Changes in spinal cord architecture after brachial plexus injury in the newborn

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Summary
Obstetric brachial plexus palsy is a devastating birth injury. While many children recover spontaneously, 20–25% are left with a permanent impairment of the affected limb. So far, concepts of pathology and recovery have focused on the injury of the peripheral nerve. Proximal nerve injury at birth, however, leads to massive injury-induced motoneuron loss in corresponding motoneuron pools and therefore limits the extent of functional recovery. In the present study, the role of spinal cord plasticity after injury and recovery from obstetric brachial plexus lesions was investigated. A selective injury to spinal roots C5 and C6 was induced in newborn Sprague–Dawley rats, leading to motoneuron loss in corresponding motoneuron pools. Recovery of extremity function was evaluated with different behavioural paradigms. Permanent changes of adjacent motoneuron pools were quantitatively evaluated by retrograde tracing and functional muscle testing. We report that the adjacent C7 motoneuron contribution to biceps muscle innervation increased four-fold after upper trunk lesions in newborns, thus compensating for the injury-induced motoneuron loss. These results indicate that, in obstetric brachial plexus palsy, changes in spinal cord architecture are an integral part not only of primary pathology but also of the subsequent recovery process. While present treatment is directed towards the restoration of neural continuity, future treatment strategies must recognize and take advantage of CNS participation in the injury and recovery process.

Keywords: nerve; injury; obstetric; spinal cord; plasticity

Abbreviations: MCMP = musculocutaneous nerve motoneuron pool; MCN = musculocutaneous nerve


Introduction
Obstetric brachial plexus palsy is caused by injury to the brachial plexus during complicated child delivery (Shenaq et al., 1998). Rouse categorized the incidence of obstetric brachial plexus palsy depending on birth weights and found incidence rates ranging from 0.9 to 2.6 per 1000 live births (Rouse et al., 1996). In a recent survey, Evans-Jones found an incidence rate of 0.42 per 1000 live births in the UK and the Republic of Ireland (Evans-Jones et al., 2003). The rate and extent of recovery can differ dramatically, depending on the extent of primary lesion. While many recover spontaneously with little clinical deficit, 20–25% of the children face a permanent impairment of the affected limb (Gilbert, 1995; Gjørup, 1966). A recent publication has documented that more than 80% of the affected children are Narakas Group I (C5–C6) and II (C5–C7) cases, whereas Narakas Group III (C5–T1) and IV (C5–T1; Horner’s syndrome) cases are rare, with little chance of spontaneous recovery (Narakas, 1987; Bisinella and Birch, 2003).

Since Smellie’s first clinical description of obstetric brachial plexus palsy in 1768, concepts of pathology and treatment strategies have focused on the injury of the peripheral nerve (Smellie, 1768; Gilbert, 1995; Strömbeck et al., 2000). Investigations of CNS events in injury and recovery were not pursued, even though Boyer had already described the dramatic CNS effects of brachial plexus birth traumas in humans as early as 1911 (Boyer, 1911; Birch, 2002).

It is of significance that injury strikes an immature nervous system. During the early postpartum period, interaction between maturing motoneurons and their target structures is
vital for motoneuron survival (Lowrie and Vrbova, 1992). Axon injury during this critical period initiates events in corresponding motoneurons, ultimately leading to their death (Fig. 1) (Schmalbruch, 1984; Oppenheim, 1991). As motoneurons mature, they gradually lose their target-dependency. As a result, injury-induced motoneuron loss is absent after comparable injuries in mature animals (Schmalbruch, 1984).

Recovery of motor and sensory function is attributed to axonal regeneration followed by original target reinnervation (Strömbeck et al., 2000). In adults, functional regeneration is constrained by time and distance. Prolonged axonal regeneration distances or delayed repair generally compromise functional recovery (Fu and Gordon, 1995). Recovery processes after brachial plexus lesions in newborns, however, are different. Periods of functional improvement are longer, often progressing up to the age of 5 years and beyond (Birch, 2002). The longer periods of recovery mirror adaptational mechanisms at the spinal and supraspinal level to overcome the initial motoneuron loss (McComas et al., 1993).

Adaptational responses affect all levels of the neuraxis, the extent depending on location, lesion pattern and developmental stage (Kolb and Whishaw, 1998; Pearson, 2000; Raineteau and Schwab, 2001). The fundamental role of the spinal cord in functional recovery after obstetric brachial plexus lesions is emphasized by the findings of Vredeveld and colleagues (Vredeveld et al., 2000). They demonstrated, that after obstetric brachial plexus lesions, spinal root C7 contributes to biceps and deltoid innervation. This effective C7 contribution is in clear contrast with the normal adult innervation pattern of the deltoid and biceps muscles, which is limited to spinal roots C5 and C6, regardless of pre- and postfixation. This suggests that, after upper trunk injuries in newborns, motoneurons of the neighbouring segment C7 take over the lost function of roots C5 and C6.

In the present study we investigated the hypothesis that injury to the brachial plexus in the newborn will lead to permanent changes in normal spinal cord architecture. Here we report that injury to C5 and C6 leads to injury-induced motoneuron loss in corresponding motoneuron pools that is, in part, compensated for by the preservation of C7 pathways. These findings emphasize the importance of spinal cord plasticity not only in primary pathology but also in the recovery process of obstetric brachial plexus injuries.

**Material and methods**

**Experimental design**

A total of 35 newborn and adult female Sprague–Dawley rats were divided into three groups (A, B, C). In 10 adult animals of group A, the normal size of the musculocutaneous nerve motoneuron pool...
(MCMP) and normative values of the C7 contribution to the MCMP were assessed with retrograde tracing techniques. In 15 animals of group B, the upper trunk of the brachial plexus was crushed within 12 h after birth. In 10 animals of group C an identical lesion of the upper trunk was carried out in mature 4-week-old animals. In groups B and C, overall motor recovery was evaluated with different behavioural paradigms throughout a 12-week survival period. After 12 weeks, functional muscle testing was used to determine the relative contribution of C7 motoneurons to the development of force in the biceps muscle. Finally, C7 motoneurons contributing to the MCMP were quantified. All experiments were performed under strict supervision of the University of Vienna ethics committee according to international regulations governing the use of laboratory animals.

*Nerve crush injury*

In group B, the right upper trunk of 15 animals was crushed within 12 h after birth. Pups were anaesthetized by hypothermia (7°C) until spontaneous and reflex movement ceased. The individual trunks (Fig. 2) of the right brachial plexus were identified using an operating microscope (magnification ×40) and the upper trunk was crushed for 5 s using jeweller’s forceps No. 5 (Bridge et al., 1994). After wound closure, animals were rewarmed and returned to their mothers for suckling.

In group C the same procedure was performed in 10 mature 4-week-old animals. Anaesthesia was maintained with a combination of Ketavet® (150 mg/kg, intramuscularly; Pharmacia and Upjohn, Erlangen, Germany) and Rompun® (4 mg/kg, intramuscularly; Bayer, Leverkusen, Germany). Crush lesions in mature animals were made following the same protocol as in newborns. After wound closure animals were returned to their cages.

**Behavioural assessment**

*Bertelli test*

Motor behaviour of the upper extremity was assessed with the Bertelli test (Bertelli and Mira, 1993). To allow postoperative recovery, testing sessions started 2 weeks after primary surgery. A voluntary, bilateral grooming response allowing exact analysis of elbow flexion and abduction was initiated with squirts of sweet syrup onto the animals’ snouts. Movement of the operated forelimb was compared with that of the contralateral healthy forelimb and subsequently graded employing a non-parametric score: grade 1 (no movement or mouth), grade 2 (region below the eye), grade 3 (eye), grade 4 (front of ears), and grade 5 (behind the ears). Compensatory movements, unstable movements and deviations from the normal plane of movement were documented. The Bertelli test was performed 2, 6, 8, 10 and 12 weeks after injury.

*Grid walk*

To uncover latent motor and balance deficits, accurate limb placement was examined using the grid walk paradigm 6 and 12 weeks after injury (Metz et al., 2000). Without postoperative training, animals had to cross an 80 cm runway raised 1 m above ground with 3.5 cm gaps between round metal bars. Animals had to cross the elevated runway a minimum of three times. The number of errors (footfalls or searching movement) within a 20-bar sector was recorded for each crossing and a mean error was calculated.

*Functional muscle testing*

In group A, total biceps muscle force was measured in vivo by supramaximal electrical stimulation (0.1 ms, 100 Hz, 10 mA) of the musculocutaneous nerve (MCN) and subsequent isometric force measurement of the biceps muscle. In groups B and C, the C7 contribution to biceps muscle force generation was assessed 12 weeks after crush injury of the upper trunk. Complex anatomy of the brachial plexus and surrounding vessels prohibited direct stimulation of spinal root C7. Instead, the upper trunk of the brachial plexus was axotomized and resected 10 days prior to muscle force measurement. After 10 days, axons originating from spinal roots C5 and C6 had degenerated. Supramaximal stimulation of the MCN was thus restricted to surviving C7 axons.

All animals were anesthetized with Nembutal® (60 mg/kg, intraperitoneally; Sanofi, Libourne, France) and stabilized in a supine position. The right MCN and biceps muscle were identified. The distal tendon of the biceps muscle was connected to a force transducer (FT-03, Grass Instruments, Boston, MA, USA) with a 4-0 silk suture. The signals generated by the force transducer were passed through a Grass 7P122 low-level DC amplifier and displayed on a personal computer. The average force of three tension measurements was calculated.

*Retrograde labelling of motoneuron pools*

Structural and numeric changes at the spinal level were quantified by retrograde motoneuron tracing (Swett et al., 1986). In control group A, the size of the MCMP was established. Animals (250–300 g) were anaesthetized with Nembutal® (60 mg/kg, intraperitoneally). The right MCN was explored, transected at biceps muscle entry, and introduced into an aqueous 5% Fluorogold® solution (Fluorochrome, Denver, CO, USA) for 60 min. To establish the contribution of C7 to the MCMP, the upper trunk was transected before MCN tracing. Thereby, retrograde tracer transport was limited to axons coming from spinal root C7. Following wound closure animals were returned to their cages.

After 48 h to allow retrograde tracer transport, animals were deeply anaesthetized with an overdose of Nembutal® followed by a left ventricular perfusion with 4% paraformaldehyde. Spinal cords were harvested from C3 to T1, postfixed, and immersed in a 20% sucrose solution for cryoprotection. Using a freezing microtome (Leica, Stuttgart, Germany), 40 μm cross-sections were cut and...
motoneurons of every section were quantified with an epifluorescence (405 nm) microscope under 20–40× magnification.

**Statistical analysis**
Statistical analysis was performed with SigmaStat® software (SPSS Science, Chicago, IL, USA). Means of motoneuron counts were subjected to analysis of variance in all experimental groups. If the analysis demonstrated significance ($P < 0.05$), a specific group mean comparison was performed for that variable using the Student–Newman–Keuls method. Means in behavioural paradigms were analysed using the Mann–Whitney $U$ test in all experimental groups. All data in this study are presented as mean ± SEM.

**Results**

**Behavioural assessment**

**Bertelli test**
Temporal analysis of the entire 12-week survival period showed differences in postinjury recovery between groups B ($n = 10$) and C ($n = 10$) (Fig. 3A). Recovery after upper trunk crush injury in animals of group C was fast and a mean score of $4.6 ± 0.25$ was reached 2 weeks after injury. On the contrary, neonatal postinjury development in animals of group B progressed continuously over the entire 12-week observation period. Comparison of the affected limb with the contralateral, healthy limb, however, revealed qualitative differences in movement patterns in all animals of group B. On the lesioned side, animals displayed deficits in aim, advance and accuracy of movement.

Comparison between the groups was statistically significant at 2 weeks ($P = 0.003$), 4 weeks ($P = 0.003$), 6 weeks ($P = 0.003$), 8 weeks ($P = 0.003$) and 10 weeks ($P = 0.018$). There was no statistically significant difference between the groups 12 weeks after injury ($P = 0.755$).

**Grid walk**

Grid walk evaluation revealed subtle deficits in complex task performance, which were not evident in free open field locomotion. The results at 6 weeks ($5.13 ± 0.53$) and at 12 weeks ($5.13 ± 0.53$) after injury in group B ($n = 10$), revealed deficits in foot placement and balance at a time when free open-field locomotion had fully recovered. Animals of group C ($n = 10$) showed almost no impairment in runway crossing 6 weeks after injury ($0.93 ± 0.44$) and 12 weeks after injury ($0.93 ± 0.44$). There was a statistically significant difference between the groups 6 weeks after injury ($P = 0.004$) and 12 weeks after injury ($P = 0.004$) (Fig. 3B).

**Functional muscle testing**
Extremity function was evaluated by functional muscle testing 12 weeks after the initial injury. Total biceps muscle force in group A was $17.96 ± 0.73$ mN ($n = 5$). In group B the C7 contribution to biceps muscle force generation after neonatal crush injury of the upper trunk was $7.50 ± 0.62$ mN ($n = 10$). In group C the C7 contribution to biceps muscle force generation after crush injury of the upper trunk in mature 4-week-old animals was $4.23 ± 0.31$ mN ($n = 5$). There was a statistically significant difference between the C7 contribution to biceps muscle force generation after an upper trunk crush injury in newborns and mature 4-week-old animals ($P = 0.004$) (Fig. 4).

**Neuroanatomical analysis**
In group A the MCMP contained $407.4 ± 19.1$ motoneurons ($n = 5$). The number of C7 motoneurons contributing to the musculocutaneous nerve was $11.6 ± 4.5$ ($n = 5$). In group B, crush injury to the upper trunk in newborns reduced the size of the MCMP to $109.0 ± 3.0$ motoneurons ($n = 5$). The C7 motoneuron number contributing to the musculocutaneous nerve was $44.9 ± 9.1$ ($n = 10$). In group C the number of C7
motoneurons contributing to musculocutaneous nerve after crush lesion to the upper trunk in mature 4-week-old animals was 1.4 ± 0.7 (n = 10).

There was no statistically significant difference between the number of C7 motoneurons contributing to the MCMP in groups A and C (P = 0.455). There was a statistically significant difference between the C7 motoneuron number in normal adults of group A and after an upper trunk crush injury in newborns of group B (P = 0.015). The difference between the C7 motoneuron numbers after an upper trunk crush in newborns compared with a crush in mature 4-week-old animals of group C was also statistically significant (P = 0.001) (Fig. 5). Comparison of means showed an almost four-fold increase in motoneuron number in adult animals after a neonatal upper trunk crush injury compared with normal animals (Fig. 6).

**Discussion**

While Smellie is credited with the first description of birth palsy in 1768, it was Duchenne de Boulogne who introduced the term ‘obstetrical’ palsy in 1872 (Smellie, 1768; Duchenne, 1872). From the very beginning, the injury of the peripheral nerve was at the centre of attention and surgical reconstruction of the nerve lesion was readily attempted (Gilbert, 1995). In 1925, however, Sever’s pessimistic report of plexus repairs brought investigations to a near halt for almost 50 years (Sever, 1925; Birch, 2002). Only with the advent of microsurgery and improved anaesthesia in the late 1960s did physicians begin to show renewed surgical interest in the disease. The initially promising results of peripheral nerve reconstruction in adults were readily transferred to the child (Birch, 2002). While CNS participation was never disputed, explanations of these centrally generated mechanisms were, in the words of Sunderland, ‘oversimplified and obscure’ (Sunderland, 1978). In addition, the effects of young age on functional outcome remained unclear and controversial (Kennard, 1936; Zalis, 1965; Sunderland, 1978). It was only in the late 1990s that cumulating experimental evidence on the vulnerability of immature motoneurons and evidence on the critical importance of the postpartum period for motor development were linked to obstetric brachial plexus palsy (Brown et al., 2000; Carlstedt and Cullheim, 2000; Noetzel and Wolpaw, 2000; Birch, 2002).

It was our aim to investigate the spinal components of brachial plexus injury occurring at two developmental time points. To accomplish our objective, we selected a crush injury rather than an axotomy or avulsion as our injury model. Axonal regeneration after an axotomy/avulsion is highly irregular and peripheral targets are reinnervated in a random fashion (Brown and Hardman, 1987). Crush injury, however, consistently results in a second-degree nerve injury. Regardless of the developmental stage, a second-degree nerve injury is characterized by a loss of axonal continuity,
while at the same time injured axons remain confined to their original endoneurial tubes (Bridge et al., 1994). With only 3% of axons misplaced, errors in projection after crush injuries are negligible (Brown and Hardman, 1987). Thus, by choosing a crush injury we were able to minimize any possible influence of a standardized peripheral recovery process on behavioural, functional and neuroanatomical assessment and limit our investigation explicitly to CNS components of brachial plexus injury.

The ability of an animal to adapt to injury and to overcome resulting deficits is ultimately reflected in the animal’s behaviour. Whereas in newborns gross movement recovered, structural damage of the spinal cord clearly limited the development of higher motor functions. Evaluation of the Bertelli test revealed a slower, but progressive improvement of upper limb function after injuries in newborns. The affected limb was well integrated into the overall body scheme, and 12 weeks after injury the animals could reach all anatomical landmarks during grooming movements. Comparison with the contralateral healthy side, however, revealed deviations from the normal movement pattern. On the lesioned side, animals displayed deficits in aim, advance and accuracy of movement.

The grid walk paradigm exposed deficits in complex tasks such as balance and limb placement, which were masked by good overall locomotor ability. Grid walk performance of the animals reflects function of the descending corticospinal and rubrospinal tracts (Schucht et al., 2002). As mature motor patterns emerge, connections between these descending tracts, interneurons, muscle afferents and motoneurons are refined (Gibson et al., 2000). The observed functional deficits suggest that the deleterious effects of neonatal injuries on the motoneuron population may impair complete structural and functional integration of all relevant components.

The overall integration of information from diverse sources through motoneurons, Sherrington’s ‘final common path’, is one of the central paradigms in motor system study. Crush injury in newborns resulted in a selective elimination of corresponding MCN motoneurons from 407.4 ± 19.1 in normal adult animals to 109.0 ± 3.0 permanently surviving motoneurons. This motoneuron loss is an essential feature of any proximal nerve injury in the early postpartum period, because feedback between motoneurons and their target muscle is critical for motoneuron survival during this vulnerable period (Lowrie and Vrbova, 1992; Oppenheim, 1996; Aszmann et al., 2002). As motoneurons mature they gradually lose their dependency on muscle-derived neurotrophic factors. Motoneuron loss is reduced to 50% when proximal nerve transections are performed 1 week after birth, and becomes nearly undetectable when axotomies are performed in 1-month-old animals (Schmalbruch, 1984). Whereas every axonal injury in adults leads to characteristic neural and non-neural reactions in the spinal cord, the fundamental feature of a proximal nerve injury in newborns—the dramatic motoneuron loss—is absent (Himes and Tessler, 1989; Aldskogius and Kozlova, 1998).

The devastating results of an early proximal nerve injury and the great extent of motor and sensory recovery in newborns may seem paradoxical. Despite evidence for extensive sensory neuron loss after neonatal nerve injury (Himes and Tessler, 1989), Anand and Birch found an excellent restoration of sensory function in patients with severe brachial plexus injuries (Anand and Birch, 2002). The authors attribute the remarkable recovery of sensory function to the immense plasticity of the CNS in children. Further studies have reported sparing of function after cortical ablation (Kolb and Whishaw, 1985) or spinalization (Robinson and Goldberger, 1986) in neonates through structures either adjacent to or remote from the lesion site. In newborns, unjured sites can readily take over function from injured areas (Nudo et al., 2001). We therefore investigated if the contribution of adjacent spinal root C7 to the musculocutaneous nerve increased after an upper trunk injury in newborns compared with an identical lesion in adults. We found an almost four-fold increase in the C7 contribution to biceps muscle innervation after brachial plexus lesions in newborns, partially compensating for the injury-induced motoneuron loss associated with a proximal nerve injury at this developmental stage.

The findings suggest that, during postinjury development, the innervation pattern of the biceps muscle is adjusted to the limitations associated with the dramatic motoneuron loss. The existence of an intact anatomical link between the C7 motor pool and the biceps muscle could serve as a basis for compensatory postinjury development (Fig. 7). Vredeveld and colleagues clearly demonstrated the existence of a C7 link in the innervation pattern in newborns—pathways which were absent in normal adults (Vredeveld et al., 2000). Feedback between these C7 motoneurons and target muscles, essential for continuous locomotor development, remained intact (Oppenheim, 1991). Therefore, C7 pathways were strengthened and stabilized as locomotor maturation progressed (Shatz, 1990). This suggests that pathways that would have normally been lost as the adult innervation pattern evolved were preserved after injury to the upper trunk in neonates. C7 pathways suddenly became vital for the development of biceps muscle innervation (Fitzgerald, 1985).

The results of the present study are in agreement with a frequent clinical observation in obstetric brachial plexus palsy patients. Irrespective of the degree of peripheral nerve injury, there is ample evidence that functional outcome is improved if the injury is confined to the upper trunk as opposed to injuries which include spinal root C7 (Narakas, 1987; Nehme et al., 2002). This is an intriguing finding because in normal adults spinal root C7 does not contribute to innervation of muscles that affect shoulder stability and elbow flexion. In the light of the present study, these clinical findings could be explained on the basis of an adaptation in spinal cord development that results in a change of the normal adult innervation pattern after obstetric brachial plexus injuries.
The results of our present study clearly show that spinal root C7 has a larger motoneuron pool after an obstetric upper trunk injury that can be used as a donor reservoir in peripheral nerve surgery. Whereas functional motor recovery must always include restoration of anatomical continuity of peripheral nerves, a more general recognition of CNS components will reveal new frontiers that therapy of obstetric brachial plexus palsy may target.

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Spinal cord changes in birth palsy

1495


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