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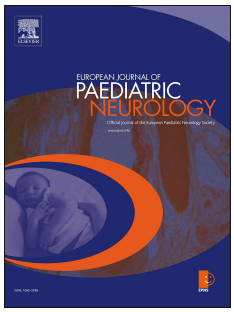

Understanding the relationship between brain and upper limb function in children with unilateral motor impairments: A multimodal approach

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PII: S1090-3798(17)30890-5

DOI: 10.1016/j.ejpn.2017.09.012

Reference: YEJPN 2312

To appear in: *European Journal of Paediatric Neurology*

Received Date: 20 April 2017

Revised Date: 18 September 2017

Accepted Date: 30 September 2017

Please cite this article as: Weinstein M, Green D, Rudisch J, Zielinski IM, Benthem-Muñiz M, Jongsma MLA, McLellend V, Steenbergen B, Shiran S, Ben Bashat D, Barker GJ, Understanding the relationship between brain and upper limb function in children with unilateral motor impairments: A multimodal approach, *European Journal of Paediatric Neurology* (2017), doi: 10.1016/j.ejpn.2017.09.012.

Understanding the relationship between brain and upper limb function in children with unilateral motor impairments: A multimodal approach

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Understanding the relationship between brain and upper limb function in children with unilateral motor impairments: A multimodal approach

Abstract

Atypical brain development and early brain injury have profound and long lasting impact on the development, skill acquisition, and subsequent independence of a child. Heterogeneity is present at the brain level and at the motor level; particularly with respect to phenomena of bilateral activation and mirrored movements (MMs). In this multiple case study we consider the feasibility of using several modalities to explore the relationship between brain structure and/or activity and hand function: Electroencephalography (EEG), both structural and functional Magnetic Resonance Imaging (sMRI, fMRI), diffusion tensor imaging (DTI), transcranial magnetic stimulation (TMS), Electromyography (EMG) and hand function assessments. Methods: 15 children with unilateral CP (ages: 9.4±2.5 years) undertook hand function assessments and at least two additional neuroimaging and/or neurophysiological procedures: MRI/DTI/fMRI (n=13), TMS (n=11), and/or EEG/EMG (n=8). During the fMRI scans and EEG measurements, a motor task was performed to study cortical motor control activity during simple hand movements. DTI tractography analysis was used to study the corpus-callosum (CC) and cortico-spinal tracts (CST). TMS was used to study cortico-spinal connectivity pattern. Results: Type and range of severity of brain injury was evident across all levels of manual ability with the highest radiological scores corresponded to children poorer manual ability. Evidence of MMs was found in 7 children, mostly detected when moving the affected hand, and not necessarily corresponding to bilateral brain activation. When moving the affected hand, bilateral brain activation was seen in 6/11 children while 3/11 demonstrated unilateral activation in the contralateral hemisphere,

and one child demonstrated motor activation predominantly in the supplementary motor area (SMA). TMS revealed three types of connectivity patterns from the cortex to the affected hand: a contralateral (n=3), an ipsilateral (n=4) and a mixed (n=1) connectivity pattern; again without clear association with MMs. No differences were found between children with and without MMs in lesion scores, motor fMRI laterality indices, CST diffusivity values, and upper limb function. In the genu, midbody, and splenium of the CC, higher fractional anisotropy values were found in children with MMs compared to children without MMs. The EEG data indicated a stronger mu-restoration above the contralateral hemisphere in 6/8 children and above the ipsilateral hemisphere in 2/8 children.

Conclusion: The current results demonstrate benefits from the use of different modalities when studying upper-limb function in children with CP; not least to accommodate to the variations in tolerance and feasibility of implementation of the differing methods. These exposed multiple individual brain-reorganization patterns corresponding to different functional motor abilities. Additional research is warranted to understand the transactional influences of early brain injury, neuroplasticity and developmental and environmental factors on hand function in order to develop targeted interventions.

Introduction

Brain injury during gestation or early childhood that leads to atypical brain development may have profound effects on motor development and subsequent independence. Cerebral Palsy (CP) is the most common physical disorder in childhood, with unilateral motor impairments evident in 30 to 40%[1-3]. Pathogenesis of unilateral CP (UCP) is varied and may include brain malformation, unilateral bias of periventricular haemorrhage, peri-ventricular leukomalacia, post-haemorrhagic porencephaly, or middle cerebral artery infarct[4, 5]. Studies exploring the brain structure and function in early infancy through to adulthood have shown the brain's remarkable capacity for reorganisation in response to injury or experience[6, 7]. Such changes include brain structures working more intensively, undertaking different 'functional' roles, re-routing of pathways, or establishing new connections between structures[6]. Transcranial magnetic stimulation (TMS) studies reveal that some children with UCP show ipsilateral connectivity of corticospinal-tract projections (CST) from primary motor cortex (M1) in the contralesional hemisphere to the affected hand while others demonstrate a mixed CST connectivity pattern, and some show a more typical contralateral motor projection from the lesioned hemisphere[8-10]. Also reported are atypical branched CST axons from the lesioned hemisphere evidenced in early in utero damage[11]. Diverse patterns of re-organisation, occurring during different developmental periods, may influence the microstructure of other brain structures, notably the corpus callosum[9] and functional connectivity of neural circuits involved in motor control[12, 13]. This may affect hand function and response to intervention[14-16].

Different neuroimaging and physiological techniques have been implemented in attempts to understand the phenomenon of neuroplasticity and its implications for

intervention[14, 17, 18]. Interpreting neuroplastic adaptations during infant and child development is confounded by variations in sample selection (natural and therapeutic environmental influences on development), tolerance of children to different procedures, and most likely also the choice of the techniques and methodologies employed. For example, Reid et al[17] recently reported on the challenges of interpreting task-focused functional magnetic resonance imaging (fMRI). They stated that activation patterns may be influenced by a number of different parameters such as attention, anticipatory motor planning, as well as adherence to the task protocol.

Heterogeneity of neuropathological profiles is also reflected at the motor level with varying severity of hand function impairments and type of movement disorder (e.g. spasticity, weakness, dyskinesis)[19, 20]. In addition to the functional deficits directly related to neuro-motor control, mirrored movements (MMs), defined as simultaneous involuntary and homologous movements accompanying voluntary movements on the opposite side of the body[21], are evident in many children with UCP. Aetiological mechanisms of MMs are as yet poorly defined with some evidence suggesting MMs appearing in the affected hand indicative of one motor cortex controlling both hands via ipsilateral connectivity from the non-lesioned hemisphere to the affected hand[22]. Also, it is still under debate if it can generally be stated that MMs negatively influence bimanual hand function. It has been shown that a subgroup of children with UCP demonstrate non-symmetrical interference and/or strategic use of MMs under specific task constraints involving divergent motor actions[23, 24].

In this multiple case series, we aimed to improve our understanding of the relationship between brain structure and hand function, focusing on the phenomena of bilateral activation and MMs, using several modalities. We undertook detailed mapping of neurological processes utilizing both neuroimaging (including structural magnetic resonance imaging (MRI), fMRI, diffusion tensor imaging (DTI)) and neurophysiological techniques (transcranial magnetic stimulation (TMS), electroencephalography (EEG), and electromyography (EMG)) alongside experimental and functional tasks. We hypothesize that different techniques and procedures will provide complementary if not alternative perspectives of neuroplasticity and bimanual control.

We describe the challenges in administration and tolerance to procedures in children as well as comparisons between the results obtained through the different modalities. The implications of these different techniques and tasks used to study neuroplasticity and hand function in childhood will be discussed.

1. Materials and methods

This study was approved by the National Research Ethics Committee (10/H0804/40/A1M01, 10/H0804/40/AM02). Fully informed consent was obtained from parents along with assent from children.

2.1 Participants

Children with UCP (ages: 9.4±2.5 years) were recruited from Child Development Centres and Paediatric Neurology units in South East England consenting to participate in a 2-week bimanual intervention in 2012 or 2014. Children were included if they had clinical signs of UCP, were attending regular education and were independently mobile. Exclusion criteria were uncontrolled seizure activity, treatment to improve upper limb movement in previous six months, and any contra-indications

to MRI. The children in the current study were part of a larger cohort of children with UCP participating in prospective studies exploring experiences and effects of therapy. Only children who consented to neuroimaging and neurophysiology assessments, and for whom at least two of these procedures were free from major confounding artefacts, were included in this paper. Data were available for 15 of 20 children. See Table 1 for childrens' clinical characteristics and baseline upper limb function.

2.2 Measures

Identical measures were collected from 2012 and 2014 cohorts, with the exception of EEG and EMG which were only performed in 2014. See supplementary file for specific details of each MRI, TMS and EEG procedures.

Baseline clinical characteristics of severity of movement difficulties were assessed by a senior occupational therapist. The *Manual Ability Classification System (MACS)* ranked ability to handle objects in important daily activities and need for any assistance or adaptation[25] and *Gross Motor Classification System (GMCS)* documented functional severity of motor disorder limiting mobility and posture[26, 27] with higher values reflecting greater difficulty or impairment.

2.2.1 Upper limb motor behavior assessments:

Jebsen Taylor Test of Hand Function (JTTHF), a standardized test of uni-manual dexterity[28], was used to quantify the capacity of each hand across 6 tasks. Maximum time to complete each task was 180 seconds for a total maximum allowable of 1080 seconds. In order to establish the difference in capacity between hands (AH= affected hand, LAH= less affected hand), a ratio score was calculated (AH-LAH)/(AH+LAH). Quotients around 0 reflect balanced capacity, values closer to +1 reflecting a disproportionate dominance of the LAH and values between 0 and -1 a dominance of the AH (unlikely).

The *Children's Hand Experience Questionnaire* (CHEQ) is a 29-item questionnaire of affected hand use and experience in daily bimanual activities[29, 30]. The number of activities performed independently was calculated. A CHEQ ratio was calculated reflecting proportion of independent activities performed with both hands (2hand/(2hand+1 hand)).

Squeezing task – A small sphygmomanometer pressure bulb (sphyg-bulb) held in each hand was used to verify actual motor actions and adherence to fMRI protocol (see below) as well as to document MMs. Pressure from the sphyg-bulb was recorded at a frequency of 20Hz during the motor fMRI task. Maximum pressure, sum of pressure and change of pressure were extracted for each block of the sequence and hand.
A similar *Squeezing task* was used, with the child seated, during EEG and simultaneous EMG recordings. The child's forearms were supported by the table with the child holding a soft plastic sponge ball (of the same dimensions as sphyg-bulb). EMG was recorded from the Extensor Digitorum Communis (EDC) muscle of each arm using self-adhesive electrodes.[1]

2.3. Transcranial Magnetic Stimulation (TMS) was used to identify the pattern of corticospinal organisation in each child (ipsilateral, contralateral or bilateral innervation). Eight suprathreshold (1.5 times AMT) motor evoked potentials (MEPs) were recorded during bilateral flexor digitorum interosseous (FDI) activation and superimposed in order to identify the earliest onset latency. Absence of a MEP was defined as no response to 5 stimuli at 100% stimulator output (if tolerated), in contracting muscle.

[1] The squeezing task during EEG followed a previous squeezing task in which children were required to initiate movements to activate a windmill via connected transducer by exerting force beyond 1.5 kg; loosening grip to approximately 1kg and repeatedly squeezing between these upper and lower thresholds within 1000ms. This task is not reported here as data output was only available for only 4 children due to technical failures.

Central Motor Conduction Times (CMCT) were calculated for contralateral and ipsilateral pathways using the F wave method. Distal motor (M) and F wave latencies were measured in the ulnar nerves bilaterally. The CMCT was calculated by subtracting the Peripheral Motor Conduction Time from the latency of the Motor Evoked Potential[31] : [CMCT=MEP–(F+M-1)/2]. Connectivity patterns were determined by the presence of MEP response to ipsi- and or contra- stimulated hemisphere.

2.4 Electroencephalography (EEG) was used to compare the mean mu-rhythm between unimanual movements and rest during the squeezing task. The amount of mu-restoration after active hand movement reflects top-down control processes to focus and prepare functional neural circuits for movement execution[32]. Signals were recorded with a 32-channel actiCap (MedCaT B.V. NL) [33, 34].

The individual mean EEG mu-rhythm (2Hz surrounding the individual mu-peak within the mu frequency of 7-13.5Hz) was extracted from the EEG over the sensorimotor cortex during rest and movement of each hand for further analysis. The percentage of mu during rest following active movement in contrast to the amount of mu during movement was calculated; reflecting the amount of total mu-restoration after voluntary hand movements for both hands (affected vs. less-affected) and above both hemispheres (contralateral vs. ipsilateral).

Table 1: Clinical Characteristics

MR Child #	Gender	Age (year)	Affected hand	MACS	GMFCS	Gestational age (weeks)	Gestational weight	Type of injury	Radiology score
1	M	7.1	L	II	I	38	3856	HIE*	12
2	M	7.0	R	I	I	40	3629	IVH	7
3	M	7.5	R	II	I	42	3447	Cystic Encephalomalacia	9
4	F	8.7	R	II	I	31	1860	IVH	9
5	M	11.0	R	I	I	42	4082	Congenital malformation	4
6	M	7.3	L	III	I	41.5	4491	MCA, mild diffuse HIE	15
7	M	10.6	R	II	II	38	4600	PWM	11
8	M	7.8	R	II	II	35	1700	IVH	11
9	M	8.1	L	III	I	36	2500	PWM	11
10	F	9.9	L	II	II	40	3524	Congenital malformation	7
11	M	7.8	R	III	II	41	4190	Infarct	17
12	M	15.8	L	II	II	40	3000	Congenital malformation	7
13	F	10.8	R	I	I	41	2980	PWM+focal infarct	4
14	F	13.2	L	II	II	42	3020	Congenital malformation	9
15	M	8.3	R	III	I	40	3970	PWM+multifocal WM changes	12

* Increased T2 signal and volume loss in basal ganglia in right hemisphere with moderate peri-regional WM changes possibly associated with HIE/ infection.

GMFCS = Gross Motor Function Classification System; HIE = Hypoxic ischaemic encephalopathy; MACS = Manual Ability Classification Scale; MCA Middle cerebral artery infarct; PWM = periventricular white matter injury; WM=white matter

The presence of MMs was determined via corresponding methods; sphyg-bulb data obtained during the fMRI task and EMG data during the EEG squeezing task. Presence of MMs was determined for each child and hand in the fMRI task by dividing the baseline pressure score of the AH during rest by the average change of pressure of the AH during the LAH's active condition and vice versa to determine a ratio (see below). Presence of MMs was calculated from the squeezing task (EMG) by dividing the EMG activity of the contralateral EDC during rest epochs by that during movement epochs[35] reflecting mirrored recruitment of homologous muscles. The EMG data was full-wave rectified, band-pass filtered (20-250Hz) and segmented for movement and rest epochs and root mean square (RMS) of the contralateral muscle activity was calculated. Ratio scores that are <1 demonstrate an increased activity in the hand when the opposite hand is moving as compared to both at rest and thus indicating MMs. MM-AH represents a mirroring in the affected-hand of the activity in the less-affected hand and MM-LAH reflects the activity of the LAH mirroring the AH.

2.5 MRI

2.5.1 Scanning parameters

Images were acquired on a 3T GE HDx scanner (General Electric Healthcare, Chicago, USA), using child friendly techniques (including access to a 'mock scanner' for acclimatization and presentation of a video throughout scanning (except during the fMRI). Total scanning time approximately one hour. (Detailed protocol in supplementary file).

2.5.2 MRI injury coding

An MRI based radiological scoring system for measurement of the extent of brain injury was performed by a senior pediatric neuroradiologist according to the scoring criteria in Shiran et al.[36] .This scoring system is based on several parameters: lobes

involved, white matter (WM) injury, cortical grey matter (GM) pathology, deep GM pathology and WM tracts disrupted[36]. The result of the scoring system is a single total radiological score (RS).

2.6 fMRI

2.6.1 Task description

A block-design fMRI motor task was used in which children clenched and extended all fingers of one hand in synchrony with 2-Hz paced tones, while a sphyg-bulb was placed in their palms to measure maximum pressure, sum of pressure and change of pressure [37-39]. Total task duration was 4 minutes, 45 seconds; alternative hands clenching with resting epochs in between. In cases where the sphyg-bulb measurement indicated discrepancies from the fMRI motor task protocol, the child's fMRI protocol was adjusted based on his actual hand movements. (Details in supplementary file)

2.6.2 Motor fMRI task data analysis

The fMRI signal in the various conditions was compared using BrainVoyager QX (Version 2.4, Brain Innovation, Maastricht, Netherlands). The functional data were analyzed using a multiple regression model (General Linear Model; GLM) consisting of predictors, which corresponded to the particular experimental conditions of each child: movement of affected hand condition, movement of less-affected hand condition, movement of both hands and rest (no hand movement).

2.7 DTI

DTI tractography analysis was used to study the corpus-callosum (CC) and cortico-spinal tracts (CST). DTI was performed using DTIStudio software (Johns Hopkins University, Baltimore, MD, USA) which uses a streamline fibre tracking method with Fibre Assignment by Continuous Tracking (FACT) algorithm[40]. The CC and CSTs

were extracted using a region of interest approach. Mean values of axial diffusion (AD), radial diffusion (RD), mean diffusivity (MD) and FA were calculated for each fibre tract.

2.8 Statistical analyses of non MRI data

Descriptive data are presented across cases. Group data are presented using parametric and non-parametric analyses of variance where appropriate. Comparisons of ordinal data were conducted using Kruskal Wallis. Pearson or Spearman rho correlations were calculated to consider trends. In view of the small sample, statistical inference is limited.

3. Results

3.1 Hand function

Table 2 outlines the characteristics of hand function across unimanual and bimanual skills and behaviours. Significant differences were seen between impairment in manual ability and capacity of the affected-hand (JTTHF-AH total, F (2,14)=5.65, p = .019); post hoc comparisons (using Scheffe for unequal samples) show children at MACS level III performing more poorly than those at MACS I (mean difference -667.7, p = .021).

Eight children, across all MACS levels, showed deficits in performance of the less-affected hand (≥ 2SD) compared to age and gender matched typically developing children. However, affected-hand performance did not correlate with performance of the less-affected (JTTHF rho.132, p =.638).

For bimanual tasks, there was a non-significant difference between MACS levels for number of independent tasks (CHEQ: H (2 14).263, p=.877) and percentage of use (CHEQ: F (2, 14) 1.11, p =.380). Significant correlations were evident between

the ratio of capacity between the affected and less affected hands on the JTTHF and ratio of use of the AH during bimanual tasks (CHEQ ratio) (r =-.550, *p*=.034).

Table 2: Hand Function

Child #	MACS	JTTHF affected hand	JTTHF less affected hand	JTTHF ratio	CHEQ # Indepen-dent		EMG task MM		Sphyg-bulb Pressure change ratio	
							AH	LAH	AH	LAH
1	II	382	34	.84	21	.62	-	-		
2	I	108	52**	.35	15	.93	1.07	1.1	1.00	**0.63**‡
3	II	394	53**	.76	17	.76	**0.65**‡	**0.67**‡	1.07	1.38
4	II	363	45**	.78	16	.88	**0.59**‡	1.06	0.97	**0.52**‡
5	I	50	36**	.16	25	1.00	1.19	**2.41**[a]	1.03	0.80
6	III	1015	59**	.89	10	.60	1.04	1.04		
7	II	795	33	.92	18	1.00	**0.67**‡	**0.69**‡	0.97	1
8	II	461	62***	.76	22	1.00	0.94	**0.56**‡	0.97	1.20
9	III	395	48*	.78	22	.64	1.09	1.06	0.96	0.9
10	II	735.2	22.2	.94	15	.93	-	-	**0.83**‡	0.96
11	III	1080	38.5	.93	17	.47	-	-	1	1.06
12	II	596.9	36**	.89	20	.85	-	-	**0.12**‡	**0.8**‡
13	I	63.9	26.2	.42	20	.90	-	-	1	1.95
14	II	270.9	38.9**	.75	20	.85	-	-	0.89	0.92
15	III	301.7	32.4	.81	16	.94	-	-		

*Score outside 1 SD of age-gender mean; **score outside 2 SD of age-gender mean; ***score outside 3 SD of age-gender mean

‡ = Mirror Movements evident; a= more movement evident at rest; EEG=electroencephalogram; MACS = Manual Ability Classification Scale; JTTHF = Jebsen Taylor Test of Hand Function; CHEQ=Children's Hand Experience Questionnaire; MM = Mirror movement

3.2 MRI- radiological scores

Type and range of severity of brain injury was evident across MACS levels: MACS level I (least severe hand function impairment) RS scores ranged from 4 to 11, MACS II, RS ranged from 4 to 12 and MACS III, 9 to 17. These differences did not reach significance (H 5.3, p = .70).

Children with MCA infarct were all at MACS III; those with cystic-encephalomalacia, hypoxic ischaemic encephalopathy (HIE) or periventricular white matter injury (PWM) were at MACS level II; children with congenital malformation were represented in MACS levels I and II; and children with IVH were represented across MACS levels.

Correlations between RS scores and affected-hand (AH) function were evident (Spearman rho) with more neurological impairment associated with slower performance on the JTTHF (rho=.599), a higher AH:LAH ratio (rho =.562) and less use of the affected-hand during bimanual tasks on the CHEQ (rho=-.553). RS was not associated with the number of overall bimanual activities that were performed independently, CHEQ # independent (rho=-.174).

3.3 TMS- CST reorganization and aetiology

Children 5, 10, 11, 12 did not undergo TMS due to epilepsy risk. Three children showed a pattern of contralateral connectivity (children 1, 2 and 4); one of whom was born prematurely. These children showed HIE and IVH with motor severity ranging from MACS I to II.

Four children showed a pattern of ipsilateral connectivity from dominant hemisphere to affected hand with no evidence for contralateral connectivity (participants 3,13,14 and 15). These children were all born at term but showed a range of brain injury patterns PWM injury with

multifocal WM changes and congenital malformation; motor severity ranging from MACS levels I to III and RS.

Child 7 showed a pattern of mixed connectivity from both hemispheres to affected hand. He was born at term and showed PWM and MACS II. In three children no motor evoked potentials could be recorded in the affected hand from either contralateral or ipsilateral stimulation (children 6,8 and 9). Two were born prematurely with IVH or PWM and MACS levels ranging from II to III. See Tables 1, 2 and 4. For CMCTs see TMS MEPs and connectivity subtypes table in supplementary file. MMs seem to be more common in children with ipsilateral & bilateral projections (3/3) than with contralateral projections (1/3)

3.4. EEG results

3.4.1 Mu restoration: Table 3 shows the amount of mu restoration after active hand movements (squeezing task) for the affected and less-affected hand separately. The EEG data indicated a stronger mu-restoration over the contralateral hemisphere when moving the affected hand in 6/7 children. One child showed a stronger mu-restoration over the ipsilateral hemisphere after actively moving the AH.

Table 3: Neuroimaging and Neurophysiology Data

	Child #	**AH**	**MACS**	**TMS**	**EEG** (active hand)				**fMRI**	
				Pattern to AH	% increase in mu during rest compared with during activity				AH moving	
					AH ipsi	AH contra	LAH ipsi	LAH contra	LI	Pattern
2014	1	L	II	Contra	-	-	-	-	-	-
	2	R	I	Contra	138.7	151.7*	104.82	143.6*	0.6	Bilateral
	3	R	III	Ipsi	-	-	-	-	-0.42	Bilateral
	4	R	II	Contra	113.4	149.6*	99.43	123.9*	-	-
	5	R	II	-	34.5	42.0*	26.61	29.4*	1	Unilateral
	6	L	III	None recorded	140	221.9*	68.95	87.0*	-	-
	7	R	II	Mixed	29.0*	14.94	50.23	54.1*	-	-
	8	R	III	None recorded	354.1	432.1*	166.2	259.2*	1	Unilateral
	9	L	II	None recorded	82.3	221.9*	31.7	43.4*	-	-
2012	10	L	II	-	-	-	-	-	-0.06	Bilateral
	11	R	III	-	-	-	-	-	-	Mainly SMA
	12	L	II	-	-	-	-	-	-0.21	Bilateral
	13	R	I	Ipsi	-	-	-	-	0.34	Bilateral
	14	L	II	Ipsi	-	-	-	-	-0.02	Bilateral
	15	R	III	Ipsi	-	-	-	-	0.55	Bilateral

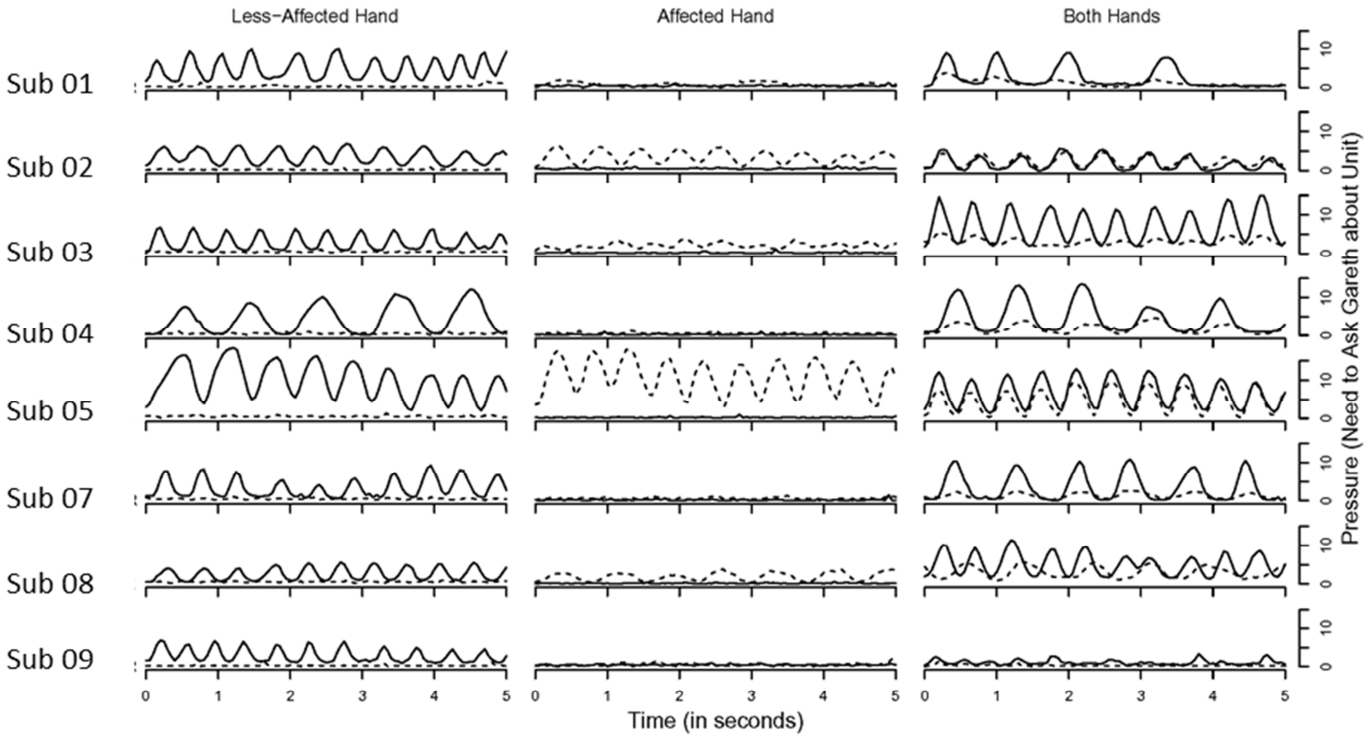
Legend: *=significant difference between hands. AH=affected hand; LAH=less affected hand; MACS=, RS=radiology score; TMS= transcranial magnetic stimulation; EEG=electroencephalogram; Ipsi=ipsilateral; contra=contralateral; fMRI= functional magnetic resonance.

3.5 Motor analysis of squeezing task: sphyg-bulb and EMG

3.5.1 Analysis of the motor task during fMRI showed evidence of MMs (from pressure changes) in the less-affected hand (when AH was active) in three children (#2, 4, 12) with child #12 also showing MMs in the AH (see supplementary file for table of sum of pressure and change of pressure values per child). Overall, squeezing actions were stronger in the LAH for most of the children with the exception of the children #2 and #5 in whom no apparent difference could be seen. Child #5 also showed an atypical MM profile on EMG with more movement at rest. Figure 1 illustrates exemplary patterns of actions per child reflecting inconsistencies in timing and frequency as well as difficulty detecting mirror movements in cases with limited capacity to perform simple clenching action. Child #9 was unable to exert sufficient pressure to perform the task with the AH and, notably, the LAH pressure was considerably less in the both hands condition than in the unimanual.

The MM calculated from the squeezing task using EMG mirrored recruitment of homologous muscles identified four additional children with MMs from the 2014 cohort (#3,5,7,8) than those identified using the sphyg- bulb; but did not identify MMs in one child who had shown these in the LAH during the fMRI task (#2) and in the AH as opposed to the LAH in another child (#4). Only one of the six children whose less-affected hand showed good capacity (within 1 SD of age-matched norms on the JTTHF) was identified as having MMs by either technique.

Figure 1 Exemplars of motor analyses during fMRI; sphyg-bulb pressure data



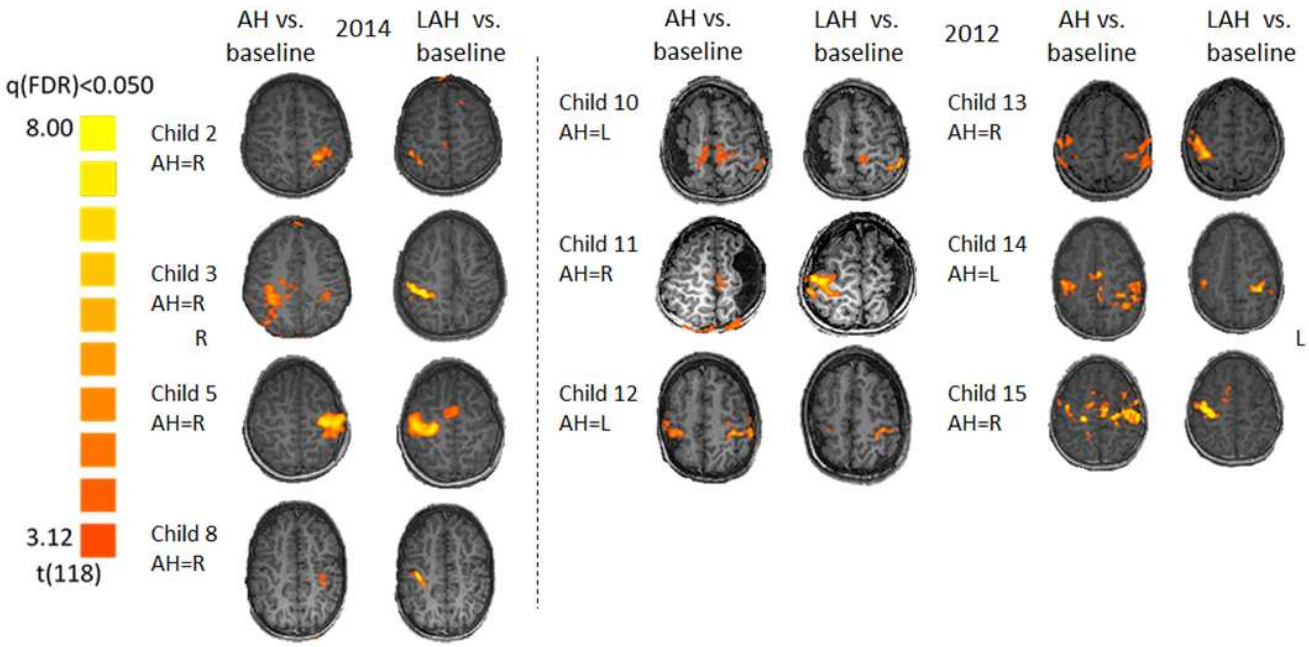
Legend: Illustrative epochs demonstrating sum of pressure when moving affected hand, less affected hand and both hands simultaneously. Taken from second tertile of first block. Solid line=less-affected hand, dotted line=affected hand.

3.6 fMRI Active motor task

fMRI active motor task data were available for 9 children (five were excluded due to head movements and one did not undergo the MRI scan). Bilateral activation when moving the affected hand was seen in the area around the central sulcus in seven children (# 2, 3, 10, 12-15); four of whom (# 3, 13,14, 15) had ipsilateral CST connectivity based on TMS (see Table 3). Of note for these seven children showing bilateral activation, all were born at term, with MACS levels I-III and RS from 7-12 and a range of pathologies (including IVH, PVWM, malformation). While two of these children showing bilateral activation (#2, #12) with evidence of MMs, an additional child (#4) without usable fMRI data due to signal noise, but with clear contralateral CST pathway on TMS, also showed MMs which differed in presentation

in the AH or LAH depending on task demands. Figure 2 reflects patterns of activation when moving AH and LAH. With the techniques we used we were not able to ascertain whether atypical branched CST axons from the ipsilesional hemisphere may also have contributed to MMs[11].

Figure 2: fMRI activation when moving AH and LAH



Legend: Axial slices demonstrating fMRI pattern of activation when moving affected hand (AH) and less affected hand (LAH).

3.7 DTI

DTI data were available for 11 participants; three participants were excluded due to head movements. In 8 participants axial diffusivity (AD) was seen to be slightly higher in the affected CST compared to the less affected CST. This trend was not observed in participant 5, with congenital malformation, where a slightly higher AD value was detected in the less affected CST. Radial diffusivity (RD) was slightly higher in the affected CST compared to the less affected CST in three participants (3,4,7) while slightly higher in the less affected CST in participants 2 and 5 (See Table 2 Supp. For

diffusivity values per child and see Figure 1 Supp. For tractography results of the CC and CST).

In the CC, RD was higher (reflecting greater diffusivity) in the midbody compared to the genu and splenium and AD was higher in the splenium compared to the genu and midbody in four participants (2,3,4,7) while child 5 demonstrated a different trend (see Table 2 Supp.).

3.7.1 Correlations between DTI and manual function

Significant positive correlations (Spearman) were found between AD and MD in the midbody and splenium of the CC with total time when using the affected hand in the JTTHF (n=11; Midbody: AD r=0.76, p=0.006; MD r=0.66, p=0.03; Splenium: AD r=0.64, p=0.04). Note JTTHF scores reflect reaction time therefore the higher the score the more impaired the hand function. For correlation graphs see supplementary files.

3.7.2 Comparison between children with and without MMs

No significant differences in radiological score or unilateral hand function were observed between children with and without MMs (Radiological score $F(1,14)=0.89$ $p=0.36$; JTTHF AH $F(1,14)= 0.08$, $p=0.78$; JTHHF LAH $F(1,14)=1.04$, $p=0.33$). There was marginally higher percent use of two hands in children with MMs (M=0.91) compared to those who didn't show MMs (M=0.75); (CHEQ percent use $F(1,14)=3.81$, $p=0.07$).

Higher FA was found in the genu, midbody and splenium of children with MMs compared to those without (Genu: $F(1,10)=15.48$, $p=0.003$; midbody: $F(1,10)=6.04$, $p=0.036$; splenium: $F(1,10)=8.08$, $p=0.019$. No significant differences were detected between other diffusivity values in the CC and CST.

Discussion

The use of a multi-model approach to study brain structure and hand function in children with hemiplegia demonstrates the complexity of brain plasticity following early brain injury with regards to hand function. Using a multiple case series analyses, our results suggest that for every child, there is a different pattern of reorganization of neural architecture subserving hand function. This is consistent with studies that have examined the association between hand function, brain lesions and CST projection types with wide variations in hand function evidenced across all motor-projection patterns[14, 41, 42]. While the extent of deep grey matter lesions has been associated with severity of upper limb movement impairments, other parameters may also influence hand functionality in children with preterm births and PWM injury. Similarly, children in our group with more extensive lesions (RS values >12) showed the most severe limitations in manual ability (MACS III) and poorest capacity of the AH on the JTTHF. Yet, overall lesion severity was not associated with use of the AH in tasks typically requiring two-hands, suggesting a number of children may use alternative strategies to achieve functionality.

In this study several modalities have been used in order to obtain comprehensive and converging information regarding brain plasticity following early brain injury. It is important to note that each method yields different information so the comparison between techniques is not straightforward and several .factors should be taken into account when choosing a method: the information it provides, risk, tolerability, feasibility and financial costs. MRI provides broad information regarding brain structure and function, without using ionized radiation, thus considered safe also for children. In this study we had a moderate success rate

in obtaining good fMRI data quality- 9/14 (64% success), however in previous studies using this method we had higher success rate. When using a child friendly environment MRI is both tolerable and feasible in children with CP while financial costs are relatively high. In our study TMS was used to probe motor function, specifically to characterize the cortico-spinal connectivity pattern. While TMS is considered non-invasive it entails neuro-stimulation which is a contra-indication to some pathologies such as epilepsy (which is common in children with CP; in our cohort 3 children had epilepsy). In our study, the majority of children tolerated the TMS well, but some had high thresholds and found the stimulus uncomfortable, (One child could not tolerate it at all and so we had to abandon the TMS for him). This method has lower financial cost than MRI. EEG was used to measure and record the electrical activity of the brain, specifically to measure the amount of mu-restoration after active hand movement. This signal reflects top-down control processes to focus and prepare functional neural circuits for movement execution. There are no counter-indications to using this method and it is in most cases tolerable and feasible (in our cohort we obtained 7/9 (77%) success rate) and relatively lower in cost.

More impaired hand function associated with ipsilateral motor-projection from the non-lesioned hemisphere has been suggested [43, 44], yet some children with this projection type in our study had fairly good hand function. In an earlier paper[45], it was reported that the timing of brain injury also affects hand function; verified by Klingels et al.[22] with respect to MMs. Specifically, those with earlier brain injury with ipsilateral CST projections from the relatively non-lesioned hemisphere showed better unilateral hand function[45] but yet stronger MMs[22]. In our cohort, the

severity of impairment on one parameter (TMS, EEG, MRI) did not necessarily correspond with hand function or MMs. Nor did identification of atypical patterns of connectivity (ipsi- or bilateral or unidentified) correspond to greater or lesser degree of hand function impairment. Of interest was a potential relationship between MMs and capacity of the less-affected (dominant) hand; five of the six children with more typical capacity of the less-affected hand were not identified with MMs by either method. Notably, of these six children, TMS studies showed two with ipsilateral connectivity from the less affected hemisphere and one with mixed connectivity to AH; one of whom showed bilateral activation on fMRI.

Brain imaging, TMS and EEG showed different profiles for each child; reflecting different aetiologies, onsets of brain injury, and developmental trajectories. This is particularly notable with respect to atypical bilateral motor activation patterns, a phenomenon consistently observed in a fraction of children with unilateral CP. Several hypotheses have been postulated to explain this phenomenon: 1) Motor brain activation as a result of ipsilateral cortico-spinal connections; 2) Lack of inhibition through the corpus callosum; 3) Atypical branched CST axons from the ipsilesional hemisphere, and, 4) Associated movements from overflow of effort.

Clear bilateral motor brain activation was observed when moving the affected hand in seven children. Four children that demonstrated this atypical motor activation pattern and had TMS data (child 3,13,14,15), showed ipsilateral CST connectivity; the other (child 2) showed contralateral innervation on TMS yet some bilateral activation on fMRI. MMs were detected in only one of these children (#12) using the sphyg-bulb which measured the actual hand movements during the fMRI motor task. Notably, DTI tractography reconstruction of the corpus callosum in

these children indicated no significant injury of the CC fibres. These findings suggest that the motor activation detected in the less-affected hemisphere may stem from ipsilateral motor connections or from simultaneous brain activation or lack of inhibition through the corpus callosum.

In typically developing children, the contralateral pathway becomes the predominant pathway and the ipsilateral pathway, although present at birth, is largely withdrawn during development and is - if retained - weaker[46] .The various types of CST connectivity found in our cohort may represent different types of brain reorganization following perinatal injury and are consistent with previous reports of different connectivity patterns in children with hemiplegia[10, 47]. In three children CST connectivity was not determined. There are several possible explanations for this. Firstly, in this age group, consistent MEPs can only be evoked in contracting muscle[48] and these children had particular difficulty in sustaining activation of their FDI muscle, which may have affected our ability to evoke a response. Another possible explanation is that some of these children may have a high threshold ipsilateral (or contralateral) pathway to the AH. Indeed in some cases, several possible responses in were recorded in the AH when stimulating the less affected hemisphere at 100% TMS output, but these were present for less than 50% of stimuli, so did not reach the criteria for threshold. Finally, TMS activates only the fastest conducting CST fibres, so a genuinely absent MEP cannot be assumed to reflect no connection, but rather that the cortico-motor-neuronal pathway mediated by the *fastest* fibres is disrupted. It is interesting that these three children showed particularly high values of Mu restoration in their resting EEG data potentially reflecting more cognitive effort required to control movement. Mu suppression data provide evidence supporting this hypothesis with excessively high values shown in

the contralateral hemisphere when the AH hand was moving for these three children, reflecting contralesional hemispheric dominance.

fMRI studies of motor related brain activation in children with hemiplegia are often based on simple motor tasks[49]. In the current study, hand movements during the fMRI tasks were measured using a pressure bulb allowing for measurement of actual hand movements rather than assuming the children moved their hands according to the protocol. This demonstrated some discrepancies, sometimes major ones, between what the children were supposed to do and what they actually did even in a simple motor task. Subsequently we designed individual protocols according to the children's actual hand movements thus avoiding misleading interpretations of brain activation which may stem from errors in task execution rather than abnormal brain activation patterns. The current study suggests that analyzing fMRI data according to a general protocol in children with disabilities is problematic and may lead to errors in attribution of associations between fMRI data and hand function and subsequent interpretation of imaging findings.

In our cohort, based on the motor analysis during the fMRI hand task, MMs were independent of CST connectivity pattern. In consideration of the impact of MMs on brain activation patterns, it is interesting to note the differences between MM identification during the squeezing task outside the scanner which identified different children, or differences in representation of AH or LAH, from the fMRI task. The task protocol for timing of squeezing was the same yet the resistance of the ball and sphyg-bulb differed. Importantly, the MM EMG data were derived from a squeezing task that came after another task in which the children had been required to squeeze to approximately 1.5 kg. It is unclear whether any motor memory or prior conditioning may have therefore influenced movement behavior in that

particular squeezing task. This has implications for testing procedures and task conditions and potential task specific nature of MMs.

There were 5 children in whom we had no clear CST connectivity data and fMRI data. Children 3,13,14 and 15 all had ipsilateral connectivity on TMS but the fMRI for all of these cases shows bilateral activation on moving the AH. This could be in keeping with ipsilateral activation of motor pathway and activation due to simultaneous sensory feedback to the contra-lesional hemisphere.

Decreased fractional anisotropy (FA) and increased AD and RD have been reported in the anterior midbody of the CC (where transcallosal motor fibers cross) in children with congenital hemiparesis with ipsilateral connectivity compared to those with contralateral connectivity[9] . For the children who had DTI data with good quality in the current study we were able to track both the affected and less affected CST and the CC. As expected, the affected CST showed higher diffusivity values/lower FA compared to the less affected CST except than in participant 5 who showed the reversed trend. This may be as a result of different type of brain damage (polymicrogryria). Bearing in mind difficulties in interpreting DTI data, caution is needed[50] particularly when tracking CST projections in view of crossing fibres. Whereas DTI tractography shows promise in mapping CSTs in children with unilateral CP[51] , larger studies are required to consider the interactions of intra- and inter- hemispheric connectivity in relation to hand function.

Overall, our results suggest multiple adaptations to early brain injury impact on brain structures and pathways as well as hand function and behaviours. Each measure and procedure provides information and mechanisms informing on different elements (e.g. anamnesis), albeit the transactional nature of these interactions remains elusive as do the clinical implications. Consistent with adult

studies in acquired CVA[52], it is unclear which neurological biomarkers are best predictors of function and a combination of techniques should be considered in the absence of higher quality studies. The different examinations used in our study varied not only in their acceptability and utility, but also suggest that under different contexts of performance, patterns of brain activation may also vary. Understanding of context specific neuroplasticity may best be explored using multiple modalities. Merging of data across studies may allow for better comparisons of differing techniques for defining current hand function and estimations of response to intervention.

There are a number of limitations to our study, not least the lack of available data across all procedures and measures for all children despite the relatively good overall sample size for this type of study. In 2012, we set a low tolerance for undertaking TMS and thus did not include any child who had had post-natal seizures. Additionally, we only used TMS to assess the pattern of CST organisation in this cohort. In future studies it would be informative to include additional TMS assessments of motor cortex excitability and/or intra-cortical inhibition, for both the lesioned and contra-lesional hemisphere [44] or to investigate connectivity using TMS-Evoked Potentials[53]. Reasons for lack of tolerance of MRI included presence of metal (n=1); intolerance to noise (n=2); anxiety (fear) (n=3)[2]. Additionally, movement artefacts were pronounced for a further 4 children, two of whom data were irretrievable. In contrast, all children who undertook EEG, tolerated the procedure albeit one child's heightened level of anxiety may have confounded

[2] At a one month follow-up the child stated that he felt he could do the MR now stating how overwhelmed he had felt.

interpretation (this child did not complete TMS or MRI and data were excluded from this paper).

Conclusion

The main conclusions of this study are that 1) each child shows a different neuroanatomical and neurophysiological profile and 2) assessments of motor parameters are not always consistent for a given individual across different techniques. These findings reflect a number of factors: a) the challenges of studying this group of children, such that a different technique may be more appropriate in a given child; b) we are not always studying what we think we are studying (eg the non-adherence to task within the MRI scanner) and c) there are many different patterns of pathophysiology, depending on the nature, extent and timing of the brain injury, the individual child's specific genetic make-up and on subsequent environmental and developmental factors and how these interact with the effects of the early brain injury. It is evident from our findings that a simplistic conceptualization of neuroplastic adaptation in the form of ipsi- and contra-lateral CST pathways, is insufficient to explain performance or predict outcomes. As further larger studies are required to accumulate more data, we envisage that a multi-modal analysis with triangulation of data, such as introduced here, is likely to become important in determining the most appropriate therapeutic path for a given individual.

Acknowledgments

We are extremely grateful to the children and families who gave so much of their time to participate in this study. We would also like to thank Amarlie Moore and therapy students from Oxford Brookes University for their support in data collection.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This project was funded by a grant from Guy's and St Thomas' Charity and supported by Breathe Arts Health Research.

VM was supported by a NIHR Academic Clinical Lectureship and a starter grant from the Academy of Medical Sciences.

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Highlights

- Different modalities exposed multiple individual brain-reorganization patterns.
- Assessments of motor parameters are not consistent for individuals across different techniques.
- Simplistic conceptualization of neuroplasticity, ipsi- vs. contra- lateral CST, do not explain function.