the transplanted cells increased the growth of host neuron and astrocyte processes as reported by others. It is possible that the magnitude of the lesion and the disruption of blood capillaries hampered regeneration. We used the weight-drop technique to produce a contusion injury that destroyed the DCST and the surrounding tissue for approximately 2.5 mm, whereas Li, et al., made a non-bleeding electrolytic lesion of approximately 500-μm diameter that preserved the architecture of the spinal cord. According to these authors, this small lesion permitted OECs to directly contact the cut axons and bridge the proximal and distal edges of the lesion, optimizing the conditions for regeneration. Although the weight-drop technique reproduces the main histopathological features of human spinal cord contusion, it creates a larger and more complex lesion that prevents close interaction between axons and transplanted cells; however, in addition to lesion size, other neurobiological factors may explain the negative results presented here. Corticospinal neurons may fail to initiate axonal growth after axotomy, or OECs may not express the appropriate phenotype to induce their growth, as is the case for Schwann cells.

Approximately two thirds of human traumatic SCIs occur in the cervical spine. Although most functional deficits reflect the interruption of axonal pathways, patients also suffer impairments caused by loss of segmental neurons. In particular, arm motor deficits arise from both segmental neuronal death and reduced input to the neurons. Forelimb (arm) motor deficits can be modeled in rodents, frequently by using the selective interruption of the CST. This results in forelimb dysfunction in reach-to-grasp movements but not locomotion impairment, at least in terms of walking velocities. Reach-to-grasp movements involve whole-body orientation and postural adjustments, a series of 10 arm and paw kinematic sequences and arpeggio movements. A rat’s reach-to-grasp movements are homologous to those of monkeys and humans, which also manifest hand dysfunction after CST lesioning. After selectively damaging the cervical DCST in rats and transplanting OECs into the lesion, reported some axonal regeneration and recovery of forelimb reaching function. Unfortunately, the movements were scored as an end point task, and their components were not studied. Because of the complexity of these movements, the use of an end point measurement as the indicator of functional recovery is insufficient. For instance, after dorsal column lesions in rats, reaching movements can be completely compensated, whereas impairments in limb synergies persist. Furthermore, the ventral CST appears to participate in functional compensation after DCST lesion, as probably other tracts do, because the execution of reaching movements is not exclusive competence of the CST.

Locomotion on a flat surface free of obstacles is highly stereotyped. It is a “simple” task, where gravity is the main factor limiting the movement of the animal. Therefore, the efficiency of locomotion is directly related to generation of force for body support and propulsion during the stance phase. Swing phase execution is, of course, indispensable for progression of the walking cycle, taking the paw to the correct place to receive the load of the accelerated body. The measurement of durations of the stance and swing phases, joint angles, and horizontal velocity provides a general idea of how locomotion is executed.

We have advanced the characterization of locomotion after C-7 mild contusion. The loss of approximately 40% of triceps brachii motor neurons is in agreement with the observed deficits in elbow extension and weight support on the forelimbs. The resultant deficits can be regarded as a segmental “C-7 motor syndrome,” because hindlimb motor function apparently remains normal, as least for locomotion at walking velocities. Axonal damage and interneuron death probably contribute to the permanent nature of the syndrome. This is suggested by the fact that more than half of triceps brachii motor neurons are spared from the lesion, and neuromuscular adaptations that compensate for motor deficits after partial denervation of limb extensors have been reported, the motor neurons of synergistic muscles receiving increased modulation by sensory feedback and/or supraspinal systems. Adaptations may also occur in the peripheral nervous system by sprouting of intact axons into denervated or synergistic muscles. A clear difference between nerve section– or cord contusion–induced partial muscle denervation is that in the latter interneurons and descending and ascending axons are injured in addition to motor neurons; the damage of those elements probably limits the activation of adaptive mechanisms. A combination of kinematic, kinetic, and electrophysiological techniques will be required to test this hypothesis. The availability of an SCI model in which permanent focal deficits may be detected after incomplete lesioning is of interest for testing repair-based strategies. In this model, neuroprotective and/or proreregenerative interventions may reveal clear functional benefits, even with little beneficial effect on the tissue and in the absence of long axon tract regeneration. Unfortunately, this was not the case after OEC transplantation.

Conclusions

Analysis of the data obtained in this study indicates that OEC transplants alone are not sufficient to promote neural repair and function recovery after contusion-induced SCI. Several factors may account for these results. Intrinsic constraints of adult cortical neurons could prevent DCST regeneration, or OECs may not possess a general proreregenerative/neuroprotective effect. Furthermore, the destruction of tissue architecture at the lesion site probably impeded the adequate interaction between axons and transplanted cells. Central nervous system repair probably cannot be effected by unspecific interventions because the highly specialized connectivity and function of the spinal
cord demands a selective approach. The three-dimension-
al reconstruction of neural tissue is a real challenge. We
hope that improved neural repair results will be obtained
by culturing growth-promoting cells on three-dimension-
al substrates for implantation into the lesion. Further stud-
ies should be conducted to determine the real utility of
OEC transplants for the treatment of human central ner-
svous system injuries.

References
1. Allen A: Surgery of experimental lesion of spinal cord equiva-
 lent to crush injury of fracture dislocation of spinal column. A
preliminary report. JAMA 57:787–880, 1919
2. Barnett S, Alexander C, Ishiiha Y, Gilson J, Crowther J, Clark L,
et al: Identification of a human olfactory ensheathing
cell that can effect transplant-mediated remyelination of demyeli-
derson D, et al: Endogenous repair after spinal cord contusion
spinal cord injury in the cat: the relation of injury intensity to
5. Bouyer L, Whelan P, Pearson K, Rossignol S: Adaptive loco-
motor plasticity in chronic spinal cats after ankle extensors
pressing olfactory ensheathing cells do not associate with my-
elinated axons after implantation into the compressed spinal
7. Bresnahan JC, Beattie MS, Todd FD III, Noyes DH: A behav-
ioral and anatomical analysis of spinal cord injury produced by
a feedback-controlled impaction device. Exp Neurol 95:
548–570, 1987
8. Brook GA, Lawrence JM, Raisman G: Columns of Schwann
cells extruded into the CNS induce growth of astrocytes to
J, et al: Spontaneous longitudinally orientated axonal regenera-
tion is associated with the Schwann cell framework within the
lesion site following spinal cord compression injury of the rat.
of several types of human spinal cord injury, with emphasis on
the astrocyte response to penetrating injuries. Adv Neurol 72:
305–315, 1997
13. Cheng H, Olson L: A new surgical technique that allows pro-
xomodistal regeneration of 5-HT fibers after complete transection
M: Motoneuron loss associated with chronic locomotion
impairments after spinal cord contusion in the rat. J Neuro-
trauma 22:544–558, 2005
15. Curt A, Dietz V: Neurographic assessment of intramedullary
motoneurone lesions in cervical spinal cord injury: consequen-
16. Fernandes KJ, Fan DP, Tsui BJ, Cassar SL, Tetzlaff W: In-
fluence of the anatomy to cell body distance in rat rubrospinal
and spinal motoneurons: differential regulation of GAP-43, tu-
bulin and neurofilament-M. J Comp Neurol 414:495–510,
1999
17. Fouad K, Schnell L, Bunge M, Schwab M, Liebscher T, Pearl-
se D: Combining Schwann cell bridges and olfactory-ensheathing
grafts with chondroitinase promotes locomotor recovery
after complete transection of the spinal cord. J Neurosci 25:
1169–1178, 2005
18. Franklin RJ, Gilson JM, Franceschini IA, Barnett SC: Schwann
cell-like myelination following transplantation of an olfactory
bulb-ensheathing cell line into areas of demyelination in the
adult CNS. Glia 17:217–224, 1996
19. Gómez VM, Averill S, King V, Yang Q, Perez ED, Chacon SC,
et al: Transplantation of olfactory ensheathing cells fails to pro-
mote significant axonal regeneration from dorsal roots into the
M, Kordower J (eds): CNS Regeneration, Basic Science and
27–54
scale purification from adult olfactory bulb, freeze preservation
and migration of transplanted cells in adult brain. Rest Neurol
Neurosci 10:25–34, 1996
22. Guest JD, Hesse D, Schnell L, Schwab ME, Bunge MB, Bunge
RP: Influence of IN-1 antibody and acidic FGF-fibrin glue on the
response of injured corticospinal tract axons to human
23. Guizar-Sahagun G, Grijalva I, Madrazo I, Franco-Bourland R,
in the spinal cord rats subjected to severe spinal cord contusion.
24. Hughes J, Brownell B: Aberrant nerve fibres within the spinal
25. Imaiizu T, Lankford KL, Waxman SG, Greer CA, Kocsis JD:
Transplanted olfactory ensheathing cells remyelinate and en-
hance axonal conduction in the demyelinated dorsal columns
26. Iwniuk AN, Whishaw IQ: On the origin of skilled forelimb
27. Lakatos A, Franklin RJ, Barnett SC: Olfactory ensheathing cells
and Schwann cells differ in their in vitro interactions with astro-
28. Levi AD, Tator CH, Bunge RP: Clinical syndromes associated
with disproportionate weakness of the upper versus the lower ex-
tremities after cervical spinal cord injury. Neurosurgery 38:
179–185, 1996
29. Li Y, Decherchi P, Raisman G: Transplantation of olfactory en-
sheathing cells into spinal cord lesions restores breathing and
30. Li Y, Field P, Raisman G: Regeneration of adult rat cortico-
spinal axons induced by transplanted olfactory ensheathing
31. Li Y, Field P, Raisman G: Repair of adult rat corticospinal tract
by transplants of olfactory ensheathing cells. Science 277:
32. Li Y, Raisman G: Sprouts from cut corticospinal axons persist
in the presence of astrocytic scarring in long-term lesions of
33. Luft A, Hatcher D, Torkko K: Enlarged motor units resulting
from partial denervation of cat hindlimb muscles. J Neurophy-
siol 59:1377–1394, 1988
model of rat spinal cord injury. J Neurosci Res 45:588–597,
1996
35. Matthews M, St Onge M, Fauve C, Gelder J: Axon sprouting
into segments of rat spinal cord adjacent to the site of a previous
36. McKenna J, Whishaw I: Complete compensation in skilled
reaching success with associated impairments in limb synergies,
after dorsal column lesion in the rat. J Neurosci 19:1885–1894,
1999
37. Metz GA, Curt A, van de Meent H, Klusman I, Schwab ME,
J. Collazos-Castro, V. C. Muñetón-Gómez, and M. Nieto-Sampedro
Olfactory ensheathing cell transplants after SCI


Manuscript received September 3, 2004. Accepted in final form July 26, 2005.
Address reprint requests to: Jorge E. Collazos-Castro, M.D., Ph.D., Neural Repair Laboratory, Hospital Nacional de Parapléjicos, Finca “La Peraleda” s/n, 45071 Toledo, Spain. email: jcollazos@sescam.org.