Unravelling cossed wires : dysfunction in obstetric brachial plexus lesions in the light of intertwined effects of the peripheral and central nervous system

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Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 26 juni 2018
klokke 16.15 uur
door

Galia Valentinova Anguelova

geboren te Sofia, Bulgarije
in 1986

Promotoren:
Prof. dr. J.G. van Dijk
Prof. dr. M.J.A. Malessy

Leden promotiecommissie:
Prof. dr. P.C.W. Hogendoorn
Prof. dr. T.P.M. Vliet Vlieland
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Prof. dr. ir. M.J.A.M. van Putten (Universiteit Twente en Medisch Spectrum Twente)

ISBN: 978-94-90858-59-9
Layout and printed by: drukkerij Mostert, Leiden
Cover: G.V. Anguelova

The research in this thesis was financially supported by the MD/PhD-track of the Leiden University Medical Centre.

Contents

Chapter 1

Introduction

Obstetric brachial plexus lesion (OBPL)

An obstetric brachial plexus lesion (OBPL) is a closed traction injury of the brachial plexus acquired during labour, with an incidence of 0.5 to 2.6 per 1000 live births.[1] In increasing order of severity the lesions concern neurapraxia, axonotmesis, neurotmesis and root avulsion.[2,7]While mild nerve damage does not exclude a full recovery, severe damage can cause permanent loss of arm function in OBPL.[11-15] Additionally, severe OBPL can cause secondary skeletal malformations, cosmetic deformities, behavioural problems,[16] and socioeconomic limitations.[13,17]

Typically shoulder abduction and elbow flexion are impaired caused by damage to the C5 and C6 spinal nerves. In more severe cases involving spinal nerves C7, C8 and Th1, extension and hand function are impaired as well.[2] Due to the nerve traction, axons are disrupted, and the distal end undergoes Wallerian degeneration.[3,4] In the majority of OBPL cases, there is no large gap between the proximal and distal nerve ends, in contrast to the situation in adults, in which the nerve ends retract, resulting in an appreciable gap.[5] The lack of a gap leads to the formation of a neuroma in continuity. This contains axons, some of which cross the lesion site and may find the empty distal basal laminal tubes.[6] The number of axons that successfully cross the lesion site is lower than the original number, and the number of available axons depend on lesion severity.

In addition to the abnormally low number of axons, the essentially random outgrowth of axons across the lesion may cause 'misrouting': axons may connect with an end organ differing from the original one. The result is that both sensory and motor nerve function can be impaired. Proprioceptive feedback may be disturbed as well as motor firing patterns.[2] Absent or inappropriate afferent input may in turn inhibit the development of central motor programs.[2,8-10] All of these aspects may contribute to sensory and motor arm dysfunction to various degrees. In turn, this can limit the ability of patients with OBPL to perform nor only straightforward daily tasks such as eating or writing, but also more specific tasks complex tasks restricting personal and professional choices.

Beyond the recognition of sensory, motor and central program dysfunction lie potential applications to influence or circumvent these limitations, with effects on the affected arm use, participation in society and quality of life.

Currently, intervening with the natural course of OBPL is done either before reinnervation is fully completed with various surgical techniques or, insufficient or erroneous reinnervation is treated symptomatically with muscle transpositions or botulinum toxin,[2] all aided by rehabilitation therapy. Although there is reasonable consensus on when to apply a specific technique, randomized controlled trials are lacking. This is partially due to an unclear effect of conservative treatment on sensory and motor function, lack of a reliable measure for misrouting extent, and what role the central nervous system plays in recovery.

The aim of this thesis was to gain a better understanding of sensory and motor function, misrouting and central motor program development in OBPL, with a focus on conservatively treated adults with OBPL.

Sensory function

Most studies in OBPL focused on motor functions, providing few details of sensory function.[18-22] This lack of attention may have been due to the prevailing perception that sensory function recovers almost completely in OBPL[18-25] in contrast to motor function. By itself, this motor-sensory discrepancy is surprising, as there are no reasons to assume that sensory and motor axons respond fundamentally different to injury in infants than in adults. As a result, widespread sensory dysfunction, such as occurs in adults after nerve injury, would be expected. To explore whether sensory function is indeed nearly normal in OBPL we assessed sensory function in a group of conservatively treated adults with OBPL, results are described in *Chapter 2*. In *Chapter 3* we compare our findings from *Chapter 2* with findings of Brown et al. in a comparable study [26] performed in conservatively treated older children with OBPL.

Motor function and misrouting

Functional recovery following OBPL depends not only on the number of outgrowing motor axons that reinnervate muscle fibres, but also on the extent of misrouting[27-29] As said, misrouting occurs when a regenerating axonal sprout grows into a distal basal lamina tube other than the original one.[2] There are indications that misrouting occurs more often in children than in adults.[27] In misrouting, an outgrowing axon may reinnervate muscle fibres in another than

the intended muscle. These fibres may lie in an agonist (e.g. an axon meant for the biceps ends up in the brachialis muscle), an antagonist (e.g. triceps instead of biceps), or a muscle with another function (e.g. deltoid instead of biceps). As regenerating axons tend to branch at the site of injury, the various branches of one axon may even end up in different muscles, thereby forming a motor unit with muscle fibres in more than one muscle.[30-33] If a sizable number of axons are misrouted, two muscles may tend to contract together, a phenomenon known as cocontraction. Misrouting in OBPL was studied exhaustively by Roth, who reported that abnormal motor connections were present in 38% of 618 investigated muscle pairs.[30] The assessment was based on the principle that stimulating any part of a neuron will excite all its branches, so stimulating nerve endings in one muscle and recording a response in another muscle suggested a motor unit with branches in separate muscles. Not all possible connections were systematically assessed by Roth, however. In *Chapter 4* we used the same principle to assess how often motor misrouting occurred in conservatively treated adults with OBPL, to link its occurrence to the site of the lesion, and to compare its presence with the degree of clinical motor dysfunction.

Cocontraction due to misrouting causes serious problems in OBPL, possibly more so than primary muscle weakness.[28,34,35] However, previous studies mainly rested on qualitative assessments; triceps and deltoid muscle cocontraction during biceps activation has not been quantified yet. One possible way to assess the quantity of misrouted axons is with electromyography (EMG). In *Chapter 5* we quantified triceps and deltoid muscle cocontraction during biceps activation in conservatively treated adults with OBPL and compared it with healthy subjects.

As EMG has some disadvantages, such as costimulation and coregistration, we explored an alternative measure possibly quantifying cocontraction at different functional force levels: this was joint stiffness originating from muscle short-range stiffness (SRS). SRS represents the resistance of a muscle against lengthening and is observed during the first 40 milliseconds or so of a rapidly stretched muscle[36], after which stretch reflexes become active, complicating stiffness and its assessment. The stiffness is proportional to the active force exerted by the muscle.[37,37] SRS is thought to be due to the elastic properties of the cross-bridges in the muscle fibres.[38] A large stiffness means much force is

needed to rotate the joint one degree. Both the agonist and antagonist muscles exhibit stiffness, so the total joint SRS is the sum of their stiffness, while the actual torque is the difference between agonist and antagonist torque.[39] Therefore cocontraction in OBPL patients is expected to increase the total joint stiffness through adding antagonist activation at a given torque compared to healthy individuals. In *Chapter 6* we quantified elbow SRS for varying flexion and extension torques and compared the results between OBPL patients and healthy subjects.

Central motor programming

As explained in the previous sections, functional recovery depends on the number of outgrowing axons and the correct routing of outgrowing axons.[40,41] Apart from the direct consequences of a peripheral nerve injury, the brain has to learn to cope with new and possibly erroneous input and output signals. Functional recovery of OBPL may therefore be additionally impaired because these central motor programs may not develop normally in young children.[40] A number of clinical observations suggested that motor programming is indeed impaired in OBPL, such as the observation that children 'forget' to flex their arm when they do not focus on using it, while they can actually flex the arm when the task at hand requires focused attention.[40,42] Some past studies collected neurophysiological evidence for such defective motor programming in OBPL.[43,44]

In *Chapter 7* we studied central motor programming in children with OBPL by systematically observing arm movements during balancing tasks and volitional movement. If the observed functional deficit in the affected arm would be wholly due to peripheral nerve, muscle or joint damage, then the deficit would not depend on whether a movement is made in a voluntary or an automatic context. Any discrepancy would suggest a central component. We reasoned that movements of the unaffected arm would serve as a control to indicate volitional or automatic action. Accordingly, we reasoned that arm movements in OBPL that can be performed volitionally by both arms, but that do not occur in the context of automatic movements of the affected arm, suggest the presence of a central deficit.

In *Chapter 8* we studied central motor programming in adult OBPL patients who were conservatively treated by measuring cortical activity during motor execution and imagery tasks with functional MRI. An expansion of motor cortical representation occurs not only at the onset of learning a new motor skill in healthy subjects, but also in patients following upper extremity injury and reconstruction.[45] While a skill is being mastered, the degree of cortical representation and excitability decrease again.[45] We used motor execution tasks to assess whether a central motor impairment in OBPL can be linked to a different motor cortical representation compared to controls. With increasing practice motor tasks become automatic and require less planning effort.[46] A decreased cortical activation has been found in the primary motor cortex contralateral to the attempted limb movement in paraplegics compared to healthy controls studied with motor imagery functional MRI, which was attributed to an increased need for attention allocation.[47] Therefore, we used imagery tasks to assess whether an increased planning effort contributes to the central motor impairment.

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Chapter 2

A cross-sectional study of hand sensation in adults with conservatively treated obstetric brachial plexus lesion

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Dev Med Child Neurol. 2013 Mar, 55(3):257-63.

Abstract

Aim Sensory function is assumed to recover almost completely in obstetric brachial plexus lesion (OBPL), and is stated to recover much better than motor function. However, there is no obvious physiological reason why this should be so. Any persistent problems with sensory innervation might contribute to disability. For these reasons, we aimed to assess sensory dysfunction resulting from obstetric brachial plexus lesions (OBPL).

Method Adults with conservatively treated OBPL (*n*=17; median age 38y; five males; lesion levels: C5–C6, *n*=7; C5–C7, *n*=7; C5–C8, *n*=2; C5–Th1, *n*=1) and healthy control persons (*n*=19; median age 23y; nine males) were investigated. Sensory function was measured using Semmes-Weinstein monofilaments, two-point discrimination, object recognition, and a locognosia test.

Results Scores of the Semmes-Weinstein monofilaments and two-point discrimination, but not object recognition or locognosia, were significantly worse in those with OBPL than in control persons.

Interpretation There may be systematic abnormalities in sensory function in adults with conservatively treated OBPL. The existence of these impairments and their contribution to functional impairment needs to be acknowledged.

Introduction

An obstetric brachial plexus lesion (OBPL) is a closed traction injury of the brachial plexus acquired during labour, with an incidence of 0.5 to 2.6 per 1000 live births.[1] Although the prognosis of OBPL was generally considered to be good in over 90% of cases, a systematic literature search showed functional deficits in 20 to 30% of cases, taking study design, population, duration of follow-up, and end-stage assessment into account.[2] Severe OBPL can cause skeletal malformations, cosmetic deformities, behavioural problems (assessed with the Pre-School Behaviour Checklist),[3] and socioeconomic limitations.[4,5]

Most of these studies focused on motor functions, and few provided details of sensory function.[6–10] This lack of attention may be due to the perception that sensory function recovers almost completely in OBPL[6–13] in contrast to motor function. By itself, this discrepancy is surprising, as there are no reasons to assume that sensory and motor axons respond fundamentally different to injury in infants. As a result, widespread sensory dysfunction, as occurs in adults after nerve injury, would therefore be expected. Our first aim was to assess sensory function in OBPL anew and to explore the reasons for the discrepancy between reported and expected results. Secondly, knowledge of the potential for sensory recovery after conservative treatment is relevant with an eye on nerve surgery: after all, should spontaneous recovery of sensation be limited, this might serve as an argument to support surgical intervention. We therefore studied sensory functions in a group of patients with OBPL who had not undergone nerve surgery.

Method

Participants

Seventeen adults with OBPL participated as well as 19 control persons. Adults were investigated instead of children for ethical reasons and because detailed sensory investigation is hardly feasible in children. Six patients had participated in earlier research projects of the Leiden University Medical Centre Rehabilitation Department and others were recruited through the Dutch Erb's Palsy Association.

Exclusion criteria for patients and controls were, firstly, the presence of any relevant disorder affecting movement or sensation other than OBPL and, secondly, when nerve repair of the brachial plexus had been performed at any age. Figure 1 shows the number of potentially eligible participants, those examined for eligibility, confirmed eligible, and included in the study. The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre. All participants provided informed consent.

Sensory assessment

Sensory function was assessed in both hands with four tests: two-point discrimination (North Coast Medical, Inc., Morgan Hill, California USA), pressure sensation with Semmes-Weinstein monofilaments,[14] locognosia (i.e. the ability to locate sites of touch),[15] and object recognition,[16,17] all detailed below. A screen prevented participants from seeing their own hand during sensory testing, while investigators could see the hand. Blinding of the observer for which hand was affected was not possible in this study owing to motor deficits in the affected arm (limited supination for example). Sensory stimuli were given to the thumb (C6 dermatome), the index and middle finger (largely C7), and the ring and little finger (C8/T1). Results are expressed quantitatively, and, as results in previous reports[6–8,10] were categorized as normal and abnormal, current results are dichotomized as normal or abnormal.

Patients' arms were categorized as affected and healthy. Hand dominance was based on the participants' opinion on the matter and corroborated by observing with which hand they wrote. In a previous study on children with OBPL, hand preference was based on the hand using for drawing;[8] we chose writing as more suitable for adults and because drawing and writing preference are highly correlated.[18]

Object recognition

We chose six common objects (a key, a paper clip, a teaspoon, a short pencil, a button, and a coin) for this test.[16,17] Participants had to name them after manipulating them while deprived of visual feedback. The objects were placed one at a time on the fingertips of the affected side for patients. Hand dominance might affect the results, which raised the question of which hand was to be used for the control persons group. As the affected side is often the non-dominant

one in OBPL,[8,19,20] the test was performed on the non-dominant side in control persons. One point was awarded for any object recognized correctly. A count lower than six was considered abnormal.

Locognosia

Participants were seated at a table with their supinated forearm resting on the table surface. As stated, a screen occluded the hand being tested from the participant's vision. A drawing of a hand was placed in front of the participants, on which fingertips were divided in numbered quadrants (Fig. 2a).[15] Separate left- and right-hand versions were used to prevent confusion. A 6.65 Semmes-Weinstein monofilament was used to touch a quadrant for about 2 seconds, and the participant was requested to state the number of the touched quadrant referring to the drawing. When uncertain, participants could request the stimulus to be repeated. No feedback about correctness of the answers was given. Each of the 20 quadrants was examined twice, in a random sequence.

Each correctly identified quadrant was awarded two points; one point was given when the touch was localized either in the correct quadrant of an adjacent finger or in the wrong quadrant of the correct finger. Any other response merited zero points. Scores were then calculated in two ways. The first involved adding points per finger for both repetitions (see Fig. 2a for quadrant numbers): thumb, quadrants 1 to 4; index finger, quadrants 5 to 8; middle finger, quadrants 9 to 12; ring finger, quadrants 13 to 16; small finger, quadrants 17 to 20. There was a maximum of 16 points per finger (four quadrants, two repetitions, two points per correctly identified quadrant). Secondly, points were added per dermatome for both repetitions: dermatome C6, quadrants 1 to 6; dermatome C7, quadrants 7 to 14; dermatome C8, quadrants 15 to 20. The number of quadrants differed per dermatome and thus the maximum score was 24 points for dermatome C6, 32 for C7, and 24 for C8. To account for these differences, percentages were calculated as follows. In patients with OBPL the affected hand score was divided by the uninjured hand score, whereas in control persons the non-dominant side score was divided by the dominant side score. Thus, if the affected hand score for dermatome C7 in a patient with OBPL was 21 points and the unaffected hand score for the same area was 27, the final percentage corresponding to dermatome C7 would be $(21/27)\times100=77.78\%$. In uninjured hands, localization ability using this test is not always perfect and

therefore the maximum score may not always be achieved.[15] The locognosia score was considered abnormal when lower than 100%.

Two-point discrimination

An NC12776 North Coast Touch-Test® Two-Point Discriminator was used. This is a plastic circular frame with two blunt pins in pairs at variable distances from each other and one unpaired pin. This frame was used to assess both static and dynamic two-point discrimination. We used a test protocol as described by Van Nes and colleagues with several adaptations. According to the protocol, the two-point discriminator was rested gently on the skin without application of any pressure, only the instrument weight. Static examination was performed by applying the ends of the discriminator arms to one point at the distal phalanx. For dynamic examination, the ends of the arms were gently moved from the proximal to the distal end of the distal phalanx, over a distance of approximately 1cm. The distance between the two ends was varied to obtain a threshold value. For this purpose, a participant had to differentiate correctly between the two points at a given distance seven out of 10 times, where catch trials were randomly applied.[21] The adaptations we made to the protocol are as follows. Various distances between the blunt pins were tested in a descending order (from 15mm to 2mm). One data-collecting series was performed for both static and dynamic assessments. On each hand the index finger (C6–C7) and the small finger (C8) were tested, resulting in eight values per participant (two fingers, two hands, static and dynamic testing). The two-point discrimination score was the smallest distance identified for the following sites: static index finger (C6–C7), dynamic index finger (C6–C7), static small finger (C8). The best possible score for each site was the smallest distance between the pins, namely 2.0mm. The scores for these sites were reported separately for the 17 affected hands of the patients with OBPL, the 17 healthy hands of the patients, and the 38 (two times 19 participants) hands of the control persons group. Abnormal sensibility was defined according to Sundholm et al., as a two-point discrimination score higher than 3mm.[8]

Semmes-Weinstein monofilaments

The A835-2 Sammons Preston monofilament kit 5PC was used to determine sensibility in six points on each hand (Fig. 2b)[14] using five differently sized monofilaments (marking number 2.83, 3.61, 4.31, 4.56, and 6.65). The

filaments were of equal length (38mm) but differed in diameter. Each filament was pushed against the skin, forcing it to bend. The thickness determines its stiffness and hence the applied pressure, being higher for thicker filaments. Participants indicated whether they perceived any touch. The filaments were tested starting from the thickest towards the thinnest. The marking number of the finest filament felt was noted for each site. These results were condensed into three Semmes-Weinstein subscores according to the corresponding dermatome: dermatome C6, the noted Semmes-Weinstein marking number of the point on the thumb in Fig. 2b); dermatomes C6–C7, the noted Semmes-Weinstein marking numbers mean of the two points on the index finger; and dermatome C8, the noted Semmes-Weinstein marking numbers mean of the two points on the small finger. The best score to be achieved for each site is the smallest possible Semmes-Weinstein marking number: 2.83. The scores for these sites were reported separately for the 17 affected hands of the patients with OBPL, the 17 healthy hands of the patients, and the 38 (two times 19 participants) hands of the control persons group. The score was considered abnormal when higher than 2.83, which is equivalent to 0.05g.[10]

Extent of OBPL

Arm motor function of all patients was examined by one of the authors (MJAM), an experienced brachial plexus surgeon. Individual muscles were graded according to the Medical Research Council scale;[22] active and passive range of motion was documented; the Mallet scale for shoulder function[23] and the Raimondi scale for hand function[24] were assessed. Subsequently motor function was used to determine the extent of OBPL, classified in three groups: group 1, C5 and C6 damage, with impaired shoulder abduction, exorotation, and elbow flexion; group 2, C5, C6, and C7, with paresis as in the first group but with additional weakness of elbow, wrist and finger extension; group 3, C5 to C8, and C5 to Th1 lesions, with additional wrist and finger weakness. This classification aids the comparison with previous reports.[6,7,9]

Statistical analysis

Statistics were analysed with SPSS 16.0 (SPSS, Inc. Chicago, IL, USA.). Differences between groups were tested with the Mann–Whitney *U* test for continuous variables or the χ^2 test for dichotomous variables. Comparisons were not performed between lesion extent groups owing to the small number

of participants in each group. We considered a 0.05 significance level too conservative as some of the tests might overlap in the sensory modalities they represent. Thus two-tailed *p* values of no more than 0.01 were considered statistically significant.

Results

Adults with conservatively treated OBPL and control persons did not differ in demographic characteristics (Table I). The unaffected hand was the dominant one in 14 of 17 adults with conservatively treated OBPL. Two of the three participants in whom the affected hand was the dominant one had a partial C5 to C6 injury; the remaining participant had a total C5 to C6 and partial C7 injury. Of adults with conservatively treated OBPL, 35% were left-handed, whereas only 10% of the control persons group were left-handed. Sensory functional scores were not normally distributed in either the OBPL or the control persons group.

Table II shows results of adults with conservatively treated OBPL and control persons for object recognition and locognosia. Scores for object recognition and locognosia did not differ significantly between the two groups. Table III shows results for two-point discrimination and Semmes-Weinstein monofilaments tests. The two-point discrimination and Semmes-Weinstein tests of the affected hand of adults with conservatively treated OBPL yielded significantly different scores than the hands of the control persons group, concerning worse function. For comparability with previous literature,[6–8,10] we additionally present our findings as the number and percentage of adults with conservatively treated OBPL with abnormal results for each of the four modalities.

Discussion

Our main finding is that sensory hand function is abnormal in adults with conservatively treated OBPL, according to the Semmes-Weinstein monofilaments and two-point discrimination test. We therefore conclude that the widely held perception that sensory recovery is generally good in these

patients should be revised. First, we will discuss a possible explanation for the apparent discrepancy between this perception and our conclusions. Second, as sensation is of paramount importance in daily tasks performance, our findings support the view that treatment should also be focused on sensation improvement. Finally, no clearly absent sensation areas were found such as may be encountered in adults with severe nerve injuries. We also present a possible explanation for this absence of major sensation 'gaps' in OBPL.

How well does sensation recover in OBPL?

Sensory function in OBPL has been reported to be excellent.[6–13] Of these reports, five presented original data.[6–10] The comparison might be affected by the inclusion of some surgically treated cases, but in four papers operated cases concerned only a small fraction of the total number of cases[6–9] and in the fifth paper cases without surgery could be identified.[10]

We suggest that the apparent discrepancy originates not so much in different results as in a difference in interpretation. For instance, the paper by Anand and Birch[10] allowed non-operated cases to be identified. These authors investigated a group of patients of whom 20 had undergone surgery and four had not. Their conclusion was that sensory function restoration was excellent, described as normal limits being found 'in all dermatomes for at least one modality in 16 out of 20 operated cases'.[10] Unfortunately, this nuanced definition of excellent sensibility and the definition of the operated group seems to have been lost in later citations of this paper. Six sensory modalities were tested (monofilaments, cotton wool, pinprick, warm sensation, cool sensation, joint position sense and vibration). Healthy participants should, however, have normal results for all six modalities in all dermatomes. The results may be rephrased to read that only three out of 20 participants (15%) had normal sensation for all six modalities. Note that these 20 cases were the operated ones; the four non-operated cases did not recover any sensory function at all.[10]

Apart from treatment options, the number of affected roots can additionally influence the results: more extensively damaged cases will most likely have worse sensory recovery. In the Anand and Birch study, all four non-operated cases had lesions of all five nerve roots.[10] Except for the paper by Sundholm and colleagues,[8] who described functional groups, all the other articles expressed the extent of the lesion through the roots which were involved. The proportion

of patients with a C5 to C6 lesion was higher in these three studies, though the proportion of patients with a C5 to C8 and C5 to Th1 lesion was higher as well. The study populations in these articles are thus more or less comparable to ours, though the conclusion was mostly drawn that sensory function had recovered excellently.[6,7,9] Of note, Sundholm and colleagues expressed caution about the purported excellent recovery of sensibility, and acknowledged the impaired tactile sensibility, especially in participants with complete plexus lesions.[8]

The nature of nerve lesions in OBPL

As mentioned, the present study did not show evident skin areas of severely abnormal sensation, and neither did previous studies. For example, the locognosia test in our study showed that localizing touch is not significantly different from the control persons group. Our findings agree with Colon and colleagues':[25] there is reasonably good sensation in skin areas in which profound deficits might be expected. The absence of such major 'gaps' in sensation in OBPL may be explained partly by the fact that in most infants with OBPL there is not an anatomical gap between two torn nerve stumps. Such a true rupture occurs frequently in traumatic brachial plexus lesions in adults. Instead, the stretched and damaged nerve in infants forms a neuroma-in-continuity, which is a tangled mass of connective scar tissue and outgrowing, branching axons.[26] Even in the most severe OBPL, at least some axons are likely to pass through the neuroma-in-continuity and reach the nerve distal to the lesion site. This ability to cross the neuroma might be attributable to the superior ability of the peripheral nervous system in infants to regenerate,[27] compared with that in adults.[26]

Most patients with OBPL have a degree of functional motor recovery, and it is well known that some motor axons form functional connections in almost all muscles in OBPL.[7,10] This is even evident at the age of 3 to 6 months, when the biceps muscle shows reinnervation even in the face of severe paresis. In short, the motor findings in OBPL exhibit a degree of continuity to all muscles, in contrast to upper plexus lesions in adults, in whom some muscles may remain paralytic for life.

In summary, spontaneous motor repair and sensory repair in OBPL show a striking similarity, in that there are no myotomes or dermatomes that remain

completely denervated. The latter pattern is what most physicians would expect in adult cases of severe nerve injury. We hypothesize that the nature of the nerve lesion in OBPL and adults, namely a neuroma-in-continuity versus partial or complete nerve rupture, gives rise to a major difference in clinical expression. In OBPL, reinnervation usually occurs to some degree. Thus, rather than concluding that motor and sensory findings differ significantly in OBPL, we contend that they share a similar clinical pattern.

Limitations and consequences

The unaffected hand was dominant in all 17 adults with conservatively treated OBPL except for three, two of them having a partial C5 to C6 injury and one having a total C5 to C6 and partial C7 injury, which confirms previous reports in the literature.[8,16,19] Prevalence of left-handedness is considered to be approximately 10% in a normal population,[19] which corresponds with our findings in the control persons group.

Possible drawbacks of this study are the small sample size. Therefore a comparison was not performed between the lesion extent groups. The high non-participation rate was probably due to patients being asked to take part in a separate study involving electrical stimuli. Also, no criterion standard exists for the assessment of the severity of the nerve lesion in OBPL.[28] A minor issue may be that sensory tests require participants to supinate their hand, and that these participants supinated the OBPL hand with their healthy hand. Future research may be directed at OBPL pathophysiology: in which dermatomes do axons passing the neuroma-in-continuity end up? Through which nerves and roots do the regenerated fibres run? Is there sensory misrouting, and can this be demonstrated and quantified? Another avenue for future research is the consequences of sensory dysfunction for the quality of life in patients with OBPL.

Acknowledgements

We thank colleagues from the Clinical Neurophysiology and Rehabilitation Department, C Jerosch-Herold for offering us her locognosia test protocol, and R Post for his Semmes-Weinstein monofilaments test protocol.

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Figure 1: Flow chart indicating the number of potentially eligible participants, those examined for eligibility, confirmed eligible, and included in the study. Potentially eligible patients were asked to participate in the current study and an associated study involving electrical stimuli as part of the same visit. This led to several participation refusals. *Competing study involving electrical stimuli.

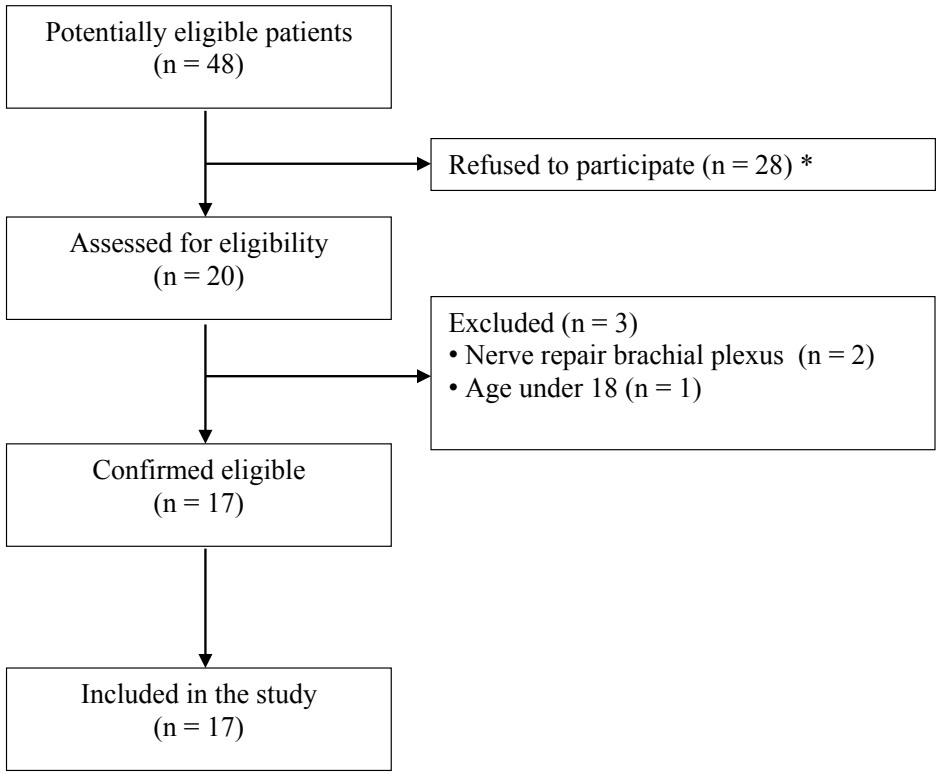

Figure 2: (a) The areas where a Semmes-Weinstein monofilament was applied to determine locognosia in each participant.[15] The different dermatomes are separated by dotted lines. (b) The six locations where the monofilaments of the Semmes-Weinstein test for sensory function were applied. The different spinal root areas are separated by dotted lines. We analyzed differences between the median for the three clusters of points: the thumb (C6), the index finger (C6–C7), and the small finger (C8).

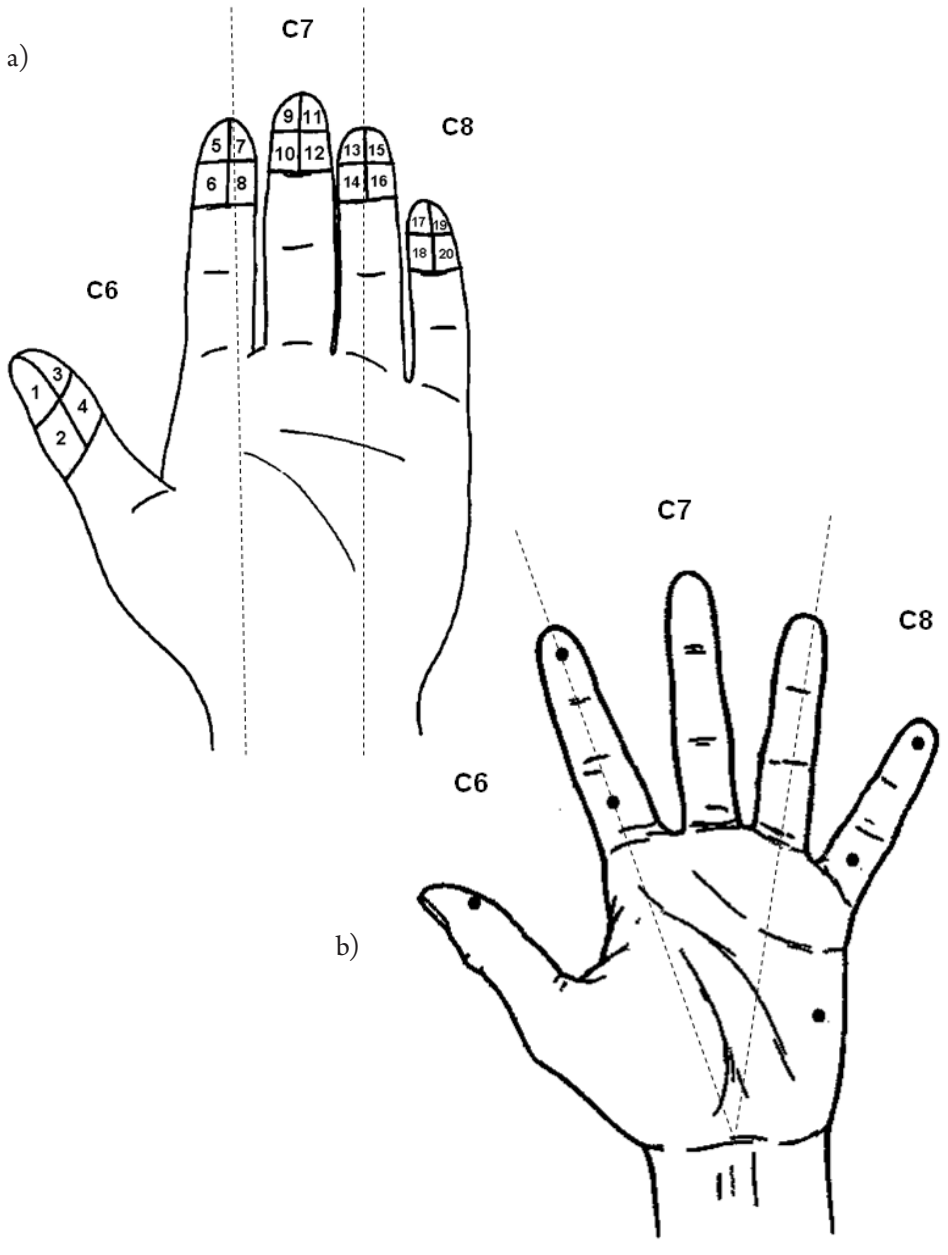

Table 1: Demographic details of adults with conservatively treated obstetric brachial plexus lesion (OBPL) participants and control persons.

	OBPL	Control persons
Total number	17	19
Male/female number	5/12	9/10
Median age years (10th–90th centiles)	38 (20–58)	23 (20–55)
Median body mass index (kg/m^2) (10th–90th centiles)	25 (18–35)	23 (19–25)
Dominant hand right/left number	11/6	17/2
Affected hand right/left number	9/8	—
Lesion level		
C5–C6	7	—
C5–C7	7	—
C5–C8	2	—
C5–Th1	1	—

Table 2: Medians (10th and 90th centiles) from the object recognition and the locognosia tests for adults with conservatively treated obstetric brachial plexus lesion (OBPL) participants and control persons. Object recognition is presented as the number of a maximum of six objects recognized correctly. Locognosia for the affected hand is presented by expressing the score of the affected hand as a percentage of that of the healthy hand. For control persons, results are presented similarly, but now the score of the non-dominant hand is expressed as a percentage of that of the dominant one.

	Control persons (n=19)	OBPL (n=17)	OBPL (abnormal number)	p
Object recognition	6.0 (6.0–6.0)	6.0 (3.2–6.0)	5	0.036
Locognosia				
Thumb (C6)	100.00 (86.94–106.98)	100.00 (80.04–122.38)	3	0.680
Index finger (C6–C7)	100.00 (88.76–104.69)	100.00 (86.26–109.02)	4	0.457
Middle finger (C7)	100.00 (100.00–116.17)	93.80 (70.06–121.00)	9	0.095
Ring finger (C7–C8)	100.00 (68.25–111.69)	100.00 (49.20–114.30)	3	0.531
Little finger (C8)	100.00 (93.45–100.00)	100.00 (76.44–123.44)	5	0.278
Root C6	100.00 (85.33–104.85)	100.00 (86.66–125.26)	4	0.756
Root C7	100.00 (91.35–114.96)	96.90 (77.62–107.76)	9	0.227
Root C8	100.00 (84.07–100.00)	100.00 (77.44–109.50)	6	0.284

Table 3: Medians (10th and 90th centiles) from the two-point discrimination and Semmes-Weinstein tests for control persons and obstetric brachial plexus lesion (OBPL) participants' healthy and OBPL sides. Two-point discrimination is presented as subscores with a minimum and best score of 2.0mm. Semmes-Weinstein is presented as subscores with a minimum and best score of 2.83.

	Control persons, both hands (n=38)	OBPL, healthy side (n=17)	OBPL, OBPL side (n=17)	OBPL, OBPL side (abnormal number)	p (OBPL side vs control persons)	p (OBPL side vs healthy side)
Two-point discrimination						
Static index finger (C6–C7)	3.0 (2.0–3.1)	3.0 (2.0–4.0)	4.0 (2.0–12.0)	13	<0.001[a]	0.104
Dynamic index finger (C6–C7)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	3.0 (2.0–5.2)	8	<0.001[a]	0.267
Static little finger (C8)	3.0 (2.0–4.1)	4.0 (2.0–6.4)	4.0 (2.0–20.0)	14	<0.001[a]	0.025
Dynamic little finger (C8)	2.0 (2.0–3.0)	3.0 (2.0–3.2)	3.0 (2.0–8.8)	6	0.001[a]	0.284
Semmes-Weinstein						
Root C6	2.83 (2.83–3.61)	2.83 (2.83–4.31)	4.31 (2.83–4.78)	13	<0.001[a]	0.028
Roots C6–C7	2.83 (2.83–3.22)	2.83 (2.83–3.68)	3.61 (2.83–4.60)	15	<0.001[a]	0.020
Root C8	2.83 (2.83–3.12)	3.09 (2.83–3.61)	3.35 (2.83–4.09)	12	<0.001[a]	0.023

[a]$p \leq 0.01$.

Chapter 3

Sensory Deficit in Conservatively Treated Neonatal Brachial Plexus Palsy Patients

Letter to the Editor

G.V. Anguelova, M.J.A. Malessy, J.G. van Dijk

Pediatr Neurol. 2016 Sep, 62:e1.

We read the article by Brown et al. on hand sensorimotor function in older children with neonatal brachial plexus palsy (NBPP) with interest.[1] The authors concluded that sensory function in NBPP may be impaired and challenge the common notion that sensory recovery is good in NBPP. These conclusions confirm our earlier ones.[2]

Brown et al. did not find significant differences using Semmes-Weinstein filaments, whereas we did, and they found differences for stereognosis, whereas we did not. This may be because their population included more severely affected NBPP patients than ours; our population was older than theirs, and test applications differed in details. Brown
et al. addressed the limitations of timing differences and concluded that a large effect size (Cohen d) indicated clinically important differences. However, Cohen d's designation of effect size need not reflect practical importance,[3] so it remains doubtful whether these timing differences impair function in daily life.

We had addressed two additional themes regarding sensory function in NBPP. The first was the origin of the common notion that sensory function is good in NBPP: this was likely due to authors overemphasizing the few unimpaired functions at the cost of many impaired ones. The second theme was why sensory deficits in NBPP do not follow the adult pattern with distinct sensory deficits following root and nerve innervation. We attributed the absence of sensory "gaps" in NBPP to a characteristic unique to NBPP: a neuroma in continuity allowing axons to reinnervate target regions, albeit with cross innervation. We had stressed that in this respect the sensory and motor abnormalities of NBPP are quite similar.

Finally, Brown et al. call attention to a possible contribution of altered central nervous system to explain the tactile impairment in NBPP. We found evidence for a central impairment affecting motor function in NBPP[4] and agree that it may also affect sensory function. However, peripheral factors are likely to explain part of the sensory impairment
in NBPP through reduced numbers of peripheral axons and extensive cross innervation.[5] The latter may well contribute to altered central processing.

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Chapter 4

Extensive motor axonal misrouting after conservative treatment of obstetric brachial plexus lesions

G.V. Anguelova, M.J.A. Malessy, E.W. van Zwet, J.G. van Dijk

Dev Med Child Neurol. 2014 Oct, 56(10):984-9.

Abstract

Aim The aim of this cross-sectional study was to systematically assess motor function and motor misrouting in adult conservatively treated participants with obstetric brachial plexus lesion (OBPL).

Method Seventeen adults with OBPL (median age 38y; five male) and 16 comparison participants (median age 26y; eight male) were investigated. Motor function in OBPL participants was assessed through passive and active motion, muscle strength of the deltoid, biceps, and triceps muscles, and Mallet aggregate score and five subscores. Motor misrouting was quantified by electrically stimulating each of 10 arm muscles and recording activity from the other nine in response to this. Motor function and motor misrouting were statistically analysed using the Mann–Whitney *U* test and Spearman correlation.

Results Motor function testing showed excellent strength but poor functional Mallet scores. Participants with OBPL had significantly more motor misrouting than comparison participants (Mann–Whitney U=31.5 [df=28], p<0.001, median difference=–4.00, 95% confidence interval [CI]=–7.00 to –1.00). Most misrouting was observed when stimulating the biceps (Mann–Whitney U=38.5 [df=31], p<0.001, median difference=–3.00, 95% CI –3.00 to –1.00), deltoid (Mann–Whitney U=68.5 [df=31], p=0.003, median difference=–1.0, 95% CI=–4.00 to 0.00) and brachioradialis muscles (Mann–Whitney U =72.0 [df=31], p=0.002, median difference=0.00, 95% CI=–3.00 to 0.00). There were no significant correlations between the presence of motor misrouting and impairment of motor function.

Interpretation There is extensive motor misrouting in conservatively treated OBPL patients. The presence of this, in addition to motor functional impairment, suggests that motor misrouting should be further studied in OBPL.

Introduction

Obstetric brachial plexus lesion (OBPL) is a closed traction injury of the brachial plexus incurred during birth, with an incidence of 0.5 to 2.6 per 1000 live births.[1] Although the prognosis is generally considered to be good, a systematic literature search has shown that there is a residual deficit in 20% to 30% of cases.[2] Severe OBPL can result in the permanent impairment of arm function, skeletal malformation, cosmetic deformity, behavioural problems, and socio-economic limitations.[3,4]

Functional recovery following OBPL is dependent not only on the number of outgrowing motor axons that reinnervate muscle fibres but also on the extent of misrouting.[5–7] There are indications that misrouting occurs more often in children than in adults.[5] Misrouting occurs when a regenerating axonal sprout grows into a distal basal lamina tube that is not the original one.[8] In misrouting, an outgrowing axon reinnervates muscle fibres in areas other than where they are intended. These fibres may lie in an agonist (e.g. an axon meant for the biceps reinnervates in the brachialis muscle), an antagonist (e.g. triceps instead of biceps), or a muscle with another function (e.g. deltoid instead of biceps; see Fig. 1). As regenerating axons tend to branch at the site of injury, the branches may even end up in different muscle groups and form a motor unit in more than one muscle.[9–12] If a sizable number of axons are misrouted, two muscles may tend to contract together, a phenomenon known as co-contraction. Misrouting in OBPL was studied by Roth,[9] who reported that abnormal motor connections were present in 38% of 618 investigated muscle pairs. The assessment was based on the principle that stimulating any part of a neuron will excite all its branches, so stimulating nerve endings in one muscle and recording a response in another muscle would suggest that there was a motor unit with branches in separate muscles. However, not all possible connections were systematically assessed by Roth.

Co-contraction causes serious problems in OBPL, possibly to a greater extent than primary muscle weakness.[6,13,14] Co-contraction in OBPL might also be due to disturbed central motor programming, owing to factors such as deafferentation,[15] misrouting, and the fact that the lesion occurs before motor programmes have been developed fully.[5,6,16] Peripheral factors may, therefore, be involved in co-contraction in addition to central factors.[5]

The aim of this study was to assess how often motor misrouting occurred in patients with conservatively treated OBPL, to link its occurrence to the site of the lesion, and to probe its clinical significance by comparing it with clinical motor function.

Method

Participants

Seventeen adults with OBPL and 16 comparison participants, all over 18 years of age, participated. Adults were investigated to reduce cooperation problems and ethical considerations in this study regarding children. It is unlikely that misrouting would disappear after childhood, and adult participants would, therefore, equally demonstrate misrouting.[9–11] Some participants with OBPL had participated in previous research and others were contacted through the Erbs Palsy Association in the Netherlands. Exclusion criteria for all participants consisted of any surgery undertaken for plexus injury and presence of any other disease affecting arm function. A flow chart indicating the numbers of potentially eligible participants, those examined for eligibility, confirmed eligible, and those included in the study, was published in a previous paper.[15] The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center. All participants provided informed consent.

Motor function assessment

Motor function in the OBPL group was assessed by a neurosurgeon with extensive experience in nerve lesions. Comparison participants were not investigated clinically, as reliable normal values were available. The assessment concerned three aspects: muscle strength, joint range of motion (ROM), and the Mallet classification. The method used to determine lesion level was described in a previous paper.[15] The patient's hand dominance was based on patient's opinion and corroborated by observing which hand they wrote with.

The muscle strength of various shoulder, elbow, wrist, and finger movements was noted using the Medical Research Council (MRC) scale, with a range per muscle of zero (complete paralysis) to five points (normal strength).[17]

The ROM of the shoulder down to the finger joints of the arm was measured during passive and active motion and noted in degrees. Glenohumeral motion was assessed and used for further analysis. The scores for active and passive movement were reported as the median with 10th and 90th centile values for three muscles representative of the upper brachial plexus: the deltoid (mostly C5), biceps (mostly C6), and triceps muscles (mostly C7). Normal values for ROM were taken from reference works.[18,19]

The five items of the modified Mallet classification were scored: global abduction, global external rotation, hand to neck range, hand on spine range, and hand to mouth range.[20] For each item grade I denotes no active motion and grade V denotes normal function. Aggregate Mallet scores were calculated by summing the grades for these five items, so the minimal score was five points and the maximal score 25 points, reported as the median (10th and 90th centiles).

To compare the degree of impairment between the three functional assessments, we expressed the median value of parameters in the OBPL group as a percentage of the corresponding normal value. Full elbow extension is normally expressed as zero degrees, which would result in division by zero to express passive and active extension in patients. To counter this, we defined extension in relation to a fully flexed arm as 145°.

Motor point stimulation

Motor point stimulation was performed in the patients and the healthy comparison participants. The latter were included to control for costimulation or volume-conducted activity from adjacent muscles, as well as for putative long-loop reflexes.[21]

Misrouting was assessed by stimulating the motor point in one muscle and recording activity in the other, non-stimulated, muscles. Ten muscles were chosen as both stimulation and recording sites. We aimed to sample all roots with an emphasis on the upper brachial plexus, the most commonly affected area in OBPL. The chosen muscles were biceps brachii, deltoid, flexor carpi radialis, brachioradialis, extensor carpi radialis, pronator teres, triceps brachii, latissimus dorsi and the thenar and hypothenar muscles.

Motor points were identified by moving the stimulator of a Medelec Synergy EMG apparatus (Oxford Instruments, Abingdon, Oxfordshire, UK) over the presumed site,[22,23] stimulating at an intensity of about 8mA and a frequency of one pulse per second, values that are based on the pilot experiments. The point at which maximal muscle contraction occurred was marked on the skin. For some muscles that were paralysed or severely atrophied no contraction was observed. In such cases literature sources and experience were used to identify the putative motor point. After identifying each motor point, two self-adhesive electrodes were placed over that zone, one over the identified point and one 0.5cm distally. These electrode pairs functioned as both stimulation and recording electrodes. Each motor point was then stimulated several times and the responses of the nine other muscles were visualized on the screen. The repetition allowed transient activity to be distinguished from reproducible evoked activity (Fig. S1, online supporting information). Two responses per stimulated motor point from each participant were saved for later comparison and analysis. Responses were acquired using a band pass filter of 10Hz to1kHz and recorded over 50 milliseconds. The procedure resulted in 90 combinations (10 stimulation sites × 9 recording sites). To avoid mistaking direct muscle stimulation for misrouting, only responses that began at least 15ms after the stimulus, during which period no there was no muscle activity, were recorded. For every stimulus–response combination, misrouting was noted as present or absent. Next, the number of comparison participants and patients with present responses for each combination was counted.

For each stimulated muscle we defined a 'muscle misrouting score' for each participant by measuring the number of muscles that showed a misrouted response, ranging from 0 (no misrouting) to 9. Adding together the scores for all 10 stimulated muscles resulted in a 'total misrouting score' per individual, with a range from 0 to 90 points. Results are presented as median values with the range.

Statistical analysis

IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The Mann–Whitney *U* test was used to test for differences between groups in baseline characteristics, motor function tests, and misrouting scores. A significance level of 0.05 was used. In addition, median differences and

95% confidence intervals CIs were calculated with the R statistical program.[24] Correlations between motor function tests and the misrouting scores of the three muscles most affected in OBPL (biceps, deltoid, and brachioradialis) were assessed with Spearman's correlation coefficient. This resulted in 45 correlations (15 motor function tests × 3 misrouting muscle scores). A Bonferroni corrected significance threshold of 0.001 (0.05/45) was used for the correlations.

Results

Median age (10th–90th centile) was 38 years (range 20–58y) in the OBPL group and 26 years (range 19–56y) in the comparison group (Mann–Whitney *U* [df=31]=114.0, *p*=0.43, two-tailed, median difference=–3.00, 95% CI=–16.00 to 5.00) There were five male participants in the OBPL group and eight in the comparison group (χ^2=1.46 [df=1, *n*=33], *p*=0.23, two-tailed, odds ratio=2.40, 95% CI=0.57 to 10.04). The right hand was affected in 9 out of 17 OBPL cases. There were six left-handed participants in the OBPL group but only 1 among 16 comparison participants (χ^2=4.16 [df=1, *n*=33], *p*=0.041, two-tailed, odds ratio=0.12; 95% CI=0.01 to 1.17). There were seven OBPL participants with lesion level C5–C6, seven with lesion level C5–C7, and three with either C5–C8 or C5–T1.

Motor function assessment

The results of functional assessment in OBPL are shown in Table I. Median values for passive ROM were the same as for active ROM in OBPL participants. Shoulder abduction was the most impaired, reaching only 67% of normal active abduction, followed by elbow extension at 86%; the range of elbow flexion was normal (100%). Muscle strength was slightly impaired for the biceps muscle (95% of normal value) while strength of the deltoid and triceps muscles was normal (100%). In contrast, the Mallet subscores showed a profound impairment, ranging from 40% to 60% of normal function.

Motor point stimulation

In the comparison group, evidence of misrouting was found in only one participant, in whom four stimulated muscles gave rise to misrouted responses

(three in biceps, one in deltoid, one in flexor carpi radialis, one in the hypothenar muscles). The results for both groups are presented in Figure 2. When responses were judged from the stimulation site, misrouted responses in the OBPL group were found for 7 out of 10 stimulated muscles. The muscles that most often gave rise to misrouting were the biceps (n=41), deltoid (n=36), and brachioradialis (n=30) muscles. For the biceps muscle, the 41 misrouted responses represent 27% of all possible instances. Three muscles never gave rise to misrouting: the triceps, latissimus dorsi, and thenar muscles. When judged from the recording point of view, the three muscles over which responses were found most often were the brachioradialis (n=22), triceps brachii (n=22), and the extensor carpi radialis muscle (n=21). Figure 3 shows the muscle misrouting scores, showing in how many cases misrouting was seen following stimulation of a given muscle.

The misrouting score had a median value of 4 (range 0–21) in the total OBPL group and 0 (0–3) in the comparison group. This total score differed significantly between the OBPL and comparison groups (Mann–Whitney U=31.5 [df=28], p<0.001, two-tailed, median difference=–4.00, 95% CI=–7.00 to –1.00); scores per stimulated muscle differed between the groups for the biceps (Mann–Whitney U=38.5 [df=31], p<0.001, two-tailed, median difference=–3.00, 95% CI=–3.00 to –1.00), deltoid (Mann–Whitney U=68.5 [df=31], p=0.003, two-tailed, median difference =–1.00, 95% CI=–4.00 to 0.00), and brachioradialis (Mann–Whitney=72.0 [df=31], p=0.002, two-tailed, median difference=0.00, 95% CI=–3.00 to 0.00) muscles.

Relation between functional assessment and motor point stimulation
Non-parametric Spearman correlations between motor function and motor misrouting within the group of OBPL participants showed no significant correlations at the stipulated level.

Discussion

The main findings of this study were that participants with conservatively treated OBPL displayed considerable functional impairment and motor misrouting; however, this contrasted with good muscle strength. Functional assessment and motor point stimulation will be discussed in further detail below.

Motor function assessment
The strength of the deltoid, biceps, and triceps muscles was excellent; the discrepancy with impaired ROM was large enough to suggest that it was not simply the result of different measurement systems for ROM and strength. The range for active abduction was less broad than for passive abduction, showing that glenohumeral malformation cannot have been the limiting factor. Pronounced muscle weakness also cannot be the explanation, in view of the good strength of the participants. Another mechanism must therefore interfere with motor function, which is most probably co-contraction.[5,25] In one study,[25] co-contraction explained abduction impairment in OBPL more often than simple weakness in muscle strength. There is the further possibility that central programming may also play a role in this.[5,9]

Motor point stimulation
Motor misrouting was most often found after stimulation of the biceps, deltoid, and brachioradialis muscles, innervated through the C5 and C6 roots, most often involved in OBPL. It is not likely that the responses were as a result of artefacts, because 'misrouted' responses were encountered in only four muscles in only one comparison participant, and in whom additional questioning revealed no known birth or motor problems. These responses concern 4 out of 1440 possible instances (16 comparison participants × 10 stimulated muscles × 9 recorded muscles).

A possible reason for the abundance of misrouting in OBPL is that traction to the brachial plexus in OBPL does not cause a true rupture of nerves as it does in adult brachial plexus traction lesions. In OBPL, a gap between two nerve stumps is very rare; instead, a 'neuroma in continuity' is formed. This may inhibit nerve regeneration and form the substrate for misrouting of crossing axons.[26]

There was an intriguing asymmetry in that the pattern of misrouting differed depending on whether a muscle was stimulated or recorded. Stimulation of the triceps did not result in misrouted responses elsewhere but the triceps responded to stimulation elsewhere frequently. In general, muscles responded with misrouted responses more often than they gave rise to them. The first explanation for this is that recording may be more sensitive than stimulation:

responses may be recorded regardless of where the active muscle fibres lie in a muscle, but only the few axons that lie near the motor point may be excited by local stimulation. Secondly, a severe lesion of the upper trunk, formed by the C5 and C6 roots, will result in branching of many axons. The deltoid and biceps muscles are innervated by these roots; therefore, misrouted responses in these muscles may stem from branched C5 fibres as well as from branched C6 fibres. For the triceps muscle the situation is different: if its C7 contribution remains intact, those axons will not give rise to motor unit activity elsewhere, but only its C6 contribution can do so.

Function and misrouting

A relation between a functional impairment and the degree of misrouting has been suggested. After nerve surgery, misrouting is thought to contribute to a lack of functional recovery.[6,13,14] However, we did not find such a correlation in patients in our study who had not undergone surgery, the strongest significance being 0.04 for the relation between biceps strength and biceps misrouting. There are several possible reasons for the absence of a relation between functional impairment and degree of misrouting. Firstly, we assessed misrouting qualitatively; however, the functional consequences of misrouting may depend on the quantitative degree of misrouting. Secondly, the limited variation of the variables in this population precluded statistical significance. Thirdly, the Bonferroni correction together with limited group size may have made it unlikely that there was significance.

Limitations and perspective

Possible drawbacks of this study are that no criterion standard exists for the assessment of the severity of the nerve lesion in OBPL,[7] although assessment in this study was done systematically by an experienced neurosurgeon. Mallet subscores and aggregate scores showed poor recovery of function; the maximum score of 5 was never assigned and the scale in practice starts at the value of 2. A limitation of the motor point stimulation test is that it results in a qualitative estimate of the presence or absence of misrouting in a muscle pair, but cannot determine what proportion of muscle fibres in a muscle are innervated by axons that do not belong there.

Conclusion

The presence of widespread motor misrouting together with motor functional impairment in conservatively treated OBPL, not explained through weakness, suggests that misrouting in OBPL deserves to be studied further.

Acknowledgements

We thank our colleagues from the Department of Rehabilitation for their help with gathering OBPL participants for the study and the technicians at the department of Clinical Neurophysiology for their help with the motor point studies.

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Table 1: Functional motor assessments

	Normal values[a]	Obstetric brachial plexus lesion measurements, median (10th–90th centile)	Difference (%)[b]
Passive range of motion (°)			
Abduction shoulder	120	60 (26–92)	50
Flexion elbow	145	145 (140–145)	100
Extension elbow	0	20 (10–36)	86
Active range of motion (°)			
Abduction shoulder	90	60 (8–90)	67
Flexion elbow	145	145 (140–145)	100
Extension elbow	0	20 (10–36)	86
Muscle strength (Medical Research Council Scale)			
Deltoid	5	5.00 (0.00–5.00)	100
Biceps	5	4.75 (3.80–5.00)	95
Triceps	5	5.00 (0.00–5.00)	100
Mallet score (modified)			
Global abduction	5	3.0 (1.0–4.0)	60
External rotation	5	2.0 (1.0–4.0)	40
Hand to neck	5	3.0 (1.8–4.0)	60
Hand on spine	5	2.0 (1.8–4.0)	40
Hand to mouth	5	3.0 (2.0–4.0)	60
Aggregate	25	13.0 (10.2–17.2)	52

The normal values and obstetric brachial plexus lesion measurements are shown for three clinical assessments: range of motion, muscle strength, and Mallet score. [a]Normal values for range of motion were taken from reference works.[18,19] [b]The column 'percentage of normal' shows the median value of the obstetric brachial plexus lesion measurement as a percentage of the corresponding normal value.

Figure 1: Schematic drawing of motor misrouting in obstetric brachial plexus lesions. Regenerating axons tend to branch, and the various branches may end up in different muscles. The reinnervated muscle fibres may lie in an agonist of the intended muscle, an antagonist, or a muscle with another function (deltoid instead of biceps, shown here). Roth's method[9–11] for measuring misrouting in obstetric brachial plexus lesions is based on the principle that stimulating any part of a neuron will excite all its branches: stimulating nerve endings in one muscle and recording a response in another muscle establishes the presence of a branched motor neuron.

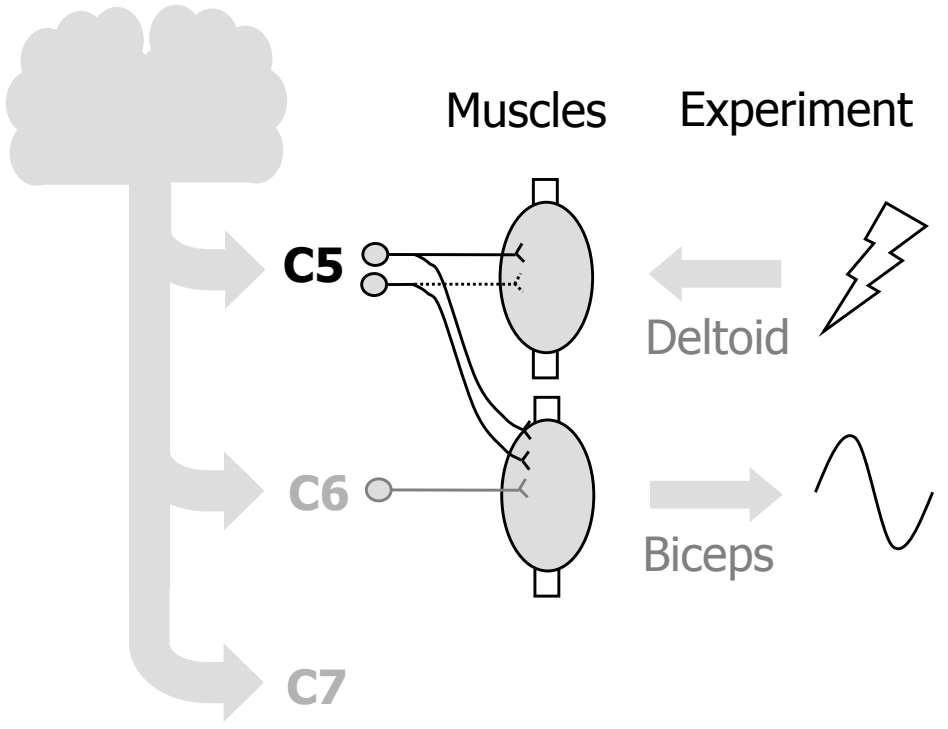


Figure 2: Motor misrouting in obstetric brachial plexus lesion participants. The horizontal axis shows the 10 muscles when used as stimulation sites, and the vertical axis shows their use as recording sites. Each node contains the number of patients with misrouting for that stimulus–response combination (maximum 17). The radius of the circle corresponds to the number of participants. Values below the columns indicate the number of recorded responses per stimulated muscle, and percentages denote the number in relation to the maximum number of recorded responses 153 (9 muscles × 17 participants). Values to the right of the rows indicate the number of cases with misrouting per recording site.

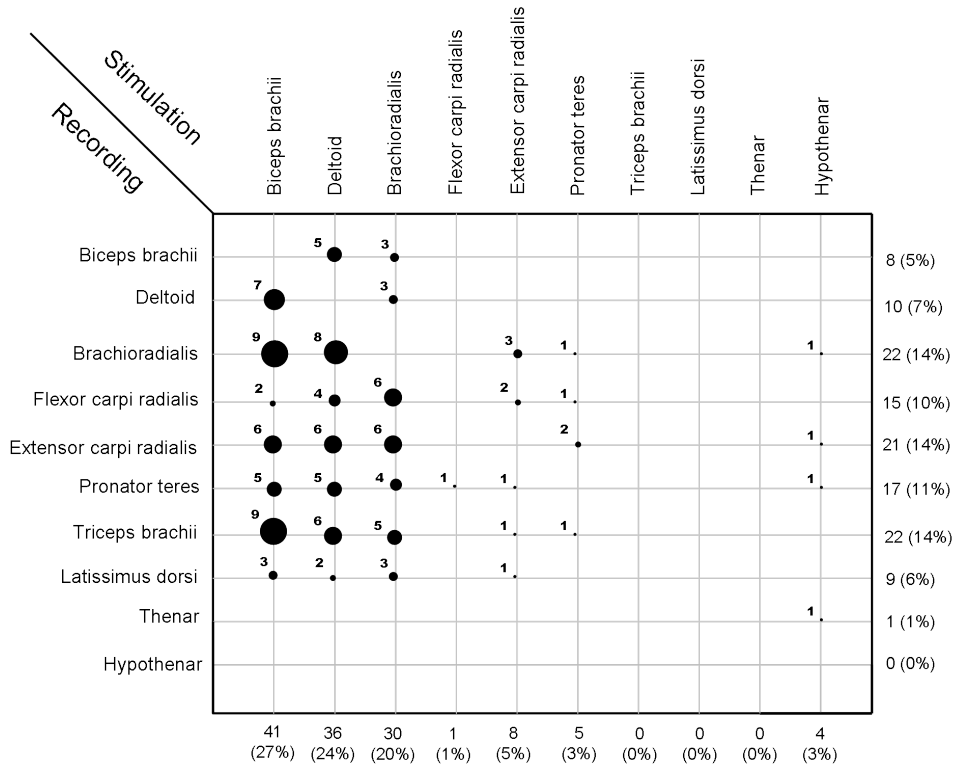


Figure 3: Motor muscle misrouting scores of participants with OBPL. Stimulation sites are shown on the horizontal axis. The vertical axis shows the subscore of number of misrouted responses. The numbers near the circles indicate how many participants reached that score. For instance, there were seven participants in whom stimulation of the biceps muscle resulted in misrouted responses in three other muscles. This is also visualized with a circle with a radius corresponding to the number of participants with the corresponding subscore. The maximum value is 17, except for the flexor carpi radialis and pronator teres, for which the values of one participant are missing.

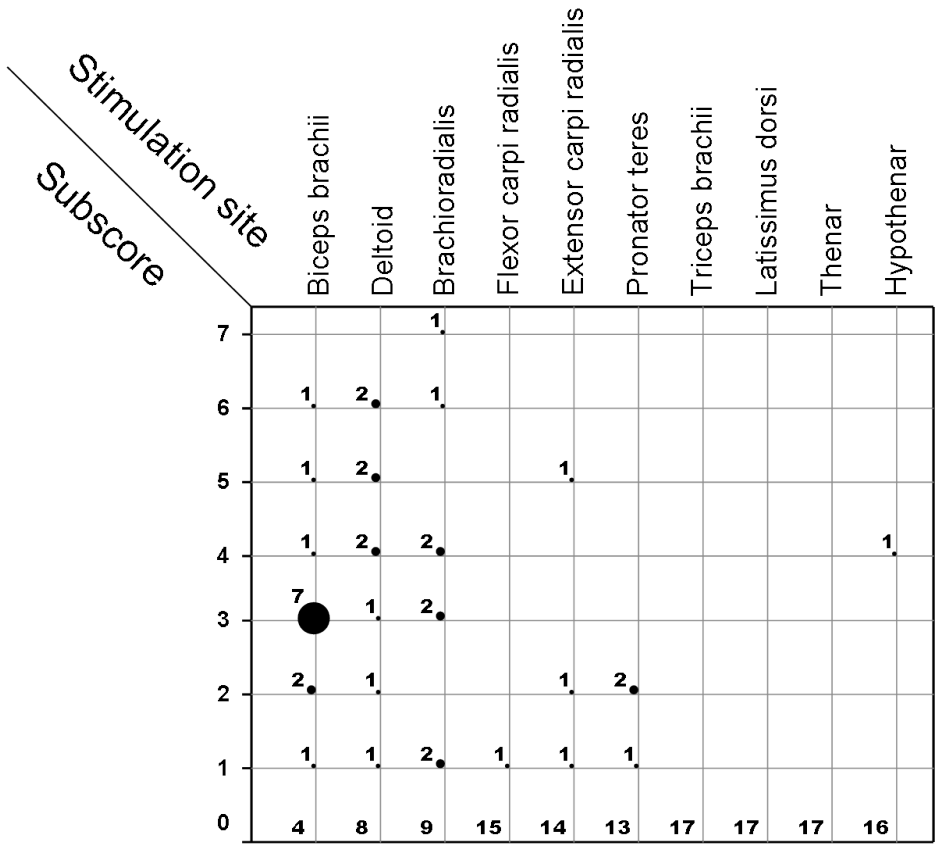
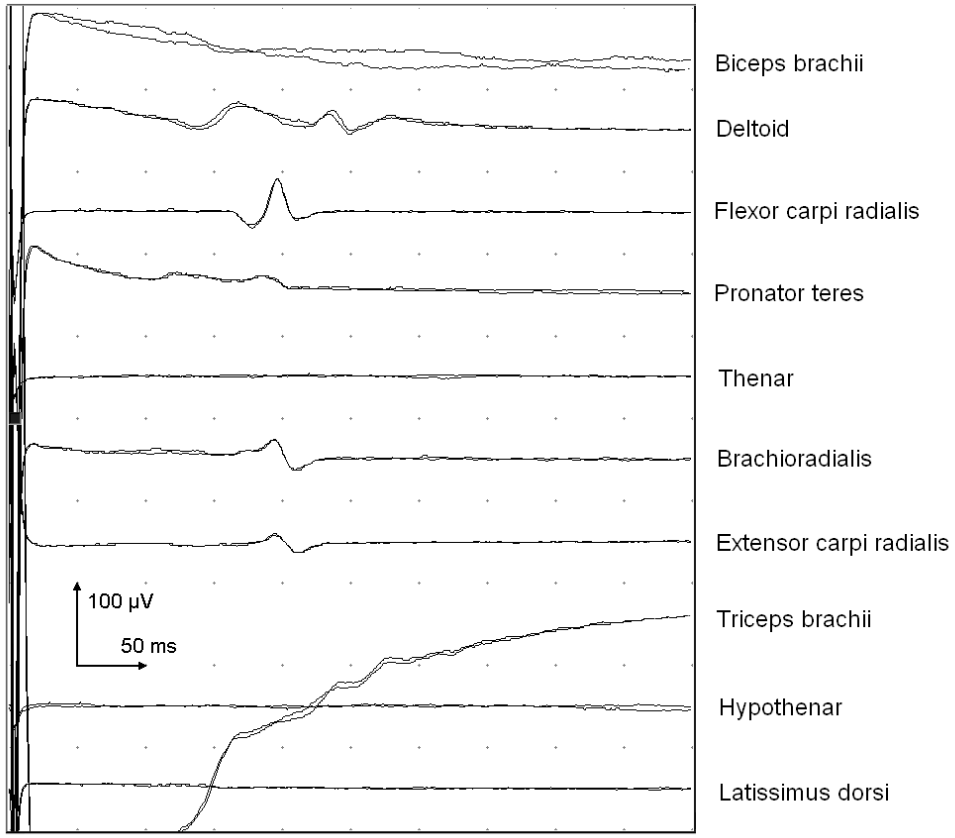


Figure S1: Representative case of biceps motor point stimulation and simultaneous recording in the other nine muscles. Two consecutive measurements in an obstetric brachial plexus lesion subject are overlaid showing reproducibility of the responses. The responses in channel two and eight (from top to bottom) have a duration of approximately 15 and 20 ms, presumably formed by summation of separate motor unit potentials.



Chapter 5

Cocontraction in adults with obstetric brachial plexus lesion

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Submitted

Abstract

Introduction Cocontraction due to axonal misrouting may contribute to the functional deficit in obstetric brachial plexus lesion (OBPL); we aimed to quantify its presence.

Method We obtained supramaximal CMAPs of the biceps muscles in 19 healthy adults (median age 23y; nine men) and 17 conservatively treated OBPL adults (median age 38y; five men) after electrical stimulation at Erb's point. We simultaneously measured CMAPs over the deltoid and triceps muscles, reflecting volume conduction as well as misrouting, with two stimulating and two recording methods. Misrouting should result in more activity measured over the deltoid and triceps muscles during biceps activation in patients than in controls.

Results A branched recording electrode resulted in the least amount of coregistration. No stimulation method was superior to the other. The cocontraction amount did not differ between patients and controls.

Interpretation The branched electrodes improved recording selectivity. None of the methods statistically proved the presence of contraction in OBPL patients.

Introduction

Obstetric Brachial Plexus Lesion (OBPL) concerns a closed traction injury of the brachial plexus during birth, with an incidence of 0.5 to 2.6 per 1000 live births.[1] A systematic literature search showed residual deficit in 20 to 30% of cases.[2] Severe OBPL can result in permanent impairment of arm function, skeletal malformations, cosmetic deformities, behavioural problems and socio-economic limitations.[3, 4] Functional recovery following OBPL depends on the number of outgrowing motor axons that reinnervate muscle fibres, but also on the extent of axonal misrouting.[5-8] The misrouted axons may innervate an agonist (e.g. an axon meant for the biceps ends up in the brachialis muscle), an antagonist (e.g. reinnervation of the triceps instead of the biceps muscle), or a muscle with another function (e.g. deltoid instead of biceps).[8] If a sizable number of axons is misrouted, two muscles will contract together, known as cocontraction. Cocontraction may cause more problems in OBPL than primary muscle weakness.[6, 9, 10] However, triceps and deltoid muscle cocontraction during biceps activation has not been quantified yet. To quantify misrouting, the effects of costimulation, e.g. stimulating unintended nerves, and coregistration, e.g. recording unwanted activity due to volume conduction, must first be minimized.

As for costimulation, electrical stimulation of the brachial plexus at Erb's point is commonly used for the evaluation of proximal nerve disorders.[11-13] The conventional stimulating method involves moving the stimulator over the skin at Erb's point, located in a triangle formed by the clavicle and the sternocleidomastoid and trapezius muscles. Compound muscle action potentials (CMAPs) of a proximal arm muscle are recorded until a stimulation site is found where the largest amplitude is obtained with the lowest current intensity. This paper focuses on the biceps muscle and its innervation through the C6 root, the superior trunk, lateral cord and musculocutaneous nerve. Stimulation in Erb's point is likely to stimulate several plexus elements simultaneously, so we designed an additional stimulation method based on stimulation of the lateral cutaneous antebrachial nerve (LCAN) as the sensory branch of the musculocutaneous nerve. Stimulating the LCAN while recording nerve action potentials (NAPs) at various sites over the plexus should reveal the precise location of axons running to the musculocutaneous nerve (Figure 1).

As for coregistration, CMAPs are conventionally recorded with one electrode over the muscle belly and one over the tendon[12], but this method also records activity of adjacent muscles through volume conduction.[14] A 'branched electrode'[15] has been shown to improve the selectivity of CMAP recordings appreciably.[16]

We first aimed to minimize the effects of costimulation and coregistration comparing two stimulation and two recording methods in healthy subjects. Secondly, the results were used to quantify triceps and deltoid muscle cocontraction during biceps activation in conservatively treated OBPL adults. We previously showed in the same patient group that misrouting was qualitatively present in over half of cases for the biceps muscle and nearly half for the deltoid muscle.[8]

Methods

Participants

Seventeen adults with OBPL and nineteen adult healthy subjects participated. Exclusion criteria were the presence of any relevant disorder affecting movement or sensation. The protocol was approved by the Medical Ethics Committee of the LUMC. All participants provided informed consent.

Stimulation methods

The conventional stimulation method to optimize biceps stimulation involved varying the stimulation site near Erb's point, with the best site defined as the one resulting in a supramaximal biceps CMAP at the lowest current intensity. For the new method, the LCAN was located in the forearm by sliding a stimulator using 8 mA stimuli along a line perpendicular to the direction of the nerve.[17] Once found, stimulating electrodes were attached and 500 stimuli of 8 mA were given, while averaging responses from four recording sites over Erb's point (Figure 1). The site with the highest nerve action amplitude (NAP) was used to identify nerve fibers running to the musculocutaneous nerve. When amplitudes were equally high at two recording sites, a site in between was chosen.

Recording methods

Two types of recording electrodes were used: a standard bipolar recording and the 'branched electrode'.[15] The latter was chosen due to its superiority and simplicity in reducing crosstalk in surface EMG recordings.[15, 16] Disposable surface electrodes of 2.2 by 3.2 cm were used for the standard recording with a distance of 0.5 cm between their edges, placed with the shorter sides of the electrodes adjacent to each other. The branched electrode consisted of three circular electroencephalography (EEG) electrodes of 5 mm diameter with distances of 0.5 cm between their rims. The two outer electrodes were connected to one another, recording a difference between the potential of the middle electrode and the mean potential at the outer electrodes. CMAPs measured with a branched electrode are half the size of those measured with a bipolar electrode.[16] Both types of recording electrodes were fastened on the muscle belly, next to one another and separated by 0.5 cm.[18] The electrode that was placed medially was varied randomly.

Quantifying misrouting

The right arm of the healthy subjects and the affected arm of the OBPL patients were fixed to the examination table to prevent movement artifacts. Supramaximal shocks were given at the standard and LCAN-derived sites to obtain biceps CMAPs. Deltoid and triceps CMAPs were simultaneously measured; these reflect effects of costimulation, coregistration and misrouting. All CMAPs were measured with both bipolar and branched electrodes. Activity was recorded over 30 ms with a band pass-filter of 20 Hz – 2 kHz using a Medelec Synergy EMG apparatus. Twelve CMAPs (2 stimulation methods x 2 recording methods x 3 muscles) were acquired per subject, and peak-to-peak amplitudes were noted.

To assess cocontraction we reasoned as follows: if the CMAP amplitude of the biceps muscle is large, mere volume conduction will cause the simultaneously measured activity over the deltoid and triceps muscles to be large as well. Over a group of subjects, a relation between these amplitudes is therefore to be expected. Misrouting should cause an additional increase of amplitude measured over the deltoid and triceps muscles. As a result, the nature of the relation of deltoid/triceps activity to biceps activity should differ between the groups: the relation in the control groups reflects costimulation and coregistration only, while that in the patient group also reflects misrouting.

Statistical analysis

IBM SPSS Statistics 20.0 (Armonk, NY: IBM Corp.) was used for statistical analysis. Demographic characteristics were compared with a Mann-Whitney *U* test. We used the natural logarithm of CMAPs for further analysis because the data were not normally distributed. A multivariate linear regression was used for three statistical analyses: 1) comparing the recording electrodes, 2) comparing the stimulation methods, and 3) comparing OBPL patients with healthy subjects.

In the first analysis the natural logarithm of triceps CMAPs was the outcome and recording electrode (bipolar or branched) the predictor with the natural logarithm of biceps CMAPs as a confounder. The analysis was performed for each stimulation method (conventional or LCAN) separately. In the second analysis the natural logarithm of triceps CMAPs was the outcome and stimulation method the predictor with the natural logarithm of biceps CMAPs as a confounder. This was performed for each recording method separately. Both analyses were performed only in healthy individuals and were repeated using the natural logarithm of deltoid CMAPs instead of triceps CMAPs as the outcome. A Bonferroni corrected significance threshold of 0.006 (0.05/8) was used considering the eight comparisons (2 muscles x 2 recording electrodes x 2 stimulation methods). In the third analysis the natural logarithm of triceps CMAPs was the outcome and patient and control group the predictor with the natural logarithm of biceps CMAPs as a confounder. This analysis was performed separately for each of the four combinations of recording and stimulation method. This analysis was repeated using the natural logarithm of deltoid CMAPs instead of triceps CMAPs as the outcome. A Bonferroni corrected significance threshold of 0.006 (0.05/8) was used. This analysis was repeated for the triceps comparing healthy subjects with a subgroup of nine patients with qualitatively present misrouting in the triceps, as determined in a previous study[8], and also for the deltoid muscle with a corresponding subgroup of seven patients with qualitatively present misrouting in the deltoid.

The natural exponential of the coefficients from the regression analyses were reported. These numbers represent how many times the triceps or deltoid CMAP would increase when changing the stimulating or recording method, or patients relative to controls. For example, when comparing patients and

controls, a natural exponential of a regression coefficient of 1.5 indicates that CMAP amplitudes are 1.5 times higher in patients than in healthy subjects.

Results

Group description

The median age (10^{th}-90^{th} percentile) was 38 (20-58) years in the OBPL group and 23 (10^{th}-90^{th} percentile: 20–55) years in the healthy subject group (p=0.24). There were five men in the OBPL group and nine in the control group (p=0.32). The right hand was affected in nine of 17 OBPL patients. There were six left-handed subjects in the OBPL group and two among 19 healthy subjects (p=0.074). There were seven OBPL subjects with a lesion level C5-C6, seven with lesion level C5-C7 and three with either C5-C8 or C5-Th1.

Costimulation and coregistration

Table 1 shows biceps, deltoid and triceps CMAP amplitudes. The branched electrode resulted in significantly smaller CMAP amplitudes compared to the bipolar one for both stimulation methods (two left columns of Table 2) and there were no significant differences in CMAP amplitude between the stimulation methods in control subjects. (two right columns of Table 2) Figure 2 shows an example of NAP measurement following LCAN stimulation in a healthy subject. In two healthy subjects NAPs revealed multiple peaks. These data were excluded from the analysis. An example CMAP recording is shown in Figure 3.

Quantifying misrouting

Triceps and deltoid cocontraction did not differ significantly between patients and healthy subjects, (Table 3) and this also held for the patients with qualitatively present misrouting.

Discussion

Costimulation and coregistration

The branched electrode proved superior to the bipolar electrode in minimizing coregistration, but the two stimulation methods did not differ in their ability to limit costimulation. As expected, CMAPs measured with the branched electrode were half the size of those measured with the bipolar electrode.[16] This lower amplitude will only present problems when absolute amplitudes are very small, but this was not the case for the 3.5-5 mV range in the present study. We advise the use of the branched electrode in similar studies to improve recording selectivity. The two stimulation methods did not differ in their ability to find the optimal stimulation site; the resulting two stimulation points were very close together.

Quantifying misrouting

There were no differences in the degree of cocontraction between OBPL patients and healthy subjects for either the triceps or deltoid muscles.

We found no significant proportion of cocontraction in the triceps or the deltoid to be due to misrouting in OBPL patients, not even in a subgroup of patients in whom in a previous study the qualitative presence of misrouting was established.[8] The apparent discrepancy with the current findings can be explained in several ways.

The first is that the number of misrouted axons causing cocontraction in our population was in fact low. Apart from misrouting, problems with the formation of central motor commands have been implied in cocontraction.[6, 9, 10, 19] In view of the present results this explanation becomes more attractive. This may mean that the present population concerned only moderately severe lesions with little misrouting. The second explanation is that we failed to suppress the effects of costimulation and coregistration sufficiently to allow cocontraction to be quantified, despite the use of a combination of conventional and novel ways to do so.

Limitations and implications

The design could not disentangle effects of costimulation and coregistration completely: activity measured over the deltoid and triceps muscles could be due to volume-conducted biceps activity as well as to activation of axons running to these muscles. The use of supramaximal electrical stimulation has the advantage of excluding the effects of voluntary activation which may be mistaken for misrouting. However, the proportion of activated misrouted axons to the triceps compared to the activated biceps axons may be higher during voluntary flexion than during supramaximal stimulation and thus may be functionally impairing. Future research should elucidate the contribution of misrouting to impairment at more functional activation levels.

Acknowledgements

We thank the technicians at the Clinical Neurophysiology department for their help with the practical execution.

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Table 1: Compound muscle action potential (CMAP) amplitudes of the biceps, triceps and deltoid muscles (median milliVolt (10^{th}-90^{th} percentile)) for the healthy subjects (n=19) and obstetric brachial plexus lesion (OBPL) patients (n=17). LCAN - lateral cutaneous antebrachial nerve

		Bipolar recording electrode		Branched recording electrode	
		Conventional stimulation	LCAN stimulation	Conventional stimulation	LCAN stimulation
Healthy subjects	CMAP biceps	7.2 (3.3-11.4)	7.4 (2.7–12.4)	5.0 (1.7–9.4)	3.5 (1.3–9.2)
	CMAP triceps	1.8 (1.0–4.8)	2.9 (0.6–8.2)	0.5 (0.2–1.6)	0.4 (0.1–3.0)
	CMAP deltoid	3.0 (0.4–7.8)	3.8 (0.8–9.4)	0.6 (0.2–2.5)	0.8 (0.2–2.1)
OBPL patients	CMAP biceps	5.6 (1.8-11.2)	5.3 (1.4-10.9)	1.2 (0.3-3.6)	1.2 (0.3-3.4)
	CMAP triceps	1.7 (0.4-4.9)	1.5 (0.2-5.5)	0.3 (0.04-1.2)	0.3 (0.05-1.2)
	CMAP deltoid	3.1 (1.4-8.8)	2.9 (1.5-10.8)	0.7 (0.09-1.8)	0.7 (0.1-2.2)

Table 2: Natural exponential of the regression coefficients comparing compound muscle action potentials between the two recording and two stimulation methods in the healthy subjects (n=19), with corresponding p-values. A significance threshold of 0.006 was used. LCAN - lateral cutaneous antebrachial nerve

	Bipolar vs. branched recording electrode		Conventional vs. LCAN stimulation method	
	Conventional	LCAN	Bipolar	Branched
Triceps (p)	0.34 (0.004)	0.25 (0.001)	0.19 (0.2)	1 (1.0)
Deltoid (p)	0.28 (0.001)	0.23 (<0.001)	1.15 (0.4)	1 (1.0)

Table 3: Natural exponential of the regression coefficients comparing compound muscle action potentials between obstetric brachial plexus lesion patients (n=17) and healthy subjects (n=19), with corresponding p-values. A significance threshold of 0.006 was used. LCAN - lateral cutaneous antebrachial nerve

	Bipolar recording electrode		Branched recording electrode	
	Conventional stimulation	LCAN stimulation	Conventional stimulation	LCAN stimulation
Triceps (p)	0.87 (0.602)	0.73 (0.286)	1.02 (0.970)	1.12 (0.779)
Deltoid (p)	1.73 (0.071)	1.25 (0.430)	1.64 (0.202)	1.12 (0.745)

Figure 1: Stimulation sites.

Left panel: The conventional way of finding Erb's point is to vary the site of a stimulation electrode in the triangle formed by the clavicle and the sternocleidomastoid and the trapezius muscles. *Right panel:* Determination of the four recording electrodes for the LCAN method, relative to the clavicle and brachial plexus. A line was drawn on the skin from the insertion of the sternocleidomastoid muscle to the clavicle to the intersection of the clavicle with an imagery line extended down the boundary of the trapezius muscle. A second line was drawn parallel to the first one, 1.5 cm in the cranial direction. Four silver-silver-chloride round electroencephalography (EEG) electrodes with a diameter of 5 mm were placed at 1 cm distances on this line with the third electrode on the middle of the line.

Figure 2: Typical measurement over the four supraclavicular electrodes after LCAN stimulation of a healthy subject with NAPs from top to bottom: 1.7, 1.9, 2.2, 2.1 μV.

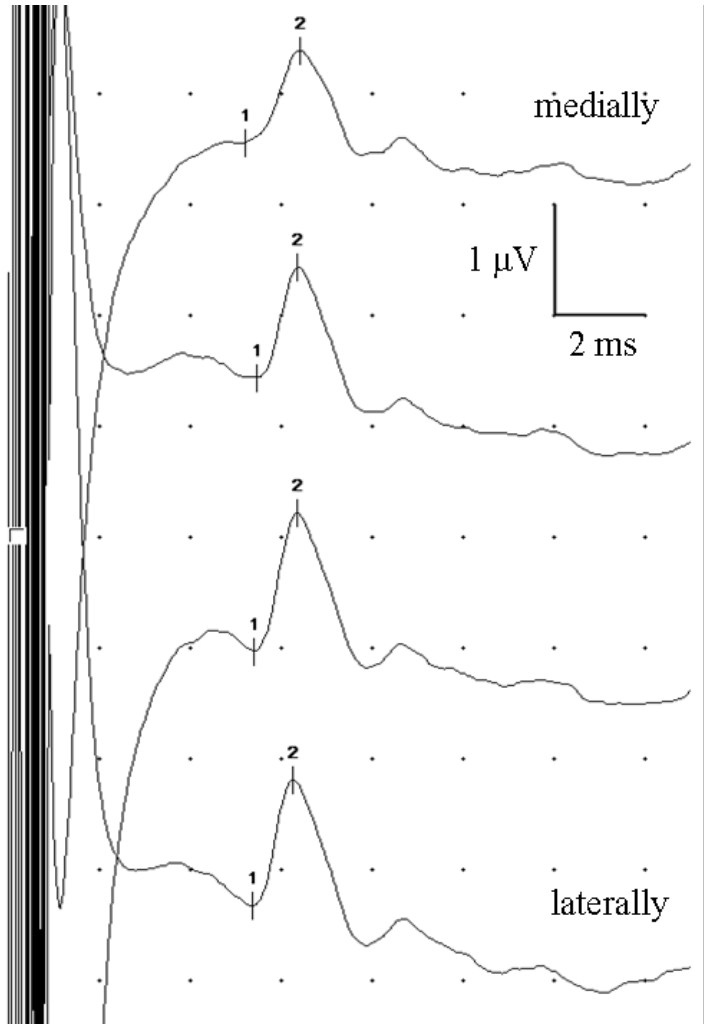
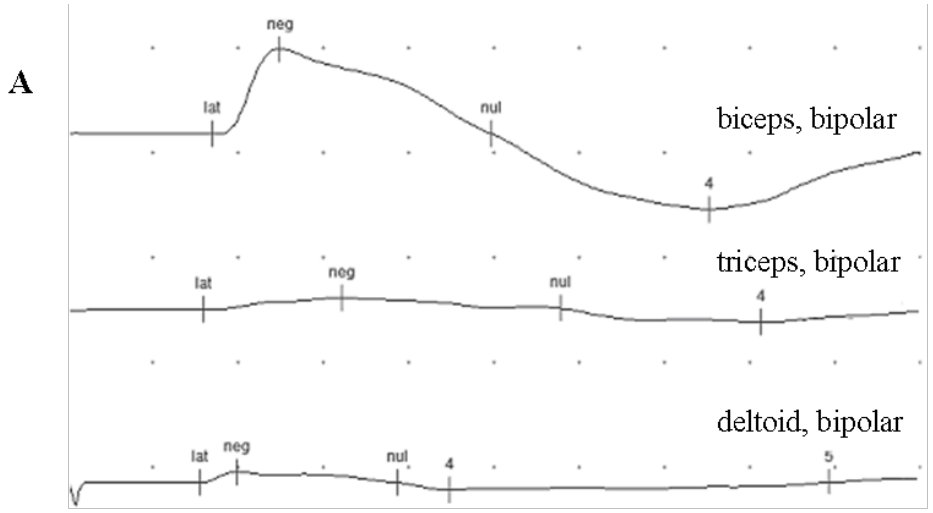


Figure 3: Typical compound muscle action potential measurement of a healthy subject with A. the bipolar and B. the branched electrode over biceps, triceps and deltoid muscles during Erb's point stimulation.



Chapter 6

Cocontraction measured with short-range stiffness was higher in obstetric brachial plexus lesions patients compared to healthy subjects

Short communication

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J Biomech. 2017 Oct, 63:192-6.

Abstract

We suggest short range stiffness (SRS) at the elbow joint as an alternative diagnostic for EMG to assess cocontraction.

Elbow SRS is compared between obstetric brachial plexus lesion (OBPL) patients and healthy subjects (cross-sectional study design). Seven controls (median 28 years) and five patients (median 31 years) isometrically flexed and extended the elbow at rest and three additional torques [2.1, 4.3, 6.4 N m] while a fast stretch stimulus was applied. SRS was estimated *in silico* using a neuromechanical elbow model simulating the torque response from the imposed elbow angle.

SRS was higher in patients (250 ± 36 N m/rad) than in controls (150 ± 21 N m/rad, p = 0.014), except for the rest condition. Higher elbow SRS suggested greater cocontraction in patients compared to controls. SRS is a promising mechanical alternative to assess cocontraction, which is a frequently encountered clinical problem in OBPL due to axonal misrouting.

Introduction

Obstetric Brachial Plexus Lesion (OBPL) concerns a closed traction injury of the brachial plexus during birth, with an incidence of 0.5 to 2.6 per 1000 live births[1]. Twenty to thirty percent of cases have a permanent functional deficit[2]. Functional muscle recovery following OBPL depends on the number of outgrowing motor axons that reinnervate muscle fibres, on how many axons are misrouted to the wrong muscles[3,4], and on aberrant central motor programming[5]. Misrouting occurs when a regenerating axonal sprout, which may also be one of several branches, elongates into a basal lamina tube different from the original one[6]. This may lead to the innervation of an antagonistic muscle and cocontraction. Cocontraction causes joint stiffness, resulting in serious functional problems in OBPL, possibly more so than primary muscle weakness[4]. Cocontraction can be assessed qualitatively using electromyographical (EMG) techniques[7], but quantifying its contribution to motor impairment is difficult due to potential EMG cross-talk[8]. Cross-talk is the unintended registration of neighbouring muscle activity. Clinical assessment (e.g. joint range of motion, muscle strength) cannot distinguish between weakness of one muscle and cocontraction of its antagonist.

'Short range stiffness' (SRS) is a promising alternative representing the state of the mechanical system including the cocontraction and/or muscle weakness. SRS, i.e. the ratio of a change in torque over change in angle, is assigned to the elastic properties of the cross-bridges in the muscle fibres[9]. Both the agonist and antagonist muscles exhibit stiffness and so the total joint SRS is the sum of their stiffness ($SRS_{joint} = SRS_{agonist} + SRS_{antagonist}$). The actual torque is the difference between agonist and antagonist torque ($T_{joint} = T_{agonist} - T_{antagonist}$)[10]. To obtain the same net flexion torque as healthy individuals, patients with biceps-triceps cocontraction will require an increased overall activation to overcome triceps cocontraction, leading to higher elbow stiffness. (Fig. 1) Hence, the aim of this pilot study was to quantify elbow SRS and compare it between OBPL patients and controls and we hypothesize that SRS will be higher in patients.

Materials and methods

Five adult patients with OBPL, recruited from the Dutch Erb's Palsy Association and earlier research projects of the Leiden University Medical Centre (LUMC) Rehabilitation Department, and seven controls participated. Exclusion criteria were brachial plexus surgery and any other relevant neuromuscular or joint disease. All patients had participated in a previous study[7]. Patients were included when they were able to flex and extend the arm against gravity with a muscle strength of at least grade 3[11]. Patients were included who had suffered a traction lesion corresponding to at least the spinal nerves C5, C6 and C7. The study was approved by the Medical Ethics Committee of the LUMC. All participants provided written informed consent.

We adapted the wrist perturbator used by van Eesbeek and colleagues for elbow use (Fig. 2) and adapted the experimental protocol with some alterations described below[10]. All variables were transformed in coordinates centred on the elbow (Appendix A). Participants were requested to generate four elbow torque levels in random order for flexion as well as extension, of 0 N m (i.e. relaxed muscles) and on average 2.1, 4.3 and 6.4 N m, depending on arm length. A ramp-and-hold rotation (0.15 radians, 4 radians/second) was automatically started when the difference between the torque generated by the participants and the target level was smaller than 2.5 % for 0.5 s. A 15 s rest period was included after each stimulus to prevent fatigue and thixotropic force reduction, a phenomenon affecting resting tension due to earlier muscle use[9]. Strain gauge signals were sampled at 5 kHz and low-pass filtered (50 Hz, 3rd order Butterworth filter). SRS was estimated during the first 0.04 s of torque, preventing stretch reflexes to affect the measurements, (Appendix B)[12] resulting in 32 trials (4 torque levels, 2 directions, 4 observations) per participant. The median of the four observations was calculated resulting in eight data points per participant for further analysis.

We adapted the wrist SRS model and data analysis[10] for the elbow joint with a varying moment arm per participant derived from the recorded forearm length. The model (Fig. 2 in van Eesbeek et al., 2010) was implemented in Simulink and the optimization was performed in Matlab (The Mathworks Inc.). In short, a dynamic nonlinear model was used to describe the recorded data (angle and

torque) consisting of two masses in series, representing the motor lever and the participants' forearm, each connected by a spring-damper element resulting in three spring-damper elements. Of the corresponding ten model parameters (Table 1) motor lever inertia, damping and stiffness, and joint damping were fixed, and the remaining six were estimated[10]. Model parameters were found by minimizing the quadratic difference between the measured and modelled torques. Goodness of model fit and parameter reliability were checked for with the 'variance accounted for' (VAF) and the normalized standard error of the mean (SEM)[10]. The median SRS of all four observations was calculated and presented as a scatter plot against the measured torque in elbow coordinates.

Additionally, we used surface EMG to assess the relative degree of agonist and antagonist activity to support our SRS measurements. Biceps and triceps activity were recorded by EMG electrodes placed over the muscle belly (DelsysBagnoli-4, 20–400 Hz band pass, 10 mm inter-electrode distance), sampled at 5 kHz, full-wave rectified, low-pass filtered (30 Hz, 4th order Butterworth). Muscle activation (A) of the biceps and triceps was calculated for each flexion and extension torque task during 0.04 second period prior to the ramp-and-hold perturbation. The mean absolute EMG signal was reduced by the mean absolute EMG at rest. Activation ratio (AR) for the biceps was calculated as follows:

$$AR_{biceps} = \frac{A_{biceps}^{flexion} - A_{biceps}^{extension}}{A_{biceps}^{flexion} + A_{biceps}^{extension}}$$, and for the triceps: $$AR_{triceps} = \frac{A_{triceps}^{extension} - A_{triceps}^{flexion}}{A_{triceps}^{extension} + A_{triceps}^{flexion}}$$

where $A_{biceps}^{flexion}$ is biceps activation during flexion, $A_{biceps}^{extension}$ is biceps activation during extension at equal absolute elbow torque conditions[13]. Calculation of the AR requires a good signal-to-noise ratio, so AR was calculated only when the value of the EMG signal of each of the three tasks was at least twice that of the EMG signal at rest.[14]

Statistical analysis

Generalized linear model for repeated measurements (Generalized Estimating Equations) was used with an unstructured correlation matrix in IBM SPSS Statistics 20.0 (Armonk, NY: IBM Corp.) for the following three statistical analyses. In the first analysis SRS was the outcome and patient and control group the predictor, with confounders: torque, flexion and extension task, the

interaction between torque and tasks, and arm mass. SRS corrected for the four confounders is referred to as the '*corrected SRS*' in the results, and uncorrected otherwise. We checked for other possible causes of stiffness such as joint deformities by comparing SRS for the zero torque level, between patients and controls with the same model. In the second analysis AR was the outcome and patient and control group the predictor with confounders: torque, flexion and extension task, the interaction between torque and tasks, and arm length. AR corrected for the four confounders was referred to as the '*corrected AR*' in the results. In the third analysis SRS was the outcome and AR the predictor with confounders: torque, flexion and extension task, the interaction between torque and tasks, arm length, and arm mass. A significance level of 0.05 was chosen.

Results

Characteristics of the groups are shown in Table 2. One patient with triceps weakness (MRC 3) was unable to produce sufficient extension torque in the trials with the highest required torques (levels 4.3 and 6.4 N m); the performed tasks were included in the analysis. A typical raw data recording is shown in Appendix B. The model parameters, their SEM and the VAF are shown in Appendix C. Fig. 3(a) shows uncorrected SRS as a function of torque for flexion and extension and Fig. 4(a) corrected SRS for patients and controls. SRS was significantly higher in patients (250 N m/rad, standard error [SE] 36 N m/rad) than in controls (150 N m/rad, SE 21 N m/rad, $p = 0.014$) and it was higher during flexion (252 N m/rad, SE 29 N m/rad) than extension (148 N m/rad, SE 16 N m/rad, $p < 0.001$). SRS increased with the level of torque both during flexion (18 N m/rad, SE 6 N m/rad, $p < 0.001$) and during extension (19 N m/rad, SE 4 N m/rad, $p < 0.001$). SRS did not differ significantly between patients and controls for torque level zero ($p = 0.185$). AR was not calculated for the torques of 0 and 2.1 N m, as the EMG signal did not exceed twice the EMG signal at rest. Fig. 3(b) shows uncorrected AR as a function of torque and Fig. 4(b) corrected AR for patients and controls. AR did not differ significantly between patients (0.19, SE 0.43) and controls (0.41, SE 0.36, $p = 0.8$). SRS was lower when AR was higher, but not significantly so (42 N m/rad, SE 77 N m/rad, $p = 0.6$).

Discussion

We were able to quantify elbow SRS in OBPL patients and controls. We confirmed our hypothesis that SRS would be higher in patients than in controls.

The amount of VAF by the mechanical model was high and the SEM was low, suggesting that the applied model was sufficiently reliable. SRS was higher during flexion than extension which fits with previous findings for the elbow joint[15-17], which may be explained by moment arm, muscle pennation angle, and muscle length differences between the biceps and triceps in healthy subjects. SRS in controls in our study was approximately five times higher than previously reported[15-17], which is likely due to the use of continuous perturbations[9,12,16] and a smaller angle between both upper arm and forearm, and upper arm and trunk in previous reports[16].

SRS was significantly higher in patients than in controls, suggesting more cocontraction in patients. Previous studies in the same subjects showed that misrouting was present in their biceps and triceps muscles[7], suggesting that cocontraction exceeding that of controls may be due to misrouting. We feel that the increased stiffness in patients is not affected by joint deformities[18], because SRS did not differ between patients and controls at torque level zero. AR did not differ between patients and controls. This may be because we did not measure brachioradialis muscle EMG which may also explain an outlying value in AR during extension, or because of the small number of participants.

The advantages of SRS compared to EMG for cocontraction measurement is that SRS represents the mechanical state of the elbow including the active contribution of all muscles affecting elbow rotation[7], is not affected by cross-talk[8,19], and has a good signal-to-noise ratio. This pilot study was potentially limited by the relatively small number of participants and large number of model parameters. When looking in more detail to the etiological factors further expansion of the mechanical model may be useful, e.g. to distinguish between the different muscular compartments of individual muscles that contribute to joint stiffness.[20,21]

We conclude that SRS is a promising mechanical parameter to quantify elbow cocontraction in OBPL patients, possibly due to misrouting. The clinical importance is that current cocontraction treatment in OBPL, injection of botulinum toxin in antagonist muscles, based on clinical measures, cannot distinguish between muscle weakness and antagonist cocontraction[22]. SRS may be a valuable alternative to tailor OBPL treatment in the future.

Conflict of interest statement
The authors declare that they have no conflict of interest.

Acknowledgements

We thank S. van Eesbeek, C.P.M. Roos, and the LUMC Departments of Instrumental Affairs and Rehabilitation. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Table 1: Mechanical model parameters. SRS – short-range stiffness

Parameter	Unit	Fixed/Estimated
Motor lever inertia	kgm^2	Fixed
Motor lever damping	Nms/rad	Fixed
Motor lever stiffness	Nm/rad	Fixed
Joint inertia	kgm^2	Estimated
Hand–handle interface damping	Nms/rad	Estimated
Hand–handle interface stiffness	Nm/rad	Estimated
Joint damping	Nms/rad	Fixed
SRS	Nm/rad	Estimated
Stiffness beyond elastic limit	Nm/rad	Estimated
Elastic limit	rad	Estimated

Table 2: Demographic details of the participants. MRC – Medical research Council scale. When biceps strength was described as MRC grade '5-', this was noted as 4.75.

	OBPL patients	Controls
Number	5	7
Median age (25^{th}-75^{th} percentile) [years]	31 (24-50)	28 (21-52)
Gender (men)	1	2
Investigated left arm	3	3
Median arm length (25^{th}-75^{th} percentile) [cm]	24.0 (22.8-25.5)	25.0 (24.0-27.0)
MRC biceps (25^{th}-75^{th} percentile)	4.75 (3.50-4.88)	-
MRC triceps (25^{th}-75^{th} percentile)	4.75 (3.88-5.00)	-
Lesion extent: number of patients	C5-C7: 4 C5-C8: 1	-

Fig. 1. For the same net flexion torque, obstetric brachial plexus lesion (OBPL) patients (right) with motor misrouting which causes increased triceps activation would have to activate the biceps more than healthy individuals (left). Top: Difference in innervation by nerve roots C6 and C7. (Not shown: theoretically also possible cross-innervation from nerve root C7 to biceps muscle in OBPL) Bottom: Difference in muscle activation, indicated by the size of the muscles and the arrow thickness (F – force, T - torque). (Not shown: there is some triceps activation in healthy individuals, presumably contributing to joint stability) In the case of absent misrouting we expect that SRS in patients for a certain torque would be within the healthy individuals range and activation ratio (AR) would be high (i.e. close to 1 as in healthy individuals). In the case of misrouting, SRS in patients would be higher than in healthy individuals and AR would be low (i.e. close to 0). In the case of paresis, certain torque levels may not be reached and SRS in patients for the lower torques are normal compared to healthy individuals and AR will be low with a tendency towards zero due to an unfavourable signal-to-noise ratio. Thus, SRS can potentially distinguish between normal function, cocontraction due to misrouting, and paresis.

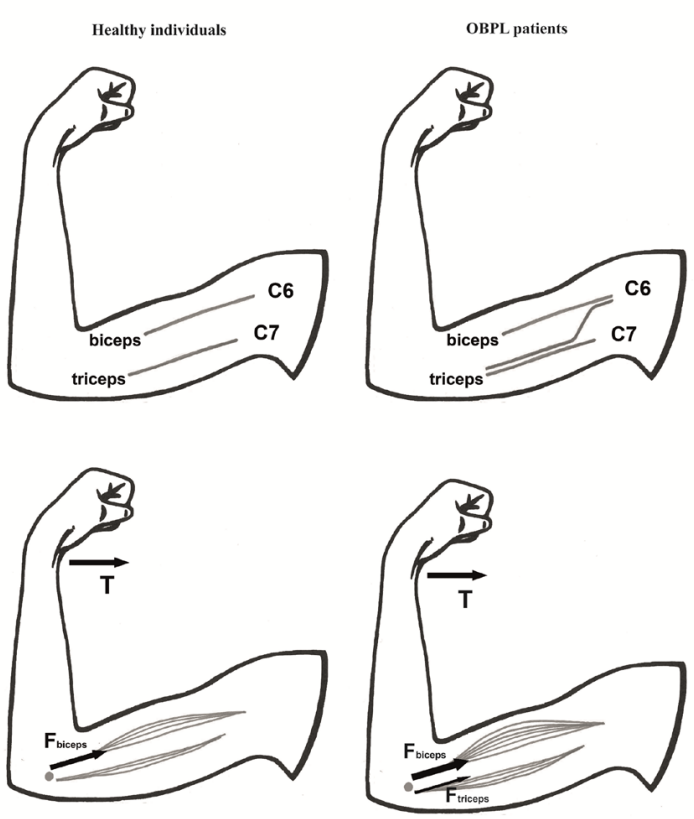


Fig. 2. Experimental set-up with arrows indicating torque (T) and rotation direction during flexion around the elbow joint. It consists of a handle driven by a position servo-controlled (50 Hz bandwidth) electrical motor delivering a torque of 1000 N m/rad[10,23]. To assure that the experimental flexion and extension tasks were within the range of motion for patients with contractures, the posture involved 90° shoulder abduction, 90° elbow flexion, with the palm of the hand facing down. The forearm was fixated at the wrist and elbow joint. The motor lever of the machine was attached to a clamp at the wrist joint, placed over the styloid processes of the radial and ulnar bones. The clamp at the elbow joint was placed over the lateral and medial epicondyles of the humeral bone. Both clamps were covered with elastic foam for comfort. The experiment was performed with the forearm aligned with the moment arm of the motor. The distance along the motor moment arm from the lever axis to the centre of rotation was 7 cm[23]. The forearm moment arm length varied per participant and was measured between the ulnar styloid process and the olecranon when the arm was in 90° shoulder abduction, 90° elbow flexion, and the palm of the hand facing down. Angular displacement of the lever was measured and torque exerted at the level of the wrist clamp was measured by strain gauges within the lever between wrist clamp and motor. Visual feedback of elbow torque was provided on a computer screen in front of the participant as described by van Eesbeek and colleagues[10].

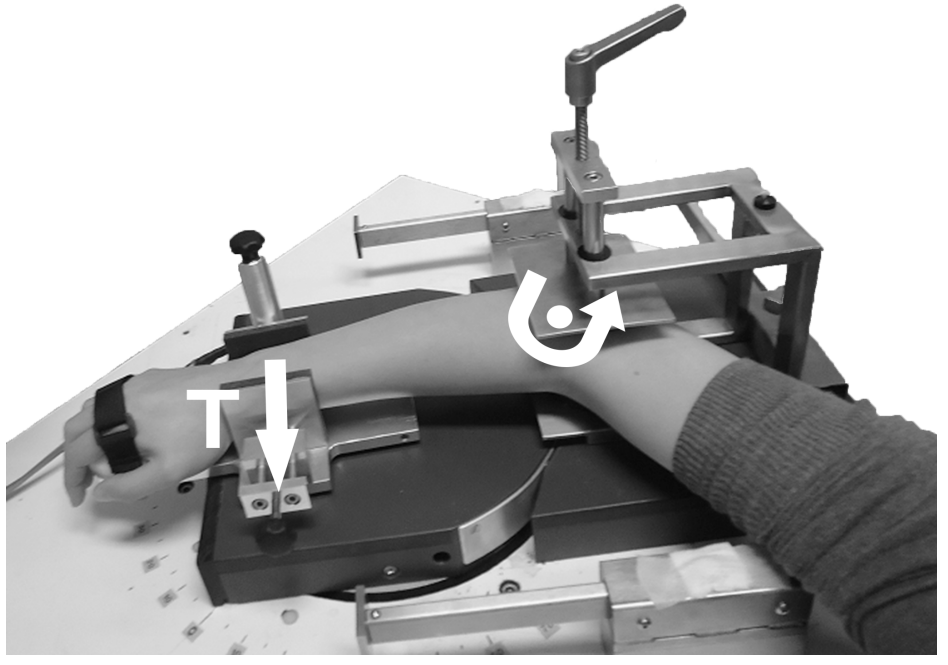


Fig. 3. Scatter plot of the uncorrected (**a**) short range stiffness (SRS) and (**b**) activation ratio (AR) against torque. The lines connect the values belonging to the same subject for flexion and extension separately. Biceps AR is coupled with extension and triceps AR with flexion. Gray circles – controls, black squares – obstetric brachial plexus lesion patients.

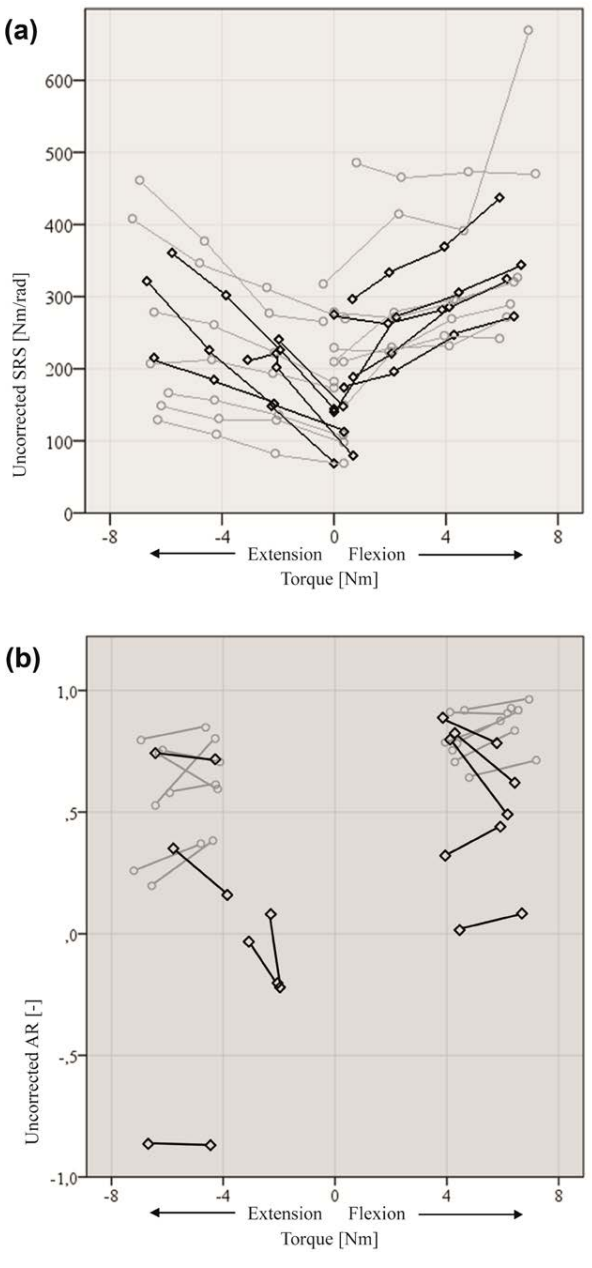
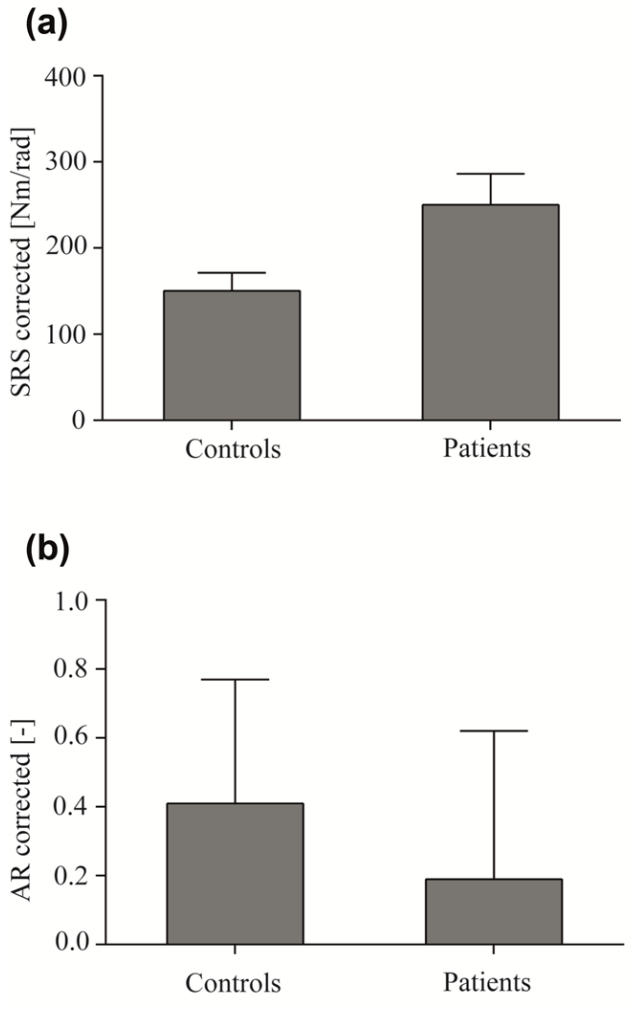


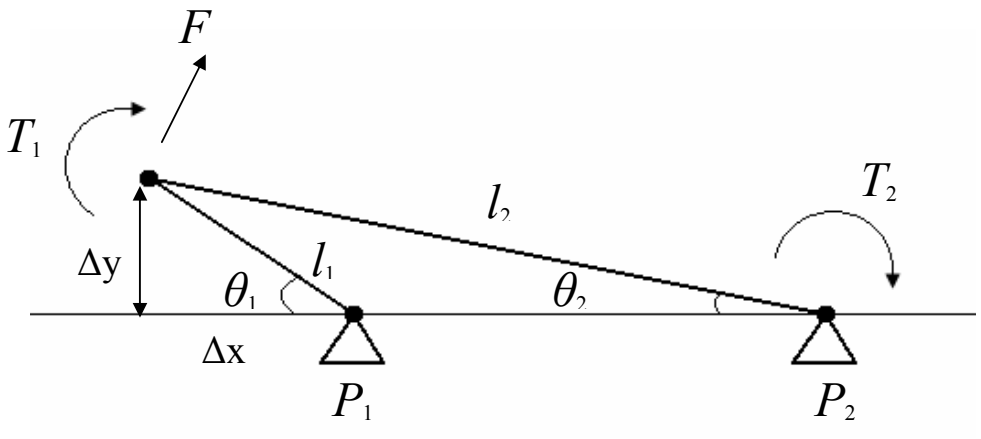
Fig. 4. (**a**) Bar plot with 95% confidence interval error bars of the corrected short range stiffness (SRS) for controls and obstetric brachial plexus lesion (OBPL) patients. **(b)** Bar plot with 95% confidence interval error bars of the corrected activation ratio (AR) for controls and OBPL patients.



APPENDIX A

Transformation variables from motor coordinates to elbow coordinates

F - applied force [N]
l_1 - lever arm perturbator [m]
l_2 - length forearm [m]
θ_1 - angle perturbator [rad]
θ_2 - angle elbow [rad]
T_1 - torque around P_1 [Nm] in motor coordinates
T_2 - torque around P_2 [Nm] in elbow coordinates
k_1 - SRS [Nm/rad] in motor coordinates
k_2 - SRS [Nm/rad] in elbow coordinates



(1) Transformation angles

For small θ_1 and θ_2 follows:

$$\tan\theta_1 \approx \theta_1 = \frac{\Delta y}{\Delta x} \approx \frac{\Delta y}{l_1}$$

$$\tan\theta_2 \approx \theta_2 \approx \frac{\Delta y}{l_2}$$

Thus:

$$\Delta y \approx l_1\theta_1 \approx l_2\theta_2$$

And the angle transformed from motor to elbow coordinates is:

$$\theta_2 \approx \frac{l_1}{l_2}\theta_1$$

(2) Transformation torques

$$T_1 = F\ l_1$$
$$T_2 = F\ l_2$$

Thus:

$$F = \frac{T_1}{l_1} = \frac{T_2}{l_2}$$

And the torque transformed from motor to elbow coordinates is:

$$T_2 = \frac{l_2}{l_1}T_1$$

From **(1)** and **(2)** we acquire the following equation of motion in elbow coordinates:

$$T_2 = I_2\ddot{\theta}_2 + b_2\dot{\theta}_2 + k_2\theta_2$$

$$\frac{l_2}{l_1}T_1 = I_2\frac{l_1}{l_2}\ddot{\theta}_1 + b_2\frac{l_1}{l_2}\dot{\theta}_1 + k_2\frac{l_1}{l_2}\theta_1$$

$$T_1 = I_2\left(\frac{l_1}{l_2}\right)^2\ddot{\theta}_1 + b_2\left(\frac{l_1}{l_2}\right)^2\dot{\theta}_1 + k_2\left(\frac{l_1}{l_2}\right)^2\theta_1$$

Thus the **parameters** in elbow coordinates can be acquired by the following transformations:

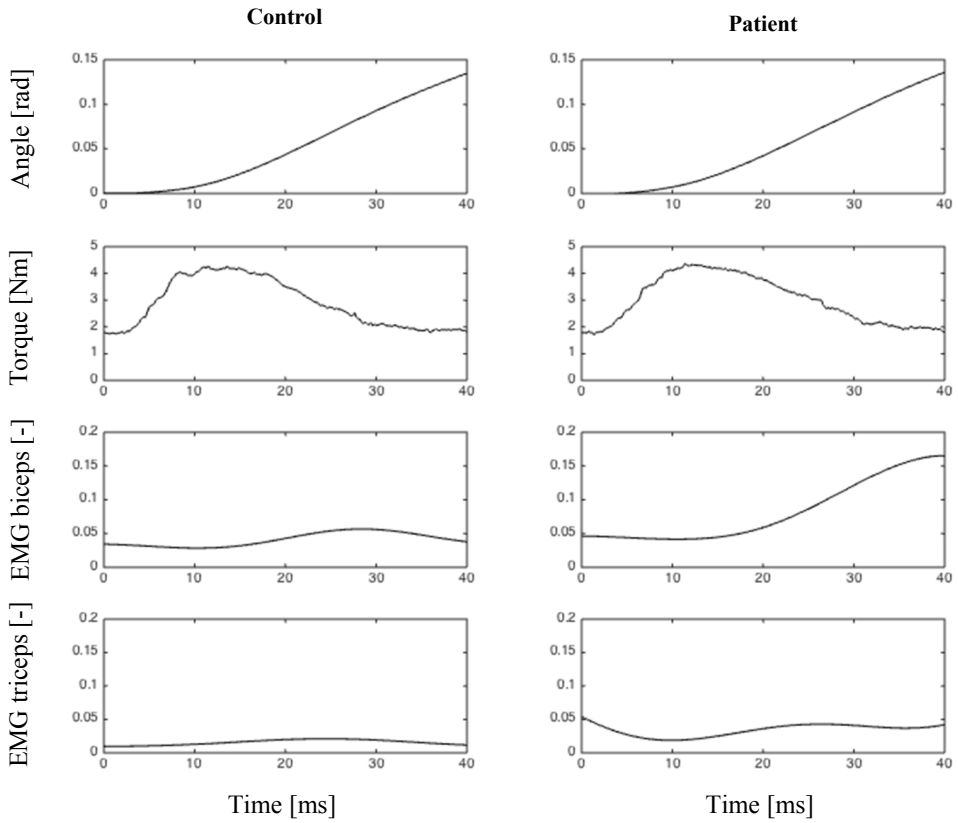
$$I_1 = I_2\left(\frac{l_1}{l_2}\right)^2 \quad \rightarrow \quad I_2 = I_1\left(\frac{l_2}{l_1}\right)^2$$

$$b_1 = b_2\left(\frac{l_1}{l_2}\right)^2 \quad \rightarrow \quad b_2 = b_1\left(\frac{l_2}{l_1}\right)^2$$

$$k_1 = k_2\left(\frac{l_1}{l_2}\right)^2 \quad \rightarrow \quad k_2 = k_1\left(\frac{l_2}{l_1}\right)^2$$

APPENDIX B

Example of a typical raw data recording during a flexion task of 6.4Nm in coordinates centred on the elbow (corresponding with 1.8Nm in coordinates centred around the motor of the perturbator as shown in the figure).



APPENDIX C

Median [25th, 75th percentile] output model parameters for controls and patients.

Controls

Parameter	Flexion	SEM	Extension	SEM
Joint inertia [kg m²]	0.055 [0.051, 0.072]	0.002 [0.002, 0.004]	0.063 [0.051, 0.082]	0.004 [0.003, 0.006]
Hand–handle interface damping [N m s/rad]	16 [15, 19]	0.002 [0.001, 0.002]	17 [15, 20]	0.004 [0.003, 0.005]
Hand–handle interface stiffness [N m/rad]	7374 [5657, 8412]	0.030 [0.024, 0.041]	3955 [3232, 4511]	0.048 [0.041, 0.063]
Stiffness beyond elastic limit [N m/rad]	328 [246, 393]	0.023 [0.018, 1615]	128 [118, 149]	0.173 [0.135, 0.194]
SRS [Nm/rad]	278 [230, 375]	0.014 [0.010, 0.017]	188 [130, 275]	0.018 [0.014, 0.024]
Elastic limit [rad]	0.05 [0.04, 0.06]	0.018 [0.015, 353]	0.10 [0.10, 0.10]	0.173 [0.135, 0.194]
VAF [%]	99.9 [99.7, 99.9]	-	99.4 [99.3, 99.5]	-

Patients

Parameter	Flexion	SEM	Extension	SEM
Joint inertia [kg m²]	0.054 [0.047, 0.056]	0.002 [0.002, 0.002]	0.053 [0.053, 0.062]	0.005 [0.004, 0.008]
Hand–handle interface damping [N m s/rad]	16 [13, 17]	0.001 [0.001, 0.002]	16 [14, 18]	0.004 [0.003, 0.004]
Hand–handle interface stiffness [N m/rad]	6536 [5284, 7586]	0.031 [0.018, 0.036]	2962 [2286, 3722]	0.057 [0.050, 0.082]
Stiffness beyond elastic limit [N m/rad]	307 [268, 346]	0.022 [0.015, 0.034]	118 [107, 130]	0.168 [0.164, 0.175]
SRS [Nm/rad]	275 [221, 324]	0.012 [0.010, 0.015]	207 [147, 230]	0.014 [0.011, 0.024]
Elastic limit [rad]	0.05 [0.04, 0.06]	0.017 [0.013, 0.024]	0.10 [0.10, 0.10]	0.168 [0.164, 0.175]
VAF [%]	99.9 [99.8, 100.0]	-	99.4 [99.1, 99.5]	-

SEM - normalized standard error of the mean; SRS - short-range stiffness, VAF – variance accounted for.

Chapter 7

Impaired automatic arm movements in obstetric brachial plexus palsy suggest a central disorder

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J Child Neurol. 2016 Mar, 31(8):1005-8.

Abstract

We aimed to find evidence for a central component of the impairment of movement of the affected arm in children with obstetric brachial plexus palsy. We performed a cross-sectional study in 19 children (median age 5 years) with obstetric brachial plexus palsy who were able to voluntarily abduct their affected arm beyond 90 degrees. They were asked to perform four tasks designed to provoke automatic arm movements to maintain balance. We assumed automatic motor programming to be impaired when two of three investigators agreed using video recordings that the affected arm did not abduct beyond 90 degrees while the unaffected arm did. Children abducted the affected arm less often than the healthy one (generalized binary logistic model of all four tasks, p=0.001). The deficit during automatic arm abduction was not observed during voluntary movements and therefore cannot be explained by a peripheral deficit, suggesting a central component.

Introduction

Obstetric brachial plexus palsy is a closed traction injury of the brachial plexus during birth, with an incidence of 0.5 to 2.6 per 1000 live births.[1] A permanent deficit in arm function affects 20 to 30% of cases.[2] Functional recovery following a nerve lesion depends on the number of outgrowing axons that successfully cross the lesion site and on their correct routing.[3-5] Theoretically, recovery of obstetric brachial plexus palsy may additionally be impaired by a disturbed development of central motor programs.

There is neurophysiological evidence supporting defective motor programming in obstetric brachial plexus palsy,[6] and the concept of impaired central motor programs in obstetric brachial plexus palsy is also supported by observations of obstetric brachial plexus palsy infants 'forgetting their arm' during automatic movements: children with obstetric brachial plexus palsy may flex the elbow on the affected side while voluntarily picking up a ball, but the same elbow may not flex during running or other tasks that rely on automatic movements, while the unaffected arm does flex at that time.[3,7] If the observed deficit in the affected arm was wholly due to peripheral nerve, muscle or joint damage, the deficit would not depend on whether a movement is made in a voluntary or an automatic context. The movements of the unaffected arm serve as a control that the task indeed demanded flexion. Accordingly, we reason that arm movements in obstetric brachial plexus palsy that can be performed voluntarily by both arms, but that do not occur in the context of automatic movements of the affected arm, suggest the presence of a central deficit. In other words, we regard the discrepancy between volitional and automatic movements as evidence for a central component. Whether volitional or automatic movements are performed worse does not in fact matter for this reasoning; clinical observation suggested that automatic movements happen to be most impaired.

Motor tasks become consolidated in central motor programs with repetition and practice.[8] Tasks that are highly practiced to the point of demanding few attentional resources are called automatic tasks.[9] Anticipatory postural adjustments of the arms during walking are in part automatic movements.[10,11] To suppress volitional influences that interfere with the automatic component in these arm movements, attention can be diverted by dual motor or cognitive tasks.

The aim of this study was to elucidate whether automatic movements are indeed impaired, suggesting that incomplete recovery is at least partially central in origin.

Methods

Participants

Twenty-three children between three and eight years of age with an obstetric brachial plexus palsy were investigated at the Nerve Centre of the Leiden University Medical Centre between August 17, 2010 and September 21, 2010. Data concerning lesion severity and any surgical intervention were taken from patient records. Parents and children provided verbal informed consent to participate after detailed information was provided. A Mallet grade four for shoulder abduction of the affected side was required for inclusion, equivalent to abduction of at least 90 degrees.[12] Any other relevant disorder affecting movement or sensation served as reason for exclusion. The study was judged by the institutional medical ethics committee to be innocuous and to not warrant a full review, conforming to Dutch law.

Procedure

The tasks were video recorded. Children were asked to perform four tasks while walking approximately 3 meters on a straight line. We searched for tasks that would lie as far as possible on the 'automatic' side of a scale from 'fully automatic' to 'fully volitional'. We used balance tasks to provoke automatic arm movements, performed to prevent falling. Automatic tasks themselves demand few attentional resources; we added dual motor or cognitive tasks of increasing difficulty to the balance tasks to make the children focus on those tasks, thereby shifting their attention away from volitional control over their arm movements. The children and parents were informed that the investigation was aimed at central motor programming, but our focus on automatic arm movements was not disclosed to avoid voluntary interference. Each task was first demonstrated by one of the authors (GVA): (a) Walking heel-to-toe towards the camera; (b) Walking on the heels with small steps; (c) Walking heel-to-toe with eyes closed; (d) The same as task (c) but with a cognitive task: count out loud or count backwards generally starting from the age of four years or to name five girl or

boy names if younger or counting was too difficult. Children were reminded to perform the task until the end of the line was reached. The investigation was stopped when children did not wish to continue.

Video records were reviewed by three authors (GVA, JGvD, MJAM). Blinding for the side of the affected arm was impossible because affected arms were often shorter and always moved differently from the unaffected side. The assessors independently scored whether either arm was abducted to at least 90 degrees in relation to the position of the trunk for each of the four tasks (Figure 1). Videos were repeatedly viewed if requested. Abduction to at least 90 degrees was considered present when at least two assessors judged so, and absent otherwise. We scored automatic movement as impaired if three conditions were simultaneously met: 1. the affected arm could be abducted on request at least 90 degrees with respect to the trunk; 2. the unaffected arm abducted at least 90 degrees during an automated balance task; 3. the affected arm abducted less than 90 degrees during the same task.

Statistical analysis

IBM SPSS Statistics 20.0 (Armonk, NY: IBM Corp.) was used for statistical analysis. A generalized binary logistic model for repeated measurements with an unstructured correlation matrix including the presence of previous brachial plexus surgery as a variable was used to test whether the rates of abduction differed between affected and unaffected arms over all four tasks. A significance threshold of 0.05 was used. The same model without correction for brachial plexus surgery was applied for the analysis of the tasks separately. A Bonferroni corrected significance threshold of 0.01 (0.05/4) was used.

Results

Four of 23 children did not cooperate and were not investigated. The median age of the 19 participants was 5 years (25^{th}-75^{th} percentile: 4-7 years); there were 12 boys. The left arm was affected in 10 cases. Five (26%) had a C5-C6 lesion, nine (47%) a C5-C7 lesion, three (16%) a C5-C8 lesion and two (11%) a C5-Th1 lesion. Fifteen had undergone surgery of the brachial plexus at a median age of 4 months (25^{th}-75^{th} percentile: 3-7) (Table 1).

The task results are presented in Table 2. Abduction over 90 degrees was present significantly less often for affected than unaffected arms of the healthy arms taking all tasks together (-1.38 (95% confidence interval (95%CI) -2.22,-0.53), p=0.001, Figure 1)). The rates did not differ between participants who had undergone brachial plexus surgery and those treated conservatively (0.49 (95%CI -0.59,1.57), p=0.371). Analysis per task showed that abduction over 90 degrees of the affected arms occurred significantly less often during task (b) (-1.57 (95%CI -2.59,-0.55), p=0.003), but not during the other tasks (task (a), -0.47 (95%CI -1.36,0.43), p=0.309; task (c), -1.12 (95%CI -2.35,0.10), p=0.072; task (d), -1.41 (95%CI -2.72,-0.10), p=0.035).

Discussion

We found that children with obstetric brachial plexus palsy abducted the affected arm over 90 degrees less often than the unaffected arm in automated balance tasks even though they were able to abduct the affected arm over 90 degrees on request. The discrepancy can therefore not be explained by incomplete peripheral nerve regeneration or joint problems. We propose that disturbed central motor programming underlies this phenomenon, at least partially.
Involvement of the basal ganglia, supplementary motor, premotor and motor cortex and the brainstem has been shown in the generation of anticipatory arm movements.[11] At the onset of learning a new motor skill in healthy subjects as well as in patients following upper extremity injury and reconstruction, there is an expansion of motor cortical representation.[13] Once a skill is mastered the degrees of cortical representation and excitability decrease again.[13] A decreased contralateral cortical activation has been found in the primary motor cortex during attempted movement in paraplegics compared to healthy controls studied with motor imagery fMRI, explained by an increased need for attention allocation.[14] Accordingly, a similar pattern of cortical deactivation in obstetric brachial plexus palsy may be the basis of our current findings.

There may be four explanations why automatic movements are impaired in obstetric brachial plexus palsy. The first concerns sensory deprivation: in children with obstetric brachial plexus palsy the connection between the brain and the affected arm is disrupted at birth, leading to muscle weakness

and diminished sensory feedback.[6] Recovery of the peripheral pathways, if present, takes weeks to many months,[15] during a period when automatic motor programs develop.[6] These programs may remain disrupted even if sensory feedback is repaired afterwards. The relevance of a critical window during motor development in obstetric brachial plexus palsy was previously suggested by Brown et al.[6] They supported their hypothesis by previous observations of poor functional recovery in visually deprived newborn kittens or human infants, or after sciatic nerve crushes in rabbit hind limbs.[6] Obstetric brachial plexus palsy likewise may concerns sensory deprivation during a critical period for the formation of automatic motor control. If so, the effects might be less severe than in the examples provided. It is possible that the degree to which movements become automated also depends on the severity of the lesion. In the present study that severity was limited because recovery had to be sufficient to allow abduction of at least 90 degrees and the central deficit may be explained by the initial afferent deficit. It is possible that a central deficit may play a larger role in obstetric brachial plexus palsy patients with less functional recovery.

The second explanation may be that automatic movement programs are formed later than normal in obstetric brachial plexus palsy, simply because the affected arm is not used often or well enough for movement automation to occur. Corresponding to the 'dual mode principle of motor skill learning', supported by experimental data,[9] tasks can become automatic when they are practiced often enough, resulting in performance that does not require direct full attentional control. If so, automatic movements in obstetric brachial plexus palsy might improve with practice and rehabilitation.

The third explanation holds that the observed decreased use of the affected arm in obstetric brachial plexus palsy during walking represents a compensatory strategy to counter any balance disrupting effects of abnormal arm movements of the affected arm. However, we feel this is unlikely for several reasons: arm swinging is useful as it decreases energy consumption,[16,17] increases stability,[18] and contributes to balance recovery after a perturbation.[19] In cerebral palsy an increased swing of the unaffected arm compensates for the increased angular momentum produced by the legs.[20]

A fourth possible explanation for decreased automatic arm movement might be that the movements are related to the mass of the arm, which is reduced in obstetric brachial plexus palsy. Again, we feel this is unlikely based on the following: adding mass to one arm has been shown to decrease movement amplitude in that arm and increase the amplitude of movements of the other arm,[20] suggesting that the opposite should hold if the mass of an arm is abnormally low. So, if the low mass of the arm would cause abnormal automatic movements, increased movements would be expected rather than decreased ones. This reasoning implies that the decreased movements impair balance causing further functional impairment.

A potential limitation of this study is that most children had undergone surgery. The lack of a difference in abduction rates between those who had and had not undergone surgery suggests that this factor is not critical. Another limitation is that three tasks appeared not specific enough to evoke abduction in healthy arms in the majority of participants. This may explain the lack of a significant difference for these tasks. The task that showed a clear difference between the affected and unaffected arms consisted of walking on the heels with small steps without additional cognitive tasks. This task may simply represent a more difficult balance act than the other ones. Alternately, our attempts to increase balance difficulty by adding cognitive tasks may not have done so as well as intended: according to the multiple resources theory, the cognitive tasks did not interfere enough with the motor acts because the motor and cognitive tasks share few resources and so cause little interference with one another.[21]

In summary, differences in automatic movements between the affected and unaffected side are present in obstetric brachial plexus palsy. These are likely caused by incomplete central program development and may contribute to incomplete arm function recovery following obstetric brachial plexus palsy.

Acknowledgements

We thank all members of the Leiden University Medical Centre brachial plexus team.

Author contribution

GVA, MJAM, SMB and JGvD substantially contributed to conception or design. GVA, MJAM, SMB, EWvZ and JGvD contributed to acquisition, analysis, or interpretation of data. GVA, MJAM and JGvD drafted the manuscript. All authors were involved in critical revision of the manuscript for important intellectual content.

Declaration of conflicting interests

The Authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

The study was judged by the institutional medical ethics committee Leiden University Medical Centre to be innocuous and to not warrant a full review, conforming to Dutch law.

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Figure 1. Task example
Example of maximal abduction range during task (b), walking on the heels; the right arm is the affected one.

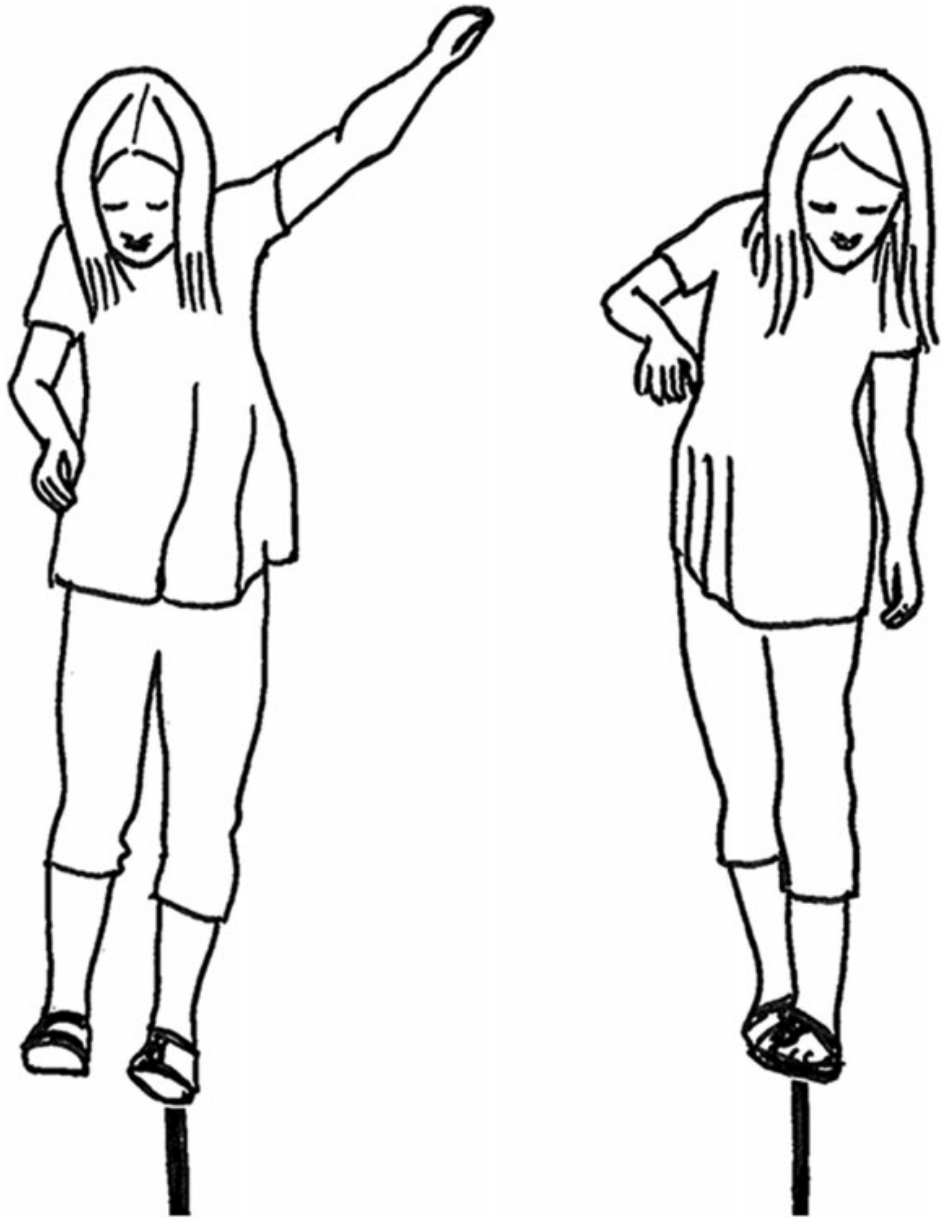

Table 1. Demographic and surgical details

Subject	Age (y)	Gender	Affected arm	Lesion	Treatment
1	5	M	L	C5 – C7	No surgery
2	4	M	R	C5 – C6	Nerve grafting ST
3	4	M	R	C5 – Th1	Transfer accessory nerve - SSN Nerve grafting C5 - PDST, C6 - ADST, anterior filaments C8, Th1
4	5	F	L	C5 – C6	Transfer medial pectoral nerve-musculocutaneous nerve
5	7	F	L	C5 – C7	Nerve grafting C5 - SSN, C5 – PDST, C6 - ADST
6	7	M	L	C5 – C7	Nerve grafting C5 - C5 and intraplexal transfer C5 - ventral filaments
7	7	M	R	C5 – C7	Nerve grafting C5 - SSN, PDST, C6 - PDST, ADST
8	8	F	R	C5 – C6	Nerve grafting C5 - SSN, C5 - PDST, C6 – ADST, neurolysis C7 - MT
9	3	M	L	C5 – Th1	Nerve grafting C5 - PDST, C6 - ADST, C7 - (PD)MT, C8, Th1, accessory nerve - SSN
10	9	F	R	C5 – C7	Nerve grafting C5 - PDST, C5 - ADST Transfer accessory nerve - SSN
11	6	M	L	C5 – C7	Nerve grafting C6 - ADST
12	7	F	R	C5 – C8	Nerve grafting C5 - motor fascicle C7, C6 - ADST, C6 - PDST
13	4	M	R	C5 – C8	Nerve grafting C5 - SSN, C5 - PDST, C6 - ADST
14	3	M	L	C5 – C7	Nerve grafting C5 - SSN, C5 - PDST, C6 - ADST
15	4	M	R	C5 – C6	No surgery
16	3	M	L	C5 – C8	Nerve grafting C5 - SSN, C5/C6 - PDST, C6 - ADST
17	5	M	L	C5 – C7	No surgery
18	4	F	L	C5 – C6	Intraplexal transfer medial pectoral nerve - musculocutaneus nerve
19	8	F	R	C5 – C7	No surgery

y: years, M: male, F: female, R: right, L: left, SSN: suprascapular nerve, ST: superior trunk, ADST: anterior division of the superior trunk, PDST: posterior division of the superior trunk, (PD)MT: (posterior division of the) middle trunk

Table 2. Arm scores for the four tasks
The tasks were: (a) Walking heel-to-toe in a straight line; (b) Walking on the heels with small steps; (c) Walking heel-to-toe with eyes closed; (d) The same as the third task but with a cognitive task, suitable for the child's age. (For the healthy arm numbers may be less than 19 because some children did not perform all tasks) * $p<0.01$

Task (a)		**Arm affected**		
		No	*Yes*	
Abduction >90°	*No*	16	17	33
	Yes	3	2	5
		19	19	38

Task (b)*		**Arm affected**		
		No	*Yes*	
Abduction >90°	*No*	7	14	21
	Yes	12	5	17
		19	19	38

Task (c)		**Arm affected**		
		No	*Yes*	
Abduction >90°	*No*	13	16	29
	Yes	5	2	7
		18	18	36

Task (d)		**Arm affected**		
		No	*Yes*	
Abduction >90°	*No*	11	15	26
	Yes	6	2	8
		17	17	34

Chapter 8

Increased brain activation during motor imagery suggests central abnormality in Neonatal Brachial Plexus Palsy

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Neurosci Res. 2017 Oct, 123:19-26.

Abstract

Neonatal Brachial Plexus Palsy (NBPP) may lead to permanent impairment of arm function. As NBPP occurs when central motor programs develop, these may be ill-formed. We studied elbow flexion and motor imagery with fMRI to search for abnormal motor programming. We compared the cortical activity of adults with conservatively treated NBPP to that of healthy individuals stratified for hand dominance, using fMRI BOLD tasks of elbow flexion and motor imagery of flexion. Additionally, resting-state networks and regional gray matter volume were studied. Sixteen adult NBPP patients (seven men; median age 29 years) and sixteen healthy subjects (seven men, median age 27 years) participated. Cortical activation was significantly higher in patients during flexion imagery compared to healthy individuals and it increased with lesion extent and muscle weakness. The contralateral and ipsilateral premotor cortex, and the contralateral motor cortex showed stronger activity during imagined flexion in the right-handed NBPP subjects compared to healthy individuals. Activity patterns during actual flexion did not differ between groups. No differences in resting-state network connectivity or gray matter amount were found between the groups. NBPP affected imagined but not actual elbow flexion, suggesting an impairment of motor planning which would indicate abnormal motor programming in NBPP.

Introduction

Neonatal Brachial Plexus Palsy (NBPP) is a closed peripheral nerve traction injury that arises most commonly, but not exclusively, from shoulder dystocia during a difficult birth. Typically, shoulder and elbow flexion is impaired because of damage to the C5 and C6 spinal nerves. In more severe cases, extension and hand function are impaired as well.[1] While mild nerve damage does not exclude a full recovery, severe damage can cause permanent impairment of arm function.[2-6] A systematic literature search showed a residual deficit in 20 to 30% of cases.[7,8]

Traction causes axonal loss of continuity with the end organ (e.g. the biceps muscle) followed by degeneration of the axon distal to the injury site (Wallerian degeneration).[9,10]. Even in severe nerve damage due to NBPP, there is usually no clear gap between the proximal and distal nerve ends. This is in contrast to adults with traumatic nerve lesions, and the difference is probably due to the smaller absolute size in infants. Instead, a 'neuroma-in-continuity' of the superior trunk is formed containing axons of which some cross the lesion site and enter empty basal laminal tubes.[11,12] Functional recovery takes place over months to years and is hampered by several factors. The number of axons that successfully cross the lesion site is reduced with increasing severity of the nerve lesion.[11] In addition, axons may connect with end organs differing from the original ones due to misrouting.[1] This may disturb proprioceptive feedback[13], as well as motor firing patterns.[1] Absent or inappropriate afferent input[13,14] occurring at the age at which central motor programs are developed may inhibit the development of these programs.[1,15,16] Neurophysiological evidence[17] and clinical observations [18] indicate that these programs are ill-formed in NBPP. The nature of central motor impairment is still unclear, however.

To assess central motor programming we investigated motor execution and imagery tasks with fMRI in conservatively treated NBPP adults. An expansion of motor cortical representation occurs not only at the onset of learning a new motor skill in healthy subjects, but also in patients following upper extremity injury and reconstruction.[19] While a skill is being mastered, the degree of cortical representation and excitability decrease again.[19] We used motor execution tasks to assess whether a central motor impairment in NBPP can be

linked to a different motor cortical representation compared to controls. With increasing practice motor tasks become automatic and require less planning effort.[20] A decreased cortical activation has been found in the primary motor cortex contralateral to the attempted limb movement in paraplegics compared to healthy controls studied with motor imagery fMRI, which was attributed to an increased need for attention allocation.[21] Therefore, to assess whether an increased planning effort contributes to the central motor impairment in NBPP we used imagery tasks. Accordingly, we expect that in the NBPP adults actual task execution does not require more central effort compared to controls, corresponding with a normal to decreased cortical activation, irrespective of muscle weakness, however planning of the movement does.

Materials And Methods

Subjects

Sixteen adult NBPP patients and sixteen healthy subjects participated in the study. Patients were recruited from the Leiden University Medical Centre data base and were looked for nationwide with the help of the Dutch Erb's Palsy Association. The minimum inclusion age was 18 years. Healthy individuals were matched to patients for sex, age (± 5 years) and handedness. Patients with NBPP had not undergone nerve surgical reconstruction of the brachial plexus or secondary surgery to improve elbow function. Further exclusion criteria were the presence of other relevant neurological diseases and MRI exclusion criteria such as claustrophobia and implanted devices. The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre. All participants provided written informed consent.

Lesion Extent

Arm function of all patients was examined by an experienced brachial plexus surgeon (MJAM). Individual muscles were graded according to the Medical Research Council scale;[22] active and passive range of motion was documented and the Mallet scale for shoulder function[23] was assessed. Subsequently, motor function was tested to assess the extent of the NBPP lesion and subdivided in four groups: group 1 concerned C5 and C6 damage, with impaired shoulder abduction, exorotation, and elbow flexion. Group 2 concerned C5, C6, and C7

damage, clinically as group 1 with additional weakness of elbow, wrist and finger extension. Group 3 had C5 to C8 damage, clinically as group 2 with absence of extension function additionally. Group 4 had C5 to Th1 damage, clinically as group 3 plus absent or minimal intrinsic hand muscle function.

Motor tasks

Motor execution tasks consisted of isometric biceps contraction, a task that even NBPP patients with a weak biceps muscle can perform; also, isometric contraction avoids MRI movement artefacts. Vacuum pillows were placed around both forearms to obtain immobilization of the forearm after air evacuation. The arm was further immobilized by a sandbag of 3.5 kilograms placed on top of the vacuum pillow. The forearms were positioned next to the body at a comfortable elbow flexion angle between 10° and 30° using cushions. The arm was supinated as far as possible without causing discomfort. Finally, a strap was placed over the middle of each forearm to prevent flexion. Subjects were instructed to lie still during the experiment. For the motor imagery task, subjects had to imagine rhythmically pushing their forearm against the strap at approximately 1 Hz. The flexion task consisted of actual isometric biceps contraction. Both tasks were performed for left and right arms separately.

Stimuli were presented using a computer running the Matlab-based PsychToolbox (The Mathworks Inc.)[24] and were projected onto a screen visible through a mirror above the eyes of the subject. To indicate movement execution, green letters were used; for the imagery condition, red letters were used. The letters 'L' and 'R' indicated that the task should be performed using the left and right arm, respectively. Thirty second task blocks were presented in a random order intermixed with 30 second baseline blocks where a fixation cross was presented. To minimize effects of muscle fatigue, the sequence of blocks was split into three 10 minute sessions. Subjects were given rest between sessions until they indicated they were ready to continue. In all subjects this was within three minutes. The tasks were performed on two occasions: once to obtain electromyographic data and once to obtain fMRI data.

Electromyography

Subjects were trained with EMG feedback to perform the tasks before scanning. To do so, subjects were in supine position with their arms immobilized as

explained above. Surface self-adhesive EMG electrodes were placed on both the left and right biceps and triceps muscle belly and 0.5 cm distally.[25] Subjects observed their EMG activity during the tasks on a screen. They were instructed to aim for activity in the agonist and reduce activity in the antagonist as much as possible during execution and not to activate both agonist and antagonist during imagery flexion. Responses were acquired using a band pass filter of 20 Hz - 2 kHz and recorded over a minimum of 100 s for each of the total four conditions (motor execution/motor imagery, right/left arm) using a Medelec Synergy EMG apparatus (Oxford Instruments, Abingdon, Oxfordshire, UK). Biceps EMG activity was not measured during MRI scanning.

fMRI data acquisition

Four brain scans were acquired: a T1-weighted anatomical scan, a high-resolution T2*-weighted scan, and T2*-weighted task-related and resting-state BOLD fMRI. Data were acquired at the Leiden University Medical Centre with a 3 Tesla Achieva scanner (Philips Medical Systems, Best, The Netherlands). An eight-channel head coil was used for all data collection.

T1-weighted images were acquired with the following scan parameters: 140 transverse slices, voxel size = 1.17x1.17x1.2 mm, FOV = 224×177x168 mm, 192x152 matrix, flip angle = 8°, TR (repetition time)/TE (echo time) = 9.7/4.6 ms. T2*-weighted images were acquired with the following parameters: 84 transverse slices, voxel size = 1.96x2.01x2.00 mm, no slice gap, FOV = 220x220x168 mm, 112x109 matrix. For the task fMRI the whole brain was covered by acquiring 38 transverse slices, voxel size = 2.75x2.75x2.75 mm, 0.275 mm slice gap, 80x79 matrix, flip angle = 80°, TR/TE = 2200/30 ms. The resting-state fMRI parameters were equal to the task fMRI except for the slice gap which was 0.272 mm.

fMRI data preprocessing

fMRI data was preprocessed with FSL version 4.1.7 (Analysis Group, FMRIB, Oxford, UK).[26] The following processing steps were applied: motion correction[27], removal of non-brain tissue,[28] spatial smoothing using a Gaussian kernel of 8 mm full width at half maximum, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with a 128 s cut-off). To register fMRI scans to standard space, functional scans of an

individual were registered to the corresponding high-resolution T2*-weighted images, which were registered to the T1-weighted images, followed by registration to MNI-152 standard space (T1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) images.[27,29] Preprocessed MRI data of patients with an affected left arm were mirrored with respect to the midsagittal plane.[30] In this way the hemisphere corresponding with the affected arm was on the same (left) side for all patients and the right hemisphere corresponded with the unaffected arm. This was also done for the corresponding matched controls.

Data analysis – task fMRI

Data analysis was carried out in three steps: 1) calculating the mean cortical response per condition per person per task block, 2) combining the three task blocks, and 3) comparing NBPP patients and controls. This is described in detail below. For every subject, four regressors, one for each of the conditions, were modelled as square-wave functions with duration equal to that of the task block. The haemodynamic responses generated by each task condition were modelled by convolving these square-wave functions with a canonical haemodynamic response function. Each subject's mean response for the three sessions was estimated with a second-level analysis (FSL FEAT version 5.98 Multi-level analysis[31]) resulting in one contrast per person proceeding in the third-level analysis. Contrasts representing the difference between each task condition and baseline were calculated and compared between NBPP patients and healthy individuals with a mixed effects third-level analysis using FSL FEAT version 5.98.[31] Additional to whole brain analysis, masking allowed to define the brain regions where cortical activity was expected and increased during the tasks. Masks were binary representations of the following regions of interest: 1) left and right motor cortex (area 4a and 4p) together[32], 2) left and right premotor cortex (area 6) together[33], which were selected from the Juelich Histological Atlas[34] in FSL. Age, extent of the brachial plexus lesion and biceps strength were normalized and added as covariates in the statistical group model. When clinical notes described biceps strength as MRC scale 5$^-$, this was noted as 4.75. The cluster threshold was set at z=2.3 and the cluster corrected significance threshold at p=0.05. As arm dominance may change due to NBPP[35], results were stratified according to the subjects' dominant side. Hand dominance was reported by the participants and corroborated

by observing with which hand they wrote. The 10 left-handed patients were compared at group level with the six healthy left-handed individuals and the six right-handed patients were compared with the remaining 10 healthy right-handed individuals. An additional comparison was performed between the dominant and non-dominant hemispheres in healthy individuals to ascertain the presence of activation differences due to arm dominance.

Data analysis – EMG

To exclude learning effects the first 50 seconds of the EMG signal for each of the four conditions (motor imagery/flexion, right/left arm) were excluded from analysis. The EMG signal was then rectified and the sum of values between 50 and 100 s of the recording was calculated. Differences in muscle activation between motor imagery and motor execution, and between the right and left arm, were tested with the non-parametric dependent samples Wilcoxon's test. Differences between healthy individuals and patients were tested with the Mann-Whitney *U* test. SPSS Statistics 20.0 (Armonk, NY: IBM Corp.) was used for statistical testing with a significance threshold of 0.05.

Data analysis – resting-state

Standard group independent component analysis (ICA) was carried out using probabilistic ICA[36] as implemented in FSL MELODIC version 3.10. Default group ICA processing steps were applied to the individual preprocessed and normalized data sets: masking out non-brain voxels, voxel-wise mean centering of the data, and normalization of the voxel-wise variance based on all data sets. Subsequently, data sets from the healthy individuals were concatenated in time to create a single 4D data set, which was then projected into a 25-dimensional subspace using principal component analysis. Next, the data set was decomposed into 25 sets of independent vectors which describe signal variation across the temporal (time courses) and spatial (maps) domains by optimizing for non-Gaussian spatial source distributions using the FastICA algorithm.[37] The values of the resulting estimated component maps were divided by the standard deviation of the residual noise and set at a probability threshold of $p>0.5$ by fitting a Gaussian/Gamma mixture model to the histogram of intensity values.[36]

Subject-specific statistical maps were created to test for differences between NBPP and healthy groups in the identified components with a dual regression

procedure.[38] In short, multiple linear regression of the z-threshold group ICA maps against the preprocessed individual 4D resampled data sets yielded a subject specific time course for each component separately. Next, multiple linear regression of these time courses was carried out against the pre-processed individual 4D data sets in the standard space resolution of 2 mm. This resulted in subject specific z-maps for each of the 25 components.

Statistical difference was assessed non-parametrically using the FSL's randomise tool version 2.8 with 5000 permutations.[39] Besides modeling regressors for each of the two groups, additional regressors describing age, lesion extent and biceps strength were added, corresponding to the task fMRI model. For each resting-state network, the resulting statistical maps were threshold-free cluster enhancement corrected for family-wise errors using a threshold of $p\leq0.05$[40] and controlled for the local false discovery rate[38] at a threshold of $q\leq0.05$.

Data analysis – gray matter regional volumes

Voxel-based morphometry analysis[41,42] was run on the acquired T1-weighted data sets, carried out with FSL tools[31]. First, structural images were brain-extracted and gray matter-segmented before being registered to the MNI 152 standard space using non-linear registration. The resulting images were flipped and averaged along the x-axis to create a left-right symmetric, study-specific gray matter template. Second, all native gray matter images were non-linearly registered to this study-specific template and modulated to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, statistical difference was assessed using the FSL's randomise tool version 2.8 as described for resting-state data analysis except for false discovery rate correction.

Results

Characteristics of the groups are shown in Table 1. Sixteen adult NBPP patients (seven men, median (25^{th}-75^{th} percentile) age 29 (22-41) years, six right-handed) and sixteen healthy subjects (seven men, median (25^{th}-75^{th} percentile) age 27 (23-41) years, 10 right-handed) participated in the study. There were four patients in group 1, nine in group 2, two in group 3, and one in group 4.

Task fMRI

During the motor imagery flexion task cortical activation was significantly (z>2.3, p<0.05) increased in NBPP patients compared to healthy individuals. Comparison of the whole brain showed increased cortical activation during motor imagery flexion of the affected arm in right- (Fig. 1a) and left-handed (Fig. 1b) NBPP patients compared to healthy individuals. Increasing lesion extent and decreasing biceps muscle force were associated with a higher cortical activation in these groups. During the imagery flexion of the healthy arm, there was increased activation only in right-handed NBPP patients, (Fig. 1c)with a similar effect of lesion extent and biceps muscle force on cortical activation as for the affected arm, as well as decreasing age. Region of interest masks, the areas where cortical activation may be expected in healthy individuals, showed that during motor imagery flexion of the affected arm the following regions were more activated in the right-handed NBPP subjects than in healthy individuals: the contralateral premotor cortex, ipsilateral premotor cortex and the contralateral motor cortex. During motor imagery flexion of the healthy arm the contralateral premotor cortex and ipsilateral premotor cortex were more activated in the right-handed NBPP subjects than in healthy individuals. No differences were found between the two groups during execution of the flexion task. There were no significant differences in cortical activation within healthy individuals between the dominant and the non-dominant hemispheres.

EMG, resting-state and gray matter

Median (25^{th}-75^{th} percentile) biceps activation (sum of samples) is shown in Table 2 with the corresponding p-values. During motor execution triceps co-contraction was higher in patients compared to controls only in the affected arm (patients 105(77-250)mV, controls 63(27-79)mV, p<0.001) and not in the unaffected one (patients 50(35-89)mV, controls 60(31-144)mV, p=0.918), corresponding with the biceps activity findings for that task: affected arm (p=0.034), unaffected one (p=0.759). There were no significant differences in the resting-state networks or the amount of gray matter between NBPP and healthy individuals.

Discussion

The main finding of this study is that NBPP patients showed more cortical activity than healthy individuals during motor imagery flexion of the affected arm. The increase was found in cortical premotor areas of both hemispheres, as well as in contralateral motor areas in right-handed NBPP patients. The findings were not restricted to the affected arm, but also account for the healthy arm, where cortical premotor areas were also more activated in right-handed NBPP patients than in controls. Additionally, higher cortical activation was associated with an increasing lesion extent and a decreasing biceps muscle force. The motor imagery findings contrast with results of the actual flexion task, during which no increase of cortical activation in NBPP patients was seen.

The EMG feedback training was included to ensure that the motor imagery tasks were executed as intended namely, a higher biceps EMG activation during motor execution than during the imagery motor task. The lack of EMG differences between the healthy side in patients and healthy individuals both during motor imagery and execution, show that patients were able to perform the different tasks appropriately. There was one unexpected EMG difference though: a higher biceps activity was recorded in the patients' affected arm during motor execution suggesting that more muscle effort was necessary to achieve the same task with the affected arm than with the healthy one. The increased triceps co-contraction during motor execution which was only observed in the affected arm in NBPP patients may be due to misrouting.

We did not find significant differences in the resting-state networks between NBPP and healthy individuals. Resting-state fMRI may reflect ongoing functional communication between brain regions during rest,[43] e.g. long term motor training may significantly increase resting-state activity within primary motor regions[44]. Functional connections of resting-state networks tend to be strongly related to structural white matter connections.[43] Accordingly, our findings may not be explained by differences in connectivity. In addition, we did not find any differences in gray matter volume.

Motor imagery

To put our findings into perspective, we will compare NBPP with some

other developmental and acquired neurological disorders. During motor imagery the representation of a given motor act is internally rehearsed within working memory without any overt motor output.[45] It comprises two parts: a representation of the body, and a representation of the goal of the action.[45] Several factors are probably necessary to form a body representation during early development; these are: proprioceptive feedback from the affected as well as the healthy limb, and visual feedback, which can be obtained from observing others (mirror neurons)[46] or possibly the affected limb in NBPP. The influence of all three factors may be enhanced by increased use or diminished by disuse of the limb.[47]

In spinal cord lesions, enhanced activation and recruitment of additional cortical regions has been reported[21], findings reminiscent of the present study. A principal difference with NBPP is however that traumatic spinal cord lesions are usually acquired later in life when motor program development is complete. A higher contralateral cortical activation was found in amputees during imagined phantom hand movements compared to healthy subjects,[48] but again the lesion is acquired later in life. A reduced somatosensory cortical representation area has been reported in patients with limb aplasia or dysmelia[47]. Contralateral cortical activation was found varying from reduced to equal to that of the healthy side during a motor task, and no activation was found during an imagery motor task.[49] However, the applicability of these findings is uncertain: the study concerned only two patients and activation was absent with the imagery task protocol in two healthy subjects.

How can the increased cortical activity during motor imagery in NBPP be interpreted?

Motor imagery has been linked to action planning.[45] Our findings suggest that in NBPP patients an increased central effort[21] is required for action planning, which increases with lesion extent and muscle weakness. When a motor task is learned, the task initially requires full attentional control. With practice the tasks become automatic and require less central effort.[20] With this in mind, the motor imagery in NBPP resembles a newly learned task, requiring much attention. In paraplegics, a similar increased need for attention allocation was suggested as well.[21]

There were no significant differences in cortical activation within healthy individuals between the dominant and non-dominant hemispheres, suggesting that effects in the patient group cannot be attributed to normal variations due to hand dominance. Yang and colleagues found that in children with right NBPP only 17% preferred to use their right upper limb for overall movements in contrast to 90% in the general population and 93% in children with left NBPP.[35] The significant differences we found only in the right-handed patients may suggest that switching hand dominance may represent a strategy to reduce central effort.

The role of ipsilateral activation

In addition to the contralateral cortical activation, we also found significantly stronger ipsilateral cortical activation in the premotor areas during motor imagery flexion of the affected arm in NBPP patients compared to healthy individuals. Increased ipsilateral cortical activation has also been found in arm amputees who lost the arm in childhood.[50] There are several explanations for our findings, in line with other studies: first, ipsilateral activation is unintentional, representing increased use of the healthy arm to increase stability and compensate for the loss of affected arm function.[51] Second, these findings might reflect central fatigue, which may cause bilateral activation: during repetitive unilateral limb movement the muscles of the contralateral limb may show increased EMG-activity, and more so as the movement requires greater effort.[51] These findings fit with the concept that motor imagery in NBPP requires much effort. Finally, a third explanation holds that pre-existing cortical connections with the ipsilateral hemisphere are either disinhibited or strengthened.[49,50,52] A similar explanation has been proposed for mirror movements observed in children with cerebral palsy.[53]

The role of the healthy arm

Besides increased cortical activity during imagery flexion of the affected arm in patients, we also found such an effect during imagery flexion of the healthy arm in the right-handed patients. Performing a unilateral task can be associated with bilateral cortical activity, applying to both sensory[54] and motor[55] activation. In view of our findings, it is plausible that the healthy arm compensating for the loss of function of the affected arm led to the increased cortical activation. In amputees, movements of the intact hand also showed increased cortical activity

in the former sensorimotor hand territory of the affected hand.[56] Accordingly, inclusion of intact hand engagement in rehabilitation has been suggested.[56] This view corresponds with the framework of neural representation formation where formation depends on sensory input from the healthy limb.[46]

Motor execution

We did not find any cortical activation differences between NBPP patients and healthy individuals during the actual flexion task. Apparently, in the adult NBPP patients we have studied, central pathways involved in elbow flexion recovered enough to result in a normal degree of activation. As said, cortical representation expands at the onset of learning a new motor skill and decreases when the skill is mastered.[19] Impaired central motor programming in children with NBPP is supported by neurophysiological evidence[17] and clinical observations[18]. Our results suggest three possibilities: firstly, in children with NBPP motor cortical representation is expanded but decreases in time due to motor learning. Secondly, because motor execution is essentially a combination of planning and actual execution, the increased cortical activity during planning masks decreased cortical activity during pure execution. A decreased contralateral cortical activation has been found in the primary motor cortex during attempted movement in paraplegics compared to healthy controls.[21] Thirdly, cortical activity in patients is increased to the level of healthy subjects due to higher biceps muscle activity in patients, suggested by the training session EMG recording.

Limitations and consequences

We did not measure biceps EMG activity during MRI scanning, so it remains possible that the tasks were carried out differently during scanning than intended. However, we feel that having an EMG-guided practice session was valuable as a quality control distinguishing motor execution and imagery. Another limitation might be that the NBPP population was heterogeneous in severity of the lesion, which may have affected our findings. However, in all patients at least a C5, C6 lesion was involved, affecting the biceps muscle as the main agonist of flexion, the focus of this study.

In conclusion, motor imagery of elbow flexion in NBPP involves increased cortical activation. The increased activity points to an increased need for task

attention, which in turn is probably caused by an interplay of motor and sensory components and the time of the lesion. It is unknown to what extent this central phenomenon affects daily functioning, and also to which extent it influences the ability to train arm function. Future studies should elucidate the role of the central nervous system in NBPP in more detail, focusing on a possibly shifting role over time. Effects of training focusing not only on the affected but also the healthy arm also deserve further study.

Acknowledgements

We thank Z.V. Angelov for his assistance with Matlab, B. Klaassens with data processing and F. Erthal for pointing out some valuable references to us.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Table 1. Demographic details of the subjects with Neonatal Brachial Plexus Palsy (NBPP) (P1 – P17) and the healthy individuals (C1 – C17). Lesion extent groups: 1. C5 and C6 roots, 2. C5, C6 and C7, 3. C5 – C8 and 4. C5 – Th1. Degree of recovery assessed using MRC scale for muscle strength (0 = no movement observed, 5 = normal muscle contraction).[22]

Subject	Age (y)	Sex	MRC biceps	Affected arm	Lesion extent group	Dominant hand	Subject	Age (y)	Sex	Dominant hand
P1	21	F	5-	L	2	R	C1	23	F	R
P2	21	F	-	R	2	L	C2	23	F	L
P3	29	F	4	R	1	R	C3	29	M	R
P4	23	F	4	R	1	L	C4	23	F	R
P5	35	F	5-	R	2	L	C5	39	F	L
P6	43	F	5-	R	3	L	C6	41	M	R
P7	29	M	5	R	4	L	C7	27	M	R
P8	21	M	5	L	1	R	C8	23	M	R
P9	53	F	4	R	2	L	C9	50	F	L
P10	30	F	3	R	2	R	C10	30	F	R
P11	24	M	5-	R	2	L	C11	24	M	L
P12	28	M	3	R	1	L	C12	26	F	L
P13	64	F	5	L	2	R	C13	50	F	R
P14	64	M	3	R	2	L	C14	50	F	L
P15	22	M	5-	L	3	R	C15	24	M	R
P16	24	M	4	R	2	L	C16	25	M	R

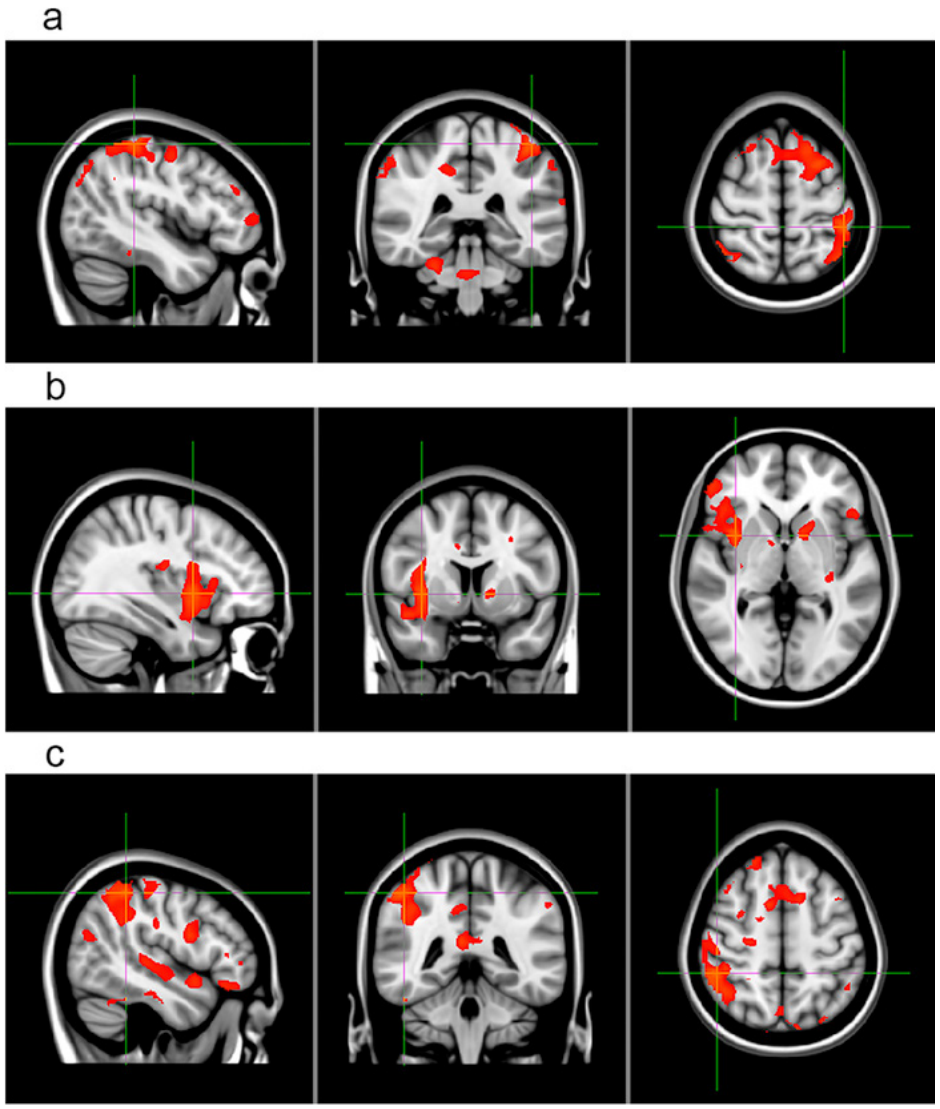
L – left, R – right, M – male, F- female, y – years

Table 2. Median (25^{th}-75^{th} percentile) biceps activation (sum of samples, in mV) during the EMG training session.

		NBPP patients	Healthy individuals	*p*-value
Imagery	Affected arm	27 (20-44)	30 (11-38)	0.822
	Unaffected arm	22 (9-43)	10 (7-79)	0.142
Execution	Affected arm	153 (102-346)	105 (46-148)	0.034
	Unaffected arm	115 (62-160)	102 (57-143)	0.759
p-value Affected versus unaffected arm	Imagery	0.278	0.074	
	Execution	0.003	0.778	
p-value Imagery versus execution	Affected arm	<0.001	0.001	
	Unaffected arm	0.001	0.001	

NBPP - Neonatal Brachial Plexus Palsy

Figure 1. Increased cortical activation during the imagery flexion of a. the affected arm in right-handed Neonatal Brachial Plexus Palsy (NBPP) patients, b. the affected arm in left-handed NBPP patients, and c. the healthy arm in right-handed NBPP patients compared to healthy individuals. The red area shows z>2.3, p<0.05, corrected. The crossing of the green lines indicates the maximal voxel: a. x=134, y=90, z=132; b. x=54, y=132, z=72; c. x=42, y=84, z=124 in MNI-coordinates.



Chapter 9

Discussion and Summary

The aim of this thesis was to gain a better understanding of sensory and motor function, misrouting and central motor programme development in patients with obstetric brachial plexus lesion (OBPL), focusing mostly on conservatively treated adults. In this Chapter we discuss our findings on these topics and some venues for future research.

Sensory function

In *Chapter 2* we found that sensory hand function was abnormal in adults with conservatively treated OBPL, based on two tests, the Semmes-Weinstein monofilament test and the two-point discrimination test, and on a comparison with healthy control subjects. Scores for object recognition and locognosia did not differ between patients and controls.

We reviewed earlier studies on sensory function in OBPL. Sensory function in OBPL had been reported to be excellent, but only five of eight sources presented original data. Results might depend on whether surgery had been undertaken, but in four papers the operated cases represented only a small fraction of the total number of cases and in the fifth paper cases without surgery could be identified. The study populations were largely comparable to ours, though the conclusion generally differed from ours: most authors reported that sensory function had recovered excellently; only one author expressed caution about this interpretation. We suggested that the apparent discrepancy between ours and earlier conclusions originated in a difference in interpretation: most studies stressed the existence of normal sensory functions, whereas we stressed that abnormal functions were in the majority. There is an obvious difference with plexus lesions acquired later in life: in adults sensory dysfunction follows well-established areas of innervation, with profound differences between normal and abnormal areas. In OBPL, in contrast, there is a degree of sensation in all innervation areas, but that does not mean that sensory function is normal in those areas. We suggested that the absence of major 'gaps' in sensation in OBPL may be explained by the neuroma in continuity in infants, that allows reinnervation to take place, much more readily than happens in a true nerve rupture in adults. As such, the sensory and motor findings show an interesting parallel in OBPL: there is a degree of function in all myotomes and dermatomes, but there also is a functional abnormality with a unique pattern not occurring in this way in adults.

In *Chapter 3* we responded to a recent study on sensory function in conservatively treated OBPL patients, one that largely confirmed our results.

Motor function and misrouting

Motor function and misrouting extent

The main findings of the studies in *Chapter 4* revealed a pattern that does not simply fit a peripheral nerve lesion: participants with conservatively treated OBPL displayed considerable functional impairment and impaired ranges of joint movement. The expected pattern for nerve lesions would be that these impairments are the result of profound muscle weakness, and yet this was absent.

Concerning ranges of motion, shoulder abduction followed by elbow extension were most often impaired, while that of elbow flexion was normal. Muscle strength was only slightly impaired for the biceps muscle, and deltoid and triceps muscle strength was normal, while the Mallet scores, assessing function, showed a profound impairment.

The abnormal range of motion could therefore not be explained through muscle weakness, as weakness was mostly absent. Another mechanism must therefore have interfered with motor function, most probably cocontraction. This is where our misrouting studies came in.

Motor misrouting was most often found after stimulation of the biceps, deltoid, and brachioradialis muscles, innervated through the C5 and C6 roots. The high rate of misrouting in patients was not due to measurement error, because apparent misrouted responses were encountered in only four out of 1440 possible instances in controls. We attribute the abundance of misrouting in OBPL to the neuroma in continuity, allowing axons, split or not, to grow into any possible pathway, including an incorrect one, causing unintentional muscle cocontraction.

Unfortunately we could not establish an association between the degrees of functional impairment and of misrouting. We suggested several explanations for this: first, statistical significance was not obtained, perhaps because of limited variable variability, the Bonferroni correction and limited group size.

More importantly, any functional impairment due to misrouting is likely to depend on the quantitative rather than the qualitative degree of misrouting, but we could only assess the latter aspect in this chapter.

Misrouting quantified

We attempted to quantify the degree of misrouting with electromyography (EMG) in *Chapter 5* using novel approaches to overcome two problems: the first is costimulation in which electrical stimulation aimed to activate one muscle unintentionally also activates its antagonist; the second is coregistration, in which surface electrodes not only record the activity of an intended muscle, but also that of unintended muscles, such as an antagonist. We designed novel techniques to disentangle these problems.

We found no differences in the degree of cocontraction between OBPL patients and healthy subjects for either the triceps or deltoid muscles. This is odd, as we reported in *Chapter 4* that misrouting was qualitatively present in the triceps in nine out of 17 patients and in the deltoid in seven out of 17 patients. The apparent discrepancy with the findings in *Chapter 5* can be explained in several ways. The number of misrouted axons may in fact have been low; a central contribution cannot be excluded. We may also have failed to suppress the effects of costimulation and coregistration sufficiently despite of our best efforts.

In *Chapter 6* we aimed to quantify cocontraction with short range stiffness (SRS) at the elbow joint. We found that elbow stiffness was significantly higher in OBPL patients (median 250Nm/rad) than in control subjects (150Nm/rad) during voluntary levels of contraction.

SRS was significantly higher in OBPL patients than in control subjects but not for torque level zero, suggesting more cocontraction in patients but not due to joint deformities. The SRS measurement method is not hampered by the entangled factors that played a role in *Chapters 4* and *5*. SRS takes all muscles contributing to flexion and extension into account. Additional advantages of SRS compared to EMG to measure cocontraction in OBPL are that surface EMG preferentially samples superficial layers of a muscle and EMG requires a good signal-to-noise ratio which makes it less accurate for low muscle force levels.

Central motor programming

Children with OBPL

In *Chapter 7* we found that children with OBPL abducted the affected arm over 90 degrees less often than the unaffected arm in automated balance tasks even though they were able to abduct the affected arm over 90 degrees on request. The discrepancy can therefore not be explained by incomplete peripheral nerve regeneration or joint problems, suggesting a central deficit.

We discussed four explanations why automatic movements are impaired in OBPL. The first concerned sensory deprivation during a critical period for the formation of automatic motor control. The second was that automatic movement programmes are formed later than normal in OBPL because the affected arm is not used often or well enough for movement automation to occur. The third held that the decreased use of the affected arm represented compensation to counter disruptive effects of abnormal arm movements, but this seemed unlikely. Finally, the lower mass of the affected arm might play a role, but adding mass to one arm decreases movement of that arm, so a lowered mass should do the opposite.

Adults with OBPL

In *Chapter 8* we found that OBPL patients showed more cortical activity than healthy individuals during motor imagery flexion of the affected arm. The increase was found in cortical premotor areas of both hemispheres, as well as in contralateral motor areas in right-handed OBPL patients. Cortical premotor areas were also more activated in right-handed OBPL patients than in controls during motor imagery flexion of the unaffected arm. Additionally, higher cortical activation was associated with an increasing lesion extent and a decreasing biceps muscle force. In contrast, the actual flexion task showed no increase of cortical activation in OBPL patients.

Our findings suggest that OBPL patients require an increased central effort to plan actions. Motor imagery in OBPL appears to be carried out as a newly learned task requiring much attention. We discussed several explanations for the increased ipsilateral cortical activation in OBPL patients during imagery flexion of the affected arm, of which the most intriguing one may be that this represents pre-existing cortical connections with the ipsilateral hemisphere. Central pathways involved in actual elbow flexion apparently evolved enough to result in a normal degree of activation.

Future research

Sensory function

One possible future research topic in OBPL may concern its pathophysiology, for instance, in which dermatomes will sensory axons passing the neuroma end up? Through which nerves and roots do the regenerated fibres run? This may be possible to visualize in the future using viral vectors and MRI-tracking.[5,6]

Having established in *Chapter 2* that sensory function is abnormal in OBPL, sensory function rehabilitation should be explored in the future. There is some evidence for a positive effect on sensory function in adult peripheral nerve injuries using sensory re-education before and after evident reinnervation.[7,8] Protocols based on observation of touch, mirror visual feedback, audio-tactile substitution or temporary anaesthesia of parts of the ipsi- or contralateral arm may prove useful after adjustment for children. The rationale for such interventions is that they prevent the shrinkage of the original sensory cortical areas in the time frame prior to reinnervation. In this light, the use of brain-machine interfaces[9] may be useful as well. In order to accomplish better sensory reinnervation, operative techniques favouring sensory function[10] and possibly using viral vectors in the future[5], deserve further study.

Is there also sensory misrouting, and can this be demonstrated and quantified? We performed an unpublished pilot study attempting to capture this phenomenon, but the attempt failed as measuring sensory misrouting was too challenging using surface EMG methods. Sensory nerves commonly overlie muscles in which motor misrouting may be present: after sensory nerve stimulation it was unclear whether any resulting potentials originated from the sensory nerve, as intended, or from the muscle, unintended, or both. However, it may be possible to quantify afferent misrouting as we have done for motor misrouting in *Chapter 6* with the SRS method by choosing a different response time frame which coincides with the latency of the afferent signal.

Another avenue for future research concerns the consequences of sensory dysfunction for the quality of life in patients with OBPL.

Motor function and misrouting

The current treatment of cocontraction with the injection of botulinum toxin

in antagonist muscles is of necessity based on fairly subjective parameters. Besides, there is a necessity for a multicentre randomized controlled trial with botulinum toxin. A method to measure cocontraction such as SRS may guide treatment efforts and may be useful in such a trial. Future research should elucidate the applicability of the SRS method in children with OBPL. The computer interface used in *Chapter 6* with adjustments to resemble a video game may be particularly useful to raise motivation in children.

Central motor programming

To investigate how central motor programmes evolve over time in OBPL, a study can be performed with the balancing tasks used in *Chapter 7* in the group of conservatively treated adults with OBPL or the same children but at an older age. Another venue to study this would be to perform an fMRI study with the same tasks as in *Chapter 8* in children with OBPL. It would be of interest as well to study whether sensory cortical processing is complicated in OBPL in a manner similar to the one we found for motor tasks in *Chapter 8*.

The effects of rehabilitation on the central component of the functional motor deficit in OBPL should be studied. To elucidate the role of the healthy arm in movements of the affected arm a functional MRI study may be useful with EMG recordings during scanning of both arms with similar tasks as we used in *Chapter 8*. The role of the healthy arm in rehabilitation deserves further study as well. Motor function improvement of an agonist muscle persistent after botulinum toxin injection in the antagonist has been proposed to facilitate central motor learning[11] and a future functional MRI study may elucidate this.

Issues regarding nerve surgical intervention

There are various surgical techniques for OBPL, depending on the lesion.[12] The selection criteria for surgery and the optimal time of surgery are debated.[2,12] There is consensus that severe cases, including neurotmesis and root avulsions, should be operated. Establishing the severity of OBPL can be difficult for various reasons, including limitations of the neurological examination in infants and apparent discrepancies between electromyographic and clinical findings.[2,13] OBPL patients are usually operated between 3 and 9 months of age.[2,12] This time represents a compromise between waiting long enough to allow spontaneous recovery to occur on the one hand and, on the other hand,

the wish to perform surgery early after the injury.[2] Unfortunately, no thorough randomized controlled trial has been performed comparing surgery with conservative treatment in OBPL. Performing such a trial may be complicated by existing beliefs about the benefits of surgery; Strombeck and colleagues found that parents interfered with the randomization process.[14]

A major issue in comparing surgical and conservative treatment is selection bias: cases selected for surgery may be more severely afflicted than those who are not operated. A systematic literature search by Pondaag and colleagues regarding the natural history of OBPL showed that seven studies met a maximum of two of the predefined four evaluation criteria: study design, population, duration of follow-up, and end-stage assessment.[15] The two prospective studies, closest to what was defined as the 'ideal study', showed that functional deficits in the cases without brachial plexus reconstruction occurred at a rate of 20-30%, much higher than the previously assumed 10%.[15]

In summary, no randomized controlled trial comparing surgery and conservative treatment is available and one may not be feasible. However, this thesis may aid in a future systematic comparison with surgery despite the small sample size and heterogeneity of the group. We studied mainly conservatively treated adults with OBPL: cases from the time when brachial plexus surgery was either not possible or not widely used. These patients were recruited from records of the Rehabilitation department of Leiden University Medical Centre and the Dutch Erb's Palsy Association. This introduces a certain selection bias: the patients with residual deficit were more likely to participate in our studies. However, this selected group may be more comparable with patients that would be operated nowadays.

Despite the identification of risk factors for OBPL such as shoulder dystocia, operative vaginal delivery, macrosomia, gestational diabetes, and breech presentation,[16,17] OBPL still occurs, and there still is a group with residual deficit despite treatment options including surgery. Therefore, future research may also be focused on prevention and a new paradigm may be necessary. In shoulder dystocia the child's shoulder is impacted behind the mother's symphysis.[18] In other words, the shoulders are the broadest part of the child relative to the mothers pelvis. We performed electrical stimulation of the accessory nerve

in one healthy adult and measured a 20% reduction of the distance between the shoulders. Theoretically accessory nerve stimulation might therefore also reduce shoulder diameter in infants, which might conceivably be of value during birth, to prevent OBPL. Whether or not this is feasible will require various preliminary steps.

Summary and clinical importance

Summary

Sensory function is impaired in adults with conservatively treated OBPL. There is widespread motor misrouting together with motor functional impairment in conservatively treated OBPL, not explained by muscle weakness. There were no differences in the degree of cocontraction between OBPL patients and healthy subjects for either the triceps or deltoid muscles during supramaximal biceps stimulation. However, elbow stiffness was approximately 1.7 times higher in OBPL patients than in control subjects during voluntary levels of contraction, suggesting a significant effect of misrouting in the patients. In children with OBPL the deficit during automatic arm abduction was not observed during voluntary movements and therefore cannot be explained by a peripheral deficit, suggesting a central component. In adults OBPL affected imagined but not actual elbow flexion suggested an impairment of motor planning.

Clinical importance

The existence of sensory impairment in OBPL and its contribution to functional impairment need to be acknowledged, as sensation is of paramount importance in daily tasks. Our findings support the view that treatment may also have to be focused on sensation improvement, with the caveats that we did not study this directly and that sensory function can in fact be improved.

The current treatment of cocontraction, injection of botulinum toxin in antagonist muscles, is of necessity based on fairly subjective parameters.[19] Clinical assessment methods such as measuring the range of motion of a joint or measuring muscle strength cannot distinguish between weakness of one muscle and cocontraction of its antagonist.[19] A method to measure cocontraction such as SRS may guide treatment efforts.

If there is a delay rather than an irreversible nonconsolidation of central motor programmes in OBPL, the component of functional deficit due to central impairment might improve with rehabilitation.

A better understanding and future improvement of both peripheral and central factors in OBPL will hopefully lead to an improvement of the affected arm use in daily tasks, and in turn remove some of the obstructions patients with OBPL face in participation in society.

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Samenvatting (Summary In Dutch)

We hebben aangetoond dat de sensibiliteit gestoord is in volwassenen met conservatief behandelde obstetrisch plexus brachialis letsels (OPBL). Onze bevindingen werden bevestigd in conservatief behandelde oudere kinderen met OPBL. Er is uitgebreide motorische verkeerde zenuwuitgroei en dysfunctie in deze groep welke niet verklaard wordt door spierzwakte. Er was geen verschil in de mate van cocontractie tussen OPBL patiënten en gezonde controlepersonen voor de triceps of deltoideus tijdens supramaximale stimulatie van de biceps. Maar de mate van elleboogstijfheid was circa 1.7 keer hoger bij OPBL patiënten dan bij gezonde controlepersonen tijdens vrijwillige spieraanspanning, wat duidt op een significant effect van verkeerde zenuwuitgroei bij de patiënten. Bij kinderen met OPBL werd een bewegingsbeperking tijdens automatische schouderabductie geobserveerd, die echter niet aanwezig was tijdens vrijwillige schouderabductie. Dit verschil kan dan ook niet verklaard worden door perifere factoren en suggereert in plaats daarvan een centrale component. Bij volwassenen leidde OPBL tot een hogere corticale activiteit tijdens imaginaire flexie van de aangedane elleboog, maar niet tijdens daadwerkelijke flexie, wat wijst op gestoorde centrale programma's voor de voorbereiding van bewegingen.

List Of Publications

1 Anguelova GV, Malessy MJA, van Zwet EW, van Dijk JG. Cocontraction in adults with obstetric brachial plexus lesion. *Submitted.*

2 Anguelova GV, de Vlugt E, Vardy AN, van Zwet EW, van Dijk JG, Malessy, de Groot JH. Cocontraction measured with short-range stiffness was higher in obstetric brachial plexus lesions patients compared to healthy subjects. J Biomech. 2017 Oct, 63:192-6.

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4 Anguelova GV, Malessy MJ, van Dijk JG. Sensory Deficit in Conservatively Treated Neonatal Brachial Plexus Palsy Patients. Pediatr Neurol. 2016 Sep, 62:e1.

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Curriculum Vitae

On September 2, 1986, Galia Valentinova Anguelova was born in Sofia, Bulgaria. She moved to the Netherlands in 1999. In 2005 she obtained her secondary school diploma at Gymnasium Haganum in The Hague. Subsequently, she started her medical training at the Leiden University Medical Centre (LUMC). In her second year of medical training she participated in an exchange programme with Karolinska Institutet in Stockholm, Sweden and received an Erasmus fund for that period. In 2006 she was selected for the LUMC's 'Excellente studenten traject' and in 2010 the MD/PhD-track which comprised two years of full-time research funded by the LUMC, eventually leading to this PhD thesis. Beside the medical training and research, she obtained an engineering masters degree in Biomedical engineering (Biomechatronics) in 2012 at the Technical University in Delft. In 2014 she received her medical doctor's degree. She is currently working as a neurology resident in Haaglanden Medisch Centrum in the Hague.