Spinal Cord (2006) 44, 605–613

www.nature.com/sc

Original Article

Intrinsic locomotor outcome in dorsal transection of rat spinal cord: predictive value of minimal incision depth

K Brechtel*[,1,6], A Tura[2,6], M Abdibzadeh[3], S Hirsch[3], S Conrad[4] and JM Schwab[5]

[1]*Department of Diagnostic Radiology, University of Tuebingen, Tuebingen, Germany;* [2]*Department of Ophtalmology, University of Tuebingen, Tuebingen, Germany;* [3]*Migragen i.I., Tuebingen, Germany;* [4]*Institute of Anatomy, University of Tuebingen, Tuebingen, Germany;* [5]*Institute of Brain Research, University of Tuebingen, Tuebingen, Germany*

Study design: Experimental, prospective, blinded, animal study.
Objectives: Subtotal transection models in rodents are widely used in spinal cord injury (SCI) research. In this model, we investigate the effect of the dorso-ventral incision depth (ID) of the spinal cord on functional locomotor outcome using the Basso, Beattie, Bresnahan (BBB) scale. We introduce the minimal incision depth (ID_{min}) and the average lesion depth (ID_{mean}) as reliable, fast and easily available predictive parameters for intrinsic locomotor function.
Setting: Tuebingen, Germany.
Methods: Dorsal over-hemisection at the level of T8 was performed in male Lewis rats. Functional outcome 4 weeks after SCI and histological analysis of the lesion were studied and correlated in 36 animals. Animals reaching weight support (BBB $\geqslant$ 9) were considered as having reached functional recovery. Data analysis was performed in linear (ordinary least squares; OLS) and nonlinear (logistic) regression models for correlation of histological parameters and functional outcome.
Results: BBB scores revealed a strong correlation with ID_{mean} and ID_{min}, showing a higher value in predicting functional outcome for the latter parameter. Based on logistic regression analysis, animals with an ID_{min} of 69% would have a 95% probability of reaching weight support.
Conclusion: These results demonstrate that histological analysis is crucial when functional outcome parameters are used in the dorsal over-hemisection SCI model. A simple and feasible histological evaluation can reliably predict spontaneous functional locomotor recovery in dorsal transection models and could provide a simple tool to identify treatment effects of new experimental therapeutic approaches.
Spinal Cord (2006) **44,** 605–613. doi:10.1038/sj.sc.3101894; published online 3 January 2006

Keywords: functional recovery; histological evaluation; tissue sparing; BBB score; spinal cord injury

Introduction

In spinal cord injury (SCI) research various animal models are used, which mimic pathological mechanisms occurring in humans after SCI.[1] Until now, the rat is the most common animal system used in experimental studies.[2] Although pathomechanisms seem to be similar to the human organism, different injury approaches address different questions and have both, advantages and disadvantages.

Blunt injury models, such as compression or contusion, are believed to induce the most relevant experimental lesion concerning the evolution of the injury over time, and also morphological aspects, such as cavity and cyst formation when compared to humans.[1–3] Furthermore, since the injury remains morphologically incomplete, different grades of white matter sparing can be present depending on the severity of the lesion.[3–5] Much effort has been focused on optimizing these models and a very high reproducibility has been achieved in spinal cord contusion, which represents more than 80% of all SCI in humans, applying almost identical injuries.[2,6]

In contrast, transection models that do not represent as relevant injury mechanisms when compared to

*Correspondence: K Brechtel, Department of Diagnostic Radiology, University of Tuebingen, Hoppe-Seyler-Strasse 3, 72076 Tuebingen, Germany
[6]These authors contributed equally to the paper

nonpenetrating SCIs cover aspects that are not represented by other models.[7] Using partial transections, high selectivity in the investigation of the functional fiber tract systems can be applied.[1,8]

Most commonly in all models, recovery is evaluated by using anatomical and functional outcomes. Antero- and/or retrograde axonal tracing of fiber tracts is used to visualize intact axonal transportation, but does not necessarily imply effects of these fibers on functional recovery.[9–11] In contrast, functional outcome measurements cover the most crucial aspect when locomotor improvement after SCI is evaluated. Besides grid walking,[6,12,13] narrow beam,[6,13,14] or inclined plane tests,[13] Basso *et al*,[15] described a locomotor scale (Basso, Beattie, Bresnahan (BBB) score) evaluating motor performance of the hindlimbs and rating scores from 0 (no movement) to 21 (normal movement of the hindlimbs). Although using a linear scale, the BBB score is rather ordinal because the different aspects of locomotor ability do not contribute equally to functionality.[16] For example, the ability of weight support might be of higher value in means of function when compared to the extent of joint movement.[6] Nevertheless, the BBB locomotor score represents a unique and reliable measuring system and was shown to correlate nicely to different grades of tissue sparing in SCI.[4,6,17]

Different groups have used a subtotal transection approach to show regeneration of fiber tracts and recovery of locomotor functions when experimental interventions have been applied.[18–21] Although the main descending fiber tracts responsible for motor function are transected, functional recovery in this model might be rather due to compensation and plasticity of spared white matter tissue than long distance regeneration of fiber tracts.[22]

Recently, Schucht *et al*,[23] showed that in incomplete transections, sparing of the ventrolateral funiculus might play a key role when functional recovery is observed, most probably due to sparing of fibers in the reticulospinal tract. The data were based on an extensively detailed histological examination of average lesion depth, amount of spared white matter tissue and percentage of spared white matter in the ventrolateral funiculus, with the latter parameter having the highest predictive value of functional recovery in dorsally applied lesions.

When using large cohorts of animals, for example in placebo-controlled treatment studies, fast accessible but reliable parameters could help to improve interpretation of study outcome, especially when locomotor function is analyzed. To implement such time saving and reliable analysis, we tried to evaluate easily assessable histological parameters and correlated them to functional outcome after thoracic over-hemisection.

For characterizing a baseline outcome, we simulated a scenario of a potential treatment that would be applied intraspinally as microinjection. Therefore, animals received an intramedullar application of phosphate-buffered saline (PBS), which was used as placebo substance in two independent studies.[24,25]

Functional locomotor outcome 4 weeks after SCI and histological examination of the lesion depth were performed. By using these outcome parameters, we addressed two issues, whether (i) recovered locomotor activities occur reproducibly in this model and (ii), whether functional outcome can be predicted by a simply accessible histological parameter that was not used earlier by other groups. Therefore, results from functional locomotor recovery according to the BBB scale were correlated with results from histological examination of the lesion site. We studied a new, fast and easily available histological parameter given by the minimal incision depth (ID_{min}), in addition to the average lesion depth (ID_{mean}).

Materials and methods

Animals

Two sets of 20 male Lewis rats ($n = 40$, 245–310 g bodyweight) underwent subtotal spinal cord transection. Both animal sets underwent the same surgical procedure with a time lap of 2 months to achieve reproducibility of data. The animals were kept in pairs under a 12/12 h dark–light cycle, with water and food available *ad libitum*. Animal care was in compliance with the International Health Guidelines under a protocol approved by the Administration District Official Committee.

Surgical procedures

Rats were anesthetized initially with diethyl ether (Chinosol) and subsequently with a mixture of ketamine (Ketanest®, 10 mg/100 g body weight, i.p.; Parke Davis, Germany) and xylazine (Rompun®, 0.24 mg/100 g body weight, i.p.; Bayer, Germany). During surgery, animals were kept on a heating pad to maintain body temperature.

The surgical procedure was performed by two different teams of experienced surgeons under identical sterile conditions according to an in-house standard operating procedure to exclude person-dependent bias. The muscles and ligaments at the spinous process of the vertebral body on thoracic level T8 were removed, and laminectomy was performed with a bone rongeur, taking care to avoid contusive injury of the spinal cord. By using iridectomy microscissors (FST, Germany), the dura mater was opened and the spinal cord was over-hemisected, just sparing parts of the ventral funiculus.

To simulate baseline groups for potential therapies applied intraspinally, an intramedullar injection of 10 μl of PBS (pH 7.4) was applied immediately after SCI. The animals were mounted on a barrel to achieve an inflected position. The dorsal process of T9 was slightly lifted and clamped to avoid movement of the spinal cord during injection caused by respiratory excursions. PBS was injected in 8–10 punctures (1.0–1.5 μl per puncture) rostral to the lesion site by using a stereotactic device in combination with a custom-made patch-clamp microcapillary for exact injection.

The musculature and skin were sutured under sterile conditions. For antibiotic treatment, enrofloxacin (Baytril®, Bayer Vital GMBH, Germany) was subcutaneously injected immediately after surgery. Animals showing signs of bladder infection in the follow-up period were separated and treated with Baytril® for 7 days, or longer if necessary. Manual compression of the urinary bladder was performed twice a day under a mild water-jet until reflex urination was re-established.

Analysis of motor function

The BBB locomotor scale[15] was used for evaluating the open-field locomotor ability of the rats weekly up to 28 days after SCI. Animals moving in an open-field (1.85 m × 0.85 m) were observed for criteria such as extent of joint movements, weight support ability and stepping/walking behavior of the hindlimbs. Functional scores ranging from 0 (no observable hindlimb movement) to 21 (normal locomotion) were assigned for both hindlimbs by two observers independently. The mean value was calculated as the main functional outcome. Scoring was performed for a maximum period of 4 min according to the guidelines of the Spinal Cord Injury Project (WM Keck Center for Collaborative Neuroscience, Rutgers State University of NJ, USA).

Main functional outcome was focused on 28 days after injury. Rats were considered to have achieved significant functional recovery at this time point when a mean BBB score of 9 or more was reached, whereas scores less than 9 were considered to represent minor spontaneous recovery.

Histological analysis

Preparation of lesion tissue Rats were deeply anesthetized with diethyl ether until breathing stopped and perfused via injection of 50–60 ml of fixative solution (4% paraformaldehyde–3% sucrose in PBS, pH 7.4) into the left ventricle. The dissected brain and spinal cord were post-fixed overnight at 2–8°C and cryoprotected in 30% sucrose at 2–8°C. An approximately 1.5 cm piece of the spinal cord, containing the lesion site at the center, was horizontally embedded in TissueTek® (Polyvinyl alcohol, Sakura, Japan) using a custom-made device to prevent oblique embedding. Tissue specimens were stored in liquid nitrogen for 1 day. Sagittal cryosections of 20 μm were serially collected onto silane-coated glass slides. After air drying for 1–2 h at room temperature, the sections were stored at –80°C.

Luxol fast blue staining To identify intact nerve fibers, luxol fast blue (LFB) staining was performed to visualize myelin sheaths. Sections were thawed, air-dried at room temperature for 30 min and dehydrated in 96% ethanol. The sections were incubated in prewarmed LFB staining solution (0.06% Solvent Blue 38, Sigma, Germany, dissolved in 96% EtOH and 0.5% acetic acid) for 30–60 min at 56°C, subsequently differentiated for 5–10 min in alternating baths of 0.01% NaOH and 96% ethanol until excess dye was removed from the gray matter and was retained only in regions of white matter. After a brief wash in distilled water, counterstaining of Nissl substance and nuclei was performed by incubating the sections in 0.1% cresyl violet for 2–3 min at room temperature. Following another brief wash in distilled water, excess cresyl violet was removed by differentiation in 1% acetic acid for approximately 1 min at room temperature. Sections were dehydrated in ethanol baths of increasing concentration (96 and 100%) and in xylol before mounting with Hico-Mic® (Hirtz & Co, Germany).

Lesion size For characterizing the severity of SCI, histological analysis was performed on sagittal sections under a light microscope (Zeiss, Germany), using an ocular with a 10 × 10 eye piece grid at 100–400-fold magnification. Lesion depth and size were reconstructed from a complete set of serial sagittal sections with an interspacing distance of 80–100 μm. Approximately 20 sections were used for characterizing the lesion. For each animal, two histological criteria were used: the ID_{mean} representing the mean value of all sections evaluated. For covering areas of low incision, which might not be expressed by the average value alone and contribute to a higher spontaneous recovery due to sparing of locomotor fiber tracts, the ID_{min} – representing the lowest ID value of all sections evaluated – was used as a second parameter.

For calculating ID values, the dorso-ventral extension of distant reference diameter spinal cord was measured 0.75 cm rostral to the lesion margin. Spared spinal cord (SSC) in the epicenter of the lesion was taken as the percentage when compared to the noninjured spinal cord. ID values were then calculated as ID = 100% – SSC (Figure 1a).

In cases of total transection (ie $ID_{mean} = 100\%$ and $ID_{min} = 100\%$; Figure 1b), additional sagittal sections were analyzed to confirm the lack of SSC.

When comparing the ID values to functional outcome, a grid system was used to distinguish different locations of identical ID_{min} values (Figure 1c). To compare the location of spared white matter in those cases, maps of neurofunctional fiber tracts were taken from the literature.[23,26,27]

Statistical evaluation

For the simple comparison of ID values and of functional outcome scores between set 1 and set 2 animals, Student's *t*-test was used to find differences in means. Values are given as mean ± SD. Differences were considered significant when $P < 0.05$.

When comparing functional outcome and ID values, it was assumed that below a certain ID value all rats would regain significant spontaneous functional recovery after 28 days in means of weight support ability (BBB score of 9).

Logistic regression with the outcome of weight support occurrence and with continuous linear explanatory variable of minimal and mean ID values (linear and quadratic terms) was used to determine the equivalent cutoff point in ID values. From the regression parameters of the logistic model, ID_{mean} and ID_{min} corresponding to 95% estimated probability of weight support was calculated. Linear regression models (OLS) with the BBB score taken as the continuous parameter were fitted as the well and compared to the logistic regression.

Multiple regression was used to investigate whether ID_{min} is an independent and superior predictor of the BBB score when compared to ID_{mean}. A P-value <0.05 was considered significant. Statistical results and graphs were generated by using GraphPad PRISM® Version 3.0 (GraphPad Software Inc., San Diego, USA) and Stata® 6.0 (Stata Corporation, College Station, USA).

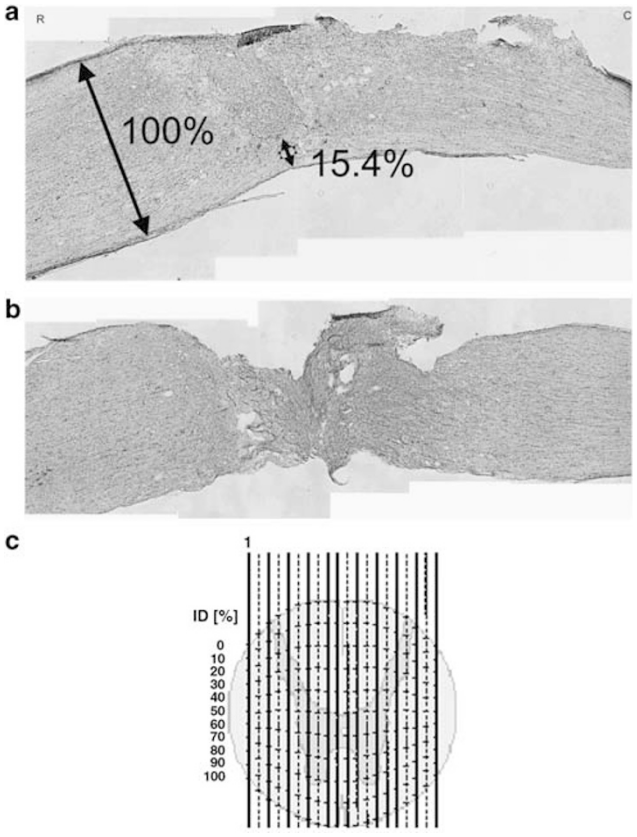

Figure 1 Examples of luxol fast blue-stained sagittal sections of rat spinal cord (T8) in cases of different ventral sparing (R: rostral; C: caudal). (**a**) A dorsal lesion. Subtotal ID was evaluated in this section as 84.6%. (**b**) An ID of 100%, where only scar tissue can be found in the lesion epicenter. (**c**) A schematic grid system in which ID values can be transferred for two-dimensional reconstruction (see also Figure 5)

Results

Inclusion and mortality

A total of 40 animals were included in the study. Three animals did not survive the complete observation period of 28 days. Owing to the poor quality of tissue preparation, one animal was excluded from histological evaluation. This animal showed a BBB score of 7 after 28 days. In total, 36 animals underwent both evaluation methods, neurofunctional and histological (Table 1).

Functional outcome

Functional outcome was measured in 36 animals (set (1) $n=17$, set (2) $n=19$) according to the BBB scale ranging from 0 to 21 points. Comparison of the BBB mean values at 1, 2, 3 and 4 weeks did not reveal significant differences between both groups at any time point (Figure 2a). The mean BBB value of all animals after 4 weeks ranged between 4 and 5, indicating the ability to move all observed joints of the hindlimbs at least slightly.

As described above, BBB scores of 9 and higher were considered as indicating remarkably high recovery of locomotor function. Taking this into account, a total number of 10 animals (28%) showed BBB scores $\geqslant 9$. Distribution of these animals between the groups was not significantly different ($P=0.25$; Table 1, Figure 2b).

Histological evaluation of incision depth

For histological evaluation, sagittal sections of all spinal cords were examined. The lesion center was characterized by destruction of axons and gray matter. Owing to the observation period of 28 days, scar tissue, was present in most cases. When compared to healthy

Table 1 Data summary for separate and pooled animal sets

	Set 1		*Set 2*		*Total*	
Animals included	20	—	20	—	40	—
Animals surviving 28 days	20/20	(100%)	17/20	(85%)	37/40	(92.5%)
Drop outs	1/20[a]	(5%)	3/20	(15%)	4/40	(10%)
ID_{mean} (%)	93.5	(8.2)[b]	93.3	(11.6)[b]	93.4	(9.8)[b]
ID_{min} (%)	82.1	(17.3)[b]	85.7	(19.8)[b]	83.8	(18.3)[b]
Weight support after 28 days	6/19	(32%)	4/17	(24%)	10/36	(28%)
Mean BBB score after 28 days	5.1	(4.3)[b]	3.9	(4.6)[b]	4.5	(4.4)[b]

[a]Histology not performed due to minor tissue quality
[b]SD

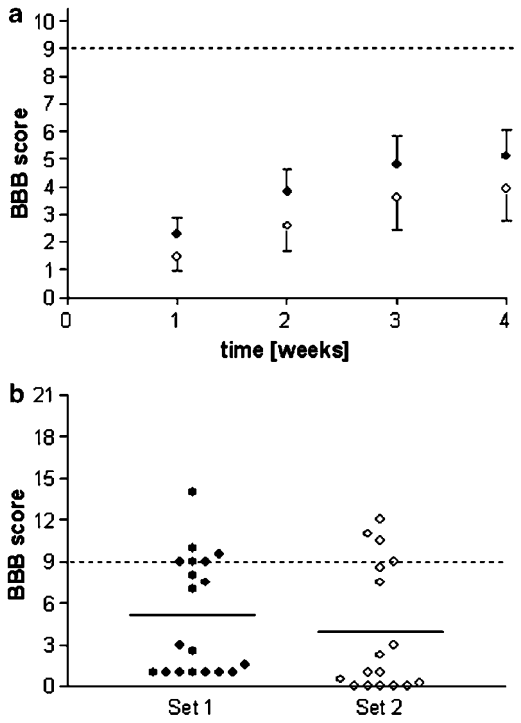

Figure 2 Time course of the functional locomotor outcomes (mean BBB scores ± SD) and comparison of the individual BBB values 28 days after SCI (dashed lines indicating weight support). (**a**) and (**b**) No significant differences were observed when set 1 and set 2 animals were compared

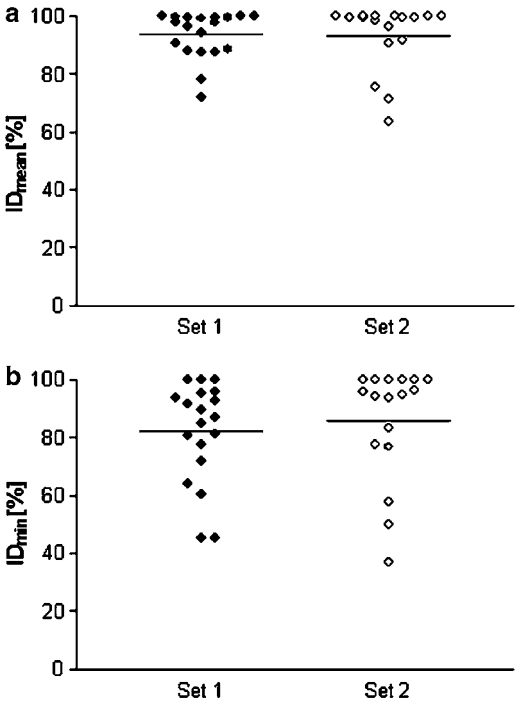

Figure 3 Comparison of ID values for set 1 and set 2 animals. Neither mean values nor distribution of both ID_{mean} (**a**) and ID_{min} (**b**) revealed differences between the groups

neuronal tissue the cellularity of the lesion epicenter was hypodense. The scar tissue surrounding the lesion area was hyperdense. Cavity formation, was observed extending from the lesion center to the rostral and caudal edges of the incision. Tissue bridges composed of longitudinally oriented nerve fibers were found in the ventral funiculus connecting the rostral and the caudal edges of the lesion (Figure 1a). In cases of complete transection no neuronal tissue bridge was found (Figure 1b).

In conclusion, spared tissue was identified as iso-cellular as seen in healthy neuronal tissue and characterized by nondisrupted fiber tracts.

According to both ID_{mean} and ID_{min} the animal sets were comparable (Figure 3a and b, Table 1). ID_{mean} values ranged from 63.7 to 100%, with a mean value of 93.4 ± 9.8% (Table 1). When evaluating the ID_{min}, the values ranged from 36.8 to 100% in cases of complete transections (mean ID_{min} ± SD: 83.8 ± 18.3%; Table 1).

As there were no statistical differences in distribution and absolute values of BBB scores and ID parameters, all further analysis was performed on data pools of set 1 and set 2 animals.

Comparison of functional outcome and histological evaluation

ID_{mean} versus BBB score Functional outcome (BBB score) and ID_{mean} were compared to determine ID thresholds for predicting remarkable functional recovery, that is, BBB score $\geqslant 9$ (Figure 4a and b). Logistic regression with outcome of weight support and ID_{mean} as continuous linear variable revealed a value of 84% for having weight support ability (95% probability; Figure 4a).

When using OLS regression analysis with the BBB score as the continuous variable, an ID_{mean} between 89 and 90% was calculated for reaching 9 on the BBB scale (Figure 4b).

ID_{min} versus BBB score The same analysis as shown for ID_{mean} was performed for the ID_{min} (Figure 4c and d). The 95% probability of having weight support revealed an ID_{min} value of 69% (Figure 4c). When using OLS regression analysis, a BBB score of 9 was achieved when ID_{min} indicated a value between 68 and 69%, which was almost identical to the result from the logistic regression (Figure 4d).

ID_{mean} and ID_{min} as predictors of the BBB score When including ID_{mean} and ID_{min} in a multiple regression model for predictivity of functional locomotor outcome after 28 days, a better fit was obtained than when including ID_{mean} alone into the analysis ($t = -2.96$, $P = 0.006$). This suggests ID_{min} as an independent, superior predictor of functional locomotor outcome after 28 days; however, R^2 only increased by 0.06 (0.73 in the univariate regression model *versus* 0.79 in the multiple regression model).

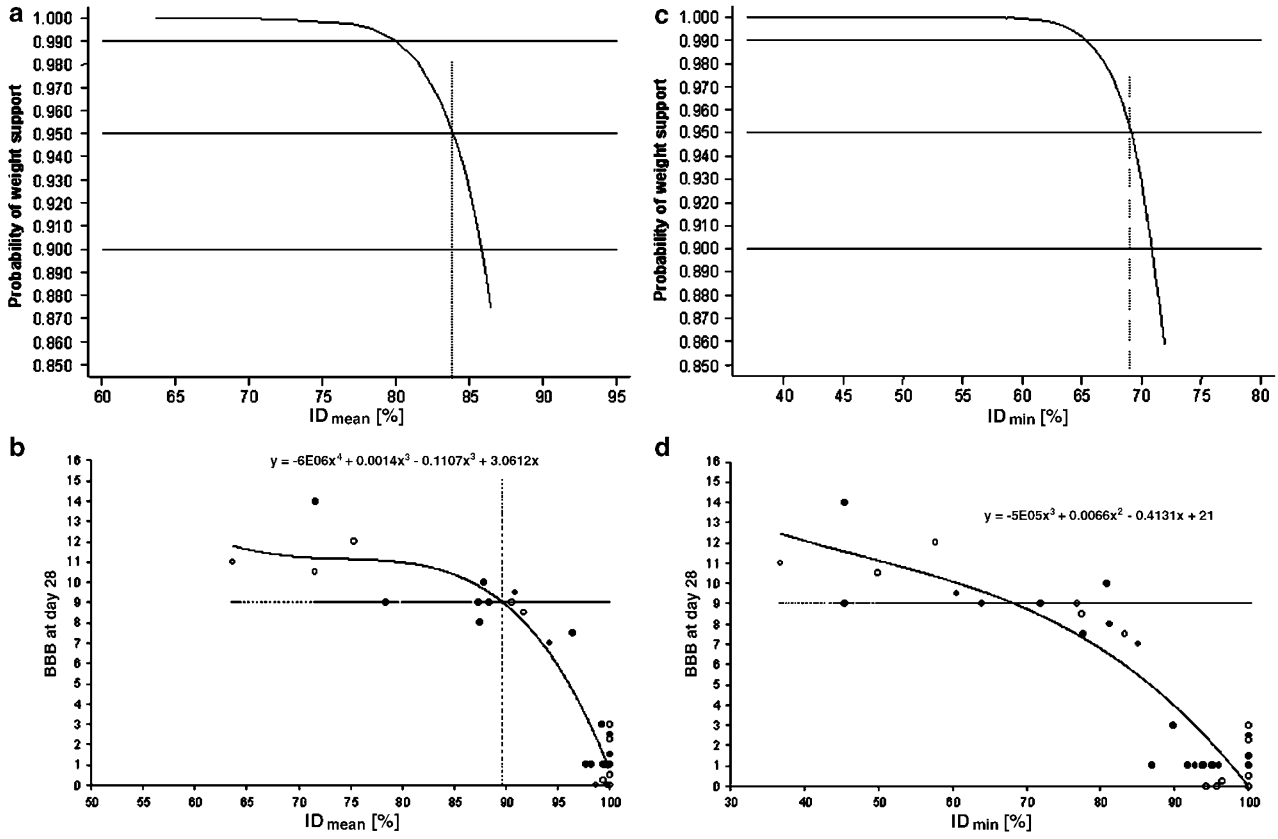

Figure 4 Logistic (**a**, **c**) and OLS (**b**, **d**) regression models for ID_{mean} and ID_{min} values *versus* BBB scores after 28 days post SCI (set 1: black dot; set 2: open dot). For ID_{mean} (**a**, **b**), regression analysis from the two models showed different results, whereas for ID_{min} (**c**, **d**), results were almost identical when weight support (BBB: 9) was taken as significant functional recovery

Sparing of lateral fiber tracts To increase the validity of the chosen parameters, we characterized variable outcomes of outliers to minimize the chance that functional outcome might implicate false-positive effects. As reported by others, the ventrolateral funiculus highly contributes to functional outcome.[23] In two pairs of animals presenting identical ID_{min} values but different functional outcomes ($\Delta BBB > 4$ or weight support yes/no), additional analysis was performed to localize sparing of functional fiber tracts (Figure 5). For the first matched pair with an ID_{min} of 45.5% (Figure 5a and c), higher lateral sparing occured in the animal presenting better functional outcome.

In a second matched pair, ID_{min} of 81% was observed almost on the same position on contralateral sides (Figure 5b and d). However, the animals presented significantly different outcomes in terms of weight support and plantar stepping (8 *versus* 10 on the BBB scale). In these cases – when animals closely reach weight support – ID_{min} might loose its predictive value.

Discussion

The present study investigated the degree of ventral spinal cord sparing on functional outcome in animals with dorsal over-hemisection of the thoracic spinal cord in order to evaluate reliability of this SCI model. We used a setup with an intramedullar injection of PBS to simulate a control baseline for interventional therapy studies, when hydrophobic or cellular compounds were used, which do not cross the blood–brain barrier by itself. We showed a strong correlation between the depth of the dorsal transection defined by histological analysis and the grade of spontaneous functional recovery, which was also shown earlier by other groups using extensive histological analysis.[23]

In detecting very simple parameters, such as ID_{mean} and ID_{min}, the transection model can be characterized as demonstrated in two independent experiments. We could establish a new parameter (ID_{min}), which is different from former approaches and turned out to be superior when compared to the mean value (ID_{mean}) in predicting functional locomotor outcome after dorsal over-hemisection SCI. Nevertheless, location of white matter sparing might play a key role when neurofunction is used as primary outcome in SCI studies.

Spared white matter tissue, SSC and average lesion depth or lesion volume are parameters often used to investigate lesion severity in penetrating and nonpenetrating SCI.[23,28,29] Some of these parameters correlate well with neurofunctional outcome in terms of BBB evaluation. Recently, Schucht *et al*[23] could demonstrate

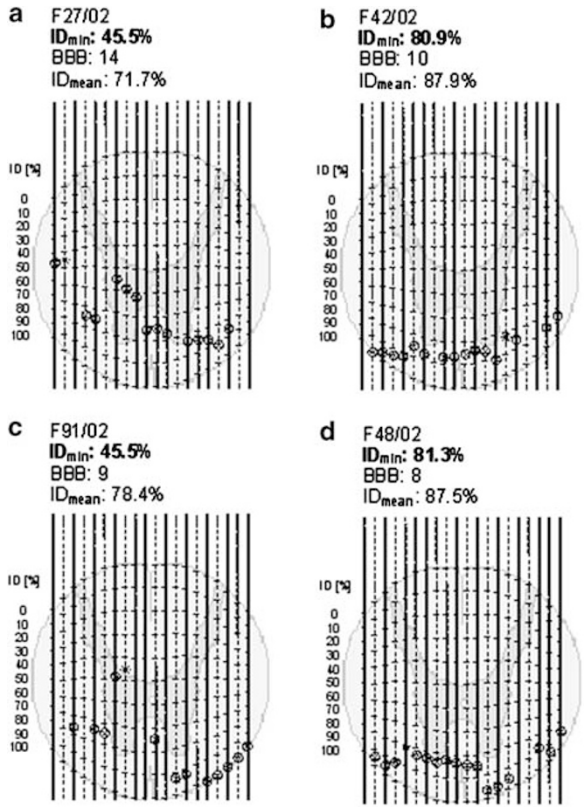

Figure 5 Two-dimensional reconstruction of lesion epicenters on a schematic grid system (see also Figure 1c). ID_{min} values are indicated as (*). Pairs were matched for low (**a**, **c**) and high (**b**, **d**) ID_{min}, but presented significant different functional outcomes. Data points are missing in sections with poor tissue quality

that in both ventral and dorsal transections of the spinal cord, different parameters can predict functional outcome of rats with sparing of the ventrolateral funiculus being more superior than SSC and spared white matter tissue. These findings were specifically true for dorsal lesions since functional fiber tracts in the ventrolateral funiculi have an extraordinary potential for contributing to functions such as open-field locomotion.[23,27] The importance of axon sparing in ventrolateral and lateral funiculus was emphasized by other studies as well, in which the reticulospinal tract as one of the critical projections descending at these positions was shown to be of highest importance for the initiation of locomotion.[22,30]

In this study, we focused on a new histological parameter as the ID_{min} value because more precise evaluation of the reticulospinal tract would require costly and time-consuming tracing procedures. When compared to the ID_{mean} as detected in our study, ID_{min} seems to have a superior predictive value on functional outcome. Therefore, ID_{mean} does not appear to be reliably describing lesion severity, since the range of spared tissue is likely to be obscured, especially in cases of assymmetric lesions. However, ID_{min} seems to be inferior when compared to evaluations of separate functional fiber tracts as performed by other groups when correlation coefficients are compared.[23] In our experiments, this was evident when large differences were observed in the BBB scores of rats that presented almost identical ID_{min} values. The detailed histological analysis of the lesion center revealed differences in the positions of ID_{min} values that were detected more towards the lateral sides of the spinal cord. In this example, ventrolateral sparing indicated better functional outcome (Figure 5a and c). However, comparison of two other animals with similar ID values, with and without weight support, were not distinguishable by analyzing tissue sparing (Figure 5b and d). In such cases, further histological analysis different from our approach might be necessary.

Our data suggest that the estimation of the ID_{min} is an easy and feasible alternative to characterize lesion severity in a high number of samples. The results from this study were reproducible in two independent sets of animals undergoing the same surgical procedure and identical assessment of locomotion abilities after 4 weeks. Comparing the mean outcome value after the observation period, there was a slight nonsignificant difference of 1 point on the BBB scale between both sets of animals. Compared to data from other groups using a similar approach, the mean outcome values were in the same range.[4,28]

The choice of an additional intramedullar injection should simulate a setup for therapies that do not cross the blood–brain barrier *per se* by taking into account that a potential, additional traumatic lesion could have been induced by the intramedullar injection in the rostral stump of the lesion. Despite the fact that microtraumatic injury of the injection device was detected histologically, unpublished data from our lab could demonstrate that these lesions were neurologically silent and there was no locomotion deficit when this application approach was used in healthy, noninjured rats (JM Schwab, personal communication). This would put the described experimental setup to the method of choice for control groups when hydrophobic therapeutic agents or even intramedullar applied cell therapies are used in transection models.

Among the 36 animals analyzed, 28% achieved a minimum BBB score of 9, corresponding to regained weight support after a period of 28 days. Weight support is necessary to perform tasks requiring body balance,[17] and hence is considered here as remarkable intrinsic functional recovery in rats. This is in accordance with data of others, which showed that in the absence of any therapy, rats can perform basic rhythmic movements of the hindlimbs, or even weight support abilities.[28] Interestingly, there is evidence that the BBB scoring system is rather an ordinal than a linear score, and parametric statistical analysis would fit the nature of this scale better.[16] Therefore, for confining a threshold in animals to superior or inferior spontaneous recovery, we assigned the ability of weight support in a logistic regression model instead of using a linear regression model as performed by others.[23] This statistical

approach enabled us to give predictive information on probabilities of achieving a positive outcome in terms of weight support. In contrast, linear and OLS regression models (also shown in this paper) do not respect the ordinal nature of the score and might lead to differences in results. By employing both analyses strategies to our data, there was a difference of 5% in ID_{mean} values for the achievement of a BBB of 9. However, the analysis of ID_{min} revealed almost identical results for both, the nonlinear and linear regression models.

Findings from the transection model are in accordance with results from other lesion models, such as contusive injury, which showed a correlation of spared peripheral tissue and functional outcome. In rats with less than 5% of tissue sparing after contusion injury (height of weight drop: 50 mm), a mean BBB lower than 9 was observed, whereas rats with only 10% sparing (height of weight drop: 25 mm) presented occasional to consistent weight-supported plantar stepping (mean BBB: 10.6)[4] suggesting that minimal tissue sparing might be sufficient to initiate or trigger segmental circuits that are essential for basic functional locomotion. As these data were taken from concentric lesions, it is very likely that in dorsal transections ventral sparing is more sensitive for intrinsic locomotor recovery, although one has to keep in mind that in any kind of incomplete injury these segmental circuits are provided if ventral sparing occurs.

However, for intrinsic locomotor recovery, the plasticity potential of central nervous system motor tracts is crucial. This potential becomes more pronounced in incomplete injuries, since a certain extent of spinal cord circuitry would remain intact.[31,32] In any SCI experiment, animals would then have different potential for developing locomotor function from the beginning of the observation period due to different grades of sparing in those areas. This could also explain very early effects in functional recovery. In interpreting outcomes after weeks with or without experimental treatment, the re-establishment and regulation of these circuits – for example induced by sprouted axons from the ventral side of the lesion – will have a disproportionately large effect on the total outcome of the study. Therefore, matching of treated and untreated animals using histological parameters such as ID_{min} would increase reliability with respect to functional outcome. By using our logistic regression model with weight support as positive outcome for neurofunction, 50, 95 and 99% probabilities can be estimated occurring at ID_{min} of 77, 69 and 66% respectively. This model could provide a tool for distinguishing between true and false-positive results in neurofunctional outcome when experimental therapies are approached with a dorsal over-hemisection SCI by using a comparably easy accessible, effective histological parameter. As mentioned before, in asymmetric lesions, lateral sparing contributes to unproportional high functional outcome, which might lead to a loss of predictivity of both ID_{min} and ID_{mean}.

In conclusion, regarding the significant correlation of injury severity in spinal cord to functional outcome, it is crucial to perform a detailed analysis of the lesion center in experimental animal studies of incomplete spinal cord transection. This is especially important in experimental models with low consistency in lesion severity as in the case of subtotal transection. This model might lead to differences in the extent of tissue sparing, and, eventually, in an unexpected high spontaneous recovery of animals in therapy or control groups. Histological analysis can be performed very easily with simple methods and spontaneous functional outcome can be predicted from these data. This might be very important for evaluating effects on locomotion of experimental therapies when a dorsal transection model is used.

Acknowledgements

We would like to acknowledge Wasima Akbari, Annelie Falk, Andreij Gerbert, Mirko Koehler, Adrian Krestel, Sabine Rawer, Reuben Siedner and Manfred Schorpp for technical assistance. Special thanks to Akos Pap and Algirdas Kakarieka for helping with the manuscript and giving valuable advice in study design and data processing.

References

1 Kwon BK, Oxland TR, Tetzlaff W. Animal models used in spinal cord regeneration research. *Spine* 2002; **27:** 1504–1510.
2 Taoka Y, Okajima K. Spinal cord injury in the rat. *Prog Neurobiol* 1998; **56:** 341–358.
3 Gruner JA. A monitored contusion model of spinal cord injury in the rat. *J Neurotrauma* 1992; **9:** 123–128.
4 Basso DM, Beattie MS, Bresnahan JC. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol* 1996; **139:** 244–256.
5 Fehlings MG, Tator CH. The relationships among the severity of spinal cord injury, residual neurological function, axon counts, and counts of retrogradely labelled neurons after experimental spinal cord injury. *Exp Neurol* 1995; **132:** 220–228.
6 Metz GA, Curt A, van de Meent H, Klusman I, Schwab ME, Dietz V. Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. *J Neurotrauma* 2000; **17:** 1–17.
7 Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Yoles E, Fraidakis M *et al*. Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. *Nat Med* 1998; **4:** 814–821.
8 Hill CE, Beattie MS, Bresnahan JC. Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. *Exp Neurol* 2001; **171:** 153–169.
9 Huang DW, McKerracher L, Braun PE, David S. A therapeutic vaccine approach to stimulate axon regeneration in the adult mammalian spinal cord. *Neuron* 1999; **24:** 639–647.
10 Kim ES, Kim GM, Lu X, Hsu CY, Xu XM. Neural circuitry of the adult rat central nervous system after spinal cord injury: a study using fast blue and the Bartha strain of Pseudorabies virus. *J Neurotrauma* 2002; **19:** 787–800.
11 Tsai EC, van Bendegem RL, Hwang SW, Tator CH. A novel method for simultaneous anterograde and retrograde

labeling of spinal cord motor tracts in the same animal. *J Histochem Cytochem* 2001; **49:** 1111–1122.
12 Goldberger ME, Bregman BS, Vierck CJ, Brown M. Criteria for assessing recovery of function after spinal cord injury: Behavioral methods. *Exp Neurol* 1990; **107:** 113–117.
13 Kunkel-Bagden E, Dai HN, Bregman BS. Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. *Exp Neurol* 1993; **119:** 153–164.
14 Hicks S, D'Amaro CJ. Motor-sensory cortex-corticospinal system and developing locomotion and placing in rats. *Am J Anat* 1975; **143:** 1–42.
15 Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995; **12:** 1–21.
16 Scheff SW, Saucier DA, Cain ME. A statistical method for analyzing rating scale data: the BBB locomotor score. *J Neurotrauma* 2002; **19:** 1251–1260.
17 Metz GA, Merkler D, Dietz V, Schwab ME, Fouad K. Efficient testing of motor function in spinal cord injured rats. *Brain Res* 2000; **883:** 165–177.
18 Bregman BS, Kunkel-Bagden E, Schnell L, Dai HN, Gao D, Schwab ME. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. *Nature* 1995; **378:** 498–501.
19 Brosamle C, Huber AB, Fredler M, Skerra A, Schwab ME. Regeneration of lesioned corticospinal tract fibers in the adult rat induced by a recombinant, humanized IN-1 antibody fragment. *J Neurosci* 2000; **20:** 8061–8068.
20 Dergham P, Ellezam B, Essagian C, Avedissian H, Lubell WD, McKerracher L. Rho signalling pathway targeted to promote spinal cord repair. *J Neurosci* 2002; **22:** 6570–6577.
21 Grandpre T, Li S, Strittmatter SM. Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature* 2002; **417:** 547–551.
22 Loy DN, Magnuson DS, Zhang YP, Onifer SM, Mills MD, Cao QL *et al*. Functional redundancy of ventral spinal locomotor pathways. *J Neurosci* 2002; **22:** 315–323.
23 Schucht P, Raineteau O, Schwab ME, Fouad K. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the spinal cord. *Exp Neurol* 2002; **176:** 143–153.
24 Cheng H, Wu JP, Tzeng SF. Neuroprotection of glial cell-line derived neurotrophic factor in damaged spinal cords following contusive injury. *J Neurosci Res* 2002; **69:** 397–405.
25 Xu XM, Guenard V, Kleitman N, Aebischer P, Bunge MB. A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. *Exp Neurol* 1995; **134:** 261–272.
26 Kuchler M, Fouad K, Weinmann O, Schwab ME, Raineteau O. Red nucleus projections to distinct motor neuron pools in the rat spinal cord. *J Comp Neurol* 2002; **448:** 349–359.
27 Tracey DJ. Ascending and descending pathways in the spinal cord. In: Paxinos G (ed). *The Rat Nervous System*. Academic Press: San Diego 1995, pp 67–80.
28 Merkler D, Metz GA, Raineteau O, Dietz V, Schwab ME, Fouad K. Locomotor recovery in spinal cord-injured rats treated with an antibody neutralizing the myelin associated neurite growth inhibitor Nogo-A. *J Neurosci* 2001; **21:** 3665–3673.
29 Rabchevsky AG, Fugaccia I, Sullivan PG, Blades DA, Scheff SW. Efficacy of methylprednisolone therapy for injured rat spinal cord. *J Neurosci Res* 2002; **68:** 7–18.
30 Pearson KG. Neural adaptation in the generation of rhythmic behaviour. *Annu Rev Physiol* 2000; **62:** 723–753.
31 Raineteau O, Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. *Nat Rev Neurosci* 2001; **2:** 263–273.
32 Schwab ME. Repairing the injured spinal cord. *Science* 2002; **295:** 1029–1031.