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MINI-REVIEW
Assessment of behavioural recovery following spinal cord injury in rats

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Abstract

Behavioural recovery is one of the primary goals of therapeutic intervention in animal models of disease. It is necessary, therefore, to have the means with which to quantify pertinent behavioural changes in experimental animals. Nevertheless, the number and diversity of behavioural measures which have been used to assess recovery after experimental interventions often makes it difficult to compare results between studies. The present review attempts to integrate and categorize the wide variety of behavioural assessments used to measure recovery in spinal-injured rats. These categories include endpoint measures, kinematic measures, kinetic measurements, and electrophysiological measurements. Within this categorization, we discuss the advantages and disadvantages of each type of measurement. Finally, we make some recommendations regarding the principles for a comprehensive behavioural analysis after experimental spinal cord injury in rats.

Introduction

The development of appropriate animal models for specific central nervous system (CNS) disorders is critical for meaningful assessments of physiology, pathology and effective therapies. Ideally, the behavioural responses of animal models should be relevant to the clinical signs seen in human patients. It is necessary therefore to have the means with which to quantify pertinent behavioural changes in experimental animals, especially as recovery of behavioural function is one of the primary goals of therapeutic intervention in both animal models of disease and in human patients.

The recent advances in transplantation technology and therapies aimed at the CNS immune response, and the subsequent widespread applications of these and other regimes to animal models of spinal injury, emphasize the need for a comprehensive review of behavioural assessments after experimental spinal injury (Kim *et al.*, 1999; Liu *et al.*, 1999; McDonald *et al.*, 1999; Popovich *et al.*, 1999; Schwartz *et al.*, 1999). The variety of methods used to assess behavioural recovery, especially motor abilities, often makes it difficult to compare results between studies. This review attempts to categorize the behavioural measures that are used to assess recovery of function after spinal injury in the rat, and provides an indication of the benefits and limitations of each measure.

Laboratory rodents perform a wide variety of behaviours, many of which are relevant to assessing the integrity of spinal pathways. These include behaviours which involve the whole body, e.g. locomotion over a flat surface or along a narrow beam, as well as behaviours involving primarily one or more individual limbs, e.g. reaching for food pellets with a forelimb or rearing on the hindlimbs. It is important to note, however, that all behaviours involve the entire body to some extent. Reaching for a food pellet, for example, requires that the animal shifts its weight onto the non-reaching limbs, using trunk muscles as well as muscles of the weight-bearing limbs (Miklyaeva *et al.*, 1997). In addition, compensation for functional deficits induced by CNS lesions may involve recruitment of limbs and muscles that are not used in unlesioned animals, and this must be taken into account during assessment of behaviour in these animals. Finally, it should be emphasized that all behaviours involve sensorimotor integration, such that impairment of sensory function, motor function or both may result in measurable deficits in any task (Miklyaeva *et al.*, 1997).

There have been several reviews which have outlined procedures for assessing function after spinal injury (Goldberger *et al.*, 1990; Wrathall, 1992; Kunkel *et al.*, 1993). Among other recommendations, these authors have emphasized the need for a battery of tests for functional assessment. This is necessary not only to completely assess the functional abilities of the animal but also to validate the effectiveness of individual tests for measuring the integrity of a particular neural pathway. The relative importance of different pathways involved in most behaviours are incompletely known and thus, measurement of an animal's performance in a number of behaviours which are thought to use some of the same neural pathways allows more reliable conclusions to be drawn regarding the integrity of those connections.

The present review attempts to make sense of the wide variety of behavioural assessments used to measure recovery in spinal-injured rats by categorizing these tests according to the type of data collected. These categories include: (i) endpoint measures, in which behaviour is scored according to some goal to be reached; (ii) kinematic measures, which can range from qualitative description of movements to continuous quantitative measurements; (iii) kinetic measurements, which quantify the force produced by a limb or limbs; and (iv) electrophysiological measurements, e.g. electromyographic evaluation of muscle activity patterns during a particular behaviour. Within this categorization, we identify measures which primarily assess whole body behaviours, e.g. locomotion, as well as measures

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TABLE 1. Rodent behaviours suitable for assessment with endpoint, kinematic, kinetic and/or electrophysiological techniques

Behaviour	Endpoint measured*	Concurrent measurements†	Example references
Whole body			
Walking on runways/ropes	Length of time, number of steps necessary to cross a runway or beam Number of errors (foot slips) crossing the beam (beams can be of various widths)	Kinematics, kinetics, electrophys.	(Kunkel *et al.*, 1993; Miya *et al.*, 1997)
Grid/ladder walking	No. of foot falls through grid/no. of steps (grids or ladders can be of various sizes)	Kinematics, electrophys	(Kunkel *et al.*, 1993; Prakriya *et al.*, 1993; Soblosky *et al.*, 1996, 1997)
Rope climbing	No. of foot slips/total no. steps	Kinematics, electrophys	(Carlini *et al.*, 1967; Z'Graggen *et al.*, 1998)
Swimming	Time to swim the length of a tank of water	Kinematics	(Gruner & Altman, 1980; Roy *et al.*, 1991)
Inclined plane	Angle at which an animal can no longer maintain its position on an inclined surface	Kinematics, electrophys.	(Rivlin & Tator, 1977; Gale *et al.*, 1985)
Reflex righting	Length of time required to move from supine to prone position	Kinematics, electrophys.	(Kerasidis *et al.*, 1987; Kunkel *et al.*, 1992; Diener & Bregman, 1998a)
Individual limb			
Forelimb reaching	Time taken to obtain or eat a fixed no. of pellets No. of pellets obtained or eaten in a fixed period of time No. of pellets eaten out of three attempts to eat pellet No. of pellets obtained from staircase test	Kinematics, kinetics, electrophys.	(Whishaw & Pellis, 1990; Montoya *et al.*, 1991; Whishaw *et al.*, 1993, 1997; Diener & Bregman, 1998a; McKenna & Whishaw, 1999a,b; Ballermann *et al.*, 2000)
Hindlimb rearing/pushing	Angle of slope at which animals can propel themselves along Time required to climb over a ledge using hindlimbs	Kinematics, kinetics, electrophys.	(Ramon-Cueto *et al.*, 1999)
Spontaneous paw use	Proportion of time each paw is used in a behaviour and/or for support during locomotion or exploration	Kinematics, electrophys.	(Liu *et al.*, 1999; Schallert *et al.*, 2000)
Sticker removal	No. of times sticker removed from limb or forehead/no. attempts to remove sticker Latency to remove or notice sticker	Kinematics, electrophys.	(Diener & Bregman, 1998b)
Paw lick	Latency to lick paws when placed on a hot surface	Kinematics, electrophys.	(Gale *et al.*, 1985)
Tail flick	Latency to flick tail away from a focused source of heat	Kinematics, electrophys.	(Gale *et al.*, 1985)
Reflex toe spread	Distance that toes spread apart after animal is suddenly lowered	Kinematics	(Blight, 1994)
Reflex withdrawal	Degree to which animal withdraws its limb following toe pinch	Kinematics, kinetics, electrophys.	(Gale *et al.*, 1985)
Reflex placing	Placement of foot on a horizontal surface after dorsum of foot contacts surface edge	Kinematics, kinetics, electrophys.	(Gale *et al.*, 1985; Kunkel *et al.*, 1993; Blight, 1994)
Reflex hopping	Replacement of limb appropriately when bodyweight is shifted by examiner (can use treadmill to move limb while holding body)	Kinematics, kinetics, electrophys.	(Kunkel *et al.*, 1993; Olsson *et al.*, 1995)
Cutaneous trunci reflex	Contraction of cutaneous trunci muscle in response to cutaneous stimulation	Kinematics, electrophys.	(Blight *et al.*, 1990; Blight, 1994)

*See example references for full description of methods. †Techniques which have been used or are possible to use to provide detailed information on the performance of each behaviour.

which might be more useful for examining function of individual limbs. Finally, we make some recommendations regarding the principles for a comprehensive behavioural analysis after experimental spinal cord injury.

Endpoint measures

Endpoint measures generally require that animals accomplish a particular goal and are then scored based on their ability to reach that goal. For behaviour involving the entire body, these measures include tasks as varied as the time required to cross a length of beam or to climb a rope (Kunkel *et al.*, 1993), the number of times a paw slips through spaces in a ladder while the animal walks over it (Soblosky *et al.*, 1996), or the angle at which the animal can no longer maintain a fixed position on a tilted surface (Rivlin & Tator, 1977). For individual limbs, endpoint measures include number of food pellets eaten in a fixed time period, or time to remove a sticker placed on a paw. A list of endpoint measures for a variety of behavioural tasks and reflexes is found in Table 1.

The use of endpoint measures to assess functional abilities of an animal has both benefits and limitations. The main advantages of endpoint measures are that they are objective, relatively simple to score, and, once the animals are trained in the task, quick to perform. One of the major limitations of endpoint measures, however, is that they provide no indication of how the task is being performed. An animal with limited use of its hindlimbs due to a thoracic spinal injury, for example, may become more proficient at using its forelimbs to pull itself along a runway, or to maintain its position on a tilted table (Cheng *et al.*, 1997). Such compensation could mistakenly be scored as 'recovery' using endpoint measures (Whishaw *et al.*, 1997; McKenna & Whishaw, 1999a,b). Animals will compensate behaviourally in complex ways to perform a particular task, and measurement of endpoints alone cannot distinguish this behavioural compensation from 'true' recovery.

Another limitation of endpoint measures is that training of the animals in the task to be performed is required. Importantly, each animal's pre-lesion competence in a task determines, to some extent, its performance post-lesion. Thus, it is necessary to have both control and experimental groups of animals at similar levels of performance prior to lesioning. When comparing results between different studies, it is also important to control for the age and strain of the animal, as performance on some tasks varies with both of these factors (Wallace *et al.*, 1980; Krauter *et al.*, 1981; Biesiadecki *et al.*, 1999).

The type of data obtained directly from most endpoint measures consist of quantitative data, e.g. time to cross a beam or number of pellets eaten in a given time period (see Table 1). In some cases, however, a scoring system is devised which produces ordinal rating scales, e.g. success in climbing a ladder at an angle of 45° may correspond to a score of 1, success at 60° corresponds to 2, etc. (Ramon-Cueto *et al.*, 1999). Similarly, the number of footslip errors made during grid-walking may be converted to an ordinal scale, e.g. 0–3 errors may correspond to a score of 1, 4–7 errors to a score of 2, etc. Results from a number of different ordinal measures have also been summed in an attempt to provide an overall indication of motor performance (Gale *et al.*, 1985; Kerasidis *et al.*, 1987).

Derivation of ordinal rating scales, though easy to perform, is not a trivial matter, and the use of such scoring systems should be treated with caution. Ordinal scales differ from continuous quantitative data in several ways (Kerlinger, 1986). Most importantly, the distinction between each category or score is arbitrarily defined, such that the intervals between the scores cannot be assumed to be equal. This produces some difficulties when satisfying the assumptions for statistical analysis (see 'Locomotor rating scales', below), but probably the most important difficulties arise with interpretation of the data. The natural tendency is to treat the scores as if they were numbers on a continuous numerical scale. Thus, for a devised ordinal scale in which 1 is the 'worst' performance and 10 is the 'best' performance, an improvement in score from 1 to 3 would be judged as the same amount of improvement as change in score from 4 to 6, which would not necessarily be the correct interpretation. Rating scales also carry a subjective bias with respect to the set of characteristics comprising each category ranging from the 'best' to the 'worst' performance. There are, of course, situations where ordinal rating scales are the only means by which to appropriately score a behaviour. For example, the measurement of many reflex responses requires the derivation of ordinal scales. Response to reflex stimulation may be judged to be present or absent, e.g. scored as either 1 or 2, or in some cases it may be necessary to make a distinction between normal, increased and decreased responses (e.g. scored on a scale of 3 or more, Gale *et al.*, 1985; Kerasidis *et al.*, 1987). Nevertheless, the derivation of ordinal rating scales, though necessary and useful in some instances, should be avoided if continuous quantitative data can be obtained.

Kinematic measures

Kinematic measures are those which describe, quantitatively or otherwise, the movement of the whole body and body segments relative to each other and/or to an external frame of reference. Those measures which have been used or are appropriate for use to assess functional abilities after spinal injury in rodents include qualitative kinematic measures, e.g. Eshkol–Wachmann movement notation (Whishaw *et al.*, 1991), ordinal rating scales devised for describing locomotor movements, e.g. the BBB scale (Basso *et al.*, 1995), and continuous kinematic data, so-called because of the continuous nature of the measurement data.

Qualitative measures

Eshkol–Wachmann (EW) movement notation was originally created for recording dance movements in humans and has been used to describe the detailed motion of rat forelimb movements during precision reaching (Whishaw & Pellis, 1990; Whishaw *et al.*, 1991, 1992). Through single-frame analysis of videotaped recordings, each behaviour is subdivided into separate movements of the component limbs and limb segments, and a rating scale is applied to each component. The strength of this approach is that the individual movements by which an animal accomplishes a task can be identified in detail. EW notation of reaching movements in animals with and without CNS lesions reveals differences that are not noted from cursory visual examination of videotapes nor can these differences be detected using endpoint measures of reaching success (Whishaw & Pellis, 1990; Whishaw *et al.*, 1991, 1992; McKenna & Whishaw, 1999a,b). Although EW notation is not a quantitative method, it has proven useful as a screening device to identify movement differences, which can subsequently be quantified using continuous kinematic measures described below (McKenna & Whishaw, 1999a,b).

Locomotor rating scales

Ordinal rating scales used for locomotor scoring, e.g. the BBB scale, have become increasingly popular methods for analysis of functional abilities after spinal cord injury (Basso *et al.*, 1995, 1996a,b). The BBB scale, and its predecessor the Tarlov scale, are 21 and 6 or 7 point scales, respectively, designed to assess recovery of hindlimb function after thoracic spinal injury (Basso *et al.*, 1995; Fehlings & Tator, 1995). Each point in the score represents a specific set of characteristics demonstrated by the animal during spontaneous open field locomotion. The order of the BBB scores (0 = complete hindlimb paralysis, 21 = normal locomotion) was modelled after the spontaneous, progressive recovery of rats with thoracic spinal contusion injuries of different severities. The details of the BBB scoring system have been well documented elsewhere (Basso *et al.*, 1995, 1996a,b). Some of the advantages of locomotor rating scales are: (i) animal training is not a requirement; (ii) the scoring system can be learned quickly and reliably by experimenters; (iii) the method provides a meaningful measure of recovery of a clinically relevant behaviour, i.e. locomotion; and (iv) it is all-inclusive, incorporating animals with a wide range of functional abilities.

There are several limitations of locomotor scoring systems, for example, the BBB scale, which have been previously documented (Basso *et al.*, 1996b). The BBB scale, like other locomotor scoring scales, is an ordinal scale and as such, has the limitations of ordinal scales noted above (see 'Endpoint measures'). Each score represents a unique stage of recovery but the intervals between the scores are not necessarily equal. Thus, functional improvement from 7 to 9 is not 'the same amount' of improvement as a change in score from 11 to 13. In addition, the BBB scale, like all ordinal scales, has no true zero (e.g. the scoring system could just as easily have been devised to start at 10 and end at 31), so that a score of 8 does not represent a rat with 'twice' the functional abilities of a rat with score 4. Although this may seem obvious at first, it is tempting for many researchers to treat ordinal scales as true continuous numerical data, with resultant misinterpretations noted above. Additionally, statistical analysis of ordinal data should, strictly speaking, be limited to non-parametric statistics. The robustness of most parametric tests, however, generally allows their use for ordinal data, but results should be interpreted with caution.

Finally, increasingly widespread use of the BBB scale has raised an important issue. The recovery of function after a particular therapeutic treatment, or from a particular lesion, may not necessarily follow the order of BBB scale, which is based on the pattern of spontaneous recovery from a dorsal contusive injury of the thoracic spinal cord in rats. Although the BBB scoring system is flexible enough to accommodate some of these differences (through the use of BBB subscores, e.g. Basso *et al.*, 1996b; Popovich *et al.*, 1999), this emphasizes the importance of applying a particular scoring system to situations for which it was designed. Many of the functional states of animals with non-contusive lesions or cervical injuries may not be represented by the BBB scale. This should not be interpreted as a failure of the BBB system but rather a demonstration that a behavioural scoring system devised for a particular lesion condition cannot necessarily be applied to different lesion types and locations. With these limitations in mind, the BBB scale is an inclusive and meaningful method with which to assess open field locomotor abilities in rats after contusive thoracic spinal injury (Metz *et al.*, 2000). It promises to serve a useful screening function for motor disabilities as well as an effective method for comparing results between laboratories.

Continuous kinematic measures

Continuous kinematic measures include a wide variety of measurements of distances, angles, velocities and accelerations of the body and limb segments. The data produced by these measures are referred to as continuous because an infinite number of values are possible within a certain range (e.g. joint angles of 70.56 °), unlike the discrete type of data produced by ordinal scales (e.g. scores of 8 or 9, not 8.4, Kerlinger, 1986). Continuous kinematic measures can be applied to any number of behaviours (Table 1). During locomotion, for example, these measurements include stride characteristics (e.g. stride length and frequency, velocity, step lengths, Clarke & Parker, 1986; Parker & Clarke, 1990; Clarke, 1991; Bem *et al.*, 1995; Cheng *et al.*, 1997) and limb joint angles (e.g. knee, ankle, hip angles throughout the stride, Molinari & Petrosini, 1993; Brustein & Rossignol, 1998; Ribotta *et al.*, 1998). Less common measures can also be devised as appropriate for a particular deficit, e.g. the angle formed between the forelimbs during swimming (Kim *et al.*, 1999; Liu *et al.*, 1999). For behaviours primarily involving one or two limbs, e.g. reaching with the forelimbs or rearing with the hindlimbs, limb segment positions and joint angles will provide a quantitative measure of the movement. The particular measurements used will depend upon the nature of the functional deficit and the behaviour being analysed.

The advantages of continuous kinematic measures are that they provide a quantitative, detailed assessment of behaviour. Unlike endpoint measures, the details of how an animal performs a behaviour or completes a particular task can be quantified, before and after lesioning and/or after therapeutic manipulation. This in turn can lead to inferences regarding 'true' recovery of function versus substitution of function for a particular behaviour.

The main limitations of continuous kinematic measurement are the equipment and time required for analysis. Video or digital cameras, VCRs capable of single-frame advance and/or specialized computer hardware and software capable of digitizing body segment and joint positions are required to record and subsequently analyse the animals' movements. Only a few kinematic measurements, e.g. stride or step length, can be obtained with a minimum of equipment (e.g. footprint analysis requiring an inkpad and paper, although see Cheng *et al.*, 1997). A second limitation is the difficulty in accurate identification of the positions of joints and limb segments in video recordings, due to the small size of rodent limbs and the bent posture of the limbs under the body. These difficulties can be avoided for some measures, e.g. stride characteristics during locomotion, by video recording the animals' steps from below as they walk over a clear Plexiglas surface. Additionally, markers placed on the skin overlying limb joints can improve identification of limb segment positions when video recording from a lateral aspect, although skin movement can introduce systematic errors in locating joint and segment positions. Skin movement errors are generally more pronounced in the more proximal limb joints, e.g. hip, knee and shoulder, although even the angle of the ankle joint cannot be measured accurately if the position of the proximal tibia, i.e. the knee joint, cannot be identified reliably. There have been attempts to make corrections for skin movement in animals, e.g. the horse and cat, where X-ray cinematography has been used to relate the position of limb bones to skin markers during locomotion (van Weeren *et al.*, 1988, 1990a,b; Boczek *et al.*, 1994, 1996, 1999; Kuhtz-Buschbeck *et al.*, 1994, 1996). Correction models have been generated for some species but are not currently available for laboratory rodents (van den Bogert *et al.*, 1990). Thus, without derivation of correction factors for skin movement, accurate analysis of joint motion in rats is limited to the distal limb. Finally, as for endpoint measures, animals may need some training depending on the task to be performed, and care must be taken to ensure an equal degree of competence in both control and experimental groups of animals. Behaviours to be measured should be relatively stereotypical for the species, as individual differences in execution of a particular movement may be detected with these sensitive methods and will contribute to variation within groups of animals.

Kinetic measures

Kinetics is the branch of biomechanics concerned with forces. Animals move by exerting forces on surfaces and objects in their environment. Quantification of these forces, using force-transducing platforms/surfaces, can provide a sensitive measure of the methods by which animals execute particular behaviours. Ground reaction forces (GRF), for example, are the forces exerted through the limb on the ground during behaviours such as locomotion. GRFs have been shown to be a sensitive and non-invasive measure of locomotor function in many species, including humans. GRFs produced during locomotion provide an indication of the role of each limb in support and propulsion. In rats, we have demonstrated that unilateral CNS lesions produce characteristic changes in the GRFs during locomotion (Muir & Whishaw, 1999a,b, 2000). Interestingly, the pattern of GRFs produced during locomotion after CNS lesioning could not have been predicted *a priori*; e.g. rats actually supported more weight on the affected hindlimb than on other limbs. Ground reaction forces have been used to examine the different roles of the fore- and hindlimbs during postural control and locomotion after spinal cord injury (Giszter *et al.*, 1998). During forelimb reaching behaviour, measurement of vertical forces exerted by the non-reaching limbs has shown that rats normally shift their weight amongst their limbs in a particular pattern (Miklyaeva *et al.*, 1997). This pattern changes in a characteristic manner after unilateral striatal dopamine depletion (Miklyaeva *et al.*, 1997). Force measurements have been used to investigate sensory discrimination during skilled reaching in rats (Ballermann *et al.*, 2000). Force measurements have also been incorporated into reflex studies. The force necessary to stimulate a forelimb-placing reaction or the strength of reflex withdrawal and hopping responses can be compared between spinal-injured and control animals (Kunkel *et al.*, 1993; Handley *et al.*, 1998; Bennett *et al.*, 1999).

There are several benefits of kinetic measurements. First, they provide novel information regarding behavioural compensation and recovery after CNS injury, information which cannot be obtained by any other method. Force measurement can also be a very sensitive method with which to detect changes in behaviour, and these methods are quantifiable. Limitations of force measurements include the requirement for special equipment – force transducers of sufficient sensitivity and appropriate size for rodent behavioural studies are not readily available commercially and thus may need to be custom-built. A second limitation is that not all behaviours lend themselves to meaningful force measurements. Even GRF measurements during locomotion, the most common use for kinetic measures, require that the animal be able to support its weight and take full plantar steps. With some forethought, however, it should be possible to incorporate kinetic measurements into a variety of behavioural situations (Table 1), thus increasing our ability to detect and quantify subtle differences in behavioural performance between animals.

Electrophysiological measurements

The previous categories of behavioural measurements involve non-invasive methods to assess behaviour. Electrophysiological assessments require the implantation of recording electrodes and perhaps stimulating electrodes into muscles or cranial sites, although transcranial magnetic stimulation has been used in rats (Metz *et al.*, 2000). These measurements range from recording of muscle activity patterns during normal movements, including reflex movements (Table 1), to activity evoked by electrical stimulation. While electrically evoked responses are not considered to be behavioural responses, they are included here because of the close relationship between reflexes and motor function.

Recording of muscle activity during behaviour can provide much insight into the methods by which animals accomplish particular movements. Recording from many muscles at once is possible with available techniques, so that a characteristic pattern of muscle activation can be determined (Loeb & Gans, 1986). Deviations from this pattern after lesioning and/or during recovery can help determine the methods by which the new movement differs from the original. This information is especially valuable if combined with kinematic and kinetic data obtained at the same time (Brustein & Rossignol, 1998). Several important issues should be mentioned here. First, because of the relatively large number of muscles crossing each joint, it is likely that several different muscle activity patterns will be able to produce a particular movement. Second, the complex and largely unknown relationship between muscle fibre activation and tendon force means that intramuscular recording of activity patterns provides incomplete information regarding the relative contribution of each muscle to a particular movement. The end result is a potentially large interindividual variation in muscle activity patterns, in both normal and lesioned animals.

Reflex behaviour, involving individual limbs or the whole body, can also be recorded and quantified using electrophysiological measures. Local spinal reflexes, e.g. the Hoffman reflex or flexor withdrawal reflexes for limbs or for the tail can be tested electrophysiologically (Thompson *et al.*, 1992; Commissiong & Sauve, 1993; Bennett *et al.*, 1999; Chen *et al.*, 1999). Electrodes can be used to record the muscle response and/or to provide reflex stimulation electrophysiologically (Bennett *et al.*, 1999). To test the integrity of long pathways in the spinal cord, electrical activity in limb muscles can be recorded in response to cortical stimulation (e.g. motor-evoked motor potentials, MEPs) or auditory stimulation (auditory-evoked motor potentials). Conversely, brain activity can be recorded in response to sensory stimulation (somatosensory-evoked potentials, SEPs). These reflexes are very sensitive to spinal injury, although they appear to involve pathways that are not necessary for locomotion (Gruner *et al.*, 1993). There are some discrepancies as to whether SEPs or MEPs correlate more closely with the course of recovery (Nashmi *et al.*, 1997; Metz *et al.*, 2000). At least part of this discrepancy may be due to differences in lesion methodology between different studies, e.g. weight-drop versus transection, which affects the number and location of damaged spinal tracts.

The advantages of electrophysiological techniques lie in direct and precise measurement of muscle activation, of reflex latency and of the relative strength of reflex responses. The disadvantages of these methods include the fact that implantation of stimulation and recording devices is required. This necessitates anaesthesia and care must be taken intra- and postoperatively to avoid infection and/or irritation, and to maintain the devices *in situ* for the duration of the study. The invasive nature of the devices means that animals may not perform some behavioural tasks normally, or may require more time to become accustomed to the devices. In addition, not all behaviours lend themselves to electrophysiological assessment.

Recommendations for comprehensive behavioural analysis after experimental spinal cord injury

The categories presented above describe several different kinds of measurements that could potentially be used to assess behavioural recovery after spinal injury in rats. Here, we attempt to illustrate how these measurements can be used to provide a quantifiable and comprehensive analysis of sensorimotor function in spinal-injured rodents. Several excellent reviews have also provided guidelines for behavioural analysis in rats (Goldberger *et al.*, 1990; Kunkel *et al.*, 1993; Whishaw *et al.*, 1998). In concurrence with these authors, we recommend a combination of functional tests for each animal. In our opinion, a comprehensive analysis should include each of the following three components: (i) a measure of motor abilities during spontaneous locomotor activity; (ii) a measure of abilities during one or more trained behavioural tasks; and (iii) an assessment of reflex function. Spontaneous movement provides an initial screening of overall motor abilities. Assessing the performance of trained behaviours will eliminate differences in motivation between animals and provides more detailed and specific measures of functional abilities. Finally, measurement of reflex function will provide an indication of the integrity of particular spinal pathways.

Spontaneous movement is probably best measured by an assessment of open field locomotion, using a scoring method, e.g. a modified Tarlov or BBB scale. As previously mentioned, these scoring systems cover a wide range of motor deficits and are useful as a method of comparison between laboratories (Basso *et al.*, 1996b). These tests also serve to classify animals according to their general functional abilities and thus will determine which trained motor tasks can be subsequently employed to measure functional abilities in more detail. In particular, there will be differences in the type of motor tasks which are possible to assess in animals which are: (i) unable to locomote overground; (ii) able to locomote but with poor limb and paw placement; or (iii) able to locomote with good limb placement. Recommended tests for each of these three situations are discussed below. Of course, the exact choice of tests for each situation will need to be determined by the examiner and will differ depending upon the type of lesion (e.g. contusion, hemisection or transection), the location of the lesion (e.g. cervical or thoracic spinal cord), as well as lesion severity.

In the case in which animals are unable to locomote overground (e.g. BBB score 0–9, Tarlov score 0–2), further testing could include tasks such as swimming, forelimb reaching and spontaneous paw preference as described in Table 1. Tests which primarily assess sensory function should also be included, e.g. the sticky paper test, paw lick and tail flick tests. A complete analysis of reflex function can be carried out on these animals, including righting, placing, hopping and withdrawal reflexes (Table 1). Both endpoint and kinematic/kinetic measures should be assessed concurrently when possible during these behaviours, in order to distinguish recovery from behavioural compensation. If necessary, the outcomes of these tests may be measured more precisely using electrophysiological techniques. Again, the tests chosen will depend upon the abilities of the animal – an animal unable to locomote due to a cervical lesion which causes near complete tetraparesis cannot be assessed on a swimming or forelimb-reaching task, but can undergo reflex testing. An animal with a thoracic lesion resulting in an inability to fully support its weight on its hindlimbs may still be able to swim.

In animals which are able to locomote but show poor limb and paw placement (BBB score 10–15, Tarlov score 3–4), any or all of the reflex tests described above could be performed. In addition, other tests of individual limbs could be used as appropriate for the lesion, including forelimb reaching, grid or ladder walking, sensory testing, pushing/climbing with hindlimbs (Table 1). Where possible, continuous kinematic measurements, kinetics and/or EMG recordings, as well as endpoint measures, should be employed to precisely document the manner in which animals accomplish the task.

In animals which are able to locomote with good paw placement (BBB score 16–21, Tarlov score 4–5), all of the above tests can be carried out. In addition, quantitative kinematic and kinetic measurements can be obtained during overground locomotion. Motor abilities should also be challenged in these animals, such that they can be trained to locomote up or down slopes, to climb ropes, or to locomote along grids, ladders and/or beams of various widths (Miya *et al.*, 1997; Soblosky *et al.*, 1997). In addition to endpoint measures with these tasks, kinematic and/or kinetic measures should be recorded in order to provide robust measures of performance and behavioural compensation.

In conclusion, we recommend a logical and rigorous approach to the analysis of behaviour after experimental spinal injury. A conscientious effort must be made to fully characterize the functional deficits arising from each spinal lesion, using a battery of measures appropriate for each deficit. Only in this way will we be able to accurately assess the effect of therapeutic intervention in experimental animals and to make progress toward improving functional recovery in human spinal-injured patients.

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Abbreviations

CNS, central nervous system; GRF, ground reaction forces; MEP, motor-evoked potential; SEP, somatosensory-evoked potential.

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