Behavioural assessment of rats with multiple cervical dorsal root avulsion and their functional recovery after transplant with olfactory ensheathing cells

Natalia Vasquez Peñuela
Supervised by Professor Geoffrey Raisman
Spinal repair unit
Institute of Neurology

Submitted as partial fulfilment of the requirements for the MSc in Clinical Neuroscience, University of London
July, 31st 2008

Institute of Neurology
University College London
Queen Square, London WC1N 3BG

Word count: 10.064 (Excluding tables and reference list)

MSc Clinical Neuroscience
2007/08

Acknowledgements

I would like to sincerely thank Professor Geoffrey Raisman for welcoming me to the Spinal repair unit and permitting me to complete this research project; Ahmed Ibrahim, for his constant help, supervision, and guidance in this process; Constaninos Kallis for his continuous and thoughtful guidance in the statistical analysis; Steve Scott-Robson for his expertise and invaluable input on the drafting of this thesis.

Finally, all the members of the MSc course, my classmates and my family for offering me constant and positive support.

Contents

List of tables and figures

Tables

Figures

Abbreviations

BPI: Brachial plexus injury

CNS: Central nervous system

CST: Corticospinal tract

DREZ: Dorsal root entrez zone

EWMN: Eskhol Wachman movement notation

OEC: Olfactory ensheathing cells

PNS: Peripheral nervous system

Abstract

Introduction *Dorsal root injuries remain a clinical challenge particularly in cases of root avulsion of the brachial plexus. Aims this project aims to design a valid and predictive behavioural instrument to evaluate sensorimotor function after multiple dorsal root avulsion and therapeutic interventions.* ***Materials and methods*** *36 adult rats were included in the study and categorised in 4 groups of 9 rats each: 3-root lesion, 4-root lesion, 4-root lesion with olfactory ensheathing cells and the control group. The instrument and scoring system was designed based on attributes predictive of sensorimotor function. Attributes included pattern of climbing, vertical successful grasps, faults, posture, and amplitude of movement. Forelimb proprioceptive deficit was tested by allowing rats to climb a frame in three behavioural tests during 3 different times within the first 6 weeks, after the lesion and after OEC transplant.* ***Results*** *Qualitative and statistical analyses clearly identified and underlined differences between the 3-root and the 4-root lesion groups. Further statistical analyses show no behavioural changes over time in the groups with no therapy; however, a slight degree of possible adaptation to the type of injury was observed. Furthermore, according to the qualitative observations over time, there was evidence of behavioural improvement in the transplanted group nonetheless this was not statistically significant.* ***Discussion*** *This behavioural assessment method allows quantifying and qualifying the proprioceptive function and recovery from forelimb sensorimotor deficits. Findings from this study will contribute to research on the clinical assessment and treatment of patients with brachial plexus injuries.*

1. Introduction

Brachial plexus injury (BPI) is one of the most severe nerve injuries of the extremities that causes functional impairment of the affected upper limb **(Figure 1.1)** (Yoshikawa et al. 2006; Nagano 1998). Spinal nerve avulsion injuries of the brachial plexus are particularly common, and have poor recovery outcomes. (Shin et al. 2005; Midha 1997). The aim of this research project is to design a valid and predictive behavioral assessment method a) to assess functional deficit after multiple cervical dorsal root avulsions in rats, and b) to identify functional improvement after therapeutic interventions such as, transplant of olfactory ensheathing cells (OEC). In order to explain the background that has led to the present research project, the following introduction will focus on: dorsal root lesions and their sensorimotor effects, the anatomical and physiological basis of dorsal root lesions, the similarities found in rats and humans, the different types of behavioral measures assessing dorsal root lesions and functional recovery after OEC transplant, and the rationale for the behavioral assessment method proposed in this project.

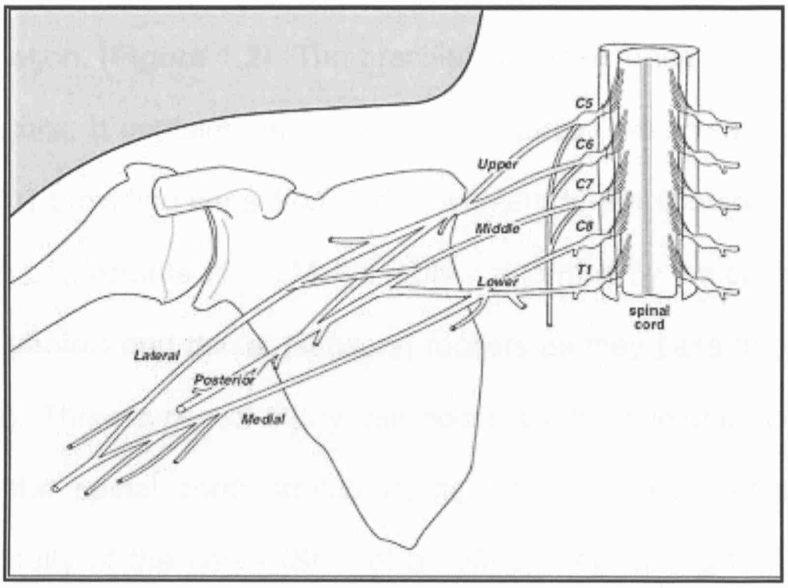

Figure 1.1 Illustration of the brachial plexus anatomy from http://www.uwhealth.org

1.1 Brachial plexus root lesion types

Dorsal and ventral root injuries remain a clinical challenge particularly in cases of root avulsion of the brachial plexus. The most frequent causes of brachial plexus injuries are motorcycle and snowmobile accidents but also can occur during a traumatic childbirth. Traumatic injuries to the brachial plexus may lead to devastating neurological dysfunction. This in turn causes a wide range of impairments including loss of sensation and motor function of the arm, hand and sometimes, severe neuropathic pain (Aldskogius & Kozlova 2002; Tang et al. 2004; Ahmed-Labib et al. 2007; Midha 1997).

The type of nerve injured after damage to the brachial plexus and the type of injury are both aspects that influence the outcome of nerve regeneration. **(Figure 1.2)** The brachial plexus extends from the spinal cord to the axilla. It contains among other structures, five roots (classically, from C5 to T1) providing sensation and movement to the shoulder, arm, forearm, and hand. (Ferrante 2004) Nerve roots are formed by the coalescence of the ventral (motor) and dorsal (sensory) rootlets as they pass through the spinal foramen. Three types of injury can occur: avulsion injuries pull the rootlets out of the spinal cord; stretch injuries; and ruptures result in complete discontinuity of the nerve (Shin et al. 2005). According to Lundborg et al. (2007) a crush lesion often results in better functional outcome when compared with avulsion injuries or complete severance of a nerve.

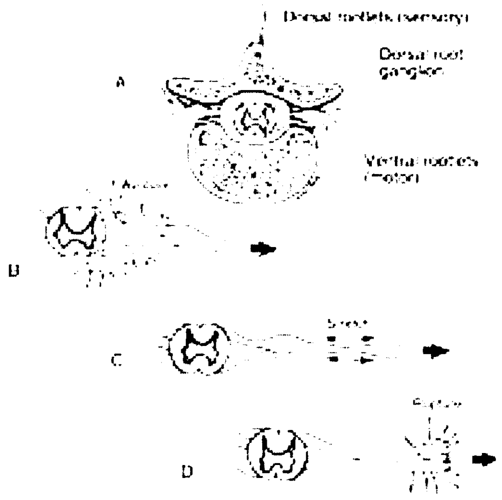

Figure 1.2 Illustration of the brachial plexus roots and types of injury. The roots are formed by the coalescence of the ventral (motor) and dorsal (sensory) rootlets as they pass through the spinal foramen (A). The dorsal root ganglion holds the cell bodies of the sensory nerves. Three types of injury can occur: avulsion injuries pull the rootlets out of the spinal cord (B); stretch injuries attenuate the nerve (C); and ruptures result in complete discontinuity of the nerve (D) (Adapted from Shin et al. 2005; Shin et al. 2005).

Dorsal root injury can result in loss of sensation of the arm and hand with severe consequences due to their critical role for humans. The complexity of the human arm, its unique and exclusive manipulative behaviour, has been well studied. In humans, evolution of grasping emerged with the use of tool and tool making (Bennett & Castiello, 1994). Moreover, sensory deficit after dorsal root lesions include loss of cutaneous, joint and muscle receptor feedback. Apart from information about the environment and limb position, cutaneous afferents also provide information necessary for the performance of skilled tasks such as grasping (Macefield et al. 1990).

Furthermore, the lack of sensory input may lead to an inability to distinguish surfaces of different textures and to a loss of regulatory input to assess load on the limbs. Finally, the role of cutaneous input appears to be crucial for the expression of spinal locomotion (Rossignol et al. 2006).

1.1.1 Anatomical and physiological correlations

BPI in humans is caused by severe traction force on the upper limb, resulting in complete or partial paralysis of the arm. Lesions in the brachial plexus are classified as preganglionic or postganglionic. It has been reported that preganglionic lesions are particularly severe, have poor prognosis of recovery and sometimes no recovery at all, instigating the need for reconstructive procedures without delay (Doi et al. 2002; Nagano 1998). Most preganglionic lesions involve an avulsion of the nerve roots. Clinical evaluation, myelographic computed tomography, sensory nerve conduction studies, and EMG studies are the principal methods used in the diagnosis of nerve root avulsion (Yoshikawa et al. 2006).

The management of BPI depends on the degree of damage, site of injury and type of roots involved and since the lesions considered for this study are preganglionic namely, avulsion of the cervical dorsal roots, these would be illustrated (Yoshikawa et al. 2006).

Varying motor, sensory, and autonomic deficit arise depending on the cervical roots involved in the lesion (Shokei et al. 1996). Clinically, patients typically show areflexia, dermatomal sensory loss, and loss of muscle function particular to the nerve roots avulsed (Shokei et al. 1996).

Recent evidence suggests significant variability of the dermatomes distribution in man (Nemecek et al. 2003). Moreover, due to anatomical variations in the nervous system of other animal models, rat dermatomes have become the basis knowledge for research relating to sensory pathways, somatotopic organisation in the CNS, and somatosensory reflexes. However, it has been found that maximal innervation areas generally overlap and that each digit is innervated by more than one spinal nerve (Takahashi & Nakajima 1996).

For the purpose of this study it was important to consider the dermatome distribution of the C6, C7, C8 and T1 dorsal roots in rats. As shown in **figure 1.3**, the root C6 supplies anterior aspect of the forelimb, the 1st and 2nd digits; C7, the anterior and middle portions of the ventral and dorsal aspects of the forelimb and the 1st, 2nd, 3rd and 4th digits of the fore paw; C8 supplies the middle and posterior portions of the forelimb, 3rd, 4th and 5th digits of the fore paw and the root T1, the 5th digit of the fore paw (Takahashi & Nakajima 1996).

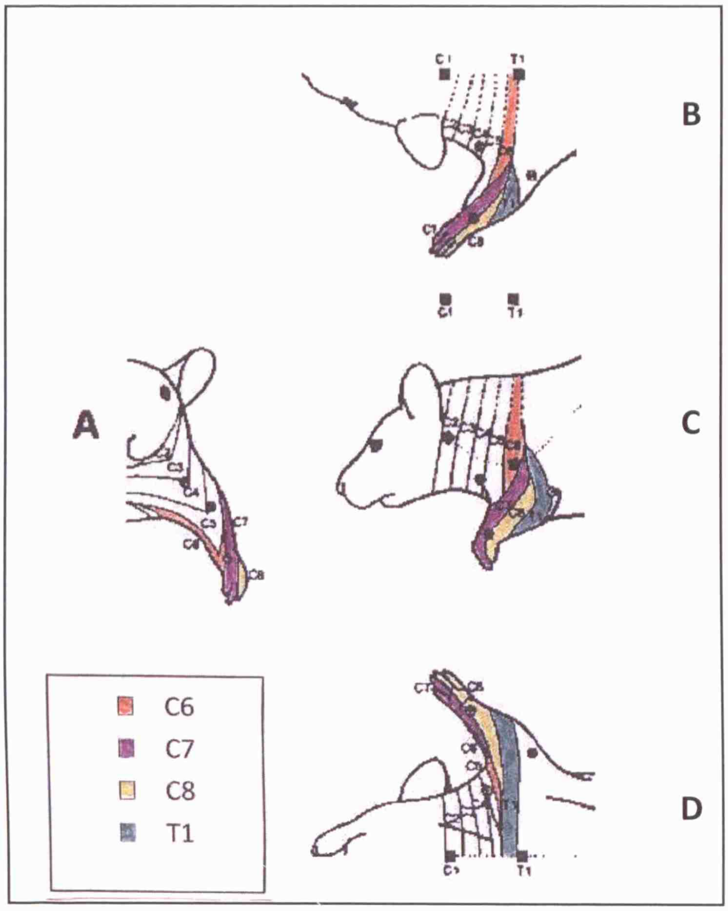

Figure 1.3 Illustration of dermatome charts from C1 to T1 (forelimb) in rats. A = anterior; B = dorsal; C = lateral; D = ventral; E = posterior (Adapted fromTakahashi & Nakajima 1996; Takahashi & Nakajima 1996).

Understanding anatomical and physiological consequences of multiple cervical dorsal root avulsions in rats, leads to understand of how sensory input contributes to adjusting movement and influencing behavior (Rossignol et al. 2006).

1.1.2 Similarities of behavior between humans and rats

The earliest studies evaluating forelimb function in rats were done in the early 1990s (Webb & Muir 2005). It is well known that for rats, grasping requires sophisticated integration of the sensory information and motor output system, and this behavior is often used as a measure of high complex sensorimotor response (Wang et al. 2008).

Grasping in rats is often used as a paradigm for the study of neural control, plasticity and recovery of function. Furthermore, it is important to note that similarities of skilled limb use in rats and primates have been demonstrated over the years. This evidence strengthens the generalizations that can be made about experimental studies on humans. The similarities in movement patterns allow the application and generalization of observations in rats' skilled reaching and grasping to humans. For instance, it was found that the components, sequence and velocity profiles of reaching movement were analogous in both species (Whishaw et al. 1992). Most studies have assessed forelimb movement by training rats to reach for food pellets, but not during a more sensorimotor demanding activity such as grasping during climbing.

It has been suggested that rodents most likely use tactile information from the vibrissae and paws to identify texture and size of the food, explaining why grasping in rats is severely decreased after dorsal root lesion (Whishaw & Kolb 1988; Whishaw & Tomie 1989). Another study suggested that vision does not contribute to direct limb movements and that olfaction is used for the control of reaching in rats (Whishaw & Tomie 1989). In addition, previous studies have also found that the initial part of the motor act in reaching does not require sensory feedback for its execution and has more a ballistic motor pattern (Zhuravin & Bures 1986). These findings confirm the importance of focusing this project on the effects of dorsal root lesions on grasping and sensory input for the execution of this behaviour.

1.2 Behavioural assessment after dorsal root lesion in rats

After cervical rhizotomy of the roots C5 toT2 in rats, reaching for food pellet performance was analysed and it was found that after the lesion, initiation of reaching was preserved whereas the modulation of the execution was lost. The fine manipulation and grasping effectiveness of these rats decreased markedly because of lost tactile information. However, this group did not describe the quality of grasping or how the lesion influenced the behaviour. The rats had visual cues to grasp and went through an extensive training programme; hence the results of the study were less reliable (Saling et al. 1992).

Another study assessed proprioceptive function after multiple dorsal rhizotomy in rats. A persistent failure to grasp was found despite improvement in both a ladder walking test and the assessment of forelimb motion while traversing a narrow beam (Ramer et al. 2002). The effects of dorsal column lesions in the rat have been of considerable interest for many (McKenna & Whishaw 1999; Webb & Muir 2003; Muir et al. 2007). Moreover recently, after axonotomy of ascending sensory fibres in dorsal columns at the C2 level, researchers found considerable contribution of these pathways in skilled forelimb movement but mainly, during over ground locomotion (Kanagal & Muir 2007). Findings in this study suggest that models with very limited lesions are not adequate for the analysis of forelimb skilled behaviours.

Skilled forelimb movement in rats has also been described as a movement dependent on the cortical spinal tract (CST). One group has analysed the behaviour during predation and skilled reaching for food pellets (Whishaw 1996b). Others have demonstrated functional recovery during food retrieval behaviour in rats, after unilateral dorsal corticospinal tract lesion at C1 level and OEC transplant (Keyvan-Fouladi et al. 2003).

A more recent study also emphasized the contribution of ascending sensory axons as well as corticospinal tract axons during skilled limb movements in rats (Kanagal & Muir 2007).

Skilled reaching and grasping in rats after unilateral sensorimotor cortex lesion has been also investigated. A staircase test used for one study provided quantitative data from ipsilateral and contralateral paws independently while rats retrieved food pellets (Montoya et al. 1991). The horizontal ladder beam system has been used to measure forelimb and hindlimb deficits after sensorimotor cortex injury in rats (Soblosky et al. 1997). Relevant findings were presented by a group that designed a behavioural assessment tool for skilled fore and hind limb stepping behaviour in mice after motor cortex lesion (Farr et al. 2006). This group combined an end-point measure with a qualitative assessment, which supports the idea that this type of strategy provides a comprehensive assessment of sensory impairments in rats and that qualitative movement analysis is a valuable tool for detecting subtle sensory deficits.

1.2.1 Types of behavioural measures

The present research project focused on the diverse nervous structures involved in grasping, as well as the variety of behavioural assessments used to measure recovery after CNS-lesioned rats. The assessment measure types have been revised by others. These measures include end-point measurements, kinematic, kinetic, and electrophysiological measurements (Muir & Webb 2000).

1.2.1.1 End-point measures

End-point measures evaluate the achievement of a particular goal. It includes evaluation of variable tasks of body behaviour variables such as time to cross or climb, number of times a paw slips or angle at which the rat can no longer maintain a position. For individual limb, it includes tasks as number of food pellets eaten in a time period or time to remove a sticker. End-point measures and the quantitative data obtained through it, allow an overall indication of motor performance. Furthermore, these measures are simple, objective and quick to administer. However, they do not provide a description or indication of how the task is being performed (Muir & Webb 2000).

1.2.1.2 Kinematic measures

Kinematic measures quantitatively describe the movement of the body and segments relative to each other. These include, the *Eskhol Wachman movement notation (EWMN),* the most common functional behavioural test nowadays used, introduced by Eshkol and Wachman in 1958. EWMN comprises details of motion of forelimb during reaching from single frame analysis (Metz & Whishaw 2000). Each behavioural activity is divided in separate movements and there is a rating scale for each component. Each movement is identified in detail revealing differences not noted in end-point measurements and it also identifies movement differences which can be quantified using continuous kinematic measures. Advantages of these type of measures are that training is not required, the scoring system can be learned quickly, it assesses spontaneous movement and can be useful for comparison between laboratories. Details of behaviour can be quantified however, equipment and time are required and it is difficult to precisely identify positions of joint and limb segments

1.2.1.3 Kinetic measures

Kinetic measures are quantifiable measures of biomechanics concerning forces. These provide information about behavioural compensation and recovery after CNS injury however, special equipment is required and not all behaviours lend themselves to force measurements.

There is evidence suggesting that most behavioural tests provide an end-point measure of motor function nevertheless, subtle deficits remain unnoticed when animals adopt compensatory movement strategies that allow them to successfully perform a task (Farr et al. 2006). As demonstrated by one study, a combination of end-point measures with qualitative assessment provides a closer insight into behavioural association of lesion induced deficits and subsequent recovery (Whishaw 2000). Therefore, a combination of end-point and kinematic behavioural assessments was used for this study.

1.3 Therapeutic interventions for repair dorsal root lesions

Many models with dorsal root lesion have been designed and have improved the understanding of the mechanisms underlying the failure of regeneration of sensory axons in the CNS (Aldskogius & Kozlova 2002). Approaches that aim to promote growth of sensory axons into the spinal cord have raised special interest among researchers. Advances have been obtained by peripheral transplant on central lesions (Raisman & Li 1994; Huang et al. 2003) as well as promising findings of functional regeneration after dorsal rhizotomy and intrathectal infusion with growth factors (Ramer et al. 2002; Ramer et al. 2000; Wang et al. 2008). Furthermore, OEC transplants have promoted regrowth of axons across the injury leading to functional recovery (Kay-Sim 2005).

There are heterogeneous cellular and molecular pathological features that present after dorsal rhizotomy and promote the regeneration barrier at the dorsal root entrez zone (DREZ). Sensory axons in the dorsal roots travel in the peripheral nervous system (PNS) and enter the CNS at the DREZ which is localized outside the spinal cord. At this site the PNS meets the dense astroglial environment and enters into the spinal cord (Aldskogius & Kozlova 2002).

1.3.1 OEC transplants and behavioural assessment

An essential strategy for dorsal root repair is to initiate the regenerative apparatus of sensory neurons (Ramer et al. 2002). OEC transplants have shown significant promise in rat models of CNS injury, encouraging the use of these cells in experimental and clinical trials (Richter et al. 2005). Several reports have suggested that transplantation of OECs after dorsal rhizotomy lesion models promote anatomical and functional regeneration of sensory fibres (Gomez et al. 2003; Gomez et al. 2003; Pascual et al. 2002).

One of the first publications demonstrating functional recovery and regeneration of adult axons after OEC transplant of acute rat spinal tract lesions illustrates the search for promoting recovery of locomotion after spinal cord injury in humans (Li et al. 1997). This and many other studies have provided evidence of the regrowth of dorsal injured sensory axons into the spinal cord after OEC grafting (Ramon-Cueto & Nieto-Sampedro 1994; Navarro et al. 1999; Pascual et al. 2002; Li et al. 2004).

Furthermore, evidence of functional recovery has been reported after ensheathing glia transplants after multiple lumbar rhizotomy (Navarro et al. 1999). However, to date no evidence has been obtained about functional recovery and regrowth at the cervical spinal cord level, after multiple dorsal root lesion and OEC transplant. The reason is still unknown; one group suggested the variability of the time frame between the transplant and the analysis, and the fact that the cervical DREZ has more inhibitory growth properties after injury compared to other levels. The extent of the lesion and the lesion site may also be determinant factors (Gomez et al. 2003).

OECs are a specialized type of glia cells present in the olfactory bulb within the CNS. Diverse properties that make OECs an exceptional type of cells have been recognized by many authors. These exist in and outside the CNS; they regenerate into the CNS and show a continuous neurogenesis throughout adult life in the absence of injury. OECs induce anatomical regeneration and functional recovery (Gomez et al. 2003; kay-Sim 2005; Li et al. 2007; Li et al. 2004; Li et al. 2003).

1.4 Our behavioral assessment method

The assessment of the effects of CNS injury and of therapeutic interventions that meet the scientific and clinical demands is essential. Namely there is a lack of instruments to measure sensorimotor function that assess the quality of movement and that are specific and sufficiently sensitive to detect small changes during a specific intervention.

The design and implementation of behavioural tests after CNS lesion in rats have been extensively studied. Nonetheless, despite the diversity of behavioural measures to assess recovery after experimental interventions, behavioural implications of this type of lesion have not yet been qualitatively described. Based on the fact that behavioral outcome should be of functional significance to the animal and should provide information that can translate to humans or other injury models (Basso 2004), this project aims to develop a behavioural instrument to evaluate sensorimotor function after multiple dorsal root lesion and after therapeutic interventions.

2. Materials and methods

A novel behavioral assessment method and a scoring system was designed, based on some attributes predictive of sensorimotor function, to evaluate the performance of multiple cervical dorsal root lesioned rats during climbing. Forelimb proprioceptive deficit was tested by allowing the rats to climb a frame. For each successful climb, the incidence of ipsilateral faults and ipsilateral vertical grasps, were counted and then divided by the number of rats in the group to obtain a mean value. Data from the pattern of climbing, amplitude of movement of the ipsilateral forelimb, and postural observations were also collected. Furthermore, each vertical successful grasp and its components was analyzed in detail. Initially, the assessment instrument was performed in three different behavioural tests during 3 different times within the first 6 weeks, after 3 and 4 dorsal left root lesions and in the last part of the study, the group with OEC transplant was also assessed.

2.1 Subjects

A total of 36 adult inbred Albino Swiss female rats, of 180 to 200gr weight, were included in the study, 9 per each group. The groups were 3-root lesions, 4-root lesions, 4-root lesions with OECs and the control group. Rats in the first three groups were behaviourally assessed three times within the first 6 weeks post lesion or post transplant and denoted firs, second and third behavioural tests. The control group was assessed once prior the surgery.

2.1.1 Surgery

Following appropriate anaesthesia, exposition of C3 to T2 laminae through a midline dorsal incision and left hemi-laminectomy of the C4-T1 vertebrae, up to four roots and associated rootlets between C6 and T1, were sectioned. All the animal interventions followed the UK Animals Act 1986, scientific procedures.

Observation and behaviour analysis of rats with no lesions, unilateral single dorsal root lesion (C6, C7, C8 or T1) and 2-root lesions (C6, C7) showed little functional deficit. Rats with 3-root lesions (C6, C7, C8) and 4-root lesions (C6, C7, C8, T1) showed more significant climbing deficits. Hence, rats with 3- and 4-root lesions were included in the current study for detailed behavioural assessment. Furthermore, to complete a pilot test of the instrument, a 4-root lesion with OEC transplant group was selected.

2.2 Functional assessment

2.2.1 Type of behavioural measure for this study

The type of behavioral assessment method designed to be tested in this study was defined as a combination of an end point and kinematic measure. It was an end point measure in the sense that it determined whether the rat successfully climbed from the bottom to the top of the frame and only successful climbs were considered, and also whether the rat accomplished vertical successful grasps. On the other hand it was kinematic, because it considered how the rat's injured forepaw grasped and how the body parts moved during climbing.

2.2.2 Climbing

An undemanding climbing frame was used to behaviorally assess the rats. It has 15 degrees of inclination, 100 cm high and bars were 1cm apart from each other **(Figure 2.1).**

Figure 2.1 Photo of the climbing frame.

The rats performance was video recorded once a week from a frontal lateral perspective to allow analysis of body posture and forelimb movements. In the process of the assessment tool development and in order to score the rats behavior all video recordings was analyzed using frame by frame inspection.

2.2.3 Behavioural attributes

The behavioural attributes were defined based on observed characteristics from an initial blinded study of multiple retrospective video recordings of injured and non-injured rats. The definition of these was based on common denominators observed in the climbing pattern as well as predictive of sensory input. After specific attributes were identified a subscoring system was used; this, with the purpose of comparing the behavior within time points as well as of revealing functional recovery characteristics.

The number of *grasps* performed with the ipsilateral forepaw to the lesion was counted for each trial. Vertical and horizontal grasps were counted as independent attributes because horizontal grasps were considered to be a different sensorimotor activity requiring different sensory input.

In order to asses the quality of the grasping pattern, this was divided into four phases **(Table 2.1),** defined in order of occurrence as follows: i) flexion of forelimb, ii) extension of digits, iii) type of grasp and iv) retraction of bar.

I Flexion of forelimb	The forelimb elevates in the midline to or above the level of the shoulder, aiming to reach the vertical bar for the next grasp.
II Extension of digits	The 5 digits of the paw are widely separated aiming to grasp the vertical grasp.
III Type of grasp	Closure of the 5 digits of the paw grasping the vertical bar, in neutral position and/or slight pronation. The pattern resembles a power grasp.
IV Retraction of bar	While holding the grasp, the uninjured forelimb advances above the level of the left paw simultaneously retracting the vertical bar. The paw should not slip or release before the uninjured forelimb is advanced.

Table 2.1. Definition of the four phases for each successful vertical grasp

Each phase of a single grasp was scored from 1 to 3 **(Table 2.2)**. Grasps were scored, summed and mean was calculated. The mean ranged therefore from 4 to 12; the higher the mean score, the worse the quality of grasping. Based on the results of the mean, 3 categories were made to describe the quality of the pattern of grasping.

Score	*Behaviour*
1	Normal
2	Impaired
3	Absent

Table 2.2 Sub-scoring system for successful vertical grasps

Pattern was defined as "good" for values from 3 to 5; "fair" for values from 6 to 8, and "poor" for values from 9 to 11. According and the characteristics seen in each category, a good pattern was defined as the most normal or closest to the normal level quality and characteristics of all four phases of the grasp; a fair pattern category indicated an impaired quality of the characteristics of the phases and a poor category, was defined as a very impaired grasp pattern.

Subsequently, this category qualification was also given to each phase of the grasp when calculating the mean for each group. It was thus possible to identify which phase of the grasp pattern was more affected in each group.

The *faults* were defined as mal-positions of the injured forelimb paw at the moment or intention of grasping. They were particularly evident when the paw passed trough the bars without grasping.

Posture and posture-related and movement-related changes during climbing and grasping were observed in rats with 3- and 4-root lesions. In all groups, the following features were observed: forelimb motion during climbing, head position, alignment of spine and body parts, sustentation base, hindlimbs movement, stability and general body compensations.

The *amplitude of joint movement* was defined based on the movement of the anterior flexion of the forelimb ipsilateral to the lesion. The parameter used to qualify this attribute was to observe whether the paw reached a height above or below the ipsilateral shoulder at the moment of reaching the vertical bar to climb. An extensive flexion of the ipsilateral shoulder to the grasping forelimb was considered normal. Because the joints were small and covered with fur, the amplitude of elbow and wrist were difficult to determine, instead, the amplitude of the ipsilateral shoulder was considered.

3. Results

3.1 Qualitative description

3.1.1 Climbing pattern

Within the normal control group eight different types of climbing pattern were recognized. One of the eight was considered as the normal one because it was more consistent than the others. Moreover, considering the eight different patterns identified, control rats climbed 47% of the times using a coordinated, and alternating pattern. This pattern started either with the right or with the ipsilateral injured left forelimb as described below.

A normal pattern of climbing could be described as a sequence of various steps. A first grasp with either forelimb is used to pull; this is followed by the advance of the contralateral hindlimb that prepares to push so the weight is supported by the ipsilateral hindlimb. While one limb provides support, the other advances in preparation for its role as the supporting limb. The **figure 3.1** illustrates the pattern identified as normal during climbing.

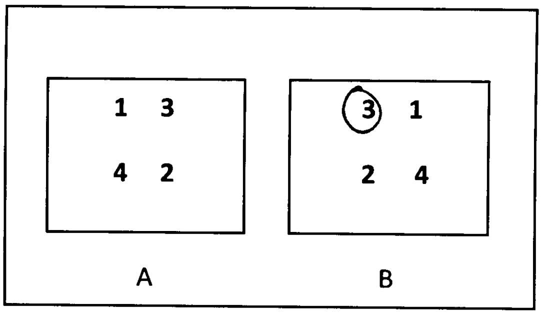

Figure 3.1 Illustration of normal pattern of climbing in rats. A = Right forelimb (1), left hindlimb (2), left forelimb (3) and right hindlimb (4); B = left forelimb (1), right hindlimb (2), right forelimb (3) and left hindlimb (4).

3.1.2 Successful vertical grasps

Control group. From the control group and 9 attempts of climbing in one time point, 100% of rats performed successful vertical grasps. The mean number of successful vertical grasps per rat, in this group, was 5.4.

Grasps quality was qualified as good in 88% of the cases and fair in 12%. All four phases of the grasp were qualified as normal, which was reflected in the mean of each of the phases.

3-root lesion group. From the 3-root lesion group all 9 rats performed successful vertical grasps in each of the three phases. The mean number of successful vertical grasps per rat in this group was 3.1, an incidence significantly higher when compared with the 4-root lesion group and very close to that in the control group **(figure 3.2)**. This result suggests that even with a multiple lesion such as the dorsal 3-root lesion, grasping can be performed.

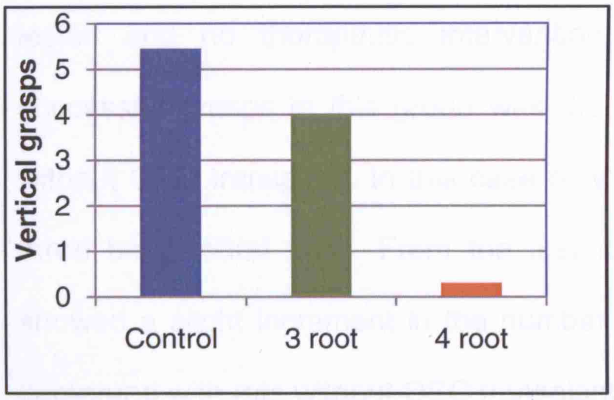

Figure 3.2 The mean number of successful vertical grasps in rats with 4-root lesion was significantly small.

Grasp quality was poor in the 37% of the cases and fair 63%. Still in this group, all 4 phases of the grasp had an impaired grasp quality even the number of grasps increased substantially and all rats completed grasps in this group.

4-root lesion group. From the 9 rats of the 4-root lesion group, only 4 rats performed successful vertical grasps. These four rats attempted 8 successful vertical grasps and the mean number for the number of successful vertical grasps in this group was 0.2. Moreover, 5 of the 9 rats did not grasp in any of the three behavioural tests. Grasp quality was poor in 100% of the attempts. The most deficient phase of the grasp was seen in the retraction of the bar; however, the first three phases were impaired. These results support the idea that an animal model with 4-root lesion clearly represents a severe sensorimotor deficit. Moreover, the lesion of the dorsal root T1 in rats from this group appears to be crucial for grasping.

Transplanted group. From the 4-root lesion group with the OEC transplant, 7 rats performed successful vertical grasps. These rats achieved twice the number of successful vertical grasps when compared with the group of 4-root lesion and no therapeutic intervention. The mean number of vertical successful grasps in this group was 0.5, slightly higher than in the group without OEC transplant. In this case only 3 rats did not grasp in any of the three behavioural tests. From the first to the third test, transplanted rats showed a slight increment in the number of successful vertical grasp when compared with rats without OEC transplant. **(Figure 3.3)**

3 time pts,

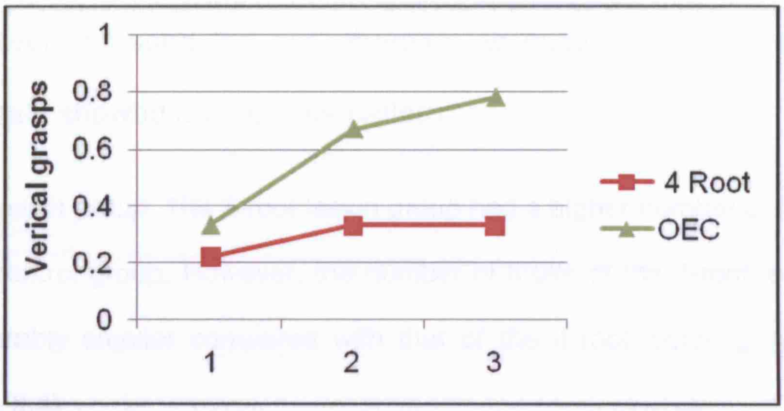

Figure 3.3 Increasing number of successful vertical grasps over time, in rats with OEC transplant.

In this group, grasp quality was poor in 33% of the cases and fair in 7%. The most deficient phases of the grasp were seen in the *type of the grasp* and in the *retraction of the bar*, this decrease may have been due to the increase in the number of grasps and to the fact that this group had the most severe type of injury in which the function of the paw was severely affected. The phases of *flexion of forelimb* and *extension of digits* were impaired.

3.1.3 Faults

Control group. From the 9 rats of the control group, only 3 had four minor faults in total within the 3 time points. The mean number of faults for this group was 0.4. The faults the rats from this group presented were subtle and immediately corrected so the grasp was still performed. This was interpreted as complete awareness of limb position as weight support was performed for each grasp during the climbing; an intact sensorimotor system is evident among control subjects which rarely registered faults and instinctively grasped the bar whereas, as expected, the 3-root and 4-root lesion rats showed an opposite pattern.

3-root lesion group. The 3-root lesion group had a higher number of faults compared to the control group. However, the number of faults of the 3-root lesion group was considerably smaller compared with that of the 4-root lesion group on average. (**Figure 3.4**)

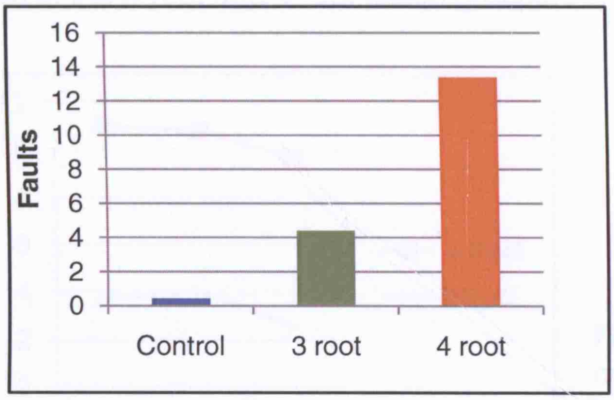

Figure 3.4 The number of faults of the 4-root lesion group was consistently higher compared with that of the 3-root lesion group and the control group

Despite a relatively severe injury, a strong presence of sensory input primarily in the paw was reflected by not only the lower number of faults but by a considerably increased number of successful vertical grasps. The type of faults the rats from the 3-root lesion presented was also different. The particularly increased activity of the injured forelimb and dissociation between the forelimb joint influenced this pattern.

4-root lesion group. Furthermore, as described previously, rats with 4-root lesion showed severe disruption of forelimb and so the incidence of faults was consistently high during the 3 tests. The most consistent type of fault observed among this group can be described as complete unawareness of the forelimb position reflected in the severe miss targeting of the paw when the rat attempted to grasp. However, a less "severe" type of fault was also present and thus, described as a touch either of the vertical or the horizontal bar without performing the grasp. This corresponds to the fact that a subtle improvement in sensorimotor function was observed in the measure of fault incidence over time. (**Figure 3.5**) An apparent biphasic pattern of behaviour with a more substantial improvement from the second to the third test was detected in the 3-root and 4-root lesion groups.

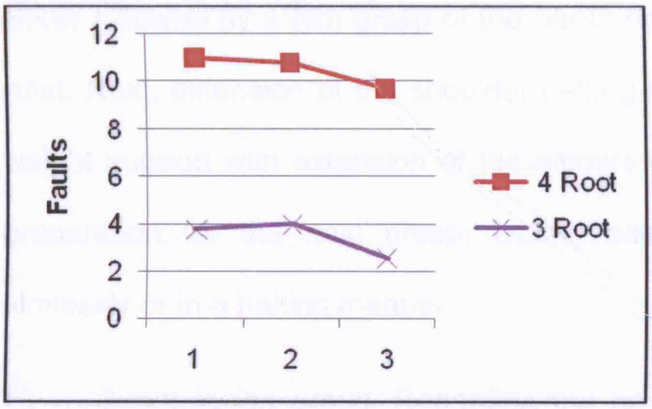

Figure 3.5 Decrease in number of faults over time in the 3-root and 4-root lesion groups

Transplanted group. Rats from the transplanted group showed a small decrease in the number of faults along the 3 tests, however slightly higher from the first to the second behavioural test.

3.1.4 Posture

Control group. The normal posture for the control group was defined as follows. The neck was moderately extended up straight and the head aligned with the body with the nose pointing up in the midline **(Figure 3.6a).**

Sporadically a slight kiphosis and a bilaterally shift of weight was seen as the rat climbed in a straight, vertical line. The sustentation base was equidistant between shoulders and hindlimb mostly. It was observed that rats occasionally, when flexion of hips was insufficient to push for the next grasp, used their hindlimb toes to grasp the vertical bar. In conclusion, rats in the control group smoothly climbed with great stability and coordination between the body parts. These rats frequently alternated the climbing pattern with jumps that evidently required a high level of dexterity and proprioception.

The normal forelimb motion during climbing was typically characterised by anterior flexion of the shoulder and semi-flexion of the elbow followed by a firm grasp of the bar in neutral or slight pronation of the wrist. Next, extension of the shoulder getting the bar closer to the body and weight support with extension of the elbow's forelimb was preceded by the preparation for the next grasp. During brief periods, rat's behave was aimlessly or in a halting manner.

3-root lesion group. Regarding the postural alterations of the 3-root and 4-root lesion groups in contrast to the normal control group, it was found that the 3-root lesion group showed an increased number of body compensations and substitutions the rat made to assist the pattern of climbing. Many of the grasps performed with the left paw were carried out with a pattern of flexion of the elbow and internal rotation of the shoulder as if the rat were 'hugging the bar'. Sometimes weight support with the left paw was seen, but without grasping.

The 3-root group also showed an increased adduction and internal rotation of the left shoulder. However, when extreme pronation of the wrist occurred, it allowed grasping with this pattern. This group was also characterised by showing major dissociation between the left limb joints, and increased movement of this limb was evident. The body was nonetheless not aligned with the head and an increased dorsal Kiphosis was also present.

4-root lesion and transplanted group. The 4-root lesion group and the transplanted group shared posture characteristics. As in the 3-root lesion group, rats showed that the injured forelimb when not aiming to grasp, adopted a position with an increased internal rotation and adduction of the shoulder, extension of elbow, increased pronation and flexion of the wrist, often with a clear extension of digits. The neck was slightly extended which partly provided posture stability; the head also frequently moved in different directions like in an exploring manner. The rat often stopped in the way up of the frame and out of the midline. A markedly increased kiphosis and left flexion of the trunk was observed, when the uninjured contralateral forelimb retracted the bar **(Figure 3.6b).** This was associated with a wider sustentation base and a constant shifting of weight as the rat climbed in diagonals, constantly changing the pattern.

Figure 3.6a

Figure 3.6b

Figure 3.6 Photographs illustrating the body position of the rat climbing the frame. (a) View of a control rat with straight and aligned body posture. (b) View of a lesioned rat with marked kiphosis.

It is important to emphasize that movement of the injured side forelimb was sometimes very limited. Body instability due to a limited ability to use the left forelimb appeared to cause many attempts to fall from the climbing frame. Often, rats were very close to the climbing frame, sometimes the nose between the vertical bars and the left forelimb trapped between the body and the frame.

Another observation of body compensations among this group included support of weight with the left forearm over the horizontal bars. Many touches of the vertical bar with no successful grasps were also a characteristic in this group. The semi-flexion of the neck showed that the rats were, at some level, taking advantage of the visual proprioceptive feedback to compensate for sensation loss in the paw. The major differences between the 4-root lesion and the 3-root lesion rats were observed in the injured forelimb activity.

3.1.5 Amplitude of movement

The normal control group showed an extensive amplitude of movement of anterior flexion of shoulders at all times, hence, each grasp was very effective during climbing. All rats in the 3-root lesion group showed extensive amplitude of movement. These rats flexed the forelimb high enough so the paw reached at least, the level of the ipsilateral shoulder thereby having more functionality during climbing. In contrast, the 4-root lesion group occasionally had the paw reaching the level of the shoulder but the amplitude was mostly defined as slight.

3.2 Statistical analysis

From all 3 groups included in the statistical analysis, data were obtained from all subjects on each variable tested during the 3 different behavioural tests; thus, no missing cases were reported for the comparisons. In order to select the adequate statistical test, variability of the data was considered surely. The test of normality provided relatively high differences between the mean and the median values **(Table 3.1).** This test also indicated skewed and kurtosis values greater than 1, suggesting a non normal distribution of the data. Therefore, a nonparametric test was selected.

	Faults		**Vertical grasps**	
	Mean	**Median**	**Mean**	**Median**
4-root lesion	-1.333	-2.000	0.111	0.000
Transplanted	-1.555	-1.000	0.444	0.000

Table 3.1 Test of normality results for the differences between the 4-root lesion and the transplanted group

At first, statistical comparisons between the 3-root lesion and the 4-root lesion groups for all the attributes during the three behavioural tests were carried out with the Mann-Whitney U test. The test showed that the two groups differed significantly during the 3 different tests for all the attributes studied. The results ($P < 0.001$) suggest, from the first test, how different the behavior was with only one root of difference in the type of lesion. The **table**

3.2 below shows the *P-value* for vertical grasps and faults, considered the most predictive variables for proprioceptive input.

Behavioural tests	***P* value**
1	< 0.001
2	< 0.001
3	< 0.001

Table 3.2 Differences between the 3-root lesion and the 4-root lesion group in the three behavioural tests for successful vertical grasps and faults.

Additional results from a second comparison, as was expected to find between groups with no therapy, were the evidence of no behavioural improvement over time. The first and the third test for the number of successful vertical grasps did not differ significantly ($P = 0.931$) nor did the number of faults ($P = 0.168$) in the 3-root lesion group. Additionally, in the 4-root lesion group vertical grasps and faults did not differ significantly ($P = 0.655$ and 0.150 respectively).

Furthermore, a comparison between the difference of scores from the first to the third behavioural test for the faults and successful vertical grasps attributes was carried out for the 4-root lesion and the transplanted group. This allowed the testing the behavioral performance after a specific therapy, in this case OEC transplant, and to observe whether functional improvement was associated with proprioceptive recovery after transplant, possibly evident within a narrow period of time. The differences of improvement along time for both attributes included for the statistical analysis were also calculated using the Mann-Whitney U test. The findings ($P > 0.05$) showed no significant difference between the first and the third behavioural tests in the transplanted group when compared with the behavioral responses of the 4 root lesion group over the same period of time.

These findings may be due to the small sample size used, the short time period and the presence of outliers and extreme values, as confirmed using the same non-parametric test. These outliers and extreme values were found likely to influence the results, by increasing the difference of the mean values between the groups. Appropriate searching for error in data recording and incorrect data entry values was done without any findings. Moreover, when excluding outliers from the analysis, the results did not change. Outliers would have influenced this comparison of groups over time less if the sample size had been increased.

From an initial qualitative description, a regular pattern of climbing was identified and described as normal, and postural observation and amplitude of movement were also qualitatively described for each group of subjects. The behavioural differences between the 3-root and the 4-lesion groups were clearly identified in the qualitative description and confirmed with a strong significant difference in the statistical analysis. Further findings from the statistical analysis show no behavioural changes over time in the groups with no therapy; however, a slight degree of possible adaptation to the type of injury was observed. Furthermore, according to the qualitative observations over time, there was evidence of behavioural improvement in the transplanted group however this was no statistically significant.

4. Discussion

The results of the instrument used in this study showed characteristic qualities for climbing and grasping in all rats belonging to the four groups studied (control, 3-root lesion, 4-root lesion without transplant, and 4-root lesion with OEC transplant groups). The assessment measure enabled to distinguish the recovery progression of each attribute assessed and to elaborate an efficient and sensitive assessment of skilled forelimb movement. Moreover, the semi-quantitative method and sub-scoring system characterizing the scale designed, allowed the quantification of improvement of the overall climbing pattern and of behavioural attributes and the definition of the different recovery responses.

Important functional differences between the dorsal root lesion groups were observed particularly in terms of vertical grasps and faults. Although an end-point was in all cases achieved, the behavior of 3-root and 4-root lesion rats during climbing was significantly altered when compared to each other and to that of control normal rats. These differences confirmed that grasping is a complex sensorimotor activity and requires CNS integrity.

Based on the qualitative observation, it was concluded that rats could achieve horizontal grasping by placing the paw over the horizontal bar without necessarily relying on sensory input. On the other hand, vertical grasp was shown to be a complex skilled behavior, highly affected after multiple cervical dorsal root lesions. All rats from the 3-root lesion group performed successful vertical grasps at the 3 behavioural tests, whereas rats from the 4-root lesion group rarely accomplished grasping, thus showing significant reduction in the number of successful vertical grasps. This last group, showed a severe functional limitation and a substantial disruption of the forelimb use, suggesting that an animal model with 4-root lesion clearly results in a severe sensorimotor deficit. The reduction in successful vertical grasps also supports the idea that the 4-root lesion group, despite severe sensory limitation, can show, through this behavioural assessment method, detectable motor changes related to the proprioceptive recovery.

In terms of vertical grasps, the study also showed a consistent behavioural progression in the mean of successful vertical grasps of the 4-root lesion transplanted group when compared to the 4-root lesion with no transplant. This observation suggests that the transplant might have had an early functional effect associated to the gain of sensory input and proprioception. At very early stages, an improvement in the quality and effectiveness of grasping was also observed. Although qualitative analysis demonstrated that the transplanted group displayed behavioural improvement for successful vertical grasps, the difference between the transplanted group and the 4-root lesion group without the transplantation did not reach statistical significance. Results for the statistical analysis however may be attributed to the presence of type 2 errors.

Differences in faults also were identified using the assessment measure. In fact, the number and type of faults differed when comparing the 4-root lesion group with the 3-root lesion group. In the third behavioural test, however, there was a slight decrease in the number of faults in both groups, which was associated to sensorimotor adaptations and probable subtle sensory input recovery after the lesion. This finding is explained by the fact that rats might have developed motor adaptations associated to the sudden lack of sensory input after the injury. However this behaviour stabilizes between the second and third test. This last result can be interpreted also, as a progressive degree of sensorimotor behavioural restoration during early stages post lesion. A possible explanation might be regeneration or axonal sprouting, or also uninjured cervical roots supplying enough innervation to initiate spontaneous sensory recovery, as reflected in the increased incidence of vertical successful grasps over time. (Wang et al. 2008).

Finally, observation of posture attributes demonstrated that different compensatory movement strategies were revealed across all the lesion groups and appeared to differ significantly from control rats. Different compensatory movement strategies were further confirmed by observations of the amplitude attributes, showing a reduction in the range of joint motion due to the loss of proprioceptive afferents impeding this movement.

The climbing frame system was considered simple and a practical test to employ. Its use allows description of functional deficits according to the severity and time of the lesion. Due to its sensitivity to demonstrate general forelimb deficits after dorsal root injury, it can be used in other laboratories. To observe, assess and score the performance of rats during climbing only requires minimal equipment, i.e. video recordings. Such equipment provides a simple method of visualizing the manner in which rats with unilateral cervical root injury behave. This system of assessment also allows the observation and scoring in detail by comparing videos taken at different times.

The value of the semi-quantitative instrument used for this study, compared to a purely subjective assessment tool, is its utility for statistical comparisons (Basso et al. 2006). Moreover, by combining an end-point measure with a kinematic measure, it was possible to assess not only behaviour but also to identify descriptive characteristics of posture and movement (Whishaw 1996a).

Another advantage of the current project is that, while most studies have assessed skilled forelimb movement in rats after CNS injury without considering the lesion type and the structure involved, our assessment method was based on multiple observations of rats with different number of roots lesioned; thus the assessment instrument presented here is reliable and specific for dorsal root lesions.

Finally, while researchers disagree about whether correct paw placement is evidence for recovery or adaptation to the deficit (Soblosky et al. 2001), the design of this instrument allows the demonstration of the importance of sensory input for grasping soon after the lesion. Furthermore, As visual feedback can be an alternative supply of proprioceptive input in the lesioned rats, rats were only assessed when they were climbing with the nose pointing up straight and not visually exploring the climbing frame.

Nevertheless, there are also a number of disadvantages that are important to discuss and that unfortunately limit the results of the study. Observation of behaviour may be appropriate for characterizing important movement alterations such as abnormalities in climbing and grasping. However, as the behaviour complexity increases with lesion, additional objective analyses become necessary. The reliability of the observations, particularly of deviations of movement and posture, was limited by the difficulty record in detail performance of rats moving quickly and the use of only one camera, thus constraining the visual field of observation.

Another disadvantage of the present study pertains to subjective bias and sample size. Each score of the sub-scoring system used to qualify grasping represented a unique stage of recovery, but the intervals were not necessarily equal. Thus, since the distinction between each category and score must be arbitrarily defined, interpretation of data from these type of measures can be difficult. Subjective bias is thus involved when observing characteristics of each category from the "best" to the "worst" performance (Muir & Webb 2000). Finally, the influence of outliers and extreme values on the comparison of groups over time concluded from the statistical analysis could be decreased by increasing the sample size.

4.1 Clinical implications

The present study on the behavioural assessment of rats with multiple cervical dorsal root avulsion and of functional recovery after OEC transplant discloses implications for research and interventions on humans affected by brachial plexus injuries. In terms of the clinical repercussions of this instrument, it can be concluded that the effects of CNS surgical procedures, either for experimental or therapeutic aims, should be continuously monitored with accurate behavioral assessment instruments. Moreover, behavioural measures should be designed to demonstrate the extent of functional recovery after a particular therapy. Sub-scoring each attribute of the overall behavior is justified by the fact that a specific therapy might affect some but perhaps not all aspects of behaviour (Basso 2004). This is particularly important in rehabilitation protocols since addressing the intervention process on the basis of the specific deficits increases the possibilities for improving functional outcomes. Furthermore, the possibility to tailor therapeutic procedures to specific goals improves not only the function but the quality of specific sensorimotor activity.

Another implication of this study is the confirmation of behavioural impairment in rats after damage to cervical dorsal roots. Impaired proprioception appears to inhibit behaviour due to diminished information about limb segments position in space. Moreover, it has been stated that in humans, nerve injury affecting sensation of the hand considerably affects the function of the hand, causing considerable social disability (Lundborg & Rosen 2007). Also in humans, sensory feedback is an integral part of the overall motor control system and is critical in modifying motor patterns in order to facilitate adaptations to the environment (Kay-Lyons 2002). Although visual feedback is useful to predict required skilled hand function, the digital tactile sensors provide critical information for the adaptation of grip forces and grasp kinematics (Jenmalm et al. 2000; Johansson 2002). The well developed feedback system between the hand and the central nervous system based on permanent proprioception and sensory input is a requirement for the regulation of grasping characteristics. Due to the fact that in humans the hand represents an organ with highly developed sensory functions, injuries affecting its function deserve special attention regarding the assessment methods. (Johansson 2002). The elaboration of a behavioural measure similar to the one designed to assess behavior and recovery in rats in this study can thus be beneficial for the evaluation of lesions and recovery of individuals as well. A clinical implication then of this study is the possible adaptation in the future of a similar assessment measurement to humans.

Further investigations assessing behaviour over longer periods of time would provide information about the long-term effects of the transplant and/or about adaptation to the lesion.

It has been established that proprioceptive afferents influence "advancing" or "delaying" components of a motor pattern (kay-Sim 2005). Therefore, assessment timing of performance is another consideration for future work. Despite having observed "delay" of behavior particularly in the rats with 4-root lesion, timing of performance was not considered for this research project.

Directions for future research also include the possibility for assessment of behaviours other than the ones that were included in this study. In fact, other attributes including contralateral grasps, horizontal grasps and jumps, were identified and included in the initial qualitative description. Yet, these were found not as predictive of proprioceptive deficit and recovery as vertical grasps, faults, posture and amplitude of movement.

Even though this data were not included for the purpose of this study, it is subject of analysis for further investigations.

In conclusion, it has been reviewed that in humans and quadrupeds alike, sensory input from limbs plays a crucial role in modifying muscle activity (Yang & Gorassini 2006). Therefore, any strategy for recovering behavioral function should include modulation of the physiological state of the spinal cord while using the proprioceptive input system as control of motor function (Edgerton et al. 2008). It can be stated that a rigorous approach to the analysis of behaviour is necessary to characterize all functional deficits arising from a specific central nervous system lesion. Using a measure that is adequate for each deficit is the only way to assess the effect of therapeutic interventions in animal models and to progress toward improving functional recovery in humans (Muir & Webb 2000). Considering the impact of cervical root lesions on quality of life and function, assessment measurements are essential, making the design of practical instruments a concern among researchers. The behavioural assessment method elaborated in this research project allows quantifying and qualifying the proprioceptive function and recovery from forelimb sensorimotor deficit. Furthermore, findings from this behavioural assessment in rats with a challenging clinical condition such as cervical dorsal root avulsion, will contribute for the research on the evaluation and treatment of patients with brachial plexus injuries.

Appendix 1

Data of differences between the 4-root lesion transplanted group and the 4-root lesion group without transplant from the first to the third behavioural test, for faults and successful vertical grasps attributes.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Vertical grasps	4-root lesion	9	9.06	81.50
	OEC	9	9.94	89.50
	Total	18		

Test Statistics

	Vertical grasps
Mann-Whitney U	36.500
Wilcoxon W	81.500
Z	-.447
Asymp. Sig. (2-tailed)	.655
Exact Sig. [2*(1-tailed Sig.)]	.730(a)

a Not corrected for ties.
b Grouping Variable: group

Ranks

	Group	N	Mean Rank	Sum of Ranks
Faults	4-root lesion	9	8.89	80.00
	OEC	9	10.11	91.00
	Total	18		

Test Statistics

	Faults
Mann-Whitney U	35.000
Wilcoxon W	80.000
Z	-.492
Asymp. Sig. (2-tailed)	.623
Exact Sig. [2*(1-tailed Sig.)]	.666(a)

a Not corrected for ties.
b Grouping Variable: group

Appendix 2

Scoring charts for each group of rats (control group, 3-root lesion, 4-root lesion and 4-root lesion with OEC transplant group). The data shown in these charts include scoring of the attributes vertical left grasps, horizontal left grasps, total of left grasps, faults, right grasps, jumps and quality of the pattern.

Subject	Group	V Grasps	H Grasps	L Grasps	Faults	R Grasps	Jumps	Pattern
B2	Control	4	1	5	0	6	4	Good
B3	Control	6	1	7	0	6	2	Good
A2	Control	6	1	8	2	6	0	Fair
A1	Control	6	1	7	1	7	0	Good
F3	Control	7	0	7	0	6	1	Good
D2	Control	5	0	5	0	7	0	Good
E1	Control	7	1	8	0	8	4	Good
E3	Control	3	2	5	1	6	3	Good
F1	Control	5	2	7	0	6	1	Good

Subject	Group	Time	V Grasps	H Grasps	L Grasps	Faults	R Grasps	Jumps	Pattern
A1	3-root	1	3	4	7	4	8	2	Fair
A1	3-root	2	1	6	7	5	10	3	Poor
A1	3-root	3	2	5	7	2	9	1	Fair
B1	3-root	1	2	4	6	2	8	4	Fair
B1	3-root	2	4	1	5	6	8	2	Fair
B1	3-root	3	4	2	6	2	5	1	Poor
B2	3-root	1	2	1	3	6	8	5	Fair
B2	3-root	2	1	4	5	7	9	1	Fair
B2	3-root	3	2	3	5	1	6	3	Poor
B3	3-root	1	4	0	4	5	6	2	Poor
B3	3-root	2	4	0	4	6	6	4	Poor
B3	3-root	3	3	0	3	3	6	4	Poor
F1	3-root	1	3	3	6	3	9	3	Fair
F1	3-root	2	5	2	7	0	6	0	Good
F1	3-root	3	4	3	7	1	6	1	Fair
F2	3-root	1	3	3	6	2	7	2	Fair
F2	3-root	2	7	2	9	2	7	2	Fair
F2	3-root	3	3	1	4	5	4	4	Fair
F3	3-root	1	3	1	4	5	6	1	Fair
F3	3-root	2	5	2	7	0	8	2	Fair
F3	3-root	3	5	1	6	3	7	2	Fair
E2	3-root	1	3	2	5	3	8	1	Poor
E2	3-root	2	2	3	5	5	8	4	Poor
E2	3-root	3	1	5	6	3	8	4	Poor
E3	3-root	1	3	1	4	4	5	4	Fair
E3	3-root	2	4	1	5	5	7	4	Fair
E3	3-root	3	2	4	6	3	8	2	Fair

Subject	Group	Time	V Grasps	H Grasps	L Grasps	Faults	R Grasps	Jumps	Pattern
Q35	4-root	1	1	0	1	10	10	0	Poor
Q35	4-root	2	0	0	0	11	9	1	No grasp
Q35	4-root	3	1	0	1	7	9	1	Poor
Q54	4-root	1	0	0	0	13	10	0	No grasp
Q54	4-root	2	0	0	0	10	10	0	No grasp
Q54	4-root	3	0	0	0	10	10	0	No grasp
Q56	4-root	1	0	0	0	10	9	0	No grasp
Q56	4-root	2	0	0	0	14	9	1	No grasp
Q56	4-root	3	0	0	0	8	8	2	No grasp
94 EN2	4-root	1	0	0	0	13	9	1	No grasp
94 EN2	4-root	2	0	0	0	11	8	2	No grasp
94 EN2	4-root	3	0	0	0	12	8	1	No grasp
Q30	4-root	1	0	2	2	9	9	0	No grasp
Q30	4-root	2	0	1	1	10	10	2	No grasp
Q30	4-root	3	0	0	0	11	8	2	No grasp
Q38	4-root	1	0	0	0	9	9	3	No grasp
Q38	4-root	2	2	0	2	10	8	3	Poor
Q38	4-root	3	2	0	2	5	7	4	Poor
Q25	4-root	1	0	0	0	13	10	0	No grasp
Q25	4-root	2	1	0	1	10	10	3	Poor
Q25	4-root	3	0	0	0	11	8	0	No grasp
Q28	4-root	1	0	2	2	9	11	0	No grasp
Q28	4-root	2	0	0	0	10	13	0	No grasp
Q28	4-root	3	0	1	1	12	12	0	No grasp
Q29	4-root	1	1	0	1	13	11	0	Poor
Q29	4-root	2	0	0	0	11	14	2	No grasp
Q29	4-root	3	0	1	1	11	12	0	No grasp

Subject	Group	Time	V Grasps	H Grasps	L Grasps	Faults	R Grasps	Jumps	Pattern
Q6	OEC	1	0	0	0	14	11	0	No grasp
Q6	OEC	2	0	0	0	12	10	0	No grasp
Q6	OEC	3	0	0	0	12	10	0	No grasp
Q7	OEC	1	0	0	0	9	12	1	No grasp
Q7	OEC	2	0	0	0	11	7	0	No grasp
Q7	OEC	3	1	0	1	8	8	1	Poor
Q8	OEC	1	1	0	1	12	9	0	Fair
Q8	OEC	2	2	0	2	11	8	0	Poor
Q8	OEC	3	5	0	5	8	11	0	Poor
Q9	OEC	1	1	0	1	16	9	2	Fair
Q9	OEC	2	0	0	0	10	6	0	No grasp
Q9	OEC	3	1	0	1	7	7	0	Poor
Q11	OEC	1	0	0	0	13	10	0	No grasp
Q11	OEC	2	1	0	1	9	9	0	Poor
Q11	OEC	3	0	0	0	15	11	0	No grasp
Q12	OEC	1	0	0	0	13	9	0	No grasp
Q12	OEC	2	1	0	1	10	9	0	Poor
Q12	OEC	3	0	0	0	12	14	0	No grasp
Q14	OEC	1	1	1	2	9	9	0	Poor
Q14	OEC	2	1	0	1	10	8	1	Poor
Q14	OEC	3	0	0	0	8	10	2	No grasp
Q15	OEC	1	0	0	0	11	9	0	No grasp
Q15	OEC	2	1	0	1	11	10	0	Poor
Q15	OEC	3	0	0	0	12	10	0	No grasp
Q16	OEC	1	0	0	0	10	10	0	No grasp
Q16	OEC	2	0	1	1	11	9	0	No grasp
Q16	OEC	3	0	1	1	11	13	0	No grasp

References

AHMED-LABIB, M., GOLAN,J.D. & JACQUES,L. (2007) Functional outcome of brachial plexus reconstruction after trauma. *Neurosurgery*, **61,** 1016-1022.

ALDSKOGIUS, H. & KOZLOVA, E. N. (2002) Strategies for repair of the deafferented spinal cord. *Brain Research Reviews* **40**[1-3], 301-308.

BASSO, D.M., FISHER,L.C. & ANDERSON.A.J (2006) Basso mouse scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. *Journal of neurology*, **23,** 635-659.

BASSO,D.M. (2004) Behavioral testing after spinal cord injury: congruities, complexities, and controversies. *Journal of neurotrauma*, **21,** 395-404.

DOI, K., OTSUKA,K., OKAMOTO,Y., FUJII,H., HATTORI,Y. & BALIARSING,A.S. (2002) Cervical nerve root avulsion in brachial plexus injuries: magnetic resonance imaging classification and comparison with myelography and computerized tomography myelography. *Journal of neurosurgery*, **96,** 277-284.

EDGERTON,V.R., COURTINE,G., GERASIMENKO,Y.P., LAVROV,I., ICHIYAMA,R.M., FONG,A.J., CAI,L.L., OTOSHI,C.K., TILLAKARATNE,N.J., BURDICK,J.W. & ROY,R.R. (2008) Training locomotor networks. *Brain Research Reviews*, **57,** 241-254.

FARR, T.D., LIU,L., COLWELL,K.L., WHISHAW,I.Q. & METZ,G.A. (2006) Bilateral alteration in stepping pattern after unilateral motor cortex injury: a new test strategy for analysis of skilled limb movements in neurological mouse models. *Journal of Neuroscience Methods*, **153,** 104-113.

FERRANTE, M.A. (2004) Brachial plexopathies: classification, causes, and consequences. *Muscle Nerve*, **30,** 547-568.

GOMEZ,V.M., AVERILL,S., KING,V., YANG,Q., DONCEL,P.E., CHACON,S.C., WARD,R., NIETO-SAMPEDRO,M., PRIESTLEY,J. & TAYLOR,J. (2003) Transplantation of olfactory ensheathing cells fails to promote significant axonal regeneration from dorsal roots into the rat cervical cord. *Journal of neurocytology*, **32,** 53-70.

HUANG, M.C., CHEN, K.C. & CHUANG, T.Y. (2003) Cervical root repair in adult rats after transection: recovery of forelimb motor function. *Experimental Neurology*, **180,** 101-109.

JENMALM, P., DAHLSTEDT,S. & JOHANSSON,R.S. (2000) Visual and tactile information about object-curvature control fingertip forces and grasp kinematics in human dexterous manipulation. *Jouranl of.neurophysiology*, **84,** 2984-2997.

JOHANSSON, R.S. (2002) Dynamic use of tactile afferent signals in control of dexterous manipulation. *Advances in experimental medicine and biology*, **508,** 397-410.

KANAGAL, S.G. & MUIR, G.D. (2007) Bilateral dorsal funicular lesions alter sensorimotor behaviour in rats. *Experimental Neurology*, **205,** 513-524.

KAY-LYONS, M. (2002) Central pattern generation of locomotion: a review of the evidence. *Physical therapy*, **82,** 69-83.

KAY-SIM, A. (2005) Olfactory ensheathing cells and spinal cord repair. *The Keio journal of medicine*, **54,** 8-14.

KEYVAN-FOULADI, N., RAISMAN,G. & LI,Y. (2003) Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, **23,** 9428-9434.

LI, Y., YAMAMOTO,M. & RAISMAN,G. (2007) An experimental model of ventral root repair showing the beneficial effect of transplanting olfactory ensheathing cells. *Neurosurgery*, **60,** 734-741.

LI, Y., CARLSTEDT,T., BERTHOLD,C.H. & RAISMAN,G. (2004) Interaction of transplanted olfactory-ensheathing cells and host astrocytic processes provides a bridge for axons to regenerate across the dorsal root entry zone. *Experimental Neurology*, **188,** 300-308.

LI,Y., DECHERCHI,P. & RAISMAN,G. (2003) Transplantation of olfactory ensheathing cells into spinal cord lesions restores breathing and climbing. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, **23,** 727-731.

LI,Y., FIELD,P.M. & RAISMAN,G. (1997) Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science*, **277,** 2000-2002.

LUNDBORG,G. & ROSEN,B. (2007) Hand function after nerve repair. *Acta Physiologica (Oxf)*, **189,** 207-217.

MACEFIELD, G., GANDEVIA,S.C. & BURKE,D. (1990) Perceptual responses to microstimulation of single afferents innervating joints, muscles and skin of the human hand. *The Journal of physiology*, **429,** 113-129.

MCKENNA, J.E. & WHISHAW,I.Q. (1999) Complete compensation in skilled reaching success with associated impairments in limb synergies, after dorsal column lesion in the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **19,** 1885-1894.

METZ, G.A. & WHISHAW,I.Q. (2000) Skilled reaching an action pattern: stability in rat (Rattus norvegicus) grasping movements as a function of changing food pellet size. *Behavioural brain research*, **116,** 111-122.

MIDHA, R. (1997) Epidemiology of brachial plexus injuries in a multitrauma population. *Neurosurgery*, **40,** 1182-1188.

MONTOYA, C.P., CAMPBELL-HOPE, L.J., PEMBERTON, K.D. & DUNNETT, S.B. (1991) The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats. *Journal of neuroscience methods*, **36,** 219-228.

MUIR, G.D. & WEBB, A.A. (2000) Assessment of behavioural recovery following spinal cord injury in rats. *European Journal of Neuroscience*, **12,** 3079-3086.

MUIR, G.D., WEBB, A.A., KANAGAL,S. & TAYLOR,L. (2007) Dorsolateral cervical spinal injury differentially affects forelimb and hindlimb action in rats. *European journal of neuroscience*, **25,** 1501-1510.

NAGANO, A. (1998) Treatment of brachial plexus injury. *Journal of.orthopaedic science.*, **3,** 71-80.

NAVARRO,X., VALERO,A., GUDINO,G., FORES,J., RODRIGUEZ,F.J., VERDU,E., PASCUAL,R., CUADRAS,J. & NIETO-SAMPEDRO,M. (1999) Ensheathing glia transplants promote dorsal root regeneration and spinal reflex restitution after multiple lumbar rhizotomy. *Annals of neurology*, **45,** 207-215.

NEMECEK,A.N., AVELLINO,A.M., GOODKIN,R., LITTLE,J. & KLIOT,M. (2003) Mapping dermatomes during selective dorsal rhizotomy: case report and review of the literature. *Surgical neurology*, **60,** 292-297.

PASCUAL,J.I., GUDINO-CABRERA,G., INSAUSTI,R. & NIETO-SAMPEDRO,M. (2002) Spinal implants of olfactory ensheathing cells promote axon regeneration and bladder activity after bilateral lumbosacral dorsal rhizotomy in the adult rat. The *Journal of urology*, **167,** 1522-1526.

RAISMAN,G. & LI,Y. (1994) Schwann cells induce sprouting in motor and sensory axons in the adult rat spinal cord. *The journal of neuroscience*, **14**, 4050-4063

RAMER,M.S., BISHOP,T., DOCKERY,P., MOBARAK,M.S., O'LEARY,D., FRAHER,J.P., PRIESTLEY,J.V. & MCMAHON,S.B. (2002) Neurotrophin-3-mediated regeneration and recovery of proprioception following dorsal rhizotomy. M*olecular and cellular neuroscience*, **19,** 239-249.

RAMER,M.S., PRIESTLEY,J.V. & MCMAHON,S.B. (2000) Functional regeneration of sensory axons into the adult spinal cord. *Nature*, **403,** 312-316.

RAMON-CUETO,A. & NIETO-SAMPEDRO,M. (1994) Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. *Experimental Neurology*, **127,** 232-244.

RICHTER,M.W., FLETCHER,P.A., LIU,J., TETZLAFF,W. & ROSKAMS,A.J. (2005) Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25,** 10700-10711.

ROSSIGNOL,S., DUBUC,R. & GOSSARD,J.P. (2006) Dynamic sensorimotor interactions in locomotion. *Physiological reviews*, **86,** 89-154.

SALING,M., SITAROVA,T., VEJSADA,R. & HNIK,P. (1992) Reaching behavior in the rat: absence of forelimb peripheral input. *Physiology and behaviour*, **51,** 1151-1156.

SHIN,A.Y., SPINNER,R.J., STEINMANN,S.P. & BISHOP,A.T. (2005) Adult traumatic brachial plexus injuries. *The Journal of the American Academy of Orthopaedic Surgeons*, **13,** 382-396.

SHOKEI,Y., RUSSELL,L. & ROBERT,I. (1996) Bypass coaptation procedures for cervical nerve root avulsion. *Neurosurgery*, **38,** 1145-1152.

SOBLOSKY,J.S., COLGIN,L.L. & CHORNEY LANE,D. (1997) Ladder beam and camera video recording system for evaluating forelimb and hindlimb deficits after sensorimotor cortex injury in rats. *Journal of Neuroscience methods*, **78,** 75-83.

SOBLOSKY,J.S., SONG,J.H. & DINH,D.H. (2001) Graded unilateral cervical spinal cord injury in the rat: evaluation of forelimb recovery and histological effects. *Behavioural brain research*, **119,** 1-13.

TAKAHASHI,Y. & NAKAJIMA,Y. (1996) Dermatomes in the rat limbs as determined by antidromic stimulation of sensory C-fibers in spinal nerves. *Pain*, **67,** 197-202.

TANG,X.Q., CAI,J., NELSON,K.D., PENG,X.J. & SMITH,G.M. (2004) Functional repair after dorsal root rhizotomy using nerve conduits and neurotrophic molecules. *European Journal of Neuroscience*, **20,** 1211-1218.

WANG,R., KING,T., OSSIPOV,M.H., ROSSOMANDO,A.J., VANDERAH,T.W., HARVEY,P., CARIANI,P., FRANK,E., SAH,D.W. & PORRECA,F. (2008) Persistent restoration of sensory function by immediate or delayed systemic artemin after dorsal root injury. *Nature.neuroscience*, **11,** 488-496.

WEBB,A.A. & MUIR,G.D. (2005) Sensorimotor behaviour following incomplete cervical spinal cord injury in the rat. *Behavioural brain research*, **165,** 147-159.

WEBB,A.A. & MUIR,G.D. (2003) Unilateral dorsal column and rubrospinal tract injuries affect overground locomotion in the unrestrained rat. *European journal of neuroscience.*, **18,** 412-422.

WHISHAW,I. (1996a) Skilled forelimb movements in prey catching and in reaching by rats (Rattus norvegicus) and opossums (Monodelphis domestica): relations to anatomical differences in motor systems. *Behavioural brain research*, **79**, 163-81

WHISHAW,I.Q. (2000) Loss of the innate cortical engram for action patterns used in skilled reaching and the development of behavioral compensation following motor cortex lesions in the rat. *Neuropharmacology*, **39,** 788-805.

WHISHAW,I.Q. (1996b) An endpoint, descriptive, and kinematic comparison of skilled reaching in mice (Mus musculus) with rats (Rattus norvegicus). *Behavioural brain research.*, **78,** 101-111.

WHISHAW,I.Q. & KOLB,B. (1988) Sparing of skilled forelimb reaching and corticospinal projections after neonatal motor cortex removal or hemidecortication in the rat: support for the Kennard doctrine. *Brain Research*, **451,** 97-114.

WHISHAW,I.Q., PELLIS,S.M. & GORNY,B.P. (1992) Skilled reaching in rats and humans: evidence for parallel development or homology. *Behavioural brain research*, **47,** 59-70.

WHISHAW,I.Q. & TOMIE,J.A. (1989) Olfaction directs skilled forelimb reaching in the rat. *Behavioural brain research.*, **32,** 11-21.

YANG,J.F. & GORASSINI,M. (2006) Spinal and brain control of human walking: implications for retraining of walking. *Neuroscientist.*, **12,** 379-389.

YOSHIKAWA,T., HAYASHI,N., YAMAMOTO,S., TAJIRI,Y., YOSHIOKA,N., MASUMOTO,T., MORI,H., ABE,O., AOKI,S. & OHTOMO,K. (2006) Brachial plexus injury: clinical manifestations, conventional imaging findings, and the latest imaging techniques. *Radiographics*, **26 Suppl 1,** S133-S143.

ZHURAVIN,I.A. & BURES,J. (1986) Operant slowing of the extension phase of the reaching movement in rats. *Physiology and behaviour*, **36,** 611-617.