MECHANISMS UNDERLYING CO-CONTRACTION DURING DEVELOPMENT AND IN PATHOLOGY IN MAN

By

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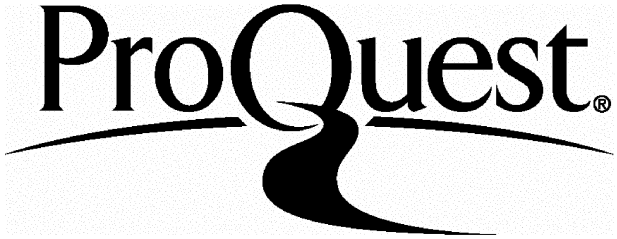

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This thesis is dedicated to the memory of Joe - a faithful and special friend.

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ABSTRACT

Postural and motor skills require the synergistic or co-ordinated action of a number of different muscles. These muscle synergies change during childhood and are disordered in developmental abnormalities. The underlying mechanisms have been investigated using a number of neurophysiological techniques. Focal magnetic brain stimulation was used to investigate the integrity and distribution of the corticospinal pathways; cutaneous reflexes and phasic stretch reflexes to investigate spinal and transcortical reflex pathways; somatosensory evoked potentials to examine afferent pathways and cross-correlation analysis of multi-unit EMG signals to look for the presence of common synaptic drive to motoneurone pools.

Investigation of children performing a unimanual task showed the occurrence of contralateral involuntary activity (mirror movements). This decreased with age. A study of a group of 4-11 year old children provided evidence to suggest that the mirror movements in children are due to a lack of interhemispheric inhibition by the corpus callosum. No bilateral activity was observed in adults unless there was contralateral background activity. In subjects with X-linked Kallmann's Syndrome (XKS) who show obligatory mirror movements, it was concluded that there is an abnormal ipsilateral projection which accounts for the occurrence of common synaptic drive to left and right homologous motoneurone pools and underlies the mirror movements in XKS. In the group of children with cerebral palsy (CP) the associated activity was not due to a common synaptic drive although in some patients there was evidence of a novel ipsilateral projection.

In the adult, antagonistic muscles can either co-contract to stabilise a joint or act reciprocally to produce force or movement about a joint. In young children, and those with cerebral palsy, agonist and antagonist muscles have been found to co-contract. The synaptic input to the antagonistic motoneurone pools was investigated using cross-correlation analysis. In adults the presence of a short duration central trough in the cross-correlogram indicated the presence of a reciprocal inhibitory mechanism. This was not observed in the young children or those with CP. Nor was there evidence of any shared excitatory input in these children.

CONTENTS

GENERAL INTRODUCTION

Motor abilities change during the normal course of development in man and this has been well documented in the clinical and behavioural literature (Illingworth, 1983). Relatively little, however, is known about the underlying mechanisms involved. Studies in animal models have been carried out by many authors and these have suggested the types of changing anatomical and functional neuronal connectivity that might be involved. Studies of motor development in man are less numerous, and have largely consisted of biomechanical and EMG recordings from various muscle groups and joint complexes during voluntary, postural and locomotor tasks (Berger *et al*, 1985 &1987; Forssberg, 1985; Leonard *et al*, 1991). Changes in reflex function have been studied by a number of groups, concentrating on locomotor reflexes (Berger *et al*, 1982), H-reflexes (Leonard *et al,* 1990), stretch reflexes (O'Sullivan *et al,* 1991; Myklebust & Gottlieb, 1993; Leonard & Hirschfeld, 1995) and cutaneous reflexes (Issler & Stephens, 1983; Evans *et al*, 1990).

In this thesis the aim has been to use a combination of neurophysiological techniques to investigate the mechanisms underlying the altering patterns of EMG activity that accompany some simple voluntary and postural tasks during development. A novel approach has been to apply cross-correlation analysis of EMG signals to look for changes in the distribution of synaptic drive shared between motoneurone pools innervating synergistic and antagonistic muscles.

A characteristic feature of the immature motor system in man, is that the distal movements of the upper limb on one side of the body are accompanied by a similar but involuntary movement on the contralateral side. Such movements are referred to as mirror movements and result from the co-contraction of bilateral homologous muscle pairs. In the first part of this thesis the changes in the prevalence of these movements is reported and the

underlying mechanisms investigated. An explanation is sought in terms of changing transcallosal interhemispheric inhibition or changes in the distribution of shared synaptic drive to bilateral homologous motoneurone pools.

Persistent mirror movements are a sign of motor disorder and can be observed in a variety of pathologies such as Klippel-Feil Syndrome (Farmer *et al*, 1990) and congenital hemiplegia (Carr *et al,* 1993). In these conditions, there is known to be a reorganisation of corticospinal pathways. In Section two of the thesis, the origin of the mirroring in subjects with X-linked Kallmann's Syndrome (XKS), a developmental disorder resulting from a genetic defect, is investigated to determine whether the mechanism is the same in these patients, or results from a persistence of the mechanisms underlying the mirror movements present in children as described in Section one.

In Section three, recordings have been made from children with cerebral palsy (CP) in which the normal process of motor development has been interrupted due to neural damage. In these individuals, the mirroring usually present during normal development is replaced by a stereotyped pattern of motor activity. The mechanism underlying this activity is shown not to be the result of changes in connectivity of the type seen in subjects with XKS, patients with Klippel-Feil Syndrome or children with hemiplegia, and is different from the mechanism producing mirror movements in normal children.

Another feature of the developing motor system is that rather stereotypical patterns of co-contraction of antagonistic muscles in the lower limb present at birth are progressively replaced by a more reciprocal pattern of activation later in childhood (Berger *et al,* 1985; Leonard *et al*, 1988). In children with CP this pattern persists. In Section four of this thesis, EMG recordings have been made from the lower limbs in normal children and children with CP. Cross correlation analysis has been applied to determine whether the co-contraction

results from common excitatory input to the motoneurone pools innervating the antagonistic muscle pair, or whether the co-contraction results from a lack of reciprocal inhibitory input.

SECTION 1

Co-contraction of left and right homologous muscle pairs in the hands of normal adults and during development.

Section 1:Chapter one: Bilateral EMG accompanies unilateral movements in man

SUMMARY

1. Electromyographic recordings were taken from the upper limbs of seven healthy subjects while making unilateral forceful self-paced index finger abductions with the preferred hand.

2. Subjects were instructed to make forceful self-paced index finger abductions with their preferred hand while maintaining a sustained voluntary contraction of 10% MVC on the opposite side. In all subjects there was an increase in the rectified averaged EMG on the non-preferred side, mean 1.4 $\pm$ 0.2% MVC ($\pm$ SEM, n=7) above the 10% MVC background EMG. The time of onset of the involuntary EMG was not significantly different from the commencement of the EMG burst of the preferred side. The increase in EMG of the non-preferred side was insufficient to cause any visible movement.

3. In a second series of experiments, recordings were made in a group of eight subjects during hand grip of a dynamometer maintained at 25%, 50% and 80% of maximum voluntary force (MVF) with the preferred hand.

4. Involuntary contralateral EMG was observed in all of the eight subjects performing the dynamometer experiment. When the grip force was increased this involuntary activity occurred earlier and was of greater amplitude than that recorded at 25% MVF.

5. In both experiments cross-correlation analysis was performed to determine if the bilateral EMG recorded was due to a common synaptic drive to the motoneurones innervating the left and right first dorsal interossei. In all cases the correlogram was flat indicating the absence of a common synaptic drive.

INTRODUCTION

Independent finger movements are generally believed to be organised by direct mono-synaptic cortico-motoneuronal connections from the contralateral sensori-motor cortex, via the lateral corticospinal tract (see Lemon, 1993 for a review). The discovery by Ferrier, and Fritsch & Hitzig in the 1870's that the cortex in the monkey and dogs was electrically excitable, anticipated the detailed study in humans by Penfield & Boldrey (1937). They showed that most movements were produced contralateral to the point of cortical stimulation, with the exception of bilateral activation of various facial, masticatory and truncal muscles.

However, there have been several reports in the literature of the occurrence of contralateral EMG accompanying unilateral movement in adults (Cernacek, 1961; Kristeva *et al*, 1991; Durwen & Herzog, 1992), and it is well established that young children produce bilateral activity when attempting to perform unilateral finger movements (Wolff *et al*, 1983; Lazarus & Todor, 1987). These involuntary movements are referred to as mirror movements or associated movements and decrease with increasing maturity.

Mirror movements maybe defined as unintentional movements occurring in homologous muscles which accompany movement in the contralateral hand. They were first described by Erlenmeyer in 1879. Such movements are also referred to as synkinesis, motor overflow and motor irradiation, and are considered to be a specific class of associated movements. The particular characteristics of mirror movements are that they result from activity in homologous muscle pairs, and the pattern of activity appears to be very similar in both hands, although generally of smaller amplitude in the mirroring hand. If such movements persist into adulthood they are considered to be pathological and have been described in certain conditions such as Klippel-Feil syndrome (Farmer *et al*, 1990) and

congenital hemiplegia (Carr *et al*, 1993). Mirror movements may be familial (Conrad *et al*, 1978) or idiopathic (Cohen *et al*, 1991; Harrison *et al*, 1993). Nevertheless, mirrored EMG activity is reported to occur in adults during complex or effortful tasks but this rarely produces a visible movement (Cernacek, 1961; Durwen & Herzog, 1992). This activity occurs predominantly during finger movements such as sequential finger-thumb opposition, increases with effort and is thought to be related to the complexity of the task (Hermsdorfer *et al* 1995). There are also suggestions that the strength of mirroring is influenced by the handedness of the individual. Using functional magnetic resonance imaging, Kim *et al* (1993a&b) have shown that subjects who are left handed show more ipsilateral cortical activation during ipsilateral movements than those who are right handed.

The question arises as to how this bilateral muscle activation might be produced by the central nervous system. Nass (1985) for example, suggested that it occurs as a result of activity in the ipsilateral uncrossed portion of the lateral corticospinal tract. Others have suggested excitation or a lack of inhibition via the transcallosal pathway resulting in bilateral cortical activation is responsible (Cohen *et al*, 1991; Danek *et al*, 1992).

It seems likely that the mechanism underlying the mirrored activity in adults differs from that underlying persistent pathological mirror movements, rather than for example being only quantitatively different along a continuum. The aim of this series of experiments was to investigate the bilateral activity which accompanies unilateral hand movements in healthy adults and to investigate the mechanisms underlying its occurrence. It is concluded that the bilateral EMG which accompanies phasic and sustained hand activities is generated via a fast conducting pathway, probably from the contralateral cortex.

A preliminary account of these experiments has been presented to the Physiological Society (Mayston *et al*, 1994).

METHODS

Subjects

Recordings were made from two groups of healthy subjects aged (20-46 years) with informed consent and local ethical committee approval.

Assessment of handedness

Hand dominance of the subjects was assessed using a modified version of the Edinburgh Handedness Inventory (Oldfield 1971, see appendix A).

Assessment of the presence of mirror movements

The presence of mirror movements in each individual was assessed according to the criteria of Woods and Teuber (1978). Each subject was asked to oppose each finger to the thumb from index to little and back, as neatly and as quickly as possible for 10 repetitions, with no instruction given about the opposite side. Unintentional movements on the opposite side were scored as follows:

0 = no mirror movements

1 = barely discernible but repetitive movement

2 = either slight but unsustained movement, or stronger, but briefer repetitive movement.

3 = strong and sustained repetitive movement

4 = movement equal to that observed in the intended hand

EMG recording

EMG was recorded using pre-gelled surface electrodes (TECA disc/bar electrodes, Medelec, Woking, Surrey, UK) attached to the skin overlying the left and right hand muscles as described in each experiment, with a centre-to-centre distance of 20mm. The EMG was amplified and filtered (-3dB at 20Hz and 5 kHz) using a 4 channel Medelec Sapphire clinical EMG machine and stored on magnetic tape (Racal Store 4, Racal Ltd., Hythe, Southampton, UK) for future analysis. In all the experiments the subjects were seated in a reclining chair with the arms well supported.

EXPERIMENT ONE:

Recordings of index finger abduction

EMG was recorded from surface electrodes attached to the skin overlying the left and right first dorsal interosseous muscles of seven subjects (3 male; 4 left hand preference). Subjects were seated at a table with their palms facing downwards and instructed to make about 120 self-paced forceful ("short and sharp") index finger abductions with their preferred hand whilst maintaining 10% of a maximum voluntary contraction (MVC) of the first dorsal interosseous muscle in the non-preferred hand. Visual feedback of the EMG signal of the non-preferred hand was provided via a root mean square (RMS) voltmeter to enable the subjects to maintain a steady contraction on that side. Verbal feedback was also given if required. Any involuntary increase in EMG above the background 10% MVC was not obvious in each sweep, therefore in order to reveal small changes the EMG signal from both sides was rectified and averaged for 100 sweeps time-locked to the beginning of the EMG burst recorded from the preferred side using averaging software (SigAvg Programme, CED).

The areas of the rectified-averaged EMG of both sides were measured and the ratio of the area of the burst of the involuntary EMG to the phasic voluntary EMG was calculated. In addition, in two subjects recordings of the EMG activity of the wrist and finger extensors were made while the subject maintained 20% MVC of extension of the wrist and fingers (Fext) while performing index finger abduction as described to determine if any contralateral activity recorded was confined to homologous muscles or was more widespread.

Cross-correlation analysis

In two subjects cross-correlation analysis was performed using surface EMG recordings from left and right first dorsal interossei muscles during i). phasic index abduction of the preferred side with simultaneous contralateral activity of 10% MVC on the non-preferred side, and ii). a sustained co-contraction. As indicated in figure 1 (an example of a sustained co-contraction), medium and large spikes were selected for analysis using a level detector (Neurolog NL200, Neurolog, Hemel Hampstead, UK). The resulting trigger pulses were passed into a microcomputer (Tandon 486) via a Cambridge Electronic Design 1401 interface (CED 1401) for processing. Cross-correlograms were constructed between the times of occurrence of motor unit spikes recorded from the left and right first dorsal interossei with a bin width of 1ms and a pre-and post trigger sweep of 100ms (Spike2 software, CED). At least 5000 spikes were used from each EMG signal; periods of silence between the phasic abductions were excluded from the analysis. The size of any central peak was estimated in terms of E/M where E is the number of spikes in the peak in excess of those expected by chance and M is the mean bin count in a 1ms bin calculated from an area away from the central peak, usually the first and last 75 bins. A central feature of less than 35ms was considered to be of short duration.

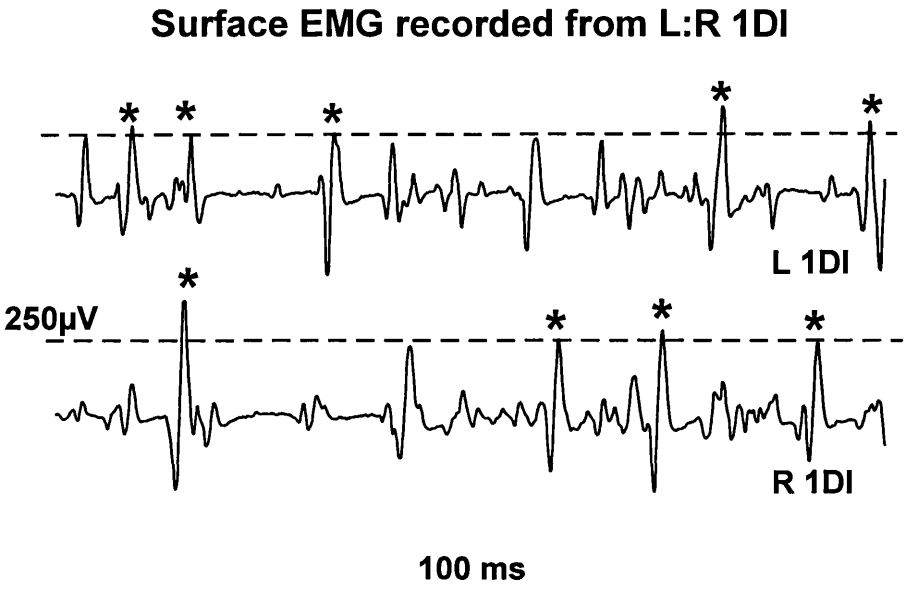


Figure 1: Surface EMG recorded from right and left 1DI in a normal adult subject. The line indicates the set level of the level detector, and the asterisks denote the spikes used as triggers and events.

EXPERIMENT TWO:

Sustained hand grasp recordings

Eight subjects (3 male, 3 left hand preference) were seated well supported as in the previous experiment. Surface EMG was recorded from the skin overlying the wrist and finger flexors of the preferred hand; the wrist and finger flexors/extensors of the non-preferred (NP) hand. The electrodes were placed proximal to the wrist, approximately a quarter of the distance between the wrist and elbow creases. Subjects were instructed to maintain a steady grip on a hand-held dynamometer (University College London Mechanical/ Electronics workshops, London, UK) for as long as possible using the preferred hand at 20%, 50% and 80% MVF. The tasks were presented in randomly. No instruction was given about the NP hand. Visual feedback of the force trace was provided on an oscilloscope (Hameg, 205-3) to enable subjects to maintain a steady grip. The EMG of the wrist and finger flexors of the gripping

hand was rectified, and any EMG of the wrist and finger flexors and extensors of the opposite side was integrated using a one second time constant (Neurolog, NL 703). The EMG and force signals were passed into a microcomputer via a CED 1401. The average rectified EMG of the wrist and finger flexors of the active side and the mean of the integrated EMG of the wrist and finger flexors and extensors of the opposite side was calculated for each 20 second frame of EMG recorded for each task in each subject (SigAvg programme, CED). The average EMG measured for each frame was expressed as a percentage of the maximum EMG recorded during a maximal voluntary grip of the dynamometer.

The EMG of the wrist and finger muscles and the wrist and finger flexors and extensors of both sides was recorded for each individual during three MVC's and the means used as the maximum for that subject. In addition the force was recorded during the MVF of the preferred side wrist and finger flexors, and the mean of the force recorded in each of three maximum grips used as the maximum force for that subject.

The subjects were given adequate rest between the sustained contractions to eliminate any effects which might result from fatigue.

Statistical analysis

Various statisitical tests were used throughout this thesis; the chosen level of significance was $P \leq 0.05$ unless stated otherwise. When the data were normally distributed, matched and of equal numbers of observations, the Student's paired *t*-test was applied. But if the data were matched for subject type but with an unequal number of observations the Student's unpaired *t*-test was applied. Linear regression was applied to the data recorded from the children who participated in the study of mirror movements to determine if there was a significant correlation between age and mirror movements for example, given by the correlation coefficient *r*, where $r=1$ is a perfect correlation. In the case of data which were not normally distributed a non-parametric test was used: the Mann-Whitney *U*-test for two sets of data.

RESULTS

Mirror movements

In all of the subjects mirror movements were either absent or weak (grade 0-1).

EXPERIMENT ONE:

Index finger abduction

Figure 2a. shows the rectified average of the surface EMG recorded from the right and left 1DI from one subject during self paced phasic index abduction of the preferred side with the contralateral side relaxed. No contralateral EMG was recorded. Figure 2b shows a single sweep of surface EMG recorded from the same muscles during the same task when 10% MVC was sustained on the contralateral side. An involuntary increase of EMG above the 10% background could occasionally be seen in a single sweep. This contralateral increase in EMG was not obvious in every sweep of EMG and is more clearly seen in figure 2c. which shows the EMG rectified and averaged for 100 sweeps. Taking the subjects as a group, the average rectified EMG signal of the preferred side consisted of a burst of EMG lasting between 192 and 256 ms, mean duration 234±9ms (± SEM, n=7). The increase in EMG seen on the contralateral non-preferred side was of similar duration and onset latency as that of the voluntary EMG burst of the preferred side. In all subjects there was an increase in the averaged rectified EMG above the background of 10% MVC on the non-preferred side lasting between 76 and 268 ms, mean 191±28ms (± SEM, n=7). The time difference between the onset of the EMG burst on the two sides was not significantly different from zero (Student *t*-test, $P>0.5$). The mean amplitude of the averaged rectified EMG on the non-preferred side ranged from 10.7-12.3% MVC, mean 11.4± 0.2%MVC (± SEM, n=7). The mean area of the averaged rectified EMG signal of the non- preferred side (10% background

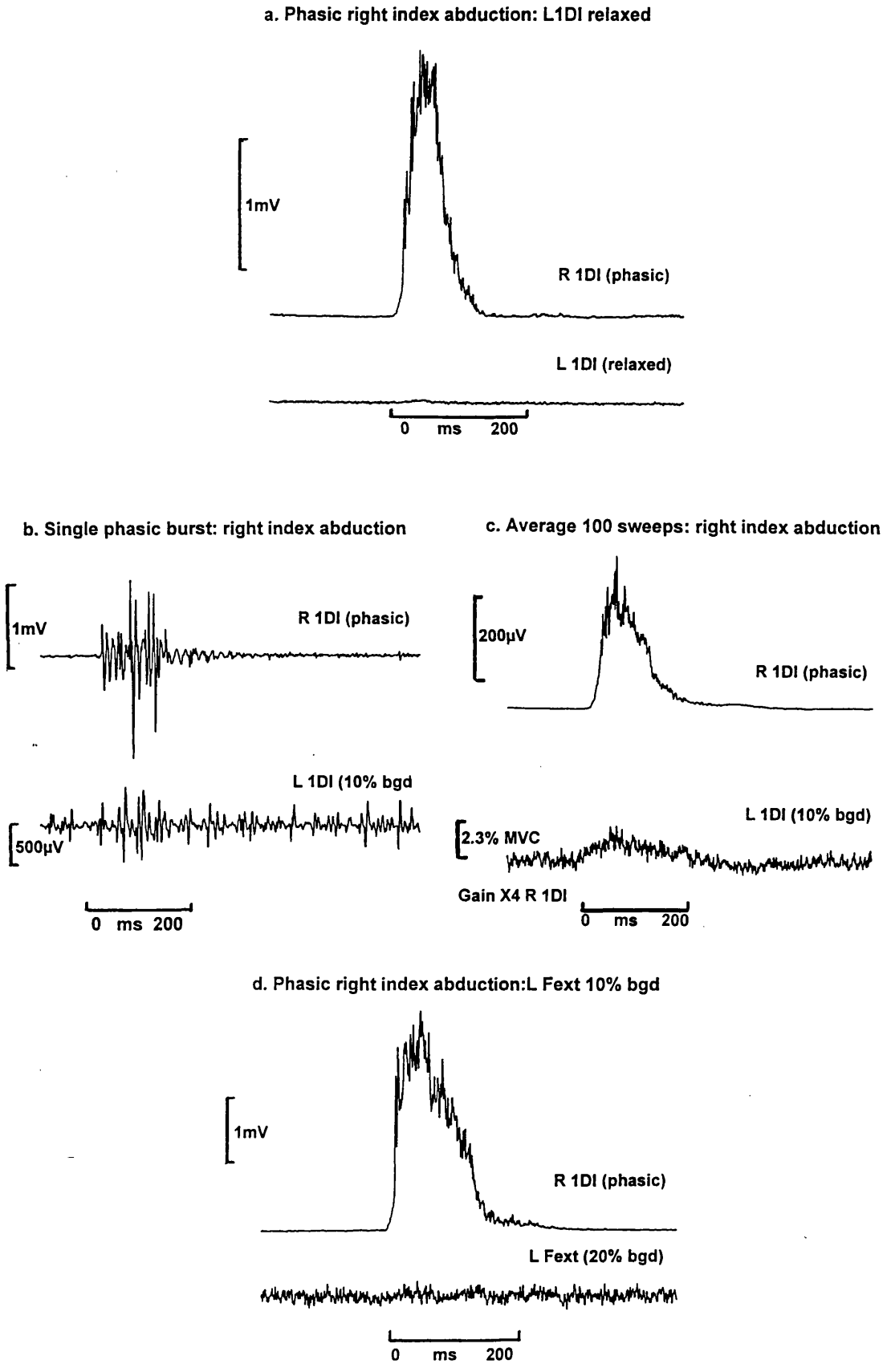


Figure 2: Subjects were instructed to make index finger abductions with the preferred hand. **a**. When the L1DI (contralateral) was relaxed no involuntary EMG was recorded in the opposite hand. But when instructed to maintain 10% background in the contralateral hand, small amplitude EMG was recorded above the 10%MVC in occasional single sweeps (**b**) and, was obvious in the rectified average of 100 sweeps of EMG (**c**). As shown in **d**. no involuntary EMG was recorded in non-homologous muscles (Fext).

EMG plus the increase associated with the voluntary burst) was 2.0-5.0% of that recorded on the *preferred side*, mean 3.1±0.5% (± SEM, n=7), but was insufficient to cause any visible movement on that side. Figure 2d. shows the rectified average of the recordings from one subject during index finger abduction while maintaining a 20% contraction of the wrist and finger extensors on the contralateral side. No increase of rectified EMG was recorded on the contralateral side.

Cross correlation analysis

Cross-correlograms constructed from the times of occurrence of spikes in the EMG signals recorded from the two sides contained no short duration central peak. The absence of a central feature in the correlogram indicates that there is no common synaptic drive to the motoneurones innervating the left and right first dorsal interossei which could account for the bilateral activation of this muscle pair (see section four on mechanisms underlying co-contraction of antagonistic muscle pairs for a detailed description of the use and interpretation of cross-correlation analysis for the detection of common synaptic input).

EXPERIMENT TWO:

Sustained grip recordings

Figure 3 shows an example of raw EMG recorded from one subject at 20, 125 and 205 seconds after the onset of the grip on the preferred side. The preferred side contraction increased with time as the subject recruited an increasing number and amplitude of motor units to maintain the required force, as seen in the lower panel of fig.3. At 20 seconds after the onset of the preferred side contraction at 25% MVF there was no involuntary activation of the contralateral wrist and finger flexors. But as shown in the upper panel of fig.3 at 125 seconds,

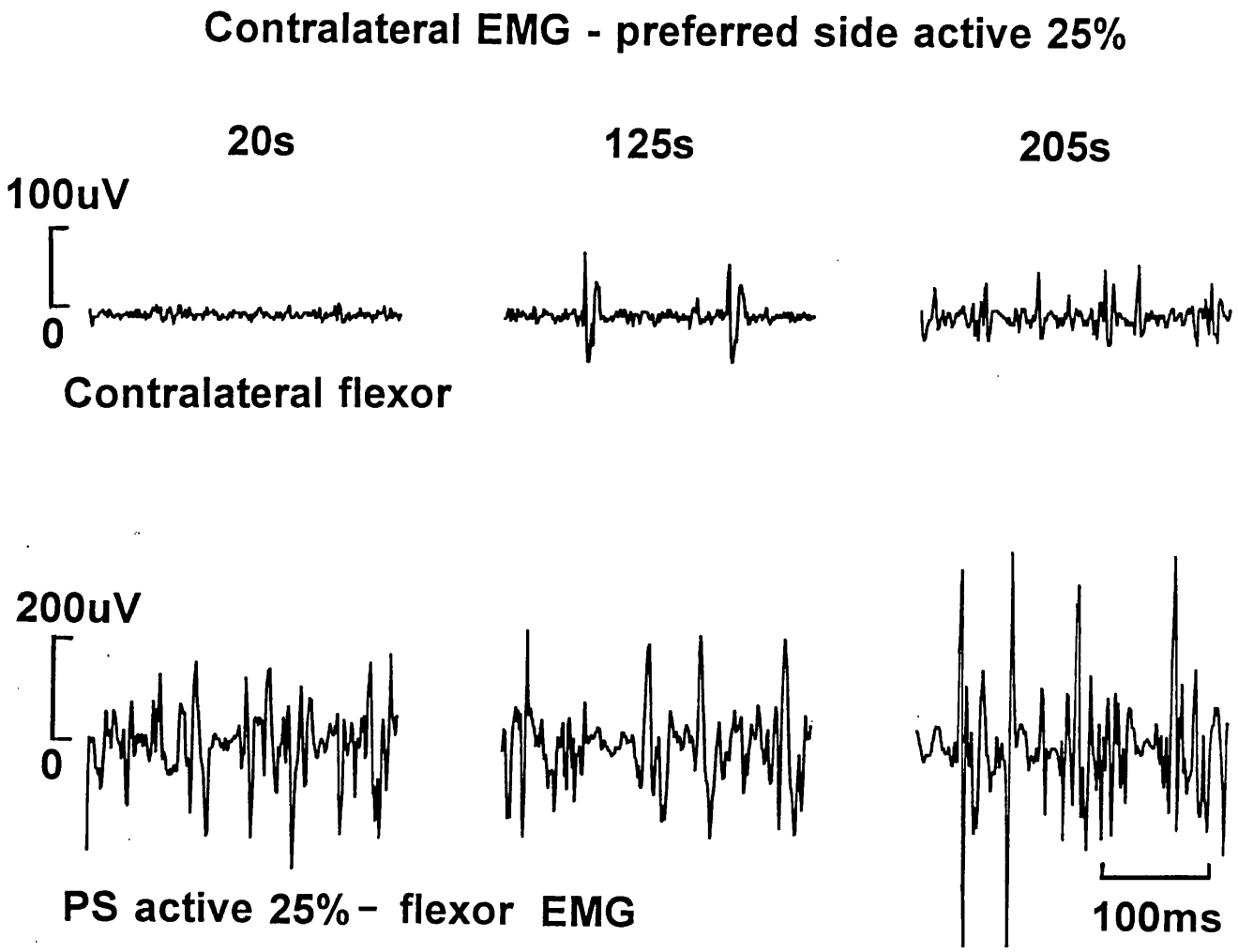


Figure 3: Subjects were instructed to grip the dynamometer in the preferred hand at 25% MVF. The upper panel of fig.3 shows the involuntary EMG recorded in the non-preferred hand. The involuntary EMG commenced at 125s in this subject. The EMG recorded in the preferred hand, shown in the lower panel, increased with time as the subject fatigued and recruited more motor units to maintain the required force.

a single unit has become active, while at 205 seconds there was activity in several motor units. All of the eight subjects showed contralateral EMG on the non-preferred side when the preferred side performed a sustained grip at 25%, 50% and 80% MVF. Involuntary EMG activity was recorded in the non-preferred side wrist and finger flexors in all subjects but only in 7 out of eight subjects in the wrist and finger extensors at 25% MVC; involuntary EMG was recorded in both muscle groups on the non-preferred side at 50% and 80% MVF.

The results for the group of eight subjects are illustrated in fig.4, which shows the time of onset and increase of the mean amplitude of the integrated EMG of the non-preferred side wrist and finger flexors and extensors at 25%, 50% and 80% MVF. The wrist and finger flexors are represented by the dashed line and the wrist and finger extensors by the solid line. On average the EMG appeared first in the non-preferred side wrist and finger flexors with a mean latency of 125±52 (± SEM, n=7) for 25% preferred side contraction, and 33.0 ±5 and 22.3 ±3 (±SEM, n=8) seconds respectively for the 50% and 80% preferred side contractions. This involuntary activity gradually increased until the preferred side contraction ceased at 197.1 ± 11.1, 102.5 ± 7.7 and 95.0 ± 6.7 seconds respectively. At this point the mean integrated EMG of the non-preferred side wrist and finger flexor muscles had reached 5.5±2.3, 7.3±2.3 and 13.1±4.8 of MVC respectively. The mean integrated EMG of the non-preferred side wrist and finger extensors had reached 6.5±3.7, 12.7±4.2 and 15.9±3.0 of MVC respectively. The time of onset and end-point amplitude of the involuntary EMG in the wrist and finger flexors and extensors is summarised in table 1.

Table 1:

Contralateral EMG : preferred side active (n=8)				
Time of onset of involuntary EMG (s)				
	Wrist & finger flexors		**Wrist and finger extensors**	
% MVF	**Mean**	**± SEM**	**Mean**	**± SEM**
25	**125.0**	**52.0**	**125.0**	**41.0**
50	**33.0**	**5.0**	**43.0**	**9.0**
80	**22.0**	**3.0**	**25.0**	**3.0**
End-point amplitude of involuntary EMG (% MVC)				
	Wrist and finger flexors		**Wrist and finger extensors**	
% MVF	**Mean**	**± SEM**	**Mean**	**± SEM**
25	**5.5**	**2.3**	**6.5**	**3.7**
50	**7.3**	**2.3**	**12.7**	**4.2**
80	**13.1**	**4.8**	**15.9**	**3.0**

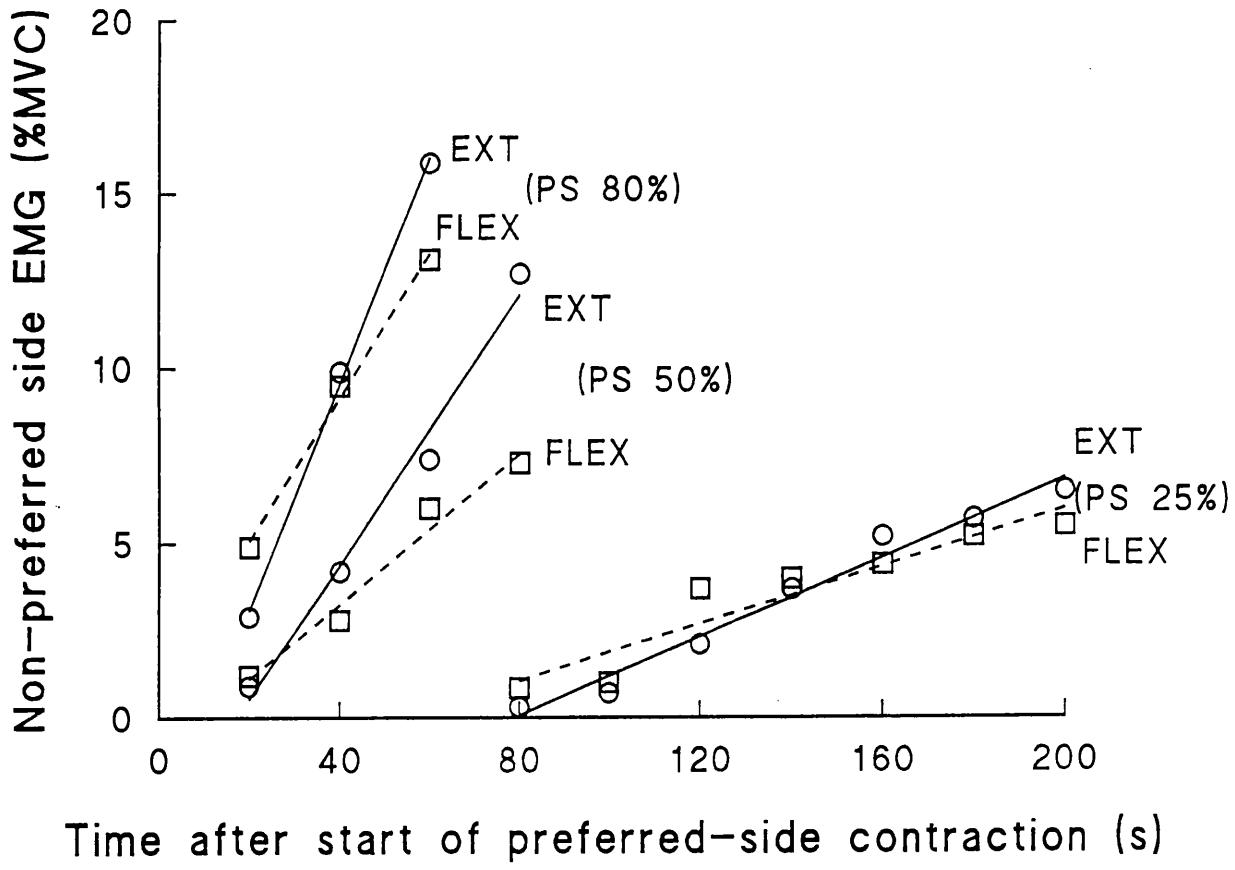


Figure 4: The mean integrated involuntary EMG, (n=8) expressed as a percentage of a maximum contraction for a 20 second sweep of EMG at each grip force 25,50 & 80% MVF is shown. The dashed lines represent flexor activity and the solid lines extensor activity. For stronger preferred side contractions the involuntary EMG commenced earlier and was of greater amplitude.

DISCUSSION

In all of the subjects studied, involuntary contralateral EMG was recorded when unilateral movements were made but this activity was only apparent in the rectified average of at least 100 phasic movements if the subjects maintained a background of 10% MVC.

The record of involuntary EMG contralateral to the voluntarily active hand in this group of adults supports the findings from previous studies (Cernacek *et al*, 1961; Durwen & Herzog, 1992). In those studies EMG was not recorded in all of the trials, but in the present study the subjects maintained a background contraction of EMG contralaterally so that the motoneurones were closer to firing threshold than in the case of the relaxed subjects and thus were more readily activated.

Various mechanisms have been put forward to account for the production of bilateral EMG activity by the CNS. Bilateral cortical activation has been observed in studies utilising measures of regional cerebral blood flow using positron emission tomography (PET) (Shibasaki *et al*, 1993; Kawashima *et al*, 1993), magnetoencephalography (MEG) (Kristeva *et al*, 1991) and functional magnetic resonance imaging (fMRI) (Kim *et al*, 1993 a&b), although such activation was recorded while subjects performed more complex tasks than in the present study. A lack of inhibition across the corpus callosum is another mechanism that has been suggested to account for bilateral activation in cases of pathology such as callosal agenesis and X-linked Kallmann's syndrome (Dennis, 1976; Danek *et al*, 1992). A further possibility to consider is that bilateral EMG could result from activity in the normally occurring uncrossed ipsilateral portion of the corticospinal tract. Studies of stroke patients find some support for this hypothesis. For example a study by Smutok *et al*, (1989), found that reaction times and finger tapping ability were impaired in the "unaffected hand" and suggested that the ipsilateral tract exerted some control over hand movements. Whilst

branching of corticospinal tract fibres to supply bilateral motoneurone pools has been proposed as the mechanism to account for the mirror movements seen in patients with Klippel-Feil syndrome and children with congenital hemiplegia (Farmer *et al*, 1990; Carr *et al*, 1993), such pathways have never been found in normal adults. Therefore, the following discussion will focus on two possible mechanisms of mirrored activity in adults, firstly the possibility of activity being conducted via a normally occurring ipsilateral pathway, and secondly bilateral cortical activation.

Activity in the normal ipsilateral corticospinal tract projection

Evidence from human studies

The ipsilateral corticospinal tract in humans accounts for approximately 15% of the corticospinal projection, but once in the spinal cord further crossing occurs and there remains about 2% which is uncrossed (Wilkinson, 1992). This small portion of the tract is thought to innervate axial and proximal muscles (Brinkman & Kuypers, 1973) and to be of slow conduction velocity. However, there is some evidence to suggest that fast conducting ipsilateral pathways could exert some control over distal finger movements.

Most of this evidence is found in studies of acquired hemiplegia in adults, in which it has been found that the function of the unaffected limb has been affected by the ipsilateral cortical lesion. Colebatch & Gandevia (1989) in their study of stroke patients observed that there was weakness of the muscles in the unaffected arm indicating that the ipsilateral tract was functionally effective. Smutok *et al*, (1989) in their study found deficits in reaction times and finger tapping function of the unaffected hand. Thus it is possible that the involuntary activity recorded in the present study could have been produced by activity in the ipsilateral corticospinal tract, or alternatively it could occur as a result of activity in brain stem pathways

which are influenced by the motor cortex.

One might anticipate that if the ipsilateral tract does exert some control over distal movements that it should be able to be activated using focal magnetic brain stimulation. This has proved difficult. There are reports of an inhibition being demonstrated ipsilaterally (Carr *et al*, 1994; Meyer *et al*, 1995b), and reports of bilateral motor evoked potentials (MEP) in proximal muscles (Basu *et al*, 1994). But Wassermann *et al*, (1994) recently demonstrated that they could evoke ipsilateral responses using Magstim. They were able to record bilateral EMG responses in the first dorsal interosseous muscle of the 6 subjects investigated, although it occurred at later latency (4.5-6.5ms) than the contralateral response. The time of onset of the involuntary activity recorded in the present study was not significantly different from the commencement of the voluntary activity, therefore it is unlikely that the ipsilateral projection or brainstem pathways are responsible for the involuntary activity recorded in the present study. Wassermann *et al*, (1994) also noted that the optimal site for evoking the ipsilateral response was near the representation of the contralateral face, suggesting that its origin is from a different cortical site than the contralaterally projecting population of pyramidal cells. A high output of the stimulator was required (65-90%). Bilateral MEP's in the distal hand muscles have only been demonstrated in the presence of pathology (Farmer *et al*, 1990 & 1991; Cohen *et al*, 1991; Carr *et al*, 1993). In the case of axial and truncal muscles, and those usually acting symmetrically such as masseter and the diaphragm, bilateral MEP's were recorded (Carr *et al*, 1994), supporting the observation of Kuypers (1987) that the projection to those muscles is bilaterally organised.

Evidence from animal studies

An anatomical study using the macaque monkey (Galea & Darian-Smith, 1994) has shown that the ipsilateral projection of the corticospinal tract has its origin in the same areas as the

contralateral projection (frontal area 4, SMA, areas 3a, 3b, 2 & 5, insular and SII). They found that 8.1% of the tract projected ipsilaterally, but that further crossing occurred below the level of the decussation. Aizawa *et al*, (1990) in their study of the monkey recorded from cells in the primary precentral cortex which exhibited activity before and during key pressing movements of the ipsilateral and contralateral digits. Intracortical stimulation of cells in this region elicited bilateral responses in the digits, suggesting that both ipsilateral and contralateral projections to the fingers originated from that site, and thus could account for bilateral activity in the distal muscles.

Taken together, evidence from animal and human studies suggest that it is possible that the ipsilateral corticospinal projection could have some influence on the control of ipsilateral hand movements. But this seems an unlikely mechanism to account for the bilateral EMG activity recorded in the present study in which the task was simple and well practised by the subjects rather than a complex sequential finger task which produced the bilateral motor cortical activity as described by Kim *et al*, (1993 a&b) and Rao *et al*, (1993). Another factor to consider is the fast conduction of the signal producing the involuntary activity such that it occurred at the same time as the voluntary activity. Ipsilateral corticospinal axons do not normally make direct corticomotoneuronal connections (Kuypers, 1973) therefore it is unlikely that the activity recorded in the present study was conducted via the normally present ipsilateral corticospinal tract projection, but rather by bilateral cortical activity.

Bilateral cortical activation

Evidence from human studies

PET and *f*MRI studies have shown that when a unilateral hand movement is intended, that

activity in the contralateral cortex is associated with this task as expected (Roland *et al*, 1982; Deiber *et al*, 1991; Sabatini *et al*, 1993). However studies of more complex finger movement sequences have resulted in significant bilateral activation of the sensorimotor cortex, in particular the supplementary motor area (Kim *et al*, 1993 a&b; Shibasaki *et al*, 1993; Kawashima *et al*, 1993; Rao *et al*, 1993).

In their study of six normal adults performing a sequential finger-thumb opposition task, unilaterally and then bilaterally, using fMRI, Kim *et al*, (1993a) found bilateral activation of the lateral motor cortex. When the right hand only was used the ipsilateral activation was 20 times smaller than when the left hand was moved. Whilst this small ipsilateral activation could have been due to inadvertent movement on the contralateral side the authors stress that the subjects were instructed to make only unilateral movements. A similar finding was obtained by Shibasaki *et al*, (1993) in their PET study of a complex finger-thumb sequential task. A more consistent finding of functional brain scanning studies is the bilateral activation of pre-motor areas such as the supplementary motor area (SMA). It has been shown that there is significant SMA activation in tasks requiring planning, such as an internally cued task, and when complex rather than simple tasks are performed (Rao *et al*, 1993; Shibasaki *et al*, 1993). In contrast to these studies, in the present study subjects were instructed to maintain a 10% MVC background in the contralateral homologous muscle thus raising the firing threshold of the motoneurone pool. It maybe possible that when an individual makes unilateral movements a small command to the "inactive" side is normally produced but is too small to be detected in relaxed muscle. Thus the use of background EMG in this study enabled the small bilateral component of the motor command to be detected.

Further evidence for bilateral cortical activation of premotor areas is found in the study of movement related cortical potentials. Field potentials have been recorded from

electrodes placed on the scalp overlying the motor cortex. The resulting movement associated potentials have been classified into four components: readiness potential, otherwise known as the Bereitschaft potential (N1); premotion positivity (P1); motor potential (P2), and a complex post-movement potential (P2). N and P denote positive and negative deflections. The readiness potential is usually seen 1-1.5 seconds prior to movement onset (Roland *et al*, 1980) and is thought to be associated with activity in the SMA. Shibasaki and Kato (1975) studied the N1 in 9 normal adult subjects during a unilateral movement but found no significant difference between contralateral and ipsilateral responses, and concluded that the readiness potential was bilateral. Tarkka and Hallett (1990) have also recorded a symmetrical readiness potential in their study. Additional support for this finding is found in the study of normal adults performing unilateral and bilateral tasks using measurement of the magnetoencephalogram. The study by Kristeva *et al*, (1991) also demonstrated bilateral premovement fields. In their study of 6 subjects, EMG was also recorded, and was never completely silent in the non-active side during unilateral hand movements. Whilst they found bilateral activation preceding a voluntary unilateral movement, the movement evoked fields occurred only over the contralateral fields during unilateral movements, although mirrored EMG activity was recorded. They suggested that the mirrored motor activity was too small to produce a movement evoked field. It would be interesting to apply the study of movement related fields to the current study. It would be anticipated that bilateral fields would be recorded during a sustained contraction of both sides, but it would be interesting to determine if a difference could be found when the subject produced phasic activity rather than sustained activity as in the current study.

Bilateral cortical activity could result from excitation via the corpus callosum. This seems unlikely since the time of transcallosal conduction time is thought to be about 9ms

(Cracco *et al*, 1989), and the EMG recorded would be similarly delayed. In the present study the contralateral involuntary EMG recorded was found to commence on average at the same time as the onset of the voluntary EMG. In any case, the transcallosal pathway is thought to be inhibitory in adults (Ferbert *et al*, 1992; chapter 3, this section). Further support for the transcallosal inhibition hypothesis is suggested by the work on premovement potentials reported by Shibasaki & Kato (1975). They found that the premovement positivity (P1) was recorded in 6/9 normal adults during unilateral movements. This P1 was recorded ipsilateral to the moving hand, was not recorded in a bilateral task and thus they concluded that the P1 was associated with an inhibitory mechanism to prevent imitative movement on the opposite side during a unilateral task.

Taken together, these reports of bilateral cortical activation are commensurate with the findings of the present study. The involuntary activity recorded must have been produced by activity in a fast conducting pathway given that there was no significant time difference between the start of the voluntary and involuntary activity. No peaks were observed in the cross-correlograms constructed from the EMG recorded from the co-contraction of left and right 1DI. Thus it is unlikely that the involuntary activity was produced by activity in descending axons branching to project bilaterally, or by activity in corticospinal axons whose cell bodies share a common synaptic input to the bilateral homologous motoneurone pools.

Mirrored activity and effort

It has been suggested that mirrored activity is more likely to be seen in activities requiring effort or repetition (Todor & Lazarus, 1986; Durwen & Herzog, 1992). In the present study, involuntary EMG was recorded contralateral to the EMG recorded during grip of the dynamometer using the preferred hand. This involuntary EMG increased with

amplitude when the subject was instructed to maintain higher grip force and thus required more effort. Similar results have been found in other studies (Cernacek, 1961; Armatas *et al*, 1994) and in agreement with the finding of the present study, suggest that the force requirement of the task influences the amplitude of the mirrored activity.

In their study of 8 normal subjects holding a dynamometer in one hand, Hopf *et al*, (1974) recorded EMG contralateral to the voluntary activation when the subjects reached maximal, or nearly maximal effort. In a similar experiment Armatas *et al*, (1994) studied the mirrored activity associated with the exertion of two different finger pressures for the index and the little finger. They studied 22 subjects, finding that greater involuntary force was recorded when a larger voluntary force was produced, and when the weaker finger (little vs index finger) was used. They made no conclusion as to the underlying mechanism of the mirrored activity, but suggested that it was most likely related to a lack of inhibition across the corpus callosum. They further suggested that there are right-left differences in the cortical control of the two hands, such that the left hemisphere is more concerned with skilled movements and the right hemisphere with holding/stabilising movements. This would result in more mirrored activity during skills performed with the left hand because functionally the left has less practice of fine skilled movements than the right. Gandevia *et al*, (1993) in a study of lower limb muscles investigating the effect of a lack of afferent feedback, noted that they recorded "inadvertent mirror contraction" in the contralateral unparalysed limb which increased during the maximal effort on the paralysed side. They made no comment as to the origin of the activity. A similar observation was made by Dimitrejevic *et al*, (1992) in their study of 17 subjects performing ankle dorsiflexion. They found co-contraction of homologous and non-homologous muscles which increased with effort and fatigue. They concluded that this occurred as the result of a non-specific spread of excitation resulting in a

general increase in excitability of the motoneurone pools, such that previously subthreshold input became effective. But in the current study the bilateral activity was only recorded in homologous muscles and did not appear to occur as a result of a general spread of excitation. As in the present study and that of Cernacek (1961), the excitation did not produce a visible movement and was only detected by electromyography.

Excitation in other muscles

The results from the present study suggest that the bilateral EMG recorded is confined to homologous muscles and does not spread to non-homologous muscles. This would appear to support a hypothesis for a bilateral command which is only transmitted to the motoneurones supplying the muscles required for the desired voluntary task. However, recordings were only made from the forearm extensors in 2 subjects. It will be necessary to repeat this section of the study with a greater number of subjects and to investigate a larger number of non-homologous muscles in order to be confident of the restriction of activity to homologous muscle pairs.

Conclusion

This study has shown that phasic and maintained unilateral voluntary movements in man are accompanied by a small activation of contralateral muscles. Whilst it is possible that a fast conducting ipsilateral tract could be associated with the involuntary activity observed, it is more likely that bilateral cortical activity underlies the bilateral EMG recorded in the present study.

Section 1: Chapter 2: Mirror movements in children : a developmental study

SUMMARY

1. Electromyographic recordings were made in the upper limbs of forty-seven normal school aged children while making self paced sequential finger-thumb opposition of the left and then the right hand, with no instruction given about the contralateral side.

2. All of the children aged 4-6 years had mirror movements. The intensity and frequency of the mirror movements decreased with age until by age 11 years, 7% of the children had no mirror movements, 79% had mild mirror (grade 1) movements and only 14 % had marked mirror movements (grade 2).

3. The EMG recorded from the left and right first dorsal interosseous muscles (1DI) was rectified and averaged for 25 sweeps, time locked to the start of the bursts of voluntary EMG. The amount of involuntary EMG showed a significant decrease with age.

4. Cross-correlation analysis was performed in 5 children aged between 5 and 10 years to determine if the bilateral EMG recorded during unilateral movements was the result of a common synaptic drive to the homologous bilateral motoneurone pools. In all cases the correlogram was flat, thus there was no evidence for a common synaptic drive that could account for the bilateral activation of the homologous muscle pairs. The most likely explanation for the mechanism underlying mirror movements in children, is that they are produced by activity transmitted by the fast conducting contralateral projection of the corticospinal tract from both left and right motor cortices.

INTRODUCTION

Associated movements describe the unintended movements associated with voluntary effort in another part of the body. They are also known as motor overflow, synkinesis and motor irradiation. When these movements occur in homologous muscles of the hand, they are known as mirror movements. Such movements are thought to be a characteristic of an immature central nervous system (CNS) and while present interfere with the ability to perform precise hand skills, particularly those requiring intermanual co-ordination. For example, when a young child voluntarily uses one hand to pick up and manipulate an object the same movement maybe observed in the opposite hand occurring involuntarily. The presence of such a movement makes the ability to grasp an object with one hand and manipulate it with the other difficult or impossible. A typical example would be the ability to cut using scissors in one hand, while holding an object in the other. In this case each hand has a different task: one to hold, the other to make opening and closing movements which if mirrored in the other hand prevents the child from holding the object.

Such movements are known to decrease in intensity and occurrence as the child matures (Fog and Fog, 1963; Abercrombie *et al*, 1964; Cohen *et al*, 1967, Connolly and Stratton 1968; Wolff *et al*, 1983; van Sant and Williams, 1986; Lazarus and Todor 1987) . They are often seen in young babies aged 6-8 months who at this stage exhibit strongly symmetrical activity in all limbs and especially in the hands thus preventing unilateral hand skills (personal observation). Associated movements have been observed to decrease markedly after 8 years of age (Wolff *et al*, 1983, Lazarus and Todor, 1987), but may be seen in older children and adults performing difficult or novel fine hand skills, during effort or under fatigue or stress (Podivinsky, 1964; Cernacek, 1961,). If significant mirror movements persist in the hands after the age of 8-10 years of age they are considered to be pathological

(Fog and Fog 1963; Abercrombie 1964; Cohen *et al*, 1967). Specific tests for the presence of these movements are used in the clinical assessment of minimal neurological dysfunction (Touwen, 1978).

Associated movements in children have been studied in a variety of ways (Connolly and Stratton, 1968; Denckla, 1974; Woods and Teuber, 1978; Touwen, 1978; Van Sant and Williams, 1986). Visual examination of the frequency and intensity of such movements during repetitive finger-thumb opposition or clip-pinch task can be scored as present or absent and a scale can be used to monitor changes in the intensity of mirror movements during development and in pathology. The most common scoring system is that of Woods and Teuber (1978), which scores the movements from 0-4; but that of Touwen is also used and scores the associated movements from 0-2. In each case 0 denotes the absence of mirror movements, the highest score in each case indicating symmetrical movements. Other tests include timed finger-thumb opposition, hand pronation-supination, finger spreading, feet to hands, stress gait (walking on heels, toes, lateral border of feet and medial border of feet) and heel-toe alternation.

Denckla (1974), in her study of associated movements in children, used timing of seven motor tasks to assess the co-ordination of a group of 156 normal right-handed children aged between 5 and 11 years. She found that the older children performed the tasks more rapidly, with significant differences between the age groups in the 5-8 year range, after which there was a relative plateau. Another study of associated movements in a group of children aged between 5 and 7 years at 6 month intervals over one year by Wolff *et al* (1983), showed that the frequency of associated movements decreased over the 12 month period with significant correlation between the different tests. Thus they suggested that an assessment of

associated movements could be used to demonstrate the child's developmental stage. The study also demonstrated differences between children of the same chronological age.

To enable a more detailed investigation of the occurrence and intensity of associated movements in children, measurements of force and/or EMG activity during the performance of clip-pinch and finger lift tasks have been undertaken. In their study of a group of children aged between 6 and 16 years, using a modified version of the clip-pinch task of Fog and Fog, Lazarus and Todor (1987) demonstrated that the magnitude of associated movements decreased with age dramatically between age 6.5 and 8.5 years, but that there was no significant difference beyond the age of 8.5 years. They measured the occurrence of associated movements as a percentage of maximum active hand force, making measurements of force bilaterally whilst the children performed the clip-pinch task at 50, 70 and 100% maximum voluntary force (MVF) respectively.

The aim of the present study was to investigate the occurrence, intensity and origin of mirror movements during development. The presence of mirror movements was scored using the criteria of Woods and Teuber (1978), and EMG was recorded simultaneously from left and right first dorsal interosseous and abductor digiti minimi muscles while each child performed a thumb-finger opposition task. Cross-correlation analysis of the EMG signals recorded from left and right co-contracting muscle pairs was performed to determine whether a common synaptic drive to homologous motoneurone pools was present.

A prelimimary account of these experiments has been presented to the Physiological Society (King *et al,* 1996).

METHODS

Subjects

Recordings were made from forty-seven children (25 males) recruited from a local primary school, with local ethical committee approval and written parental consent. In addition verbal consent was sought from the children when appropriate. The children were aged between 4 and 11 years and had no known neurological abnormalities. Four of the children were left handed and the subject group was of mixed ethnic origin.

Assessment of handedness

All children were assessed using a modified version of the Edinburgh Handedness Inventory (Oldfield, 1971). See Appendix B. In contrast to adults who are generally certain about handedness, the children when asked to indicate hand preference verbally could be unsure. Therefore the children were asked to show the examiner how they performed activities such as writing, using a spoon and cutting with scissors. Both hands were tested. They were also asked to demonstrate how they kicked a ball as a further assessment of laterality (Peters 1988).

Assessment of mirror movements

The presence and degree of mirror movements were assessed in each child and graded according the criteria of Woods and Teuber (1978). The child was instructed to sit with their hands in a supinated (palms facing up) relaxed posture on their knees. The child was instructed to oppose each finger to their thumb sequentially. The instruction to the child was: "Tap each finger to your thumb in turn, starting with your index (pointer) finger going down to your little finger and back again, as quickly and as neatly as possible." Demonstration and

verbal encouragement during the task was given when required. Both hands were tested. The mirror movements were scored on a scale of 0-4 (see Chapter one, this section). It was noted that some children held the index finger or all of the fingers of the contralateral hand in extension instead of, or in addition to showing mirror movements; a record was made of the occurrence of such activity.

Assessment of bilateral integration of hand activity

This was assessed in each child who was tested at the school. Each child seated on a chair was asked to place their hands on their knees, one facing down (pronation) and the other facing up (supination). They were then asked to simultaneously move them over and back as quickly and as neatly as possible. Demonstration was given if required. The ability to perform the task in this asymmetrical manner without reverting to performing it symmetrically or clumsily was scored as follows (Njiokiktjien *et al* 1986):

Grade 0 = unable to perform the activity asymmetrically

Grade 1 = able but with some difficulty

Grade 2 = able but with slight hesitation

Grade 3 = able to perform the activity asymmetrically

Electromyographic recordings

Each child sat at a table with their forearms resting on it. The skin overlying the left and right first dorsal interossei (1DI) and the left and right abductor digiti minimi (ADM) were prepared using an alcohol wipe (Sterets). Bipolar surface electrodes (Teca, see Chapter one) were attached to the skin overlying these muscles, and cut down for use with the smaller children so that the centre to centre inter-polar distance was 20 mm. In the very small

children, the active electrode was placed over the muscle belly and the reference on an adjacent area such as the metacarpophalangeal joint of the index finger. The electrodes were held in place with micropore tape.

Each child was instructed to perform 25-40 sequential finger-thumb opposition movements as they had done for the assessment of mirror movements before the electrodes were applied. The child was given verbal instruction and encouragement with demonstration if required, which was usually the case for the younger children. They were instructed to concentrate on the active hand and to forget the other one, which was placed in a mid-pronated position. In some children in whom no contralateral EMG was recorded an attempt to provide background EMG was made by asking the child to hold the roll of micropore tape between the thumb and index finger. Both hands were tested.

The EMG recordings were made using a clinical EMG machine and stored on magnetic tape as in previous experiments (see Chapter one). The signal was amplified and filtered as in previous experiments, but in this case the low cut filter was set at 100Hz to reduce movement artefact from the leads. All of the analysis was performed off-line.

The EMG signal of the left and right 1DI was rectified and averaged time locked to the onset of the voluntary EMG burst taking the most phasic 25 sweeps using averaging software (SigAvg programme, see chapter one). The averaged rectified areas of the voluntary and any involuntary occurring activity were measured and the ratio of involuntary to voluntary areas calculated. The duration of the involuntary and voluntary busts were also measured. The data from left and right ADM were not analysed because the ongoing EMG in the active side did not enable the start of individual phasic bursts to be differentiated. When possible an average was made of those bursts when there was a clear onset of contralateral EMG to enable the latency of the involuntary EMG to be calculated more accurately. In addition the incidence

of sweeps of bilateral EMG was calculated by noting the number of sweeps where a burst of bilateral EMG similar to that on the active side could be clearly seen and expressing this as a percentage of the total number of sweeps. In addition a calculation was made of the number of sweeps where there was bilateral EMG. This was calculated in the following way: when ongoing EMG or an observable burst was present contralaterally it was expressed as a percentage of the total number of sweeps of EMG. In adults performing the same movement with the contralateral hand relaxed, ongoing EMG or bursts of EMG were not observed contralaterally during unilateral phasic finger movements.

Cross-correlation analysis

In five of the children cross correlograms were constructed from the multi-unit EMG recordings from left and right 1DI (see chapter one). The children were instructed to maintain a steady contraction in the left and right 1DI muscles by gently pushing the left and right index finger against resistance, or in the younger children holding a dowel with stickers on it to maintain their interest. Only those parts of the record which contained spikes on both channels were used in the analysis.

RESULTS

In accordance with other studies which have reported that the intensity and frequency of mirror movements decreases markedly after 8 years of age (Wolff *et al*, 1983)), the children were grouped in age bands as follows:

4-6 years: n = 11

7-8 years: n = 19

9-10 years: n = 10

11 years: n = 7

Mirror movements

The mirror movements ranged from marked to absent (graded 0-3, n=88 hands) and were observed to decrease with age. Fig.1 shows a bargraph of the incidence and grade of mirroring in the children for both hands. The small size of the sample did not allow adequate comparison to be made between the left and right sides, nor between the left and right handed subjects. All children aged 4-6 years had mirror movements, 15% with grade 1, 40% with grade 2 and 45% with grade 3. The intensity and frequency of occurrence of mirroring decreased with age until by age 11, 7% had no mirror movements, 79% had grade 1 and 14% grade 2.

It should be noted that the scoring of mirror movements was often made difficult by the occurrence of sustained involuntary extension rather than repetitive flexion movements of the fingers. An example of this extension activity is seen in fig.2a & b. Figure 2a shows a typical example of a 10 year old child with mirror movements. As the active hand (right) moves the fingers of the left hand move into flexion. But in fig.2b, the active hand (left) of this younger child is accompanied by extension of all of the fingers of the right hand.

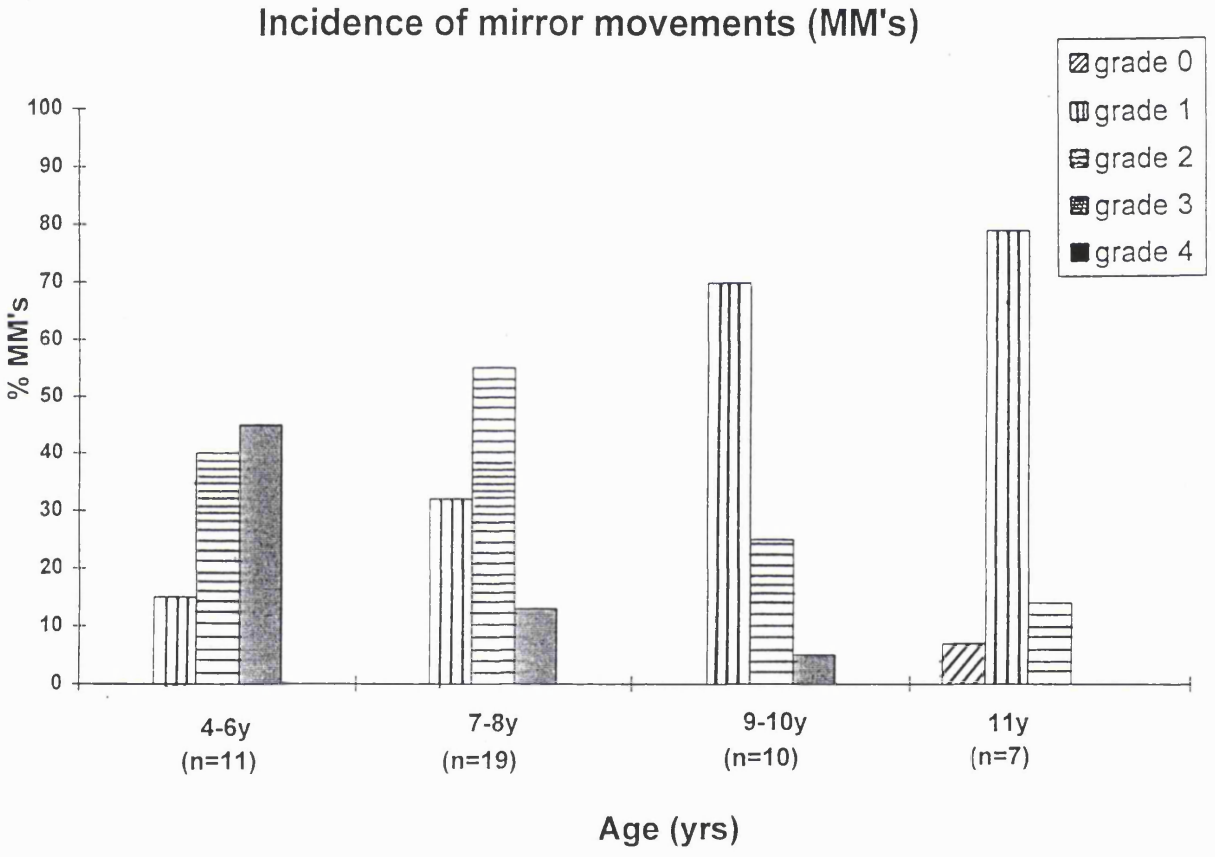
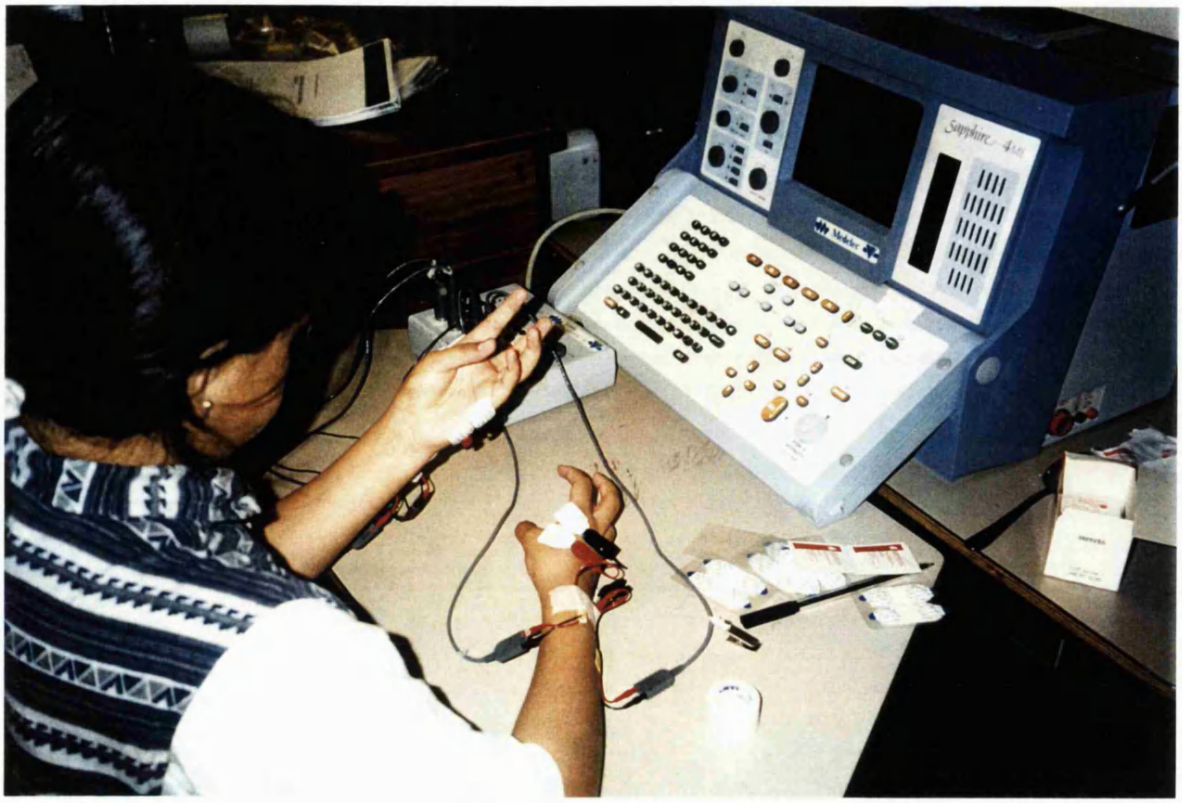


Figure 1: The incidence of mirror movements decreased in frequency and intensity with increasing age. All of the children aged 4-6 years had mirror movements, grade 1 (15%), grade 2 (40%), grade 3 (45%). By age 11 years 7% had no mirror movements, 79% had grade 1 and 14% had grade 2 mirror movements.

a.



b.

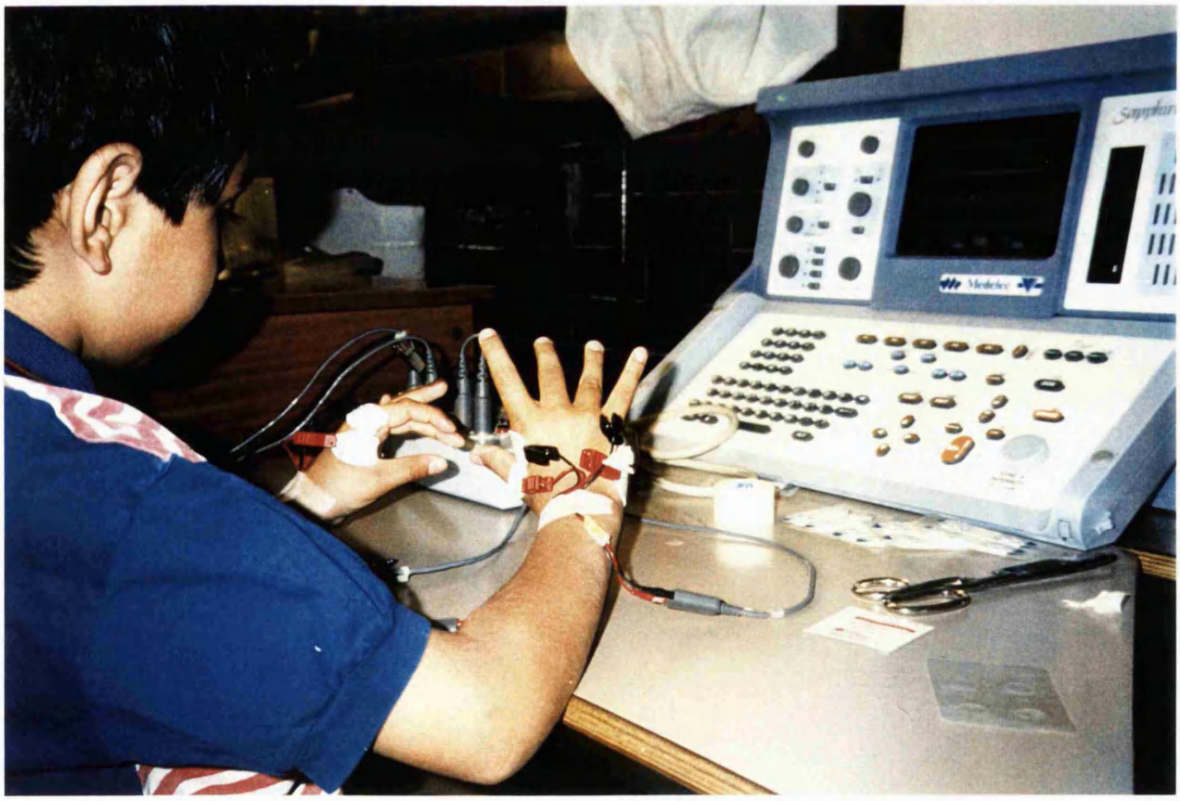


Figure 2: In 66% of the older children aged 11 years mirror movements were seen as flexion of the fingers of the contralateral hand (**2a**). But in 75% of the younger children aged 4-6 years, flexion of the voluntary hand was accompanied by extension of one or all of the fingers of the contralateral hand (**2b**).

The number of children showing extension of one or more of the fingers of the contralateral side decreased with age. The incidence of extension of the fingers in the 4-6 year age group was 75% in contrast to the 11 year age group when the incidence of such activity was 36% . The incidence of extension in the 7-8 year old group and the 9-10 year old children was 55%.

Integration of bilateral hand activity

The ability to perform asymmetrical movements increased with age as seen in fig. 3 The gradient of this relationship is close to unity ($r=0.73$, $P<0.05$, d.f. = 35). Only one out of nine children (11.1%) in the 4-6 year age group could perform the activity correctly compared with 5 out of 7 children (83.3%) in the 11 year old age group.

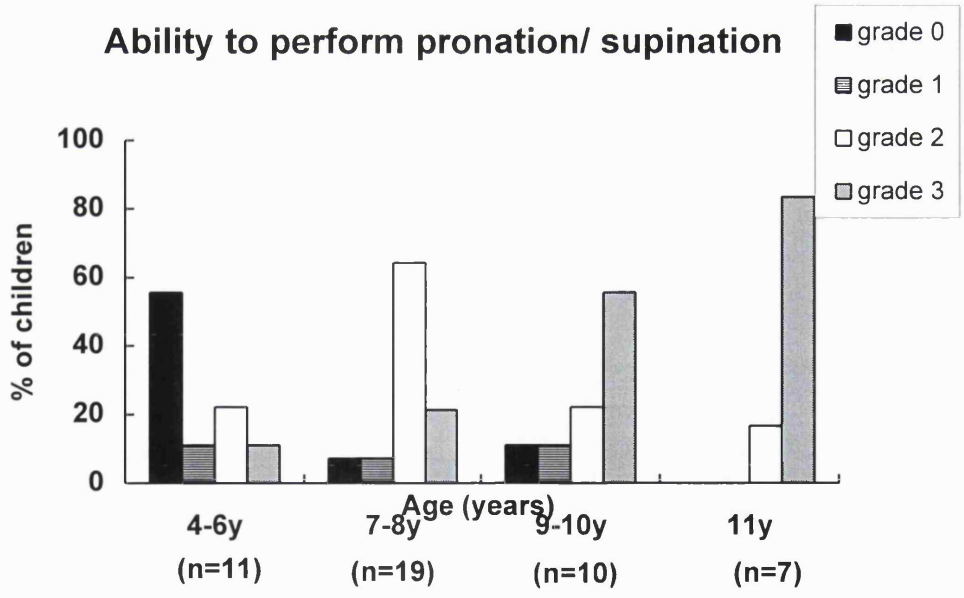


Figure 3: The ability to perform asymmetrical supination-pronation movements increased with age. One out of nine could perform the task correctly in the 4-6 year olds (grade 3, 11.1%) in contrast to 5/7 in the 11 year old group (grade 3, 83.3%).

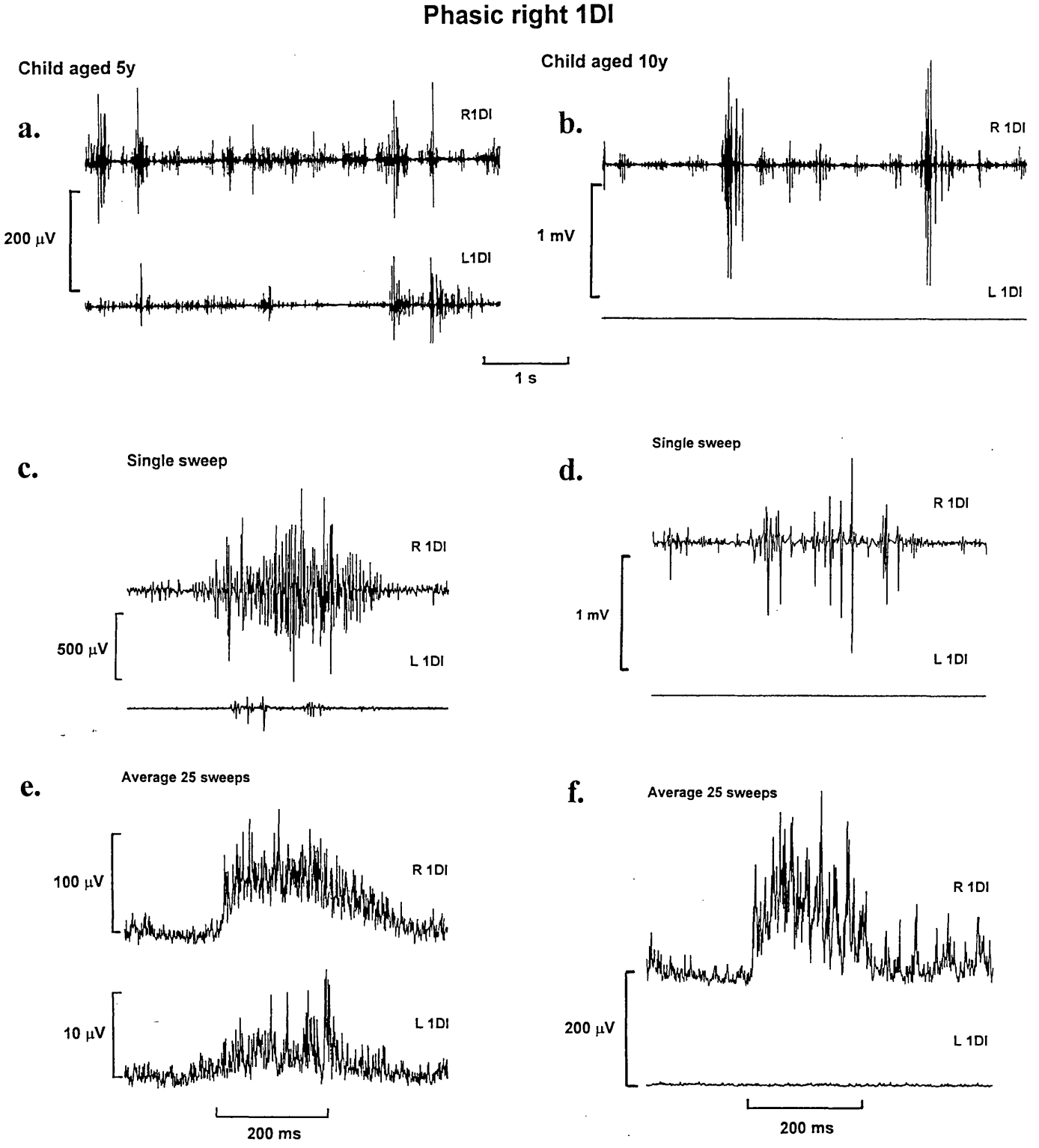


Figure 4: Surface EMG recorded simultaneously from L & R 1DI during sequential finger thumb opposition of the right hand. **a**) 5 year old child; each large burst represents a finger-thumb opposition and is accompanied by EMG on the L side. **b**). 10 year old child. Each burst of 1DI recorded during finger thumb opposition is not accompanied by contralateral EMG. EMG recorded in a single sweep in 5 year old (**c**.) & 10 year old (**d**.). The voluntary and involuntary EMG has been rectified and 25 sweeps averaged time locked to the start of the voluntary burst of EMG of R1DI in the 5 year old (**e**.). In contrast, in the 10 year old no contralateral involuntary EMG was recorded (**f**.).

Occurrence of bilateral EMG

Figure 4a. shows the EMG from several successive phasic bursts on the right (preferred) side in a child who is aged 5 and had marked mirror movements. The upper panel shows the EMG burst recorded during voluntary finger-thumb opposition. The large bursts represent finger-thumb opposition (this child performed double index finger-thumb taps as shown by the adjacent large bursts). The second panel shows the involuntary EMG on the contralateral side. In contrast in figure 4b. recorded from a child aged 10 years, the phasic burst on the active side was not accompanied by contralateral involuntary EMG and appeared to be similar to the adult pattern of activity (see chapter one, this section). A single sweep of EMG recorded from the 5 year old and the 10 year old are shown in fig. 4c. and 4d respectively. Figure 4e. shows the average of 25 sweeps of rectified EMG recorded from left and right 1DI time locked to the onset of phasic right finger thumb opposition in a 5 year old child. The voluntary burst in right 1DI is accompanied by an involuntary increase in EMG in the left 1DI. In contrast, in fig. 4f showing the EMG recorded from a 10 year old child performing the same task, no involuntary contralateral activity was seen during right phasic finger thumb opposition.

The tendency to produce bilateral EMG appeared to decrease with increasing age. Evidence for this is found by looking at the incidence of the occurrence of sweeps of bilateral EMG.

The bargraph in fig.5a shows the incidence of sweeps of EMG in which the voluntary phasic burst was accompanied by an observable burst of involuntary contralateral EMG. In the 4-6 years age band, 14.9% of the phasic bursts were accompanied by similar bursts of smaller amplitude on the opposite side which ranged from 0% to 35.3%. In contrast, in the 11 year age band only 3.5% of the phasic bursts were accompanied by contralateral involuntary EMG.

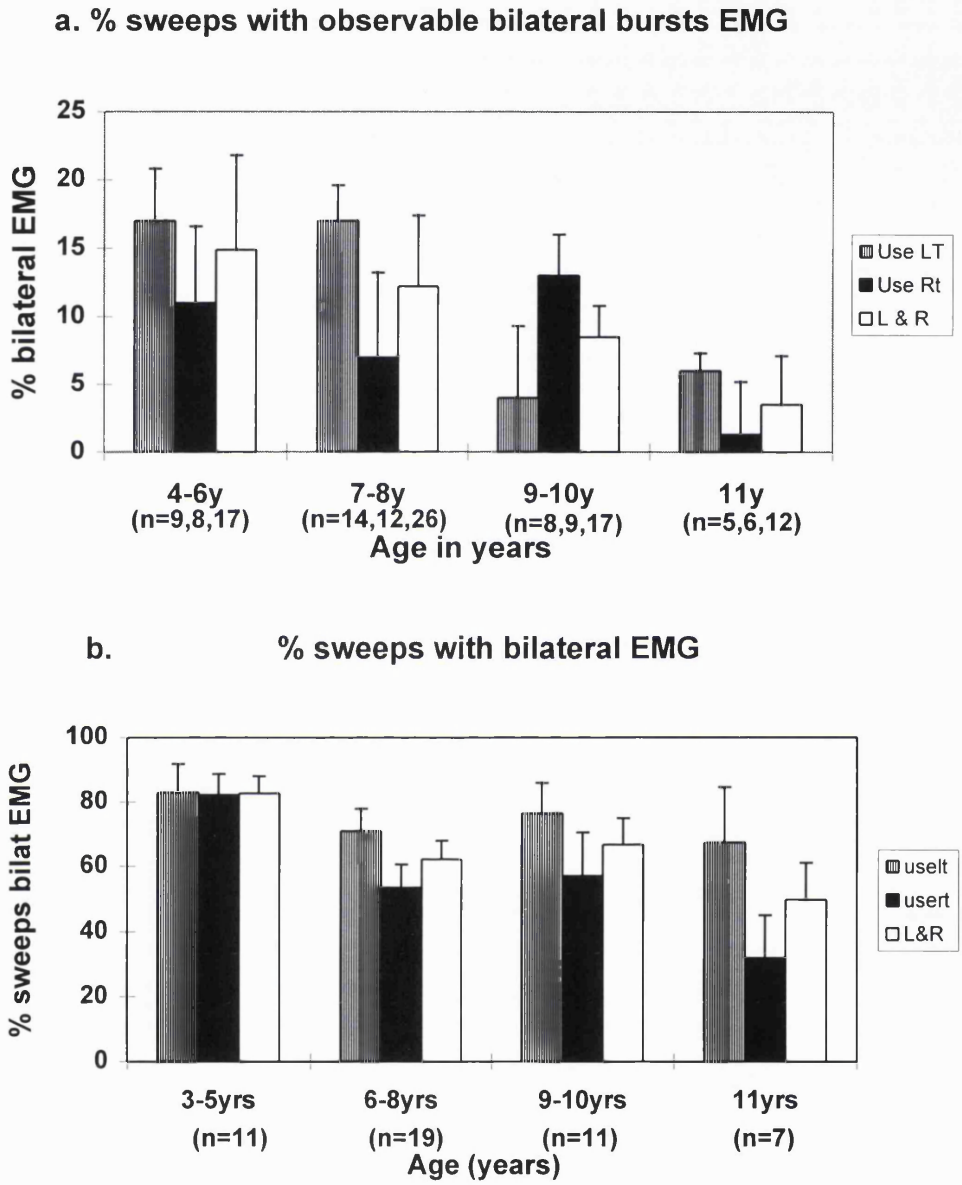


Figure 5: The incidence of bilateral EMG decreased with age. **5a.**Visible bilateral bursts of EMG accompanied 14.9% of the voluntary bursts in the 4-6 year age group in contrast to 3.5% of the voluntary bursts in the 11 year old group. **5b.** Bilateral EMG was present in 82.6% of sweeps in the 4-6 year age group, in contrast to 49.7% in the 11 year old group. One SEM is given.

When considering all sweeps with contralateral EMG, whether ongoing or phasic, the incidence of bilateral EMG was 82.6±5.4 %, (mean ± SEM, n=22 hands) in the 4-6 year age group, in contrast to 49.7±11.4 %, (n=14 hands) in the 11 year age group.The ratios of involuntary to voluntary EMG decreased with age (*r=0.29*) and are summarised in table 1. Fig. 6 shows a bargraph of the ratio of involuntary to voluntary EMG for either hand and both hands together.

AGE	NUMBER OF HANDS	RATIO: INVOL/VOL		TIME OF ONSET OF INVOLUNTARY EMG		
Years		I/V	SEM	(ms)	Range	SEM
4-6	12/18	0.030	0.009	8.2	-21.0 - 40.0	4.5
7-8	18/28	0.027	0.007	19.5	-10.0 - 50.0	4.3
9-10	9/18	0.009	0.003	10.4	-15.0 - 31.5	4.8
11	3/14	0.009	0.006	11.5	9.5 - 15.0	1.8

Table 1: The ratio of involuntary to voluntary EMG decreased dramatically after 8 years of age (0.027 at 7-8 years to 0.009 at 9-10 years of age). The time of onset of the involuntary activity showed greater variation in the ages between 4 & 8 years, with the 4-6 year group having a range between -21.0 and 40.0ms (61ms) and the 11 year old group having a range between 9.5 and 15.0ms (5.5ms).

When the right hand was active the size of involuntary mirrored EMG activity showed a significant decrease with age (*r=0.35,* P<0.05). In the 4-6 year age group 6 out of the nine children produced sufficient phasic EMG bursts to calculate a ratio of involuntary to voluntary activity. The involuntary activity ranged from 0.5 to 3.0% of the activity on the voluntary side, mean 2.2% ± 1.3% (SEM, n=9). Only one of the 11 year age group produced sufficient phasic EMG to determine a ratio of involuntary to voluntary EMG. In this individual the involuntary activity was 3.3% of the activity on the active side. When the left

hand was active, there was also a decrease in the involuntary/voluntary ratio with age but this was not significant ($r=0.26$, P>0.05).

The size of the involuntary/voluntary ratio although greater when the left hand was used, was not significantly different from that obtained when the right hand was voluntarily active (Mann-Whitney *U*-test, P > 0.05).

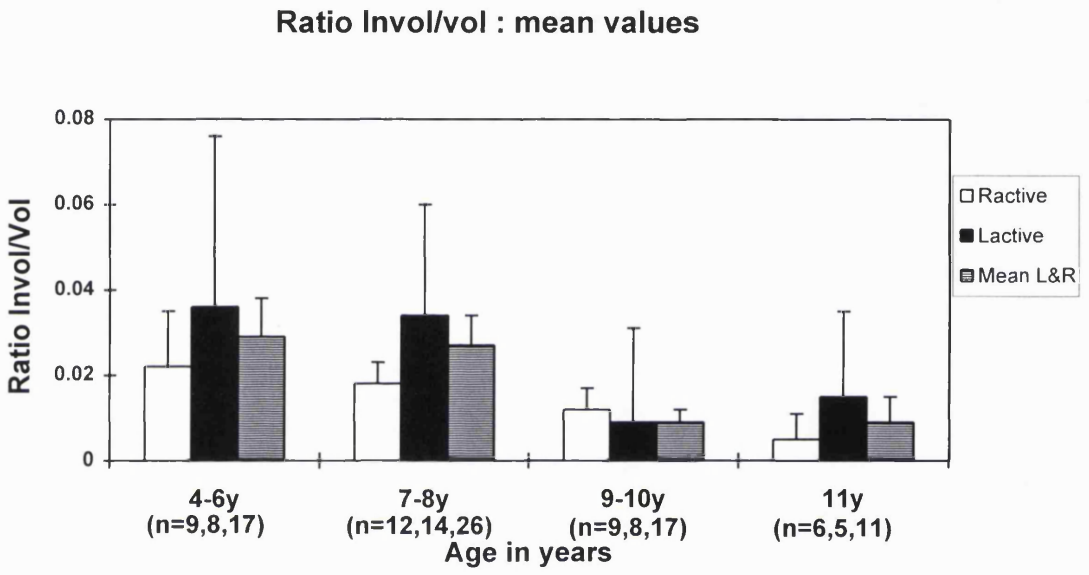


Figure 6: The open bars show the ratio of involuntary to voluntary EMG when the R hand was active. This decreased significantly from 0.022 in the 4-6 year olds to 0.005 in the 11 year olds (r = 0.35. P<0.05). When the L side (black bars) was active a decrease was seen with age but this was not significant (P>0.05). One SEM is given.

The time of onset of the involuntary activity

The time of onset of the involuntary EMG showed great variation in all age groups and in each child. The results for the groups are shown in table 1. In all age groups except the 11 year olds, the EMG could occur earlier than the EMG recorded on the voluntary side (earliest onset -21.0ms in 4-6years; -10.0ms in 7-8years; -15.0ms in 9-10 years), The mean onset latencies of the involuntary activity were 8.2±4.5, 19.5±4.3, 10.4±4.8 and 11.5±1.8 ms respectively. The mean for the group was 12.6±2.3ms.

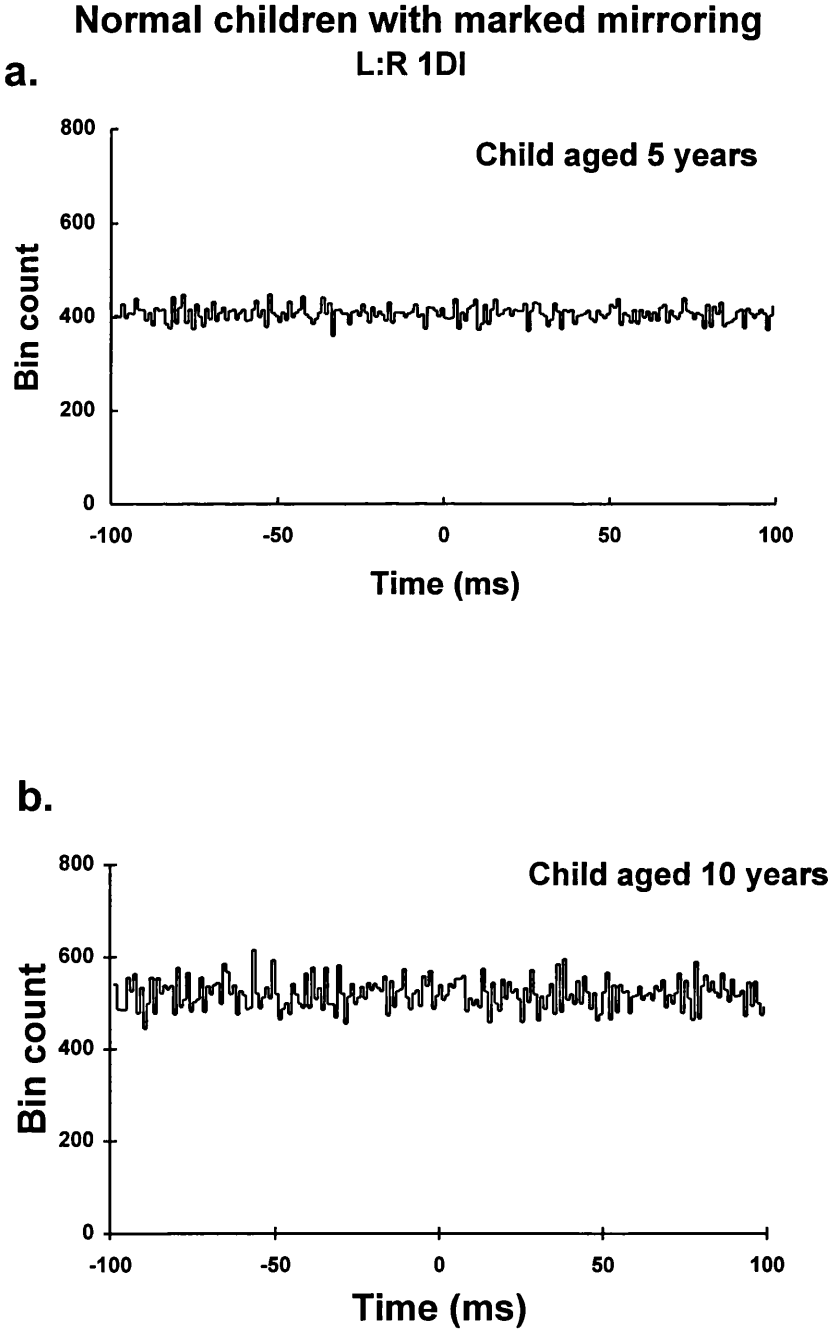


Figure 7: Cross-correlograms constructed from motor unit spikes recorded from left and right 1DI while both hands performed isometric index finger abduction. Each correlogram was constructed from at least 5000 spikes from each side. In two children with marked mirroring aged 5 **(a.)** and 10 **(b.)** years the correlogram was flat.

Cross-correlation analysis

Cross-correlograms constructed from the times of occurrence of spikes in the EMG signals recorded during co-contraction of left and right 1DI during simultaneous index finger abduction contained no short duration central peak. An example from a child aged 5 years and one aged 10 years are shown in fig. 7a&b. In both cases the correlogram is flat, indicating that there is no common synaptic drive to the motoneurones innervating the left and right first dorsal interossei which could account for the bilateral activation of this muscle pair.

DISCUSSION

In the present study it has been shown that the occurrence and intensity of mirror movements decreases with increasing age. No preactivation of muscles was found to be necessary as in the adults (this section, chapter one) to enable these movements to be seen in the majority of the children. In the older children aged 11 years the mirrored activity could only be detected in the rectified average of the EMG recorded from 25 sweeps of finger-thumb opposition. In contrast in the younger children contralateral involuntary EMG could be seen in individual sweeps. No significant difference was found between the left and the right hand, but the sample size is too small to enable an adequate discussion of left-right differences.

Intensity and occurrence of mirror movements

The mean grades of mirror movements decreased with age. In the 11 year age group 86% showed slight or no mirror movements (79% slight; 7% none), whereas the 4 year age group all showed mirror movements (15% slight; 40% sustained; 45% marked). The occurrence of bilateral EMG also decreased with age.

However the scoring of the mirror movements was made difficult by the presence of extension of one or all of the fingers of the contralateral hand which masked the overt mirror movements usually observed. The occurrence of contralateral finger extension showed a decrease with increasing age in a similar way to the mirror movements (75% aged 4-6years; 55% 7-8years; 55% 9-10 years; 36% 11 years), and seems likely to be related to the degree of difficulty the child had in performing the task. In the majority of children in whom this occurred, it was not present initially but occurred with repetition, but in some cases the extension occurred immediately (25% of the children). Activity in non-homologous muscles

has also been reported by Fog and Fog (1963) and Lazarus and Todor (1987). In their study, Fog and Fog do not quantify the incidence of the occurrence of extension rather than flexion of the fingers, but the bar graph of their results (see fig.1, p.53 in Fog and Fog, 1963) indicates that the extension is less apparent in the older children in a light task, but is more readily demonstrated when the child is asked to use a greater pressure of index finger thumb opposition. In contrast to the findings of that and the present study, Lazarus and Todor found that extension of the thumb and/or index finger occurred only in 1.67% of the trials, 50% of which occurred in the 16 year age group. They suggested that this maybe a compensatory strategy to override the mirror movements. Fog and Fog (1963) also used a finger-thumb pressure test but the details of the task are insufficiently described to know if a direct comparison of theirs and the study of Lazarus and Todor (1987) can be made. As discussed in chapter one, adults performing a similar task with the hand grip dynamometer showed greater involuntary EMG at higher grip force, and so this would be the most likely explanation of the result reported here, in which it seems a higher degree of difficulty is consistent with the occurrence of extension in the contralateral fingers. However in both these studies the subjects were performing the clip-pinch task at various magnitudes of force which is a different movement from the self-paced phasic finger-thumb opposition in this study. Interestingly, a similar phenomenon has been observed in a stroke patient who had great difficulty in performing finger-thumb opposition in the affected hand. As she tried to perform this difficult task the fingers of the opposite unaffected hand extended (personal observation).

Duration of the phasic burst

The duration of the phasic burst was longer in the younger children who generally found the task more difficult than the older children, and longer than the adults when the

group was taken as a whole. The duration of the phasic burst decreased as age increased, ranging from 332.6ms in the 4-6 year age group to 237.1ms in the 11 year age group. There was a significant difference in the duration of the phasic burst in the 4-6 year old group and the 11 year old group (Mann Whitney *U*-test, P>0.05).

The longer duration of the phasic burst recorded in the younger children appeared to be related to the difficulty they experienced in performing the task. It was observed that the younger children found the finger tapping task difficult, and appeared to perform it more slowly, less forcefully and could not easily reverse the sequence at the index and little finger, often performing double taps.

The size of the mirrored activity

The ratio of the area of involuntary to voluntary (I/V) EMG decreased as a function of age, with a significant decrease occurring between the 7-8 year age group and the 9-10 year age group. This finding was similar to that of Lazarus and Todor, (1987) who found a significant decrease in the occurrence of mirror movements accompanying clip-pinching at forces of 50, 70 and 100% MVF between 6.5 and 8.5 years of age, followed by a plateau at 9 and 10 years of age.

It has been suggested that there are differences in the amount of mirroring according to whether the right or the left hand is active (Todor & Lazarus 1986; Lazarus & Todor, 1987;1991). In the present study, a gradual decrease in the size of the I/V ratio can be observed when the right hand is active. This is in contrast to the finding of a significant decrease in the I/V ratio between 7-8 years and 9-10 years when the left hand is active. It is however difficult to directly compare use of the right and left hands in the present study as the size of the phasic burst when the left hand was active was significantly smaller (P< 0.05).

The time of onset of the involuntary EMG

In all age groups there was a wide range of onset latencies, the range of which decreased markedly after 8 years of age (see table 1). In each subject the involuntary activity could occur at the same time as the voluntary activity suggesting that it could be transmitted via a fast conducting pathway, such as the corticospinal tract which is known to be responsible for the production of individual finger movements.

But the mean time of onset of the involuntary EMG was 12.6ms later than the start of the voluntary burst. This suggests that the activity could be transmitted via a slower conducting pathway such as the ipsilateral corticospinal tract, or via less direct pathways eg. cortico-reticulospinal projections. A more likely explanation for the delay in the onset of involuntary EMG could be that the motoneurones of the contralateral homologous muscle pool take longer to reach firing threshold. No significant latency difference was found in the onset of involuntary activity in the adults studied in chapter one who maintained a background contraction contralateral to the phasically active hand. However the children performed the task with the contralateral muscle relaxed and the motoneurones were at a lower resting membrane potential, therefore taking longer to reach firing threshold. In the younger children who found phasic activity more difficult than the older children the range of latencies was longer (-21.0 - 40.0 ms 4-6 years; 9.5-15.0 ms in the 11 year age group).

Nass (1985) suggested that function in the uncrossed pathways may be the cause of the mirror movements observed during childhood. She suggested that these pathways gradually are inhibited by the contralateral hemisphere via the transcallosal pathway. She reached this conclusion after studying mirror movements in 18 subjects with hemiplegia, aged 6-20 years. She suggested that the mirror movements were due to an enhanced ipsilateral tract from the surviving cortex, and that this pathway could not be inhibited due to

the absence of callosal fibres from the damaged cortex. This explanation is difficult to accept in the light of other investigations of children with congenital hemiplegia and mirror movements. In their study Carr *et al*, (1993) demonstrated that the corticospinal fibres projecting from the surviving cortex had branched to innervate left and right motoneurone pools of homologous muscles, thus resulting in mirror movements. In this case there is an abnormal ipsilateral projection rather than an enhancement of the normally occurring ipsilateral tract. This explanation seems more logical than Nass's explanation, which on the other hand would require that during development the ipsilateral tract is inhibited via activity from the opposite hemisphere. This could only occur if both motor cortices were active (see figure 2, p.1061, from Nass 1985).

Studies using blood flow measurement have shown that the premotor areas are bilaterally active during learning of a task, or during the planning and execution of a complex task. This raises the possibility that in the child the premotor area distributes a bilateral command. In the absence of transcallosal inhibition this would result in the bilateral activation of homologous and heterologous muscles, resulting in the involuntary activity associated with mirror movements during development. According to this mechanism we can suppose that as the child's neural development progresses and as a particular skill is practised, the neural control of the task becomes more selective and lateralised.

Further evidence for bilateral cortical activity is found in the study of the developmental changes in movement related cortical potentials by Chisholm & Karrer (1988). They studied readiness potentials (RP) in 13 children who were further sub-divided into age groups, and 5 adults performing a finger lift task. From their previous studies they had hypothesised that increased positivity of the preresponse readiness potential components was related to a lack of motor control. They applied this hypothesis to their subject group and

found that a slow positivity overlapped with the negativity of the RP. They concluded that this positivity might be related to the effort required to inhibit the associated movements. The mirror movements recorded in the present study are a sign of a lack of motor control in that they prevent the performance of efficient bimanual tasks. Thus the study described by Chisholm & Karrer (1988) would appear to be in agreement with the findings of the present study, in which the mirror movements decreased with age in line with the reduction in the size of the positivity related to the inhibition of associated movements reported in that study.

The origin of the mirrored activity

In agreement with other studies, the results from the present study have shown that mirror movements decrease with intensity and frequency during development. The cross-correlogram constructed from the times of occurrence of motor unit spikes in the left and right 1DI were flat indicating that the bilateral EMG was not due to common synaptic drive to the bilateral homologous motoneurone pools. Indeed the results from the cross-correlation analysis indicate that there are separate sources of synaptic drive which produce the bilateral EMG recorded in the present study. Taking these observations together it seems most likely that the simultaneous activity in the left and right hand occurs as a result of bilateral cortical activity.

Conclusion

The results of this study show that the mirror movements which occur during development decrease with frequency and intensity during development. The most likely mechanism to explain their occurrence is that they are produced by activity transmitted by the fast conducting contralateral projection of the corticospinal tract from both left and right

motor cortices. The activity of the ipsilateral cortex is probably suppressed during development as a result of the development of interhemispheric inhibition. This becomes more effective due to myelination of the callosal fibres and is investigated in the following chapter. In addition to maturation of these fibres, the development of more precise motor programmes as a result of practise could contribute to more precise lateralised patterns of neural control to produce the skilful activity which emerges during development.

Section 1: Chapter 3 A study of interhemispheric inhibition in adults and children using focal magnetic brain stimulation.

SUMMARY

1. In the first of a series of two experiments, two magnetic stimulators were used, one to deliver a conditioning stimulus to the motor cortex controlling the non-preferred hand and the other to give a test stimulus to the contralateral cortex in a group of 5 adults and 5 children. The conditioning shock was given at several delays (7-15ms in the adults; 10-20 ms in the children) before the test stimulus to the opposite cortex to determine if there was a decrease in the size of the resultant magnetically evoked potential (MEP).

2. When the test stimulus was preceded by a conditioning shock to the contralateral cortex a reduction in the size of the MEP produced by the test stimulus was found in all of the adults when the delay was of 7ms or longer. This was not found in the children.

3. In the second series of experiments, one magnetic stimulator was used to investigate the effect of a test shock to the ipsilateral cortex on a sustained contraction of the first dorsal interosseous muscle in a group of 5 normal adults and one subject with complete callosal agenesis, confirmed by functional magnetic resonance imaging (fMRI).

4. In all of the adults a decrease of ongoing EMG was found at a mean latency of 40.8 ms and lasted for 25.1 ms (mean, n=5). In the subject with callosal agenesis no such decrease in ongoing EMG was observed when either motor cortex was stimulated.

5. Taken together, the results show that the interhemispheric inhibition described in these experiments is most likely produced by activity in the transcallosal pathway. In children less than 11 years of age, interhemispheric inhibition maybe absent, or if present occurs at a longer latency than in adults.

INTRODUCTION

The results of the experiments described in the previous two chapters raise the possibility that the transcallosal pathway may play a part in the mechanism underlying the presence of the bilateral EMG activity recorded during unimanual tasks in adults and children. Some have suggested that excitation via the transcallosal pathway could account for the bilateral activity recorded from homologous muscle pairs during a unimanual task (Britton *et al*, 1991). Others have suggested that a lack of inhibition via this pathway is responsible. For example, Rothwell *et al*, (1991) using the conditioning-test paradigm described by Ferbert *et al*, (1992), could not demonstrate the inhibitory effect that magnetic stimulation of one motor cortex has on the magnetically evoked potentials (MEP's) evoked from the other cortex in the hand muscles of a patient with agenesis of the corpus callosum and mirror movements. They concluded therefore, that this inhibitory effect was mediated by the corpus callosum, and that the lack of interhemispheric inhibition via this pathway was responsible for the mirror movements observed in this patient. A number of authors have also reported bilateral activity in patients with callosal agenesis, but not all patients with callosal agenesis have mirror movements (Meyer *et al*, 1995a).

In their study Ferbert *et al*, (1992) used two Magnetic Brain stimulators connected via a Bi-stim module and performed a condition-test technique to determine whether excitation of one cortex (conditioning stimulus) delivered at various times before excitation of the opposite cortex (test stimulus) could affect the size of the MEP's evoked in the hand muscles by the test shock. They demonstrated that the effect of the conditioning stimulus was inhibitory when delays longer than 5-6 ms were used, compatible this effect being mediated through the corpus callosum which has a conduction time of about 8-9 ms (Cracco *et al*, 1989). They also investigated the effect of a cortical shock on a sustained voluntary

contraction. While the subject maintained 10-15% MVC, magnetic stimuli were delivered to the hand area of the motor cortex ipsilateral to the contraction. A reduction in ongoing EMG in 1DI to 25% of control level was observed at a latency of 13.4 ± 3.2ms (mean ± SD) after the minimum conduction time from brain to muscle. Recently Meyer *et al*, (1995b) have also investigated the effect of ipsilateral cortical stimulation on ongoing EMG in the distal hand muscles (left and right 1DI). They studied 10 normal subjects, 7 subjects with callosal agenesis, and 3 with acquired abnormalities of the corpus callosum. In all of the normal subjects a reduction in ongoing EMG was observed ipsilateral to the cortical stimulus at a latency of 36.1 ± 3.5 ms (mean ± SD). In contrast, an inhibition was not seen in all of the patients with the anterior half of the corpus callosum absent, and the inhibition was weak or of different latency in the remaining subjects suggesting some abnormality of transcallosal transmission.

In the present study, the techniques of Ferbert *et al*, (1992) and Meyer *et al*, (1995b) have been used to determine whether the inhibitory effects of ipsilateral cortical stimulation are present in young children showing mirroring. Recordings have also been made in one subject with complete callosal agenesis.

Some of the results of these experiments have been presented to the Physiological Society (King *et al*, 1996).

METHODS

Subjects

Recordings were made from a group of 5 healthy adult volunteers aged between 20 and 46 years of age (2 male) with informed consent and local ethical committee approval. A group of five children aged between 6 and 10 years of age were also studied and written parental consent was obtained in addition to the child's consent when appropriate. One patient with complete congenital callosal agenesis confirmed by MRI was also studied.

Assessment of the presence of mirror movements

The presence of mirror movements was assessed in each individual according to the criteria of Woods and Teuber (1978) as in previous experiments (see chapter one of this section).

EMG recording

Surface EMG was recorded from pre-gelled electrodes attached to the skin overlying the first dorsal interosseous muscle of the dominant hand in each subject (one left hand dominant). The signals were amplified and filtered and stored on magnetic tape for future analysis.

Investigation of interhemispheric inhibition via the callosal pathway

Recordings from adults

Focal magnetic brain stimulation was performed using two double-coned coils (each coil 70mm diameter) with two Magstim 200 Stimulators and a Bistim Module (The Magstim Company, Dyfed, UK). One coil was used to condition the response evoked by discharging the second (test) coil according to the protocol of Ferbert *et al*, (1992). One coil was placed over the hand area for 1DI of the left motor cortex (test) and the other over the 1DI

representation on the right motor cortex (condition) according to the modified 10-20 system as described by Carr *et al*, (1994); see section 2, chapter one. For each coil the threshold for a response in 1DI was determined. The threshold for a response was defined as that output of the stimulator required to produce 5 sequential responses in the contralateral muscle at a gain of 200μV per division. The output of the stimulator was then increased to +5% for the test stimulus and to +10% for the conditioning stimulus. Using the Bistim Module the conditioning stimulus was randomly presented at intervals of 3, 7, 10, 12 and 15 ms before the test stimulus, and for each delay interval 20 conditioned and 20 non-conditioned stimuli were presented in random sequence. The responses were rectified and the areas of the conditioned and non-conditioned responses were measured. Unpaired *t*-tests were used to determine whether there was a significant difference between the size of the conditioned and non-conditioned responses. The areas were also expressed as a percentage of the mean conditioned to mean non-conditioned, when the mean non-conditioned response equals 100%. Stimulator output ranged from 40-65%. Threshold determined in relaxed muscle.

Recordings from the children

The procedure was carried out as in the adults, but in the case of the children different condition-test intervals were used to account for the assumption that late myelination of the corpus callosum (Yakovlev and Lecours, 1967) would extend the usual conduction time of around 10ms. Therefore intervals of 10, 15 and 20 ms were presented randomly. The children were instructed to maintain a 10% contraction of the R 1DI as it is rare to elicit motor evoked potentials (MEP's) in children (Koh & Eyre, 1988). They were provided with visual feedback from a root-mean-square (RMS) voltmeter. Measurements were made as for the adults but because background EMG was used, the response was normalised by dividing the area of the response by the mean amplitude of the rectified background EMG. Output range: 40-80%.

Recordings from the subject with callosal agenesis

One female subjects aged 30 years whose MRI scan revealed that there was a complete callosal agenesis was studied using the protocol of Meyer *et al*, (1995b). In addition 5 normal controls were studied. The normal subjects were instructed to maintain a steady background of 20% MVC of the preferred hand. A figure of eight coil was used to deliver magnetic shocks to the cortex to evoke responses in the 1DI of the non-preferred hand, that is to the cortex ipsilateral to the preferred hand. The output of the stimulator was set at the threshold to produce 5 consecutive responses at 200μV, plus 20%. An average of 20 responses was obtained and any decrease in ongoing EMG calculated as a percentage of background. Both sides were tested in the patient with callosal agenesis.

The control subjects were aged between 20 and 45 years (3 male). The threshold was determined in relaxed muscle and the stimulator output was between 30 and 50% for the controls but 70% was used for the subject with callosal agenesis.

RESULTS

Mirror movements

None of the normal adults had mirror movements. The mirror movements in the children were mild to marked (grade 1-2). The patient with callosal agenesis did not show any mirror movements.

Interhemispheric inhibition using Bistim

Recordings from the adults

Figure 1a & b shows an example of the result obtained from one right handed subject at a 10 ms delay between the conditioning shock and the test stimulus. Fig. 1a shows the average of 20 sweeps of EMG in which only the test stimulus was applied to the left motor cortex (area = 1.4mV.ms). Fig. 1b shows the average of 20 responses in which the test stimulus was preceded by the conditioning stimulus. It is smaller than the average rectified area of the test only condition 0.2mV.ms; con/ncon = 12.3%). The results obtained from the group of normal adults are shown in table1 and fig.1c. In the adults a significant difference was found between the conditioned and non-conditioned responses at 7 and 10ms ($P<0.05$). There was much inter- and intra-subject variability, with the coefficient of variation ranging from 24.4 to 155.9% in the area of the responses obtained. The graph in fig. 1c shows the individual results; the asterisk denotes those results in which a significant difference (unpaired t-test, $P< 0.05$) was found between the mean area of the conditioned and non-conditioned responses.

Delay (ms)	Mean con/ncon%	Range	SEM
3	95.4	73.1-121.5	8.1
7	70.0	20.2-95.4	13.5
10	53.7	12.6-95.5	13.9
12	65.4	25.9-103.1	16.2
15	79.9	27.0-116.9	15.9

Table 1: (n=5 at all delays used)

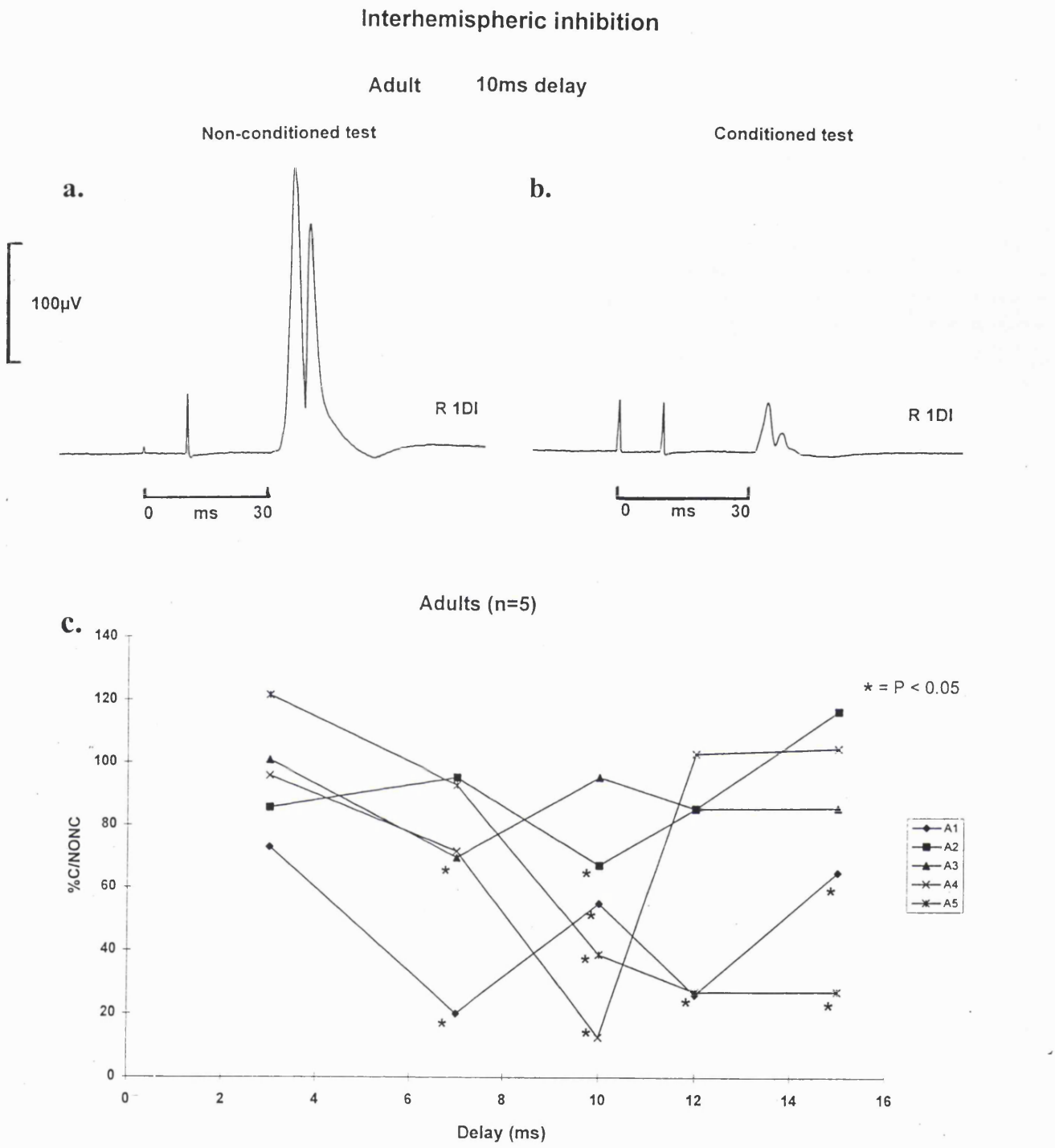


Figure 1: Responses evoked in R1DI in a normal subject using Bistim. **a.)**response when test stimulus only given; **b**.) response when test stimulus is conditioned by stimulus to the opposite hemisphere resulted in a significantly smaller response (average, n=20). **c**.) graph of the individual results at each delay. Considerable variation in the size of the conditioned and non-conditioned responses was found.

Recordings from the children

The children showed a similar degree of variation to the adults, COV = from 14.8 to 129.2 %, but no significant reduction in the size of the conditioned response was measured. Fig. 2a &b shows the average of 20 non-conditioned responses. Fig. 2b shows that in contrast to the adults no reduction of the mean area of 20 conditioned responses was recorded. Fig. 2c shows a graph of the individual results for the children compared with the group means for the adults. The 7 year old child showed a decrease in the conditioned responses at 15 and 20 ms delays, and the 9 year old child at 20ms delay. In the remaining children and intervals, the conditioned response was larger than the non-conditioned response.

Stimulation of the motor cortex ipsilateral to the hand sustaining 20% MVC:

In all of the adults tested there was a decrease in ongoing EMG in the hand ipsilateral to the stimulated cortex, ranging from 24.8-56.3% (37.9 $\pm$ 5.4; mean $\pm$ sem, n=5) which lasted for 25.1$\pm$2.8ms. In contrast to the normal adults, in the patient with complete callosal agenesis, there was no evidence of a reduction in ongoing EMG. Fig.3 a&b shows examples of the results obtained from a normal adult and the patient with callosal agenesis. Fig. 3a shows that in the normal adult there is a decrease (56.3%) in ongoing EMG at a latency of 38ms, 18ms later than the MEP in the left 1DI, suggesting that the magnetic stimulus has resulted in a reduction of the activity from the opposite cortex presumably via the transcallosal fibres. Fig.3b shows that there is no decrease in the ongoing EMG when the motor cortex is stimulated, suggesting that the conditioning stimulus to the ipsilateral cortex did not affect the output of the contralateral cortex, indicating that the absence of the transcallosal pathway prevents the interhemispheric inhibition observed in the normal adults.

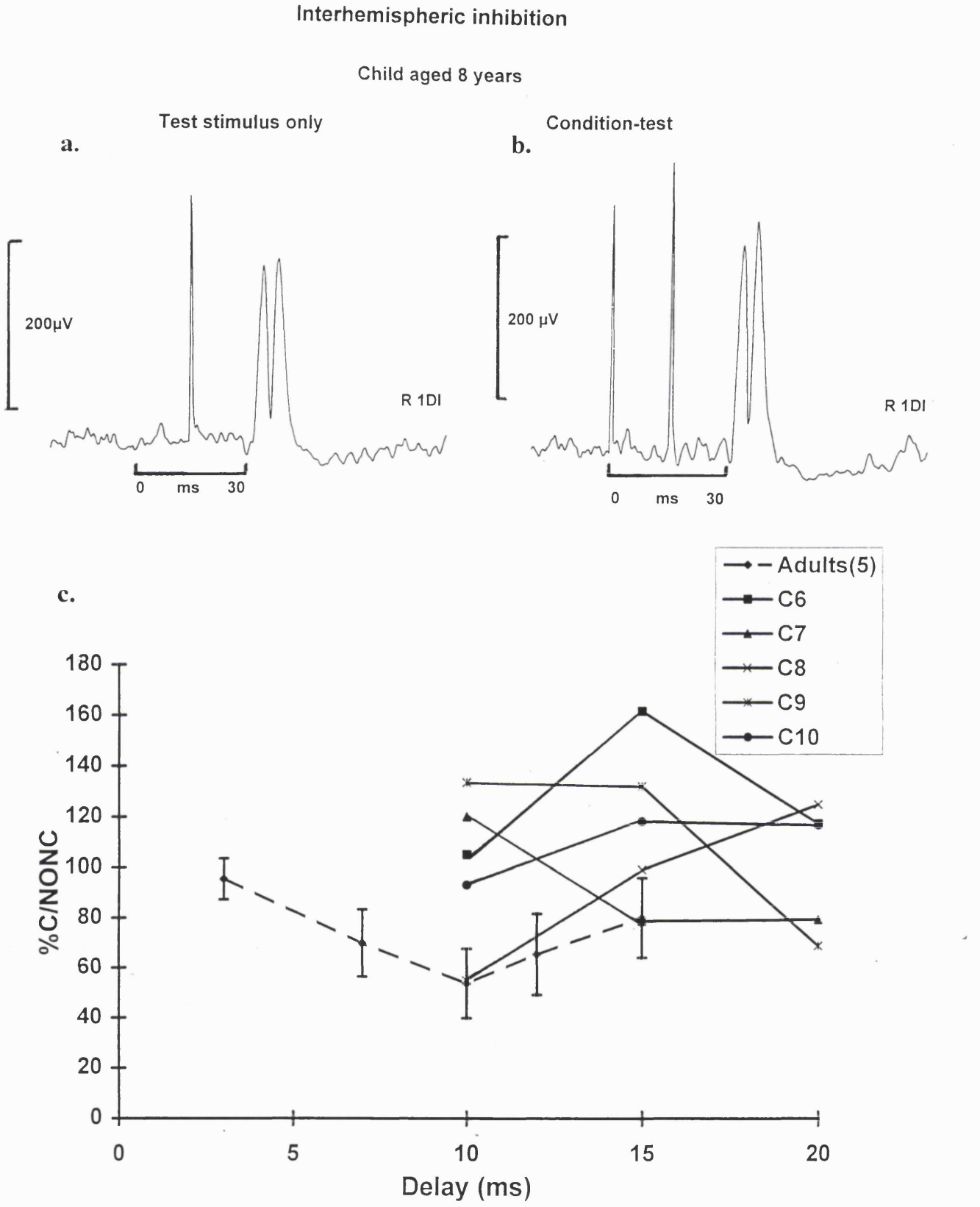


Figure 2: Responses evoked in preactivated R1DI using Bistim in child aged 8 years at 15ms delay. There was no significant difference in the mean area of 20 responses when the test stimulus only (**a**) was presented and when the test stimulus was preceded by, **b**) 15ms with a conditioning shock to the opposite cortex. In **c**) the mean of the %con/ncon responses for the adults (±SEM) are shown with the individual children's results.

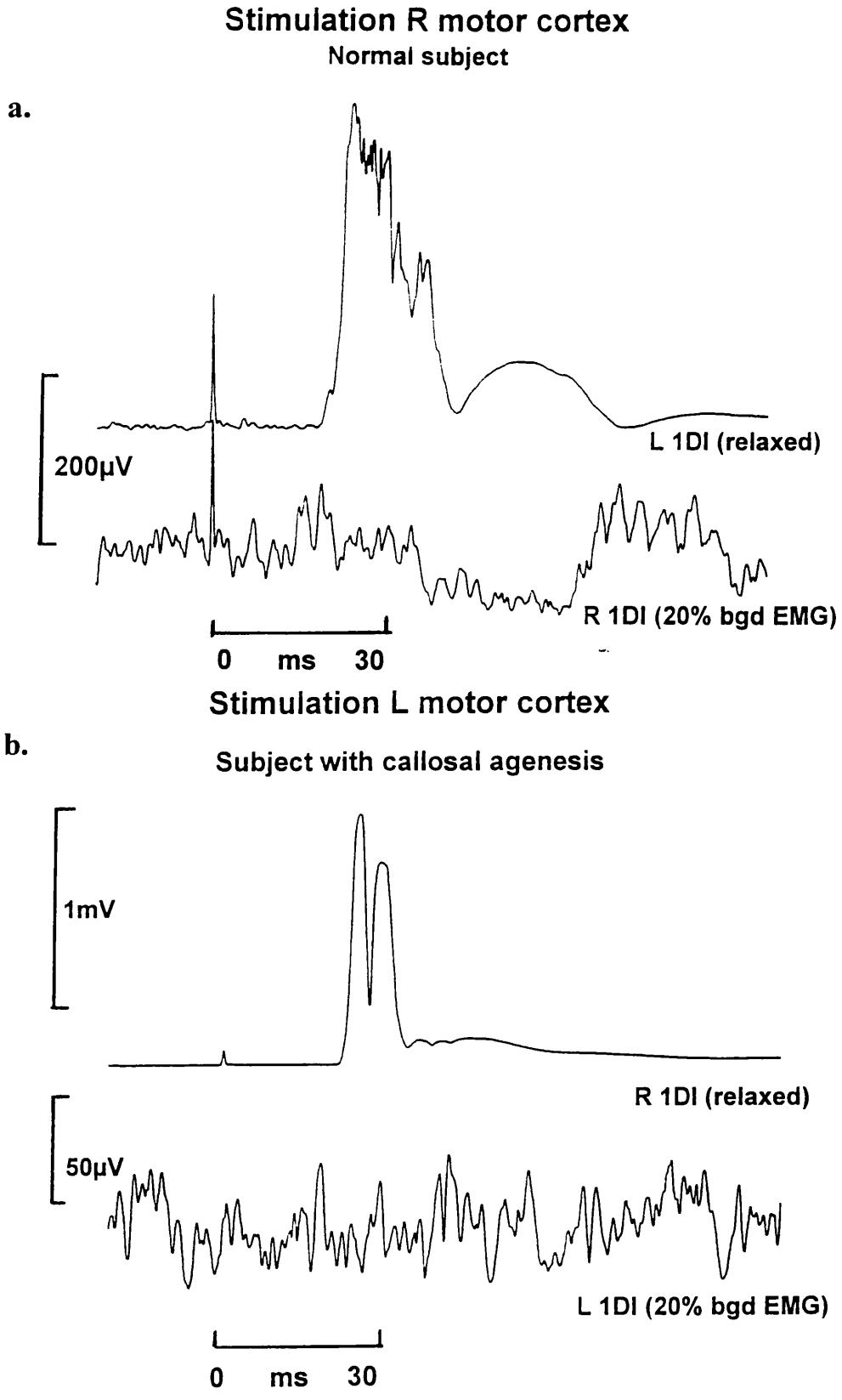


Figure 3: Responses evoked in L & R 1DI, following stimulation of the right motor cortex. **a**) Shows an inhibition of ongoing EMG recorded in a normal subject at a latency of 38ms, **b**) shows that no inhibition was recorded in the subject with callosal agenesis when the left motor cortex was stimulated.

DISCUSSION

In the adult subjects a significant decrease in the MEP's evoked by stimulation of one motor cortex in response to the conditioning stimulus of the other motor cortex was found at delays of 7 - 15 ms and was greatest at the 10ms delay (53.7 ± 13.9%, mean ± SEM, n=5). This is consistent with an effect transmitted via the transcallosal pathway which is reported to take 8-9 ms and have a duration of 7-15 ms (Cracco *et al*, 1989). The inability to demonstrate a similar response in the normal children and the subject with callosal agenesis would also suggest that the decrease in the size of the MEP's in 1DI is produced by activity in the fibres of the corpus callosum. These fibres are thought to be of slow conduction velocity in children due to late myelination, and absent in patients with total agenesis of the corpus callosum.

Meyer *et al*, (1995b) in their study of patients with complete and partial agenesis of the corpus callosum found some evidence for inhibition in one patient who had the anterior half of the trunk of the corpus callosum present, but no inhibition in the 5 subjects in whom the anterior half was absent. According to the results of labelling studies in the monkey, performed by Pandya & Seltzer (1986), the fibres connecting the primary motor cortices are situated in the anterior half of the trunk of the corpus callosum. Thus it might be assumed that activity mediated via these fibres is responsible for the inhibition found in the present study and that of Ferbert *et al*, (1992); Meyer *et al*, (1995b).

The inability to demonstrate interhemispheric inhibition in the children at similar latency to the adults provides evidence to support the hypothesis set out in chapter two of this section, that bilateral activation of both motor cortices occurs during unimanual tasks in children. The bilateral activation may result from a lack of transcallosal inhibition and in this is way could account for the mirror movements observed in young children. But the children were tested while performing a 10% MVC, and Ridding *et al* (1993) have shown that this pathway is suppressed during voluntary activity and thus could explain the present results. In the future it would be useful to test a group of children using Meyer's protocol to examine the interhemipsheric pathway during voluntary activity.

SECTION 2

An investigation of mirror movements in X-linked Kallmann's Syndrome

Section 2, Chapter 1: A neurophysiological investigation of mirror movements in X-linked Kallmann's Syndrome.

SUMMARY

1. EMG recordings have been made in the upper limbs of fourteen male subjects with X-linked Kallmann's Syndrome (XKS) and one female carrier. Thirteen of the males had pronounced mirror movements of the hands. The data from previous experiments on the upper limbs of normal subjects (see Section 1, chapter 1) were used as control data. In one of the subjects with XKS and pronounced mirror movements, EMG recordings were taken from the distal muscles of the lower limbs.

2. Focal magnetic brain stimulation was used to assess the integrity and distribution of the corticospinal tract. In the control subjects, the subject with XKS and no mirror movements and the female carrier, stimulation of the hand area of either motor cortex evoked motor responses in the contralateral hand only. In all subjects with XKS and pronounced mirror movements stimulation of either cortex evoked bilateral responses. Stimulation of the left motor cortex evoked larger ipsilateral than contralateral responses in 8 out of 13 subjects and stimulation of the right cortex evoked an equal or larger ipsilateral than contralateral response in 6 out of 13 subjects. In 3 out of 13 subjects the threshold for stimulation of a motor response was higher than in normal controls. These subjects also found relatively independent finger movements difficult.

3. Cross-correlograms were constructed from motor unit spike trains recorded simultaneously from a number of hand and forearm left and right muscle pairs during steady voluntary contraction of both muscles. In 12 of the 13 subjects with XKS and pronounced mirror movements a short duration central peak was found in the cross-correlograms constructed from the spike trains of simultaneously contracting left and right first dorsal interossei. Similar recordings were made from homologous muscle pairs of other upper limb muscles and a distal to proximal gradient was found. These peaks were not found in the control subjects, the female carrier, or the XKS patient without mirror movements.

4. Cutaneous reflex responses have been recorded in 1DI following electrical stimulation of the digital nerves of the index finger. In normal subjects, the reflex is only present on the stimulated side and has three components producing a short latency increase in EMG (E1), followed by a decrease (I1), followed by a second increase (E2). In 9 out of 12 XKS subjects with pronounced mirror movements, responses were present bilaterally following stimulation of one finger. In 3 out of 12, only the short latency (E1) component was present on the stimulated side, while I1 and E2 (but not E1) were present on the non-stimulated side. In one subject with XKS the E1 component was present bilaterally.

5. Phasic stretch reflexes were studied in 6 out of the 14 subjects with XKS to determine if there was abnormal organisation of short latency spinal reflex pathways. In 2 of the subjects a longer latency response was recorded on the contralateral side in addition to the short latency response on the stimulated side. Such a response was never found in control subjects.

6. The study found evidence for abnormal organisation of fast conducting corticospinal pathways. This abnormality varied within the group, ranging from a *more* normal predominantly contralaterally projecting corticospinal pathway, through to a strong ipsilateral projection, with a group of individuals with both contralateral and ipsilateral projections more evenly distibuted from each cortex. It is concluded that in subjects with XKS and mirror movements, there is an abnormal ipsilateral corticospinal projection which accounts for the occurrence of the common synaptic drive to left and right homologous motoneurone pools, and that it is this which underlies the mirror movements in X-linked Kallmann's Syndrome. It is proposed that this abnormal organisation has resulted from an aberrant decussation of the corticospinal tract during development as a consequence of a lack of the Kallmann gene product (KAL).

INTRODUCTION

Kallmann's Syndrome is defined by the association of hypogonadotrophic hypogonadism with anosmia. Although these related characteristics were first described by Maestre de San Juan in 1856, the first familial cases were reported by Franz Kallmann in 1944, and subsequently the syndrome has been referred to as Kallmann's Syndrome. Since then several modes of transmission have been identified including X-linked, autosomal recessive and autosomal dominant (Sparkes *et al*, 1968; Santen & Paulsen, 1972; White *et al*, 1983; Chaussain *et al*, 1988). The incidence has been estimated to be at 1 male in 10,000 with 5-7 times the number of males than females affected (Jones & Kemman, 1976).

It is known that the Kallmann gene product (KAL) is necessary for the migration of olfactory neurones and those synthesising gonadotrophins. But it has also been suggested that KAL is important for the development of non-neural tissues such as the kidney (Wegenke *et al*,1975; Franco *et al*, 1991; Hardelin *et al*, 1993; Duke *et al*, 1995). In their study, Duke *et al*, (1995) have shown that unilateral renal agenesis and mirror movements are additional features of the X-linked form of the syndrome (XKS). The latter are seen in approximately 90% of the patients with X-linked Kallmann's syndrome (Bouloux, unpublished observation).

Mirror movements, as described in section 1, chapter 1&2, are found in young children during development and decrease in intensity and frequency with increasing age (Connolly & Stratton, 1968). Although the origin of such movements is unknown, they are most likely due to unintended excitation of the opposite cortex. One mechanism to account for this maybe a lack of inhibition across the corpus callosum as suggested by Nass (1985), and supported by the results of the study reported in this thesis section 1, chapter 3. In contrast to the findings in normal adults, this study of 5 children aged 6-10 years, using the

Bi-stim technique as described by Ferbert *et al*, (1992), showed that there was no significant difference in the size of the conditioned and non-conditioned responses to Magstim at the delays used. Thus it was concluded that a lack of inhibition across the corpus callosum could account for the mirror movements observed during development.

If mirror movements are obligatory and persist into adulthood, they are considered to be pathological; such is the case for those patients with XKS. The association between KAL and axonal guidance within the olfactory system (Franco *et al* 1991), suggests that other axonal pathways could also be affected. Thus the mirror movements observed in patients with XKS could result from a defect in axonal guidance within the sensorimotor system. Bearing in mind the hypothesis put forward to account for the mirror movements in children, this could be a failure of callosal fibres to cross the midline. Alternatively, some corticospinal fibres could fail to decussate at the medulla resulting in a considerable abnormal ipsilateral projection in addition to the normally occurring ipsilateral projection. In the present study, neurophysiological techniques have been used to test these different hypotheses.

A preliminary account of some of this work has been presented to the Physiological Society and at the 25th Annual Meeting of the Society for Neuroscience (Mayston *et al*, 1995 a&b).

METHODS

Subjects

Recordings were made with ethical approval and informed consent from 14 male patients (aged 16 to 60 years) derived from 6 pedigrees with X-linked Kallmann's Syndrome as evidenced by family history or by demonstration of a mutation of the KAL gene (see appendix C). Thirteen of these patients have mirror movements. Recordings were also made from a female carrier, the mother of one of the subjects with mirror movements (K2).

Assessment of mirror movements

The presence and degree of mirror movements were assessed in the following ways:

i) Each subject was instructed to sequentially oppose each finger to the thumb from the index to little finger and back again. This was repeated several times as quickly and as neatly as possible, using the right hand and then the left. The mirror movements were assessed according to the criteria of Woods and Teuber (1978) as described in section 1, chapter one.

ii) The hands were held horizontally with the fingers extended over a box which was 4 cm high. The subject was instructed to flex each finger in turn and the involuntary excursion of the opposite side was measured. The procedure was performed with one hand and then the other. Any involuntary movement produced by the contralateral homologous muscles was expressed as a percentage of a full deflection of 4 cm. The direction of the movement was also noted.

iii) The subject was instructed to flex both elbows to a right angle and to perform pronation/supination of one forearm and then the other. Any involuntary movement was noted.

The ability to perform relatively indepedent finger movements (RIFM) was also noted.

0 = no RIFM; 1 = slight RIFM, but no functional use; 2 = moderate RIFM, some functional use; 3 = normal

EMG recordings

Surface EMG was recorded from left and right homologous muscle pairs using pre-gelled electrodes placed 20mm apart on the skin overlying the muscles studied as described in chapter one, and stored on magnetic tape for future analysis. Recordings were made from various left and right muscle pairs: left and right first dorsal interossei (1DI); left and right abductor digiti minimi (ADM); left and right forearm extensor compartment (Fext), over the index finger extensor muscle; left and right triceps (Tri) over the medial belly, and left and right deltoid (Del) over the middle belly. The EMG was amplified and filtered as previously described (Section 1, chapter one).

In one patient (K6), recordings were made using concentric needle electrodes (Medelec, DFC 25, with a recording area of 0.019 mm^2) in left and right 1DI, left and right index flexor and left and right Fext. In this case the signal was filtered at -3dB at 2kHz and 5kHz.

In this subject surface EMG recordings were also taken from left and right tibialis anterior (TA) and extensor digitorum brevis (EDB) during simultaneous dorsiflexion of the feet.

Instruction to subjects during phasic index finger abduction

The subjects sat at a table with their forearms supported, their palms facing down and their fingers flat on the surface. They were instructed to perform approximately 60 voluntary self-paced index finger abductions (the command was to make the movement "short and sharp"), keeping the other fingers relaxed, while EMG was recorded simultaneously from the left and right 1DI. The EMG was rectified and averaged for 50 sweeps time-locked to the onset of the EMG of the voluntary burst using averaging software (SigAvg, CED, Cambridge, UK). The areas of the rectified average of the EMG recorded from both sides were measured and the ratio of the area of the burst of the contralateral involuntary EMG to the phasic voluntary EMG was calculated.

Magnetic brain stimulation

I. Investigation of the laterality of magnetically evoked potentials (MEP's)

Transcranial focal magnetic brain stimulation (Magstim) was carried out using a Magstim 200 stimulator (The Magstim Co.) with a figure-of-eight coil (70mm diameter each coil) to investigate the laterality of the corticospinal projections. The coil was placed with the handle pointing laterally. Surface EMG was recorded from left and right homologous muscle pairs (left and right 1DI; left and right Fext; left and right Tri). The site of stimulation for each muscle pair is indicated in Table 1, as determined by a previous study by Carr *et al*, (1994). The threshold for a response was that output of the stimulator required to evoke 5 sequential responses in the contralateral muscle at a gain of 200μV per division. The subject was instructed to relax and the output was increased by 10% of maximal output. When possible 10 consecutive responses were recorded without background EMG. The magnetically evoked potentials were rectified and the area of each response measured and the average of 10 responses calculated. The ratio of the mean area of the ipsilateral response to the mean area of the contralateral response was determined.

Muscle	Anterior to Cz (%)	Lateral to Cz (%)
1DI	7.0	13.0
Fext	2.0	13.0
Tri	0	10.0

Table 1. Optimal sites for MEP's according to Carr *et al* 1994. The sites of focal magnetic brain stimulation were measured from the vertex (Cz) as a percentage of the interpolar distance between the nasion and inion (anterior to Cz) and the external auditory meati (lateral to Cz).

ii. Mapping of the cortical representation of left and right 1DI using Magstim.

In subjects K2, K8, K9 and K10, and in 4 age-matched controls aged 25-35 years (1 female), the cortical representation of left and right 1DI was mapped using focal magnetic brain stimulation. EMG was recorded as above. All subjects were right hand dominant. The figure of eight coil was positioned over the hand area at the expected site of optimal stimulation (as determined by Carr *et al*, 1994) for eliciting responses from left and right 1DI. Ten responses were recorded as above at each site lateral and medial to the "hot-spot"(site of optimal stimulation), and anterior and posterior to that site at 1 cm intervals. The area of each MEP was measured and the average of 10 consecutive responses calculated.

iii. Investigation of the trans callosal pathway

Two double-coned coils and a Bi-stim module were used as described in section 1, chapter 3. Two patients with XKS and mirror movements were studied (K7 and K11).

Cross-correlation analysis

Medium and large spikes were selected for analysis using a level detector (Neurolog NL 200, Neurolog, UK) from each multi-unit recording during a weak sustained voluntary isometric contraction of left and right muscle pairs. Index and/or little finger abduction against resistance for 1DI and ADM; wrist and finger extension for Fext; extension of the elbow for Tri and abduction of the shoulder with the arm extended for Del. In some subjects unilateral left then right index finger abduction was performed with the instruction to relax the opposite side. Cross-correlograms were constructed from the spike trains recorded from the voluntary and involuntary activity occurring in left and right 1DI.

Cross-correlograms were constructed using about 5000 spikes from each train with a bin

width of 1ms and a pre- and post-trigger sweep of 100ms (Spike2 software, CED). Spikes from the left muscle were arbitrarily selected to be the trigger spikes when recordings were made during simultaneous contraction of each muscle pair. The size of any central peak was estimated in terms of E/M where E is the number of extra counts in the peak above those expected by chance alone, and M is the mean count in a 1ms bin. The mean bin count was calculated at an area away from the central peak, usually the first and last 75 bins. The index E/M is known to be sensitive to the discharge frequency of the contributing spike trains, therefore the mean firing rate in each train was determined using spectral analysis and the index adjusted to a firing rate of 10Hz (Harrison *et al*, 1991): E/M x f/10. The half widths for any central peaks (left and right 1DI) were also calculated according to the criteria of Kirkwood & Sears (1991).

Cutaneomuscular reflexes

Surface EMG was recorded simultaneously from the left and right first dorsal interosseous muscles during sustained abduction of the left and right index fingers. The digital nerves were stimulated using ring electrodes (Medelec E/DS-K16639) attached to the index finger. The subjects were instructed to maintain about 10-20% MVC of the left and right 1DI against resistance, with the assistance of visual feedback from a RMS voltmeter, while the digital nerves were stimulated at 3pps (pulse width 100μsec, with a constant current stimulator) at approximately twice threshold for perception. Such stimulation is not painful and readily produces an identifiable response after a short period of averaging. The rectified EMG signals were averaged, time-locked to the stimulus for 500 sweeps (SigAvg averaging software). Both sides were tested.

Tendon taps

A tendon hammer was used to elicit a stretch reflex while simultaneously recording EMG from the left and right 1DI in 6 out of 14 subjects. The subject was instructed to activate the muscle on the contralateral side, while the examiner held the outstretched thumb and stretched the index finger towards the middle finger and tapped the 1DI at its insertion. In 4 subjects the stretch reflex was also examined in the left and right forearm extensors and in one subject the left and right forearm flexors were tested. The subject was again instructed to contract the contralateral side when the relaxed side was tapped at the tendon at the level of the wrist. Both sides were tested.

RESULTS

Mirror movements

i) Thirteen of the 14 subjects had mirror movements ranging in intensity from slight (grade 1) to marked (grade 3). Mirror movements were absent in one subject with X-linked Kallmann's syndrome and the female carrier; see Table 5.

ii) When individual finger flexion was tested, in all of the 13 subjects with mirror movements the involuntary activity occurred in homologous muscles of the index and middle fingers, but in 7 out of 13 subjects with XKS, flexion of the ring or little finger was accompanied by abduction (5/7) or adduction (2/7) of the contralateral ring or little finger.

iii) Eight out of 13 subjects with mirroring also showed involuntary supination/pronation. In subject K13 this was marked, but only slight in K1, K2, K4, K7, K9, K10, and K11.

iv) Three of the subjects with XKS (K1, K2 & K10) and the female carrier had difficulty with RIFM. K2 appeared to have clumsy fine-hand skills, but none of the other subjects reported any difficulties. Subject K9 had learnt to play the guitar.

EMG during phasic finger abduction

Figure 1A shows the surface EMG recorded during self-paced index finger abduction whilst recording from left and right 1DI of a normal control subject. Each abduction of the right index finger is accompanied by a burst of EMG in the right 1DI, but there was no visible movement of the left index finger and no EMG was recorded in the left 1DI. In contrast, figure 1B shows surface EMG recorded from a patient with X-linked Kallmann's Syndrome with mirror movements performing the same activity. In this subject each abduction of the right index finger is accompanied by a burst of EMG in R1DI, and in addition bursts of EMG are apparent in L1DI as a result of the mirror movements produced by voluntary activity of the right index finger. A single burst of EMG recorded from R1DI and the mirrored activity

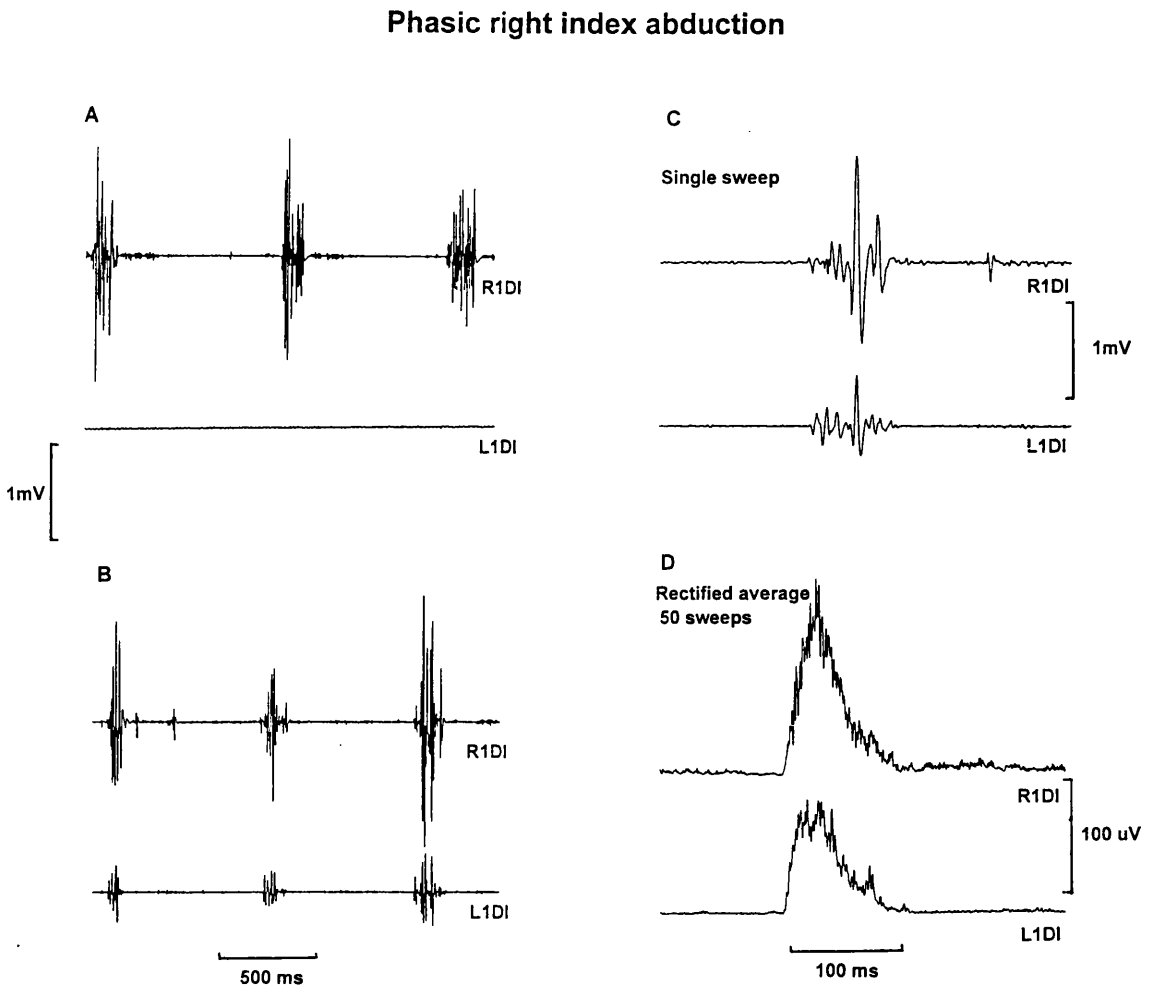


Fig.1 Surface EMG recorded simultaneously from L and R 1DI during self paced R index finger abduction. (**A**) Normal control subject; 3 bursts of EMG are present on the right side, each burst represents a single abduction of the R index finger, but there is no involuntary EMG on the L side. (**B**). Subject K7 with XKS and mirror movements similarly showing 3 bursts of EMG recorded during R index finger abduction but simultaneous bursts of involuntary EMG were also recorded. (**C**) EMG recorded from R and L 1DI during a single abduction of the R index finger. (**D**) Same subject with XKS; the EMG has been rectified and averaged time locked to the onset of the voluntary burst of EMG of R1DI for 50 sweeps

in left 1DI is shown in figure 1C. Figure 1D shows the average of 50 consecutive bursts, in which the EMG has been rectified and averaged time locked to the start of the voluntary burst.

All subjects with XKS and mirror movements produced movements of the contralateral finger with accompanying EMG, but in some subjects this did not occur with every phasic index finger abduction. The variation in the occurrence and amount of involuntary EMG is partly related to the strength of the phasic burst which showed great variation as seen in figure 1B. In 5 subjects at times there was no visible movement, but involuntary EMG was recorded contralateral to the active finger, and at other times no movement was observed and no involuntary EMG was recorded. These 5 subjects were those who showed the least mirroring (grade 1-2).

Group analysis of the time of onset of the involuntary EMG recorded from the mirrored activity in left 1DI during right index abduction ranged from 38.0ms before to 34.0ms after the start of the EMG in right 1DI with a mean onset time of 2.3ms later (SEM 5.1ms, n=13). When voluntarily abducting the left index finger, the time of onset of the EMG recorded from right 1DI on the mirroring side ranged from 33.0ms before to 21.0ms after the onset of EMG in left 1DI. On average the involuntary EMG on the mirroring side commenced 6.8ms later (SEM 4.3ms). Measurement of the time of onset of individual bursts of EMG in each subject during voluntary right and involuntary left EMG revealed that there was no significant difference in the time of onset in one subject (*t*-test, $P>0.05$); in 4 subjects the involuntary EMG preceded the voluntary EMG ($P<0.05$), and in the remaining 8 subjects the involuntary EMG began significantly later than the voluntary EMG ($P<0.05$). When the voluntary activity occurred on the left side, there was no significant difference in the time of commencement of the voluntary and involuntary EMG in 3 subjects ($P>0.05$) and for the

remaining 10 subjects, the involuntary EMG commenced significantly later than the voluntary EMG ($P<0.05$). When the subjects were grouped together there was no significant difference ($P>0.05$) in the times of onset of voluntary and involuntary EMG presumably due to the wide variation between individuals in the times of onset of involuntary EMG activity.

The ratio of involuntary to voluntary EMG varied within the group. Figure 1D shows the rectified averaged EMG recorded during 50 phasic index finger abductions. In this subject who had marked mirroring the ratio of the area of the involuntary EMG to the area of the voluntary burst of EMG was 0.7. The area of the involuntary burst of EMG to the area of the voluntary EMG burst ranged from 0.006 to 3.3 (n=13) when the right index finger was voluntarily activated, and from 0.01 to 1.2 (n=13) when the left index finger was voluntarily abducted. No contralateral EMG was recorded from the subject with XKS and no mirror movements, but in the female carrier who found RIFM difficult involuntary EMG was recorded. When using the right hand, the ratio of the area of the rectified and averaged EMG recorded from the involuntary and voluntary activity was 0.02 (50 sweeps) and the involuntary activity commenced 13.0ms after the voluntary activity. When using the left hand the involuntary/voluntary ratio was 0.09; time of onset of the involuntary EMG was 2.5ms after the start of the voluntary burst.

Focal magnetic brain stimulation

I. Investigation of the laterality of the corticospinal projection

Left and right 1DI

When background EMG was present stimulation of either motor cortex over the hand area evoked responses contralaterally and ipsilaterally in the 13 subjects with mirror movements. There was no significant difference in the latency of these bilaterally occurring responses (paired *t*-test, $P>0.05$). Only a short latency contralateral response was evoked in the subject

without mirror movements and the female carrier, as recorded in normal subjects but the threshold to stimulation in the female carrier was high (75% of the maximum stimulator output).

The size of the ipsilateral and contralateral evoked potentials showed considerable variation between the subjects. In most subjects bilateral responses were recorded whether the right or left motor cortex was stimulated without preactivation of 1DI, but in 1 out of 13 subjects, background EMG was required to see contralateral responses when stimulating the left cortex and in a further two, background EMG was needed to see ipsilateral responses when stimulating the left cortex. An example of stimulation of the left and right motor cortex of one subject is shown in figure 2A and B. In this subject stimulation of the left motor cortex evoked a larger ipsilateral than contralateral response (fig 2A) and stimulation of the right motor cortex evoked a larger contralateral than ipsilateral response (fig 2B). In 8 out of 13 subjects the ipsilateral response was larger than the contralateral response when stimulating the left motor cortex. The ratio of the size of the ipsilateral to contralateral responses (13 subjects, average area of 10 rectified responses) ranged from 34.0 to 0.004 (pre-activation contralateral to stimulated cortex in K1).

When stimulating the right motor cortex, in 6 out of 13 subjects an equal or larger ipsilateral than contralateral response was evoked. The ratio in this case ranged from 256.7 to 0.05 (pre-activation needed in 1/13 subjects to see contralateral responses in 1DI).

Left and right forearm extensors

In all the subjects with mirror movements short latency bilateral motor evoked potentials were recorded from left and right Fext (12/13 subjects with mirror movements tested) when stimulating either the left or right motor cortex. Preactivation of the Fext was required in one subject. The ratio of the ipsilateral to contralateral ranged from 16.7 to 0.02 when stimulating

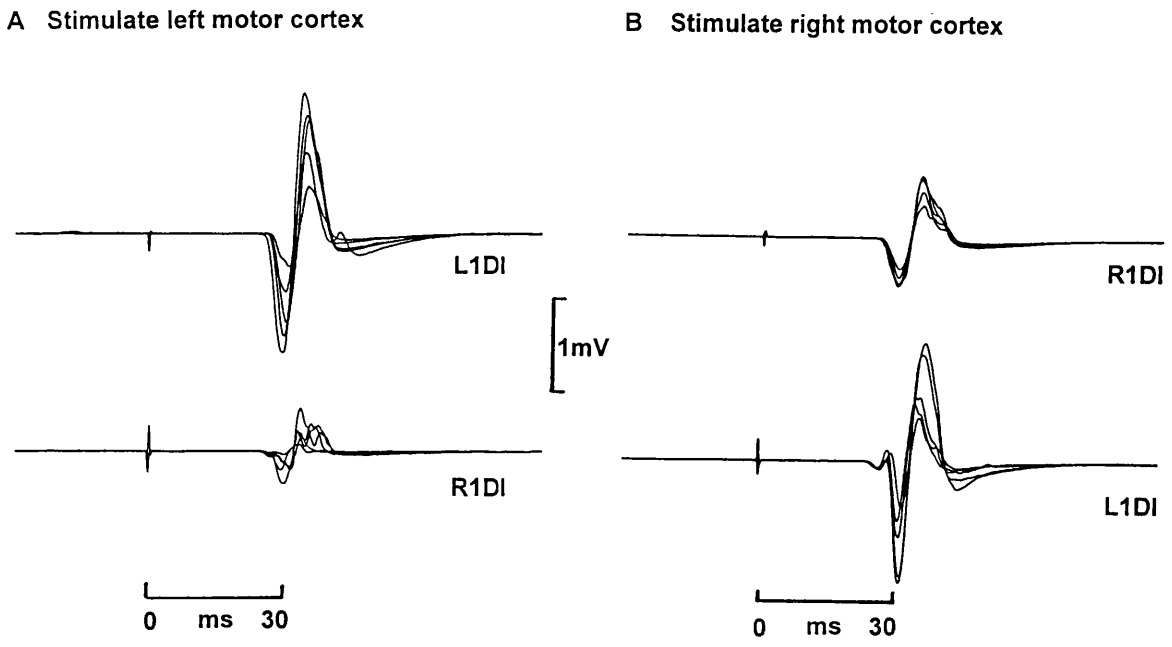


Fig. 2 Surface EMG recorded simultaneously from L & R 1DI during focal magnetic stimulation of the hand area of the motor cortex of subject K7 with XKS and mirror movements. 5 superimposed responses. (**A**) Stimulation of the L motor cortex; for this subject the ipsilateral response recorded from the L1DI is larger than the contralateral response recorded from R 1DI. (**B**) Stimulation of the R motor cortex of the same subject; the ipsilateral response recorded is smaller than the contralateral response recorded from L1DI.

		BILATERAL MEP	RATIO OF AREAS (n=10) ipsi/contra range	LATENCY (ms)					
				contra			ipsi		
				range	mean	SE	range	mean	SE
L & R	stim L	13/13	0.004-34.0	22.0-32.3	24.7	0.8	19.3-26.5	23.6*	0.6
1DI	stim R	13/13	0.05-256.7	21.0-26.8	22.9	0.5	20.7-25.0	22.9*	0.4
L & R	stim L	12/12	0.02-16.7	17.0-25.8	19.5	0.7	17.5-22.8	19.6*	0.5
Fext	stim R	12/12	0.03-2.0	16.6-20.8	18.7	0.4	17.0-22.5	19.7**	0.5
L & R	stim L	3/7*	2.50-0.03	13.5-185*	16.0		15.0-19.5	16.7*	
Tri	stim R	4/7*	0.03-0.3	13.5-15.5*	14.5		12.8-17.0	14.8*	

* only includes those with bilateral MEP's

★ bilateral background EMG present in 2 subjects

* paired t-tests contra and ipsi MEP latencies, $P>0.05$

** paired t-test contra and ipsi MEP latencies, $P<0.05$

Table 2: Summary of data from focal magnetic brain stimulation to left and right motor cortices. L & R first dorsal interosseous (1DI), 13 subjects, L & R forearm extensor compartment (Fext), 12 subjects; L & R triceps (Tri), 7 subjects. For each subject and for each trial, 10 stimuli were presented and the ratios of the areas of the rectified and averaged ipsilateral to contralateral MEP were calculated. In addition, the latencies of the contralateral and ipsilateral MEP's are given; there was no significant difference between these except when recording from L and R Fext and stimulating the right motor cortex.

the left motor cortex, and from 2.0 to 0.3 when stimulating the right motor cortex. See Table two.

Left and right Triceps

Recordings from left and right Tri were obtained from 7 out of 13 subjects with mirror movements while stimulating the motor cortex. Three of these subjects had bilaterally evoked potentials in Tri when stimulating the left cortex and 4 had bilateral responses when stimulating the right motor cortex. Pre-activation of Tri bilaterally was required for 2 of these subjects. When the left motor cortex was stimulated the ratio of the size of the ipsilateral to the contralateral ranged from 2.5 to 0.03 (n=3) and when the right motor cortex was stimulated this ratio ranged from 0.3 to 0.03 (n=4), see Table 2.

ii. Cortical representation of left and right 1DI

In the normal subjects unilateral scalp stimulation of either motor cortex using Magstim evoked contralateral responses only in left and right 1DI.

In contrast, in the four subjects with XKS who were studied, unilateral scalp stimulation evoked bilateral responses in relaxed left and right 1DI in 3 of the 4 subjects, and in all of the subjects with preactivation of left and right 1DI. The latency of these bilateral responses was not significantly different ($P>0.05$). In the 4 normal subjects only contralateral responses were recorded. Figure 3A shows the amplitude of the MEP's recorded from relaxed left and right 1DI at points of stimulation lateral to the vertex resulting from stimulation of the left and the right motor cortex in subjects K2, K9 & K10. On the left hand side of the map the amplitude of MEP's recorded contralateral and ipsilateral to the point of stimulation of the left cortex are shown, and the right hand sides shows both contralateral and ipsilateral MEP amplitudes for stimulation of the right cortex. In 1 out of 4 subjects with XKS the site of

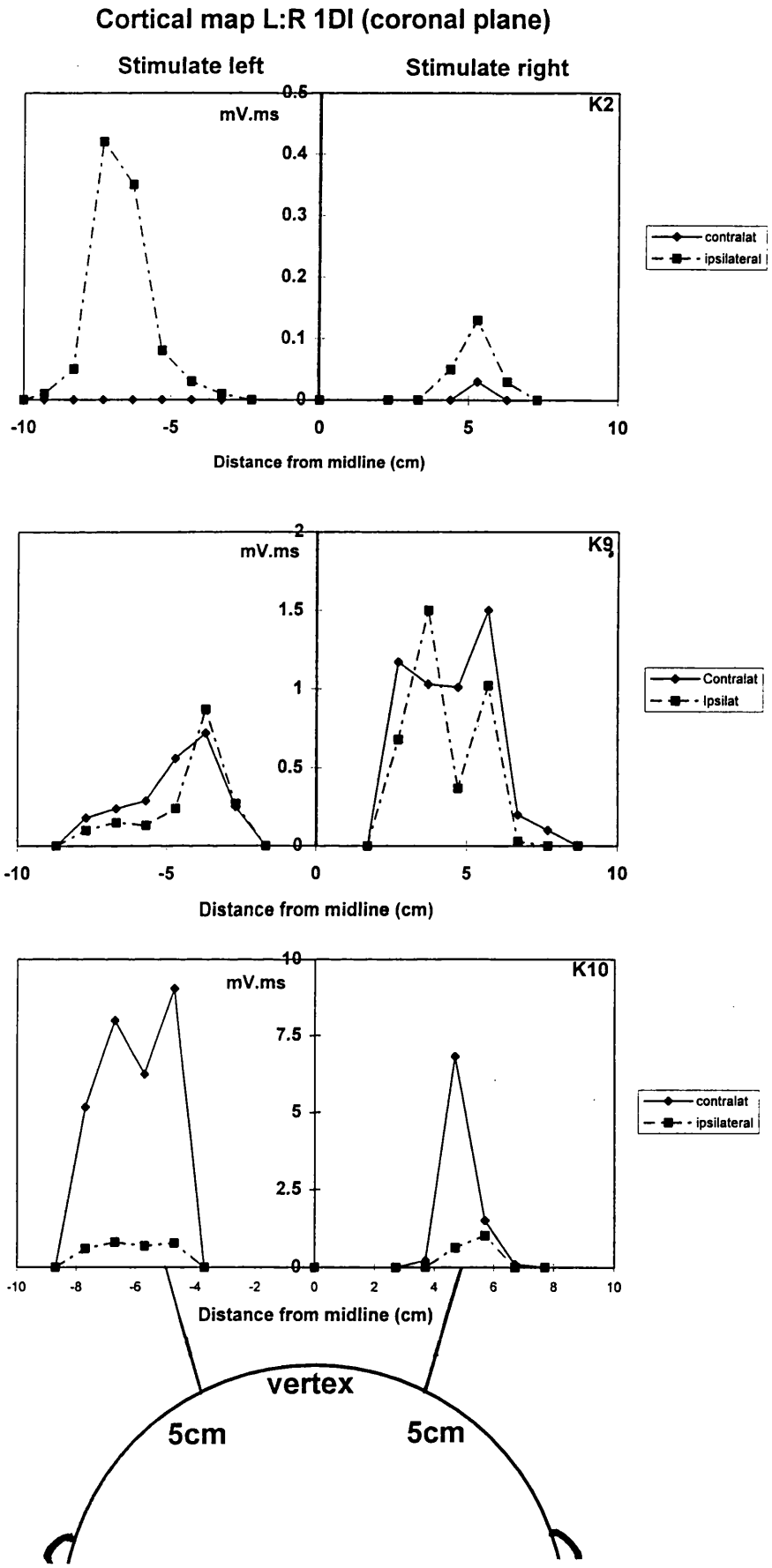


Figure 3A: Bilateral motor evoked responses were elicited from the same point of stimulation using focal magnetic brain stimulation.The upper panel shows that the ipsilateral response was always larger than the contralateral response in K2. In subject K9 the contralateral and ipsilateral responses were approximately the same size (middle panel) but in subject K10 the contralateral response was always larger (lower panel). The amplitude of the responses changed in a similar way in all subjects tested but there was great inter-subject variability.

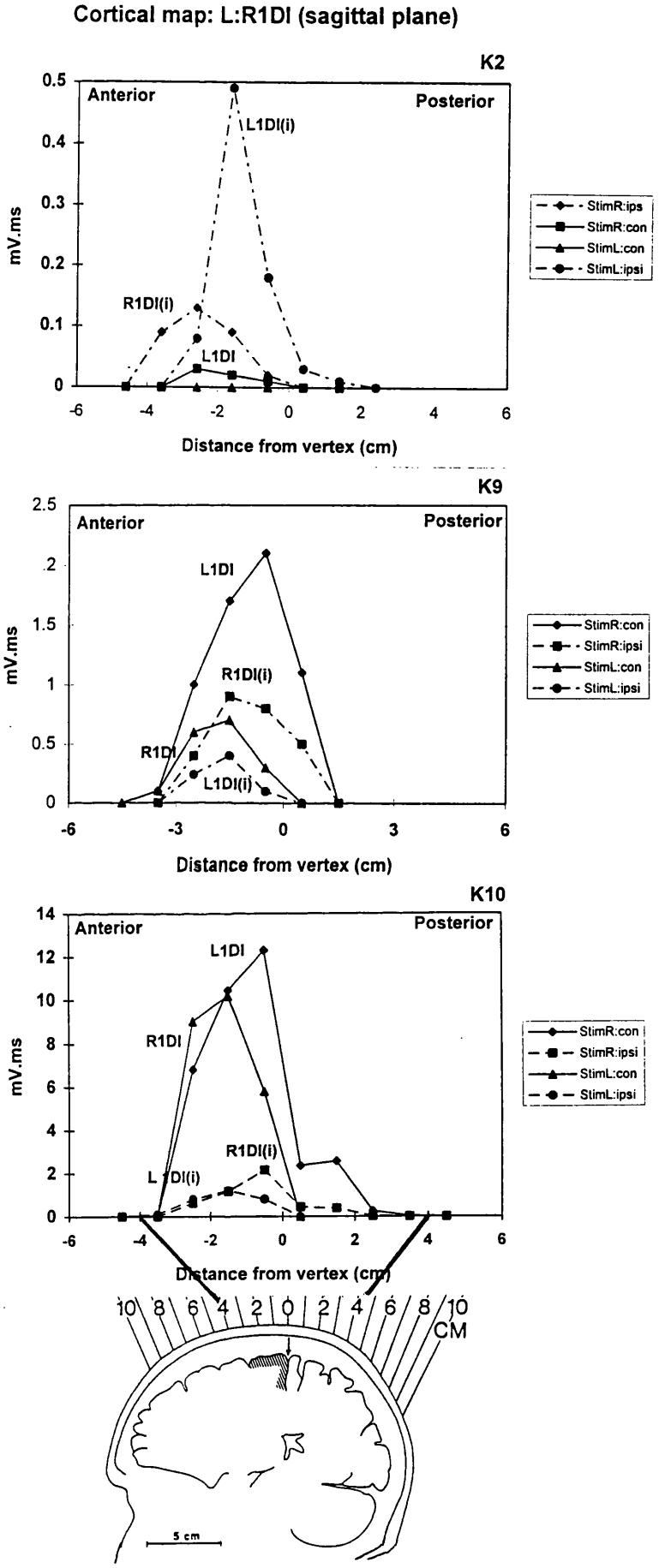


Figure 3B: As in the previous figure, the size of the motor evoked responses from each point of stimulation changed in a similar way. In subject K2 the ipsilateral response was always larger (upper panel), but in K9 the ipsilateral and contralateral responses were of similar size (middle panel). In subject K10 the contralateral response was always larger (lower panel).

stimulation at which the greatest amplitude MEP was recorded was not at the expected point of stimulation as described by Carr *et al*, 1994. In subject K2, the ipsilateral response was always larger than the contralateral response whichever site on the hand area of the cortex was stimulated

Figure 3B shows the cortical representation of left and right 1DI in the sagittal plane at 1 cm intervals anterior and posterior to the vertex when the left and then the right motor cortex was stimulated. In all of the subjects with XKS it can be seen that the contralateral and ipsilateral responses although of different amplitude, follow a similar pattern of modulation at points away from the site at which the greatest amplitude MEP was recorded. In the case of K2, stimulation of the left motor cortex evoked only an ipsilateral response in left 1DI, and preactivation was required in order to see the contralateral response recorded in right 1DI.

In both planes for each cortex, there was no significant difference between the area of representation in both groups of subjects (unpaired t-test, $P>0.05$), but the subjects with XKS demonstrated bilateral cortical hand representation.

iii. Investigation of the trans-callosal pathway

The Bi-stim technique for studying activity in the transcallosal pathway could not be easily applied to all of the subjects with XKS and mirror movements due to the presence of an abnormal ipsilateral corticospinal projection. The presence of this projection resulted in the conditioning magnetic stimulus affecting the excitability of the motoneurones which were the target of the test stimulus. This had an effect on the size of the MEP recorded in response to the test stimulus. However the Bistim technique was able to be successfully performed in subject K11 who had a predominately contralateral projection from the left motor cortex (see table 5). It was only possible to use two time intervals between the conditioning stimulus and

the test stimulus, 7 and 10ms. At both of these intervals the conditioned response was significantly smaller than the non-conditioned response (unpaired *t*-test, $P<0.05$). For the normal subjects (see section 1, chapter 3), when using a 7ms time interval, the conditioned response varied from 20.2 to 95.5% of the non-conditioned response. At this time interval in subject K11 with XKS, the conditioned response was 6.0% of the non-conditioned response. When a 10ms delay was used, the conditioned response varied from 12.7 to 95.5% of the non-conditioned response in the control subjects and was 6.2% of the non-conditioned response in subject K11.

Cross-correlation analysis

Cross-correlation analysis of multi-unit EMG recorded from voluntarily co-contracted left and right 1DI muscles was performed for all the subjects with X-linked Kallmann's syndrome and the female carrier. Figure 4A shows the correlogram constructed from the data recorded from the XKS subject without mirror movements. It can be seen that the correlogram is flat and cannot be distinguished from those constructed from data recorded from normal subjects (Carr *et al*, 1994). Fig 4B shows the cross-correlogram constructed from the multi-unit EMG recorded during voluntary co-contraction of left and right 1DI in a subject with XKS and marked mirror movements (grade3). In contrast to the normal subjects and the XKS subject without mirror movements, there is a short duration central peak. A short duration central peak was seen in twelve out of the 13 subjects with mirror movements, the size of which showed great variation. A further example is given in Fig 4C, while fig 4D shows the cross-correlogram from one subject with mirror movements but for whom a flat correlogram was obtained from the recordings made from left and right 1DI. The size of the central peak estimated using the index E/M (E = number of spikes in excess of those expected

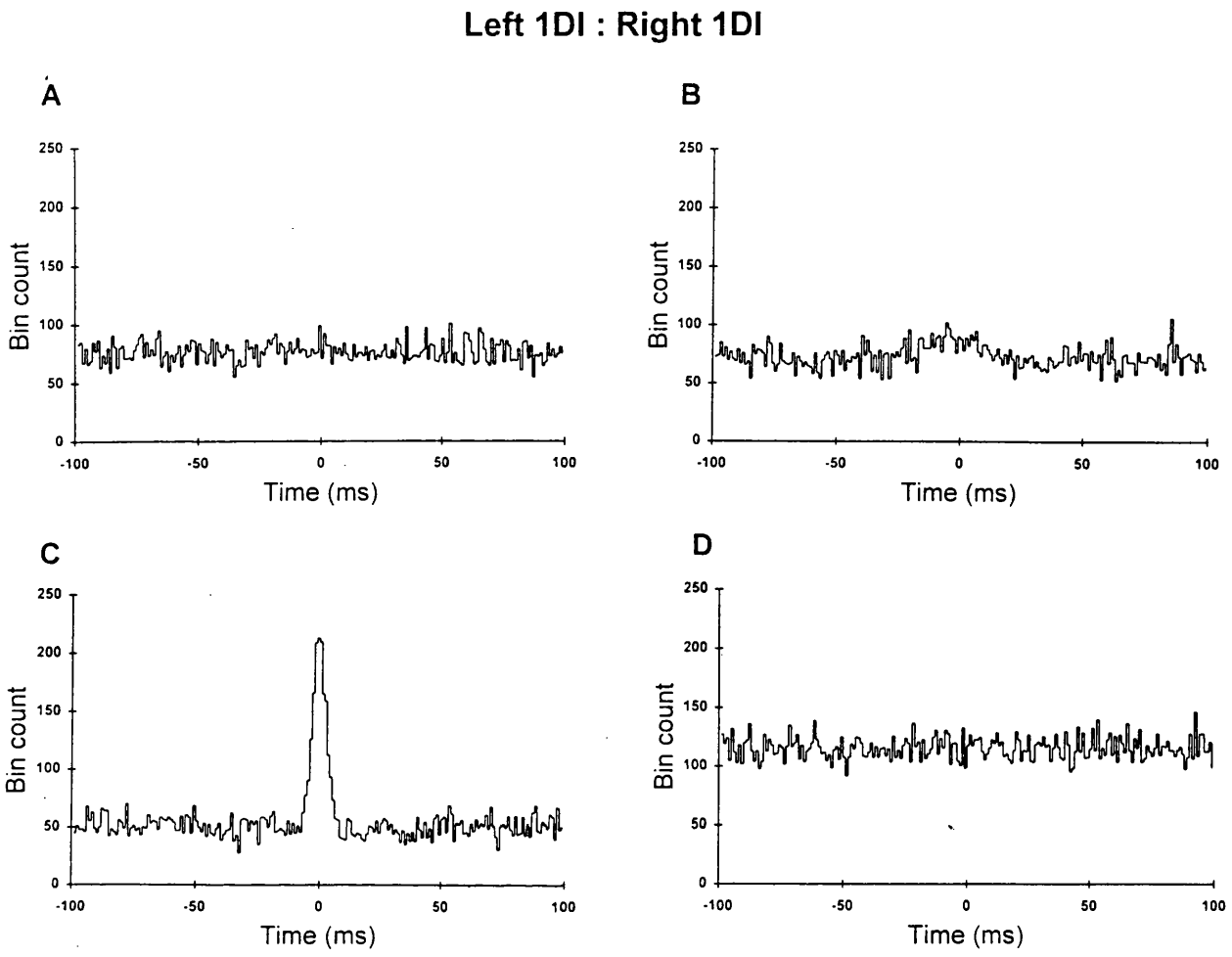


Fig.4 Cross-correlograms constructed from multi-unit surface EMG recorded during voluntary sustained L & R index finger abduction. Each correlogram comprises approximately 5000 trigger spikes (L1DI) and 5000 event spikes (R1DI). Bin width 1ms. (**A**) Subject K14 with XKS but *no* mirror movements; the correlogram is flat (**B**) Subject K1 with XKS and mirror movements; there is a small broad central peak (**C**) Subject K7 with XKS and mirror movements; there is a large short duration central peak centred around time zero (**D**) Subject K13 with XKS and mirror movements; the correlogram is flat.

Muscle pair	Correlogram peak present (n)	Duration	± SEM	Range (ms)	E/M	± SEM	Range	Half width (ms)	±SEM	Range
L:R 1DI (both active)	12/13	16.5	1.4	12.0-28.0	5.7	1.1	3.1-17.9	10.9	1.1	7.0-19.0
L 1DI active R 1DI relax	7/7	16.1	1.5	12.0-27.0	7.0	1.5	2.4-11.3	9.4	1.6	4.0-15.0
R 1DI active L 1DI relax	7/7	15.6	1.6	10.0-21.0	5.9	1.1	1.9-10.3	10.0	1.3	7.0-17.0
L:R ADM	7/8	17.6	1.4	13.0-23.0	4.8	1.4	1.2-5.8			
L:R Fext	10/12	14.4	0.8	10.0-19.0	3.2	0.7	1.2-6.7			
L:R Tri	5/9	13.6	0.8	12.0-15.0	3.1	0.8	1.5-5.8			
L:R Del	2/9	13.0			2.3		2.2-2.4			

Table 3: The duration, size and half-width for the short duration central cross-correlogram peaks constructed from co-contracting muscle pairs, showing a distal to proximal gradient in the size and occurrence of common drive to upper limb homologous muscle pairs, which was greatest in the distal muscle pairs.

by chance and M = mean bin count in a 1 ms bin) ranged from 3.1 to 17.9 as shown in table 3 and 5. The duration of the peak ranged from 12.0 to 28.0 ms(mean 16.5ms, ±1.3ms, n=12). The half-width ranged from 6.0ms to 19.0ms (mean 10.9ms, ± 1.1, n=12).

In subject K6 cross-correlograms were constructed from the EMG recorded from left and right tibialis anterior and extensor digitorum brevis. Unlike the cross-correlograms constructed from the EMG recordings of the distal muscles of his upper limbs in which peaks were found, the correlograms were flat indicating that there was no common drive to the motoneurone pools of the homologous lower limb muscle pairs.

Cutaneomuscular reflexes

A typical CMR recorded from the right 1DI during stimulation of the digital nerves of the right index finger of a subject with XKS is shown in figure 5A. A triphasic modulation of EMG can be observed ipsilateral to the stimulus. This modulation comprises a short latency increase in EMG (E1 component) followed by a decrease in EMG (I1 component) followed by a second larger increase in EMG (E2 component). However in 10 out of the 13 subjects with XKS, reflex modulation was also seen contralateral to the side of stimulation (figure 5B). Table 4 shows the CMR distribution when the left and then the right digital nerves were stimulated. In 3 subjects, only an E1 component was recorded ipsilateral to the stimulus (figure 5C), whereas both the I1 and E2 components were recorded contralateral to the stimulus (figure 5D, table 5).

No reflex was recorded contralateral to the stimulus when recording from the XKS subject without mirror movements, and the female carrier.

Like the results from using Magstim, the CMR's showed inter-subject variability as shown in the following tables for stimulation of the digital nerves of the index finger of either hand. The subjects in the upper part of the table only the E1 was recorded on the stimulated side when the

left side was stimulated, but the I1 and E2 were recorded contralateral to the stimulated side. The result was the same when the right side was stimulated (table 4b). The ticks in the table indicate that component clearly present, the 'x' denotes that the component is absent and the gaps in the table indicate those components which could not be clearly identified. For example, in subject K9 no reflex modulation of ongoing EMG was found in response to the cutaneous input which the subject could perceive and clearly identify.

Table 4

a. Stimulate left:

Subject	Stimulated side			Contralateral side		
	E1	I1	E2	E1	I1	E2
K1	✓	x	x	x	✓	✓
K2	✓	x	x	x	✓	✓
K3	✓	x	x	x	✓	✓
K4	✓	✓	x	x	✓	✓
K5	✓	✓	x	x	✓	✓
K6	✓	✓	✓	x		✓
K7	✓	✓	✓	x	✓	✓
K8	✓	✓	x	x	✓	✓
K9	x	x	x	x	x	x
K10	✓	✓	✓	x	x	x
K11	✓	✓	✓	x	✓	✓
K12	✓	✓	✓	x	x	x
K13	x	✓	✓	✓	x	x

b. Stimulate right

Subject	Stimulated side			Contralateral side		
	E1	I1	E2	E1	I1	E2
K1	✓	x	x	x	✓	✓
K2	✓	x	x	x	✓	✓
K3	✓			x	✓	✓
K4	✓	✓	✓	x	✓	✓
K5	✓	✓		x	✓	✓
K6	✓	✓	✓	x	✓	✓
K7	✓	✓	✓	x	✓	
K8				x	✓	✓
K9	x	x	x	x	x	x
K10	✓	✓	✓	x	✓	✓
K11	✓	✓	✓	x	x	x
K12	✓	✓	✓	x		
K13	✓	✓	✓	x	x	x

Tendon taps

A short latency response was recorded from 1DI following a tap to the tendon of the 1DI muscle using a tendon hammer. Figure 6A shows the average of 10 short latency reflex responses. Only ongoing voluntary EMG was recorded and there was no response contralateral to the side of stimulation. A similar result was found for all but 2 of the subjects tested when a modulation of EMG contralateral to the stimulus was also recorded at latencies which were 9 and 19 ms later than the ipsilateral reflex response. In 4 subjects, the tendons of the forearm extensors were also stretched and short latency responses were recorded ipsilateral to the tap in all of them. In one subject in whom the forearm flexors were tested, a reflex was also recorded bilaterally, the latency of the contralateral response being 19.0 ms longer than that of the ipsilateral response. (figure 6B).

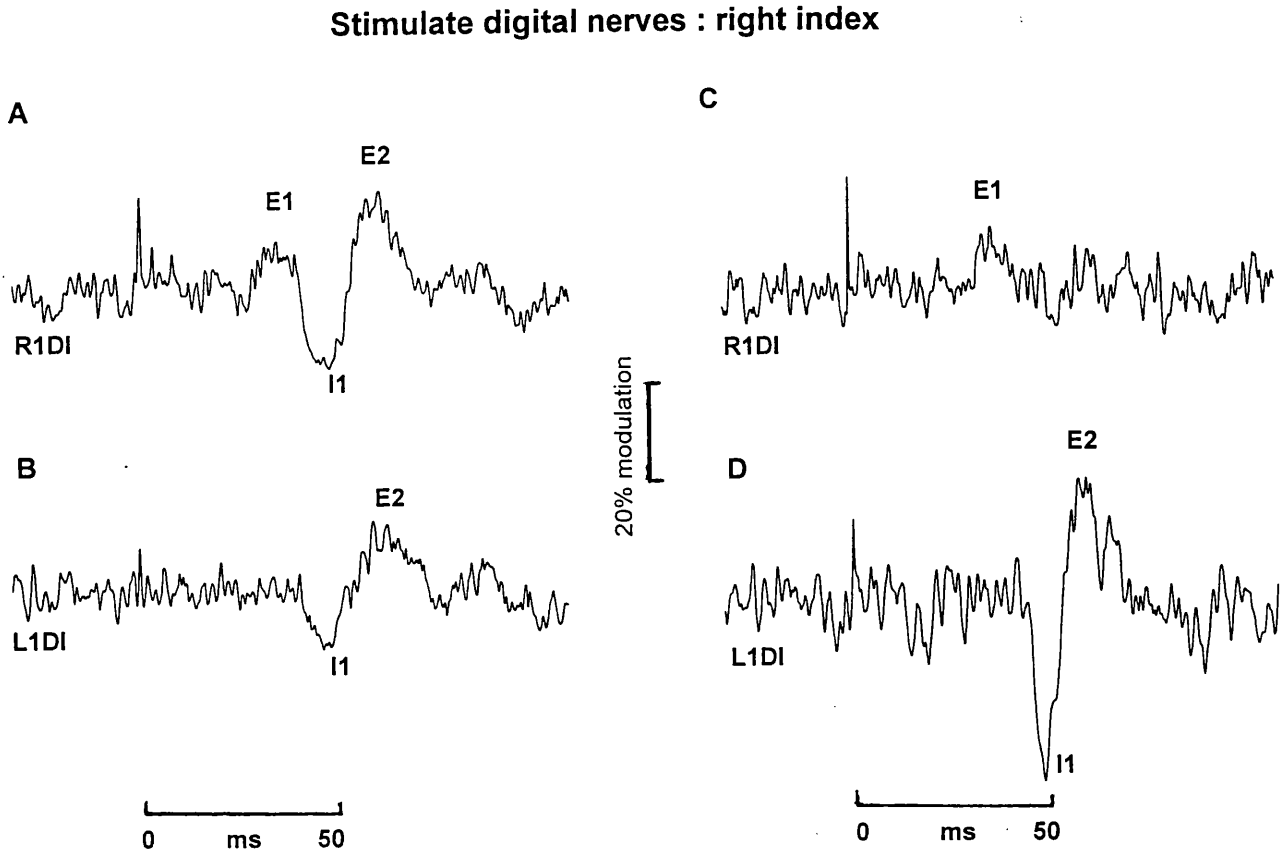


Fig. 5: Cutaneomuscular reflexes (CMR) recorded from R and L 1DI following stimulation of the digital nerves of the right index finger at 3^{sec-1} at a stimulus strength twice threshold for perception during simultaneous sustained voluntary isometric abduction of right and left index fingers. Surface EMG rectified and averaged for 500 sweeps time locked to the stimulus. (**A**) & (**B**) CMR recorded from K6, with XKS and mirror movements. The response recorded ipsilateral to the stimulus (**A**) comprises E1, I1 and E2 components, similar to that recorded from normal subjects. In contrast to normal subjects a reflex is also seen contralateral to the stimulated side, (**B**). This contralateral reflex comprises I1 and E2 components, both believed to be of supraspinal origin. (**C**) & (**D**) CMR recorded from subject K2, with XKS and mirror movements. The response recorded from R1DI ipsilateral to the stimulus only comprises an E1 component, thought to be of spinal origin, but the reflex recorded contralateral to the stimulated side in L1DI comprises I1 and E2 components.

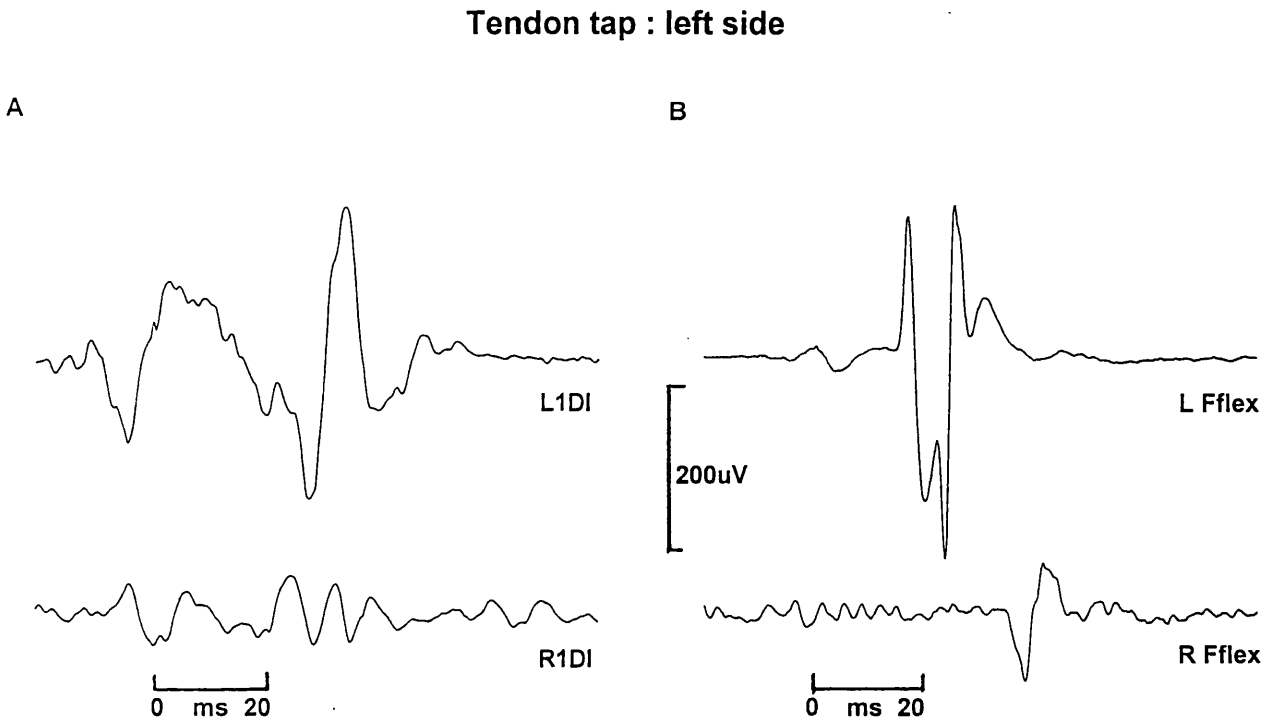


Fig. 6: Tendon taps recorded from 2 subjects with XKS and mirror movements. (**A**) subject K8, surface EMG recorded simultaneously from L and R 1DI following a tap to L1DI at its insertion. EMG averaged, time locked to the stimulus, 10 sweeps. A short latency reflex response is shown in L1DI; there is no reflex contralateral to the stimulated side. (**B**) Subject K7, surface EMG recorded simultaneously from L & R Fflex following a tap to L tendon at wrist. EMG averaged, time locked to the stimulus, 10 sweeps. A short latency reflex (15.0ms) response is shown in L Fflex; there is also a small reflex response at longer latency (34.5ms) in R Fflex contralateral to the stimulus

DISCUSSION

This study has used neurophysiological techniques to investigate the mechanisms underlying the mirror movements observed in patients with XKS. In all of these patients, voluntary movements of the fingers of one hand were accompanied by involuntary mirroring movements of the fingers of the contralateral hand. These movements were usually associated with activity in homologous left and right muscle pairs, but in 7/13 patients ring or little finger flexion was accompanied by contralateral finger abduction or adduction. The amplitude of the mirror movements varied between individual subjects and in some subjects they were asymmetrical, with greater mirroring present when the left hand was used. Taking the group as a whole, surface EMG recordings from left and right 1DI muscles during voluntary phasic unilateral index finger abduction revealed that there was no significant difference in the latency at which EMG commenced on the voluntary and involuntary mirroring side. The ability to perform independent finger movements, such as index finger abduction, is thought to be dependent on the presence of monosynaptic fast-conducting cortico-motoneuronal projections which are associated with fast conducting corticospinal axons derived from the contralateral cortex (Lawrence and Hopkins, 1976). With this in mind, it may be supposed that in the XKS patients with mirror movements, the simultaneous command to the voluntarily and involuntarily moved index finger both travel via a fast conducting pathway.

This involuntary EMG could be produced by a number of different mechanisms, but the results of the present study indicate the presence of an abnormal fast conducting ipsilateral corticospinal tract in patients with XKS and mirror movements. The evidence for this will be discussed by considering each of the neurophysiological tests.

Focal magnetic brain stimulation

Unilateral cortical stimulation of the hand area of the motor cortex of all the subjects with XKS and mirror movements evoked bilateral responses in the distal muscles. A similar result has been described by Danek *et al*, (1992) in their study of XKS and mirror movements. Mirror movements have also been studied in patients with Klippel-Feil syndrome (Farmer *et al*, 1990), congenital hemiplegia (Carr *et al*, 1993), in subjects with idiopathic mirroring (Konagaya *et al*, 1990; Cohen *et al*, 1991; Harrison *et al*, 1993) and in normal children (section 1, chapter 2, of this thesis).

It is generally agreed that magnetic brain stimulation of the motor cortex excites fast conducting corticospinal axons (Hess *et al*, 1987). This can occur both directly and indirectly (Edgley *et al*, 1990, Werhahn *et al*, 1994). Evidence for direct stimulation has been provided recently by Baker *et al*, (1994) who succeeded in recording the pyramidal volley in the conscious monkey following transcranial magnetic stimulation.

In the present study of XKS subjects with mirror movements, the ipsilateral response occurred at the same latency as the contralateral response, indicating that the conduction velocity of the ipsilaterally projecting axons is similar to that of the normally projecting contralateral axons. The amplitude of the contralaterally and ipsilaterally evoked potentials showed a similar distribution when the cortical representation of left and right 1DI was mapped. The ratio of the size of the ipsilateral response to the contralateral response showed considerable variation between subjects and within subjects when considering each motor cortex (tables 2 and 5). In 8 of the 13 subjects the ipsilateral response was equal to or larger than the contralateral response when the left motor cortex was stimulated but in only 6 when the right motor cortex was stimulated.

Origin of an abnormal ipsilateral projection:

i.presence of interhemispheric connections

It seems unlikely that activity in the ipsilateral tract is due to a spread of excitation from the contralateral hemisphere via the transcallosal pathway, since conduction time via this pathway is expected to take about 10ms (Cracco *et al*, 1989), and the onset of involuntary EMG should be similarly delayed. There was no significant difference ($P<0.05$) in the latency of the ipsilateral and contralateral MEP's recorded in all of the subjects in the present study.

Danek *et al*, (1992) have argued that mirroring in subjects with XKS might be the result of a lack of interhemispheric inhibition resulting from a defect in the migration of callosal axons. But 9 of the 13 subjects with XKS in the present study have undergone an MRI scan which has shown the corpus callosum to be present and of normal morphology.

When the bilateral Magstim condition-test technique was applied to the subjects with XKS, the conditioning stimulus to the ipsilateral motor cortex produced bilateral MEP's, thereby inhibiting the response to the test stimulus which could not be readily seen. In one subject in whom the corticospinal projection from the left motor cortex was largely contralateral, it was possible to perform the experiment and to unequivocally demonstrate the presence of interhemispheric inhibition.

ii.normally occurring ipsilateral pyramidal tract

The involuntary EMG recorded in subjects with XKS could have resulted from activity in the normally projecting ipsilateral component of the corticospinal tract. Evidence from anatomical (Galea & Darian-Smith, 1994) and physiological studies (Colebatch & Gandevia, 1989; Smutok *et al*, 1989), suggest that in normal individuals the ipsilateral corticospinal tract may have some influence on the control of both distal and proximal muscles. Anatomical tracing experiments using the macaque monkey have revealed ipsilateral projections from the motor

cortex which have a similar pattern of projection to the contralateral projection. But this projection is relatively small and only constitutes about 10% of the corticospinal tract (Galea & Darian-Smith, 1994). Further evidence for an ipsilateral pathway has been provided by Aizawa *et al*, (1990). They made recordings from motor cortical cells in the awake monkey and showed that there are neurones which are active during bilateral hand movements and some that are only active during an ipsilateral hand movement. There is also some clinical evidence in man. Colebatch and Gandevia, (1989) in their study of adults with an acquired hemiplegia demonstrated a weakness of the "unaffected side" in both distal and proximal muscles. Further support for this hypothesis is found in the study by Smutok *et al*, (1989). In their study of sensorimotor deficits in subjects with hemiplegia poor performances were found in the reaction times of the distal movements of the "unaffected" side suggesting some influence of the ipsilateral tract. In addition there are a number of reports in the literature based on imaging techniques which describe activation of the ipsilateral cortex during a unilateral task (Kim *et al*, 1993 a&b; Kawashima *et al*, 1993). More recently Wassermann *et al*, (1994) have reported that they were able to record ipsilateral EMG responses from distal muscles using magnetic brain stimulation. However these ipsilateral responses were considerably smaller than the contralateral responses, were of a longer latency and required a high output (65-90%) of the stimulator to be elicited. Not only were the ipsilateral responses smaller, but they were elicited from a different site from which the contralateral response was evoked. This was not the case in the present study. Bilateral responses could be evoked at a stimulus strength similar to normal subjects. Also, unlike in normal subjects the ipsilateral motor evoked response was larger than the contralateral in 8/13 subjects, the latencies of the ipsi- and contralateral responses were the same and both responses were elicited from the same site of stimulation. Thus the ipsilateral responses recorded in the present study in distal muscles are unlikely to result from an enhancement of the normally

occurring ipsilateral corticospinal tract projection.

iii. branching of corticospinal tract fibres

An ipsilateral corticospinal projection could also result from branching of some or all of the contralaterally projecting fibres. This conclusion was reached by Farmer *et al*, (1990) for a patient with Klippel-Feil syndrome, and by Carr *et al*, (1993) for children with congenital hemiplegia and obligatory mirror movements. Carr *et al*, (1993) argued that unilateral damage of the brain which occurred early in development resulted in abnormal development of the corticospinal tract from the surviving cortex. This led to the branching of the surviving axons which then innervated homologous left and right motoneurone pools. This interpretation is supported by experiments performed by Kuang and Kalil (1990) in the hamster. In that study, they made early unilateral lesions of the cortex and found that corticospinal axons branched in the spinal cord. It does not necessarily follow that this would be the case in patients with XKS. O'Leary and Koester (1993) from their work in the rat, have reported that the growth cones of primary layer 5 axons do not bifurcate. This, along with recent work by Halloran and Kalil (1994), suggests that it is unlikely that branching would occur at the level of the pyramidal decussation since they found that bifurcation of growth cones in developing axons was rare (in that case callosal axons). Dent *et al*, (1995) have now demonstrated that branching of axons is elicited by membrane bound cues from spinal targets as the axons near their final destination. Bilateral branching of corticospinal axons could account for the present results in subjects with XKS, but the fact that Magstim revealed that the ipsilateral projection is stronger than the contralateral projection in half of the subjects tested, seems to indicate that at least part of the ipsilateral projection must be from non-decussating, non-branched axons.

iv. lack of decussation of the corticospinal fibres at the pyramid

Conrad *et al*, (1978) in their study of XKS subjects with mirror movements, suggested

that a lack of decussation of corticospinal fibres was the cause of the mirror movements found in these patients, a hypothesis supported by the findings of the current study. The mapping of left and right 1DI showed that the amplitude of the ipsilateral and contralateral MEP's changed in a similar fashion as the coil position was changed. This was interpreted to indicate that both the ipsilateral and contralateral corticospinal tract projections originated from cell bodies lying close to each other in the cortex and receive common synaptic input. Thus it is likely that a partial pyramidal decussation could be responsible for the aberrant organisation of the corticospinal tract.

It has been demonstrated by Schwanzel-Fukuda *et al,* (1989), that XKS is characterised by a failure of axons of the olfactory, vomeronasal and terminal nerves to pass through the meninges overlying the cribriform plate to synapse with second order neurones in the developing forebrain. Migration of neurones which originate in the olfactory epithelium and synthesise gonadotrophin releasing hormone is also arrested. It has been suggested that the gene which is affected in XKS, known as KAL, codes for a protein which shares homology with molecules involved in cell adhesion and axonal pathfinding (Franco *et al,* 1991). Thus a molecular deficit in this gene could affect neuronal migration in subjects with XKS. More recently, Lutz *et al,* (1994) have identified several sites of KAL expression in a 19 week old foetus. These areas included the cerebellum, dorsomedial thalamus, the cortical plate and migrating neurones of the cerebral cortex. Thus it might be supposed that the KAL gene could affect the migration of corticospinal axons as well as olfactory axons since the pyramidal decussation is first seen at postovulatory day 57, and the lateral olfactory tract at post ovulatory day 52 in the human foetus (O'Rahilly and Muller, 1994). The developmental abnormalities associated with XKS such as unilateral renal aplasia and cerebellar dysfunction have been suggested to be attributed to developmental abnormalities of central nervous system structures (Legouis *et al,* 1991), and so a problem with

crossing the midline of the developing corticospinal tract at the pyramidal decussation is a possibility in XKS. The variation of the degree of lack of decussation may be the result of intersubject variability regarding the exact timing of migration. Alternatively it could be due to other homologous proteins which are able to compensate for the genetic defect produced by the KAL gene. For example, subjects K7 and K12 are brothers who have the same genetic defect. In K7 Magstim revealed a predominantly ipsilateral corticospinal tract projection when the left motor cortex was stimulated (ipsi/contra=2.5) and a more predominant contralateral projection when the right motor cortex was stimulated (ipsi/contra=0.6). However, K12 had a predominantly contralateral projection from the left (ipsi/contra=0.1) and the right (ipsi/contra=0.01) motor cortices.

There maybe a relationship between Kallmann's Syndrome and the Klippel-Feil sequence (Zlotogora, 1995). This is interesting because it has been shown in autopsy that in one patient with Klippel-Feil Syndrome that there was an incomplete pyramidal decussation which could result in a motor command being distributed bilaterally to the motoneurone pools (Gunderson and Solitaire, 1968). An incomplete decussation could also explain a predominantly ipsilateral projection to the distal hand muscles which was revealed by Magstim in subjects K1-7 in the present study. Thus it seems likely that a lack of pyramidal decussation could be at least in part responsible for the mirror movements observed in XKS.

Cross-correlation analysis

A short duration central peak was present in all but one cross-correlogram constructed from multi-unit EMG recorded during voluntary co-contraction of left and right 1DI of the subjects with XKS and mirror movements. Short duration central peaks were also present in all but two cross-correlograms constructed from EMG recorded from co-contracting left and right

Fext, 5/9 subjects for left and right Tri and 2/9 subjects for left and right Del. No peaks were present in the cross-correlograms constructed from the data recorded from left and right homologous muscle pairs in the subject without mirror movements, the female carrier, or from the lower limbs in the one mirroring subject tested, or in any of the normal adult subjects (Carr *et al*, 1994).

i.branched last order neurones

The size and the occurrence of the peaks showed a distal to proximal gradient. The size of the central peaks in some of the subjects with XKS, in particular subject K7, was as large as that seen for motor units recorded from the same muscle (Bremner *et al*, 1991 a&b) or from synergistic co-contracting muscles that share a common joint (Gibbs *et al*, 1995a).

The presence of a short duration central peak in a cross-correlogram results from the near simultaneous arrival of excitatory post-synaptic potentials (epsp's) at the two motoneurone pools, indicating that there is a common drive. Such common drive may result from activity in last-order branched neurones presynaptic to the motoneurones (Sears and Stagg 1976, Kirkwood and Sears, 1978) and/or from presynaptic synchronisation (Kirkwood *et al*, 1982; Kirkwood and Sears, 1991). Kirkwood and Sears emphasised that only the narrowest of peaks (half width never more than 2.2 ms) could confidently be interpreted as resulting from activity in last order branched fibres. In the current study, the peaks in the cross-correlograms constructed from EMG recorded from left and right 1DI had a mean half width of 10.9 $\pm$ 1.1 ms (n=12, $\pm$ SEM), and ranged from 6-19ms. However the cross-correlograms were constructed from multi-unit data which could lead to an increase in the duration of the central peak but the extent of this broadening is unclear. Farmer *et al*, (1991) have suggested that the use of multi-unit EMG data will contribute to a broadening of a central feature in the cross-correlogram by as much as 10.0-12.0ms due to differences in conduction time. This might be the case when making single unit

recordings in which the needle maybe distant from the motor point of the motor-unit, but when making surface EMG recordings, the surface EMG electrode is well positioned to record the earliest appearance of the motor unit action potential. Farmer *et al*, (1991) could have over-estimated the contribution of the use of surface multi-unit EMG to the broadening of the central feature in the cross-correlogram. Thus while it is possible that the correlogram peaks in the present study could have resulted from activity in last order branched fibres, taking together the size of the half-widths, and the large ipsilateral MEP's in 8/13 subjects with mirror movements this seems unlikely. It would be necessary to perform cross-correlation analysis using single unit recordings to determine an accurate duration of the half-width to be confident in an interpretation of branching of last order inputs as being responsible for the central cross correlogram peak. The smallest half width was 4ms, obtained from the cross correlogram constructed from the multi-unit EMG recorded from one subject during sustained voluntary activity of left 1DI and the involuntary mirrored activity recorded in right 1DI, but in the remaining subjects the half width was longer, ranging between 6.0 and 17.0 ms.

Furthermore it is also difficult to envisage what the stimulus to branching might be in the subjects with XKS. In the children with hemiplegia, no corticospinal tract projection from the damaged cortex was revealed using Magstim (Carr *et al*, 1993), thus it is possible that the projection from the surviving cortex could branch and innervate bilateral homologous motoneurone pools. But in the subjects with XKS, Magstim revealed bilateral projections from both motor cortices and a stimulus to branching is difficult to envisage in their case.

ii.presynaptic synchronisation

The central cross-correlogram peaks could be the result of synchronisation in the firing of separate presynaptic input fibres originating from neighbouring neurones within the motor cortex, and innervating motoneurones supplying the homologous left and right muscles. In the

group of XKS subjects studied, neurones projecting contralaterally are close to neurones projecting ipsilaterally since the present study has shown that both ipsilateral and contralateral MEP's can be evoked from the same point of cortical stimulation and change in a similar way when the coil is moved to different sites on the motor cortex.

There is evidence for synchronisation in the firing of cortical cells (Allum *et al*, 1982; Smith and Fetz, 1989; Fetz *et al*, 1990). In their recent study of rat cortical cells, Thomson and Deuchars (1994), found that the most powerful connections between pyramidal neurones is between cells in the same column, a column in this case referring to a narrow cylindrical volume of cortex perpendicular to the pial surface. They found that in the deeper layers, some single axon pyramidal-pyramidal neurone epsp's were extremely large (up to 9mV), and could readily elicit post-synaptic spikes. When action potentials in one pyramidal neurone activated a large relatively fast epsp in another, the neurones were found to be separated laterally by less than 100μm. Slower and smaller epsp's were recorded when the two neurones were more widely separated. It is also known that there are inhibitory interneurones present in the rat somatomotor cortex that can influence pyramidal neurones (Thomson & Deuchars, 1994). Perhaps the KAL gene affects the guidance of the axons of these cells resulting in a reduced level of inhibition leading to a stronger synchronisation of neighbouring corticospinal neurones, thus causing a greater degree of synchronisation than that observed in normal subjects when recordings are made from two nearby synergistic muscles of one limb (Gibbs *et al*, 1995a).

The study by Bremner *et al*, (1991b) of motor units in the same muscle (1DI) showed that there was a common drive to different motor units in 1DI, presumably due to branching. The common synaptic input was shown to decrease when motor units from synergistic muscles were tested (1DI and 4DI). If in XKS there is an aberrant decussation it might be anticipated that the axons projecting bilaterally were actually destined to target the same muscle. It is possible that

common synaptic input to the hand muscles could originate from a cortical source, which is still acting despite the fact that one axon has decussated normally but its neighbour has projected ipsilaterally. Recently Baker and Lemon (1995), using a computer simulation based on that of Powers (1993), investigated the post-spike effects in various cell types using the model of Ashby and Zilm, (1982): those having direct monosynaptic connections to motoneurones and fired independently of other cortical cells, others making such connections but synchronised together to produce a cross-correlation peak of 15ms (Smith & Fetz, 1989), and a third type making no connections to the motoneurones, but receiving the same common input. Using spike triggered averaging of the rectified EMG they showed very different post-spike effects for each cell, and concluded that the post-spike effects produced by presynaptic cortical synchronisation make estimation of the strength of the monosynaptic connection between the corticospinal neurone and the motoneurone difficult. Whilst these conclusions are made on the basis of spike triggered averaging, these results suggest that it is difficult to estimate the degree to which last order branching and /or shared common excitatory synaptic input at the cortex contribute to the central feature of the cross-correlgram peak. Work in monkey (Cheney & Fetz, 1985) has shown the presence of last-order branching of axons innervating the motoneurone pools supplying the small hand muscles, and it is also suggested to be the same in man (Bremner *et al*, 1991 a&b), but it is not known to what extent common presynaptic input contributes to the size and duration of the central cross-correlogram peak.

Taken together, these results suggest that synchronisation of cortical cells could account for the common drive detected in the homologous muscle pairs tested.

iii.presynaptic synchronisation of the two motor cortices

Mayer *et al*, (1995), Danek *et al*, (1992) and Cohen *et al*,(1991) in their studies of patients with congenital mirror movements (non XKS), showed that the mirror movements in

these subjects are accompanied by bilateral activation of left and right motor cortices. The studies of movement related cortical potentials reported by Shibasaki and Nagae (1984) in their study of a patient with XKS give support to the hypothesis of bilateral cortical activation. On this basis one would argue that the central peaks in the cross-correlograms were the result of common synaptic inputs driving corticospinal neurones in the left and right cortex projecting to right and left lower motoneurone pools respectively. Evidence for such a drive that would produce synchronisation in the firing of cells in left and right motor cortex has yet to be sought.

While the origin of the common drive to left and right motoneurone pools remains uncertain, it is likely that synchronisation of cortical cells in the same cortex is an important consideration, but bilateral cortical activity cannot be excluded. It is possible that synchronisation of cortical cells in the same cortex in addition to bilateral cortical activation may together be responsible for the mirror movements present in subjects with XKS.

Reflex studies

Stimulation of the digital nerves of the index finger resulted in a readily identifiable reflex modulation of ongoing EMG of 1DI on the stimulated side in 12 out of the 13 subjects with XKS in this study, as observed in normal individuals. The lack of observable reflex responses in the remaining subject probably indicates that the reflex pathway is less excitable in this individual. Indeed tendon jerks were also difficult to elicit in this subject.

In 9 of the subjects with an ipsilateral response, reflex responses were also recorded contralaterally. A similar finding was described for a patient with Klippel-Feil syndrome and children with hemiplegia, all of whom had marked mirror movements (Farmer *et al*, 1990; Carr *et al*, 1993). There was considerable inter-subject variation, paralleling that revealed by Magstim. In those subjects in whom a predominantly ipsilateral corticospinal tract projection was revealed using Magstim (K1-3), only the spinal short latency component of the reflex (E1), was recorded

ipsilateral to the stimulus. In contrast to normal subjects, in these 3 subjects with XKS, the I1 and E2 components were only recorded contralaterally. Thus the present results would indicate that the E2 component which is known to be dependent on the integrity of the dorsal columns, sensorimotor cortex and the corticospinal tract (Jenner & Stephens, 1982) is produced by activity in cutaneous afferents that are relayed normally to the contralateral sensory cortex, but unlike in normal subjects, the efferent pathway descends ipsilaterally.

In an earlier study, it was concluded that the crossed E2 component found in a patient with Klippel-Feil Syndrome and in children with congenital hemiplegia, was due to the presence of corticospinal axons that had branched to innervate left and right motoneurone pools (Farmer *et al*, 1990; Carr *et al,* 1993). A similar explanation could account for the finding in the present study. But if the axons were branched one might anticipate that the E2 component would be present bilaterally. This was not the case in all of the patients with XKS. Alternatively, the crossed E2 could result from a separate population of corticospinal axons which project ipsilaterally due to a failure of decussation at the pyramid. This is also consistent with the findings of Magstim which suggested that neurones projecting ipsilaterally are intermingled with those projecting contralaterally. In this case, both sets of neurones could be simultaneously excited by the cutaneous input, and in the case of an almost exclusive ipsilateral projection, it is conceivable that the contralateral projection is so small that it is not sufficient to produce any visible modulation of ongoing EMG in the muscles of the index finger which is stimulated.

The I1 component was also seen contralateral to the stimulus in all the XKS subjects who had a crossed E2, and was observed in some of the children with hemiplegia (Carr *et al*, 1993). In the original study this component was believed to be of spinal origin (Jenner & Stephens, 1982). However it is difficult to explain the contralateral response in terms of spinal processing in this subject group. There are several reasons for this. In three of the subjects with XKS (K1-3)

all of whom have a predominantly ipsilateral corticospinal projection revealed by Magstim, only the E1 was recorded on the same side of the stimulus, the I1 and E2 only occurring contralateral to the stimulus. One might anticipate that the I1 would be seen on the stimulated side if it results from spinal processing. It has been argued by Carr *et al*, (1993), that the latency of the I1 component is such that there is time for the reflex pathway to be transcortical. It is possible that the I1 (a decrease in ongoing background EMG) is the result of a reduction in excitability of the motor cortex rather than an inhibition of motoneurones mediated via excitation of spinal inhibitory interneurones by a descending cortical volley.

Stretch reflex testing only produced modulation of ongoing EMG contralaterally in 2/6 subjects tested, indicating that the short latency reflex pathway of the spinal circuit is normal for most of the subjects with XKS. For the 2 subjects with contralateral responses the latency was longer than that of the ipsilateral response (9 and 19 ms), but still compatible with a spinal origin. For the subject with the contralateral response at 9 ms, a contralateral CMR response at E1 latency was also seen when the left side was stimulated (K13). For the other subject, K7 in whom the contralateral response was 19ms later, this could be explained by either spinal or even cortical reflex pathways. It is known that the KAL gene is expressed in the human spinal cord at day 45 following fertilisation (Duke *et al*, 1995). At this time corticospinal axons have not yet entered the cord, but it is known that the KAL gene has been detected in the human foetus during week 19, indicating that expression of the KAL gene can occur at various times during development and thus may affect development of migrating axons at different stages.

The abnormal contralateral reflex responses could also result from an abnormal afferent pathway. However, the results from the somatosensory evoked potentials recorded bilaterally in response to unilateral median nerve stimulation in the subjects with XKS did not reveal any differences in those recorded from normal subjects (section 1, chapter 2). Thus no evidence could

be found to support the existence of abnormally projecting ipsilateral afferents that could account for the presence of the contralaterally recorded reflex components in the subjects with XKS in the present study.

Activation of the motor cortex and voluntary movement

Given that the KAL gene can affect neuronal migration and taking together the results of the present study, it seems most likely that the gene deficit has resulted in a lack of decussation of a varying number of corticospinal fibres in the subjects in this study. This is illustrated in figure 7a-c, which provides a scheme with which it is possible to speculate which motor cortex might control unilateral hand tasks in this situation.

All of the XKS patients with mirror movements were able to select which hand should be voluntarily activated, despite the bilaterality of the execution of the motor command. Thus it is interesting to speculate which motor cortex is active when the subject attempts to make a unilateral movement. The ratio of ipsilaterally to contralaterally projecting corticospinal axons as revealed using focal magnetic brain stimulation showed considerable intersubject variation. Table 5 is organised according to the size of the ipsilateral projection, represented by the mean of the ratio of ipsi- to contralateral mean MEP area when the left and then the right motor cortex is stimulated. Subject K1 at the top has the largest ipsilateral projection while K13 at the bottom has the largest contralateral projection. Assuming that the cell bodies of the contralaterally and ipsilaterally projecting neurones lie close together in the motor cortex, then whichever cortex is stimulated, will result in bilateral activation of the motoneurone pools supplied by those neurones. This activation will be simultaneous because these neurones share a common synaptic drive as evidenced by the presence of short duration central peaks in the cross-correlograms constructed from the recordings obtained simultaneously from left and right homologous muscle

Table 5

		Xcorr peak		MAG ipsi/contra			Invol/Vol EMG		CMR	
Subject	**MM**	**E/M**	**Duration (ms)**	**Stim L**	**Stim R**	**Mean**	**R active**	**L active**	**ipsi**	**contra**
K1	2	3.9	28	34.0	256.7*	145.3	0.1♦	0.1♦	E1	I1 E2
K2	1-2	3.3	25	10.0*	3.6	6.8	0.5	0.01♦	E1	I1 E2
K3	2	4.6	15	8.2	2.5	5.4	0.1	0.2	E1	I1 E2
K4	2	7.9	16	1.5	4.0	2.7	0.8	0.6	E1 I1 E2	I1 E2
K5	2-3	3.2	17	3.8	1.2	2.5	0.1	0.6	E1 I1 E2	I1 E2
K6	2-3	7.0	15	3.1	0.3	1.7	3.3	0.6	E1 I1 E2	I1 E2
K7	2-3	17.9	14	2.5	0.6	1.6	0.8	0.8	E1 I1 E2	I1 E2
K8	2-3	7.7	14	1.3	1.0	1.1	1.0	1.2	E1 I1 E2	I1 E2
K9	2	6.4	12	0.7	0.8	0.7	1.0	0.1	***	
K10	1	3.1	14	0.1	0.3	0.1	0.2	0.3	E1 I1 E2	I1 E2
K11	1	6.1	13	0.01**	0.2	0.1	0.004♦	0.2	E1 I1 E2	
K12	1-2	3.1	15	0.1	0.01	0.06	0.2	0.5	E1 I1 E2	I1 E2
K13	2	0.0		0.004**	0.05	0.02	0.006♦	0.04♦	E1 I1 E2	E1 ****

* with background EMG contralateral to the stimulated cortex

** with background EMG ipsilateral to the stimulated cortex

*** no reflex present

**** on stimulating the L digital nerves, a contralateral modulation of ongoing EMG was seen at E1 latency *and* following a tendon tap to the L1DI, a contralateral modulation of ongoing EMG was seen at 24ms (ipsilateral reflex latency was 15ms)

♦ some frames without contralateral EMG

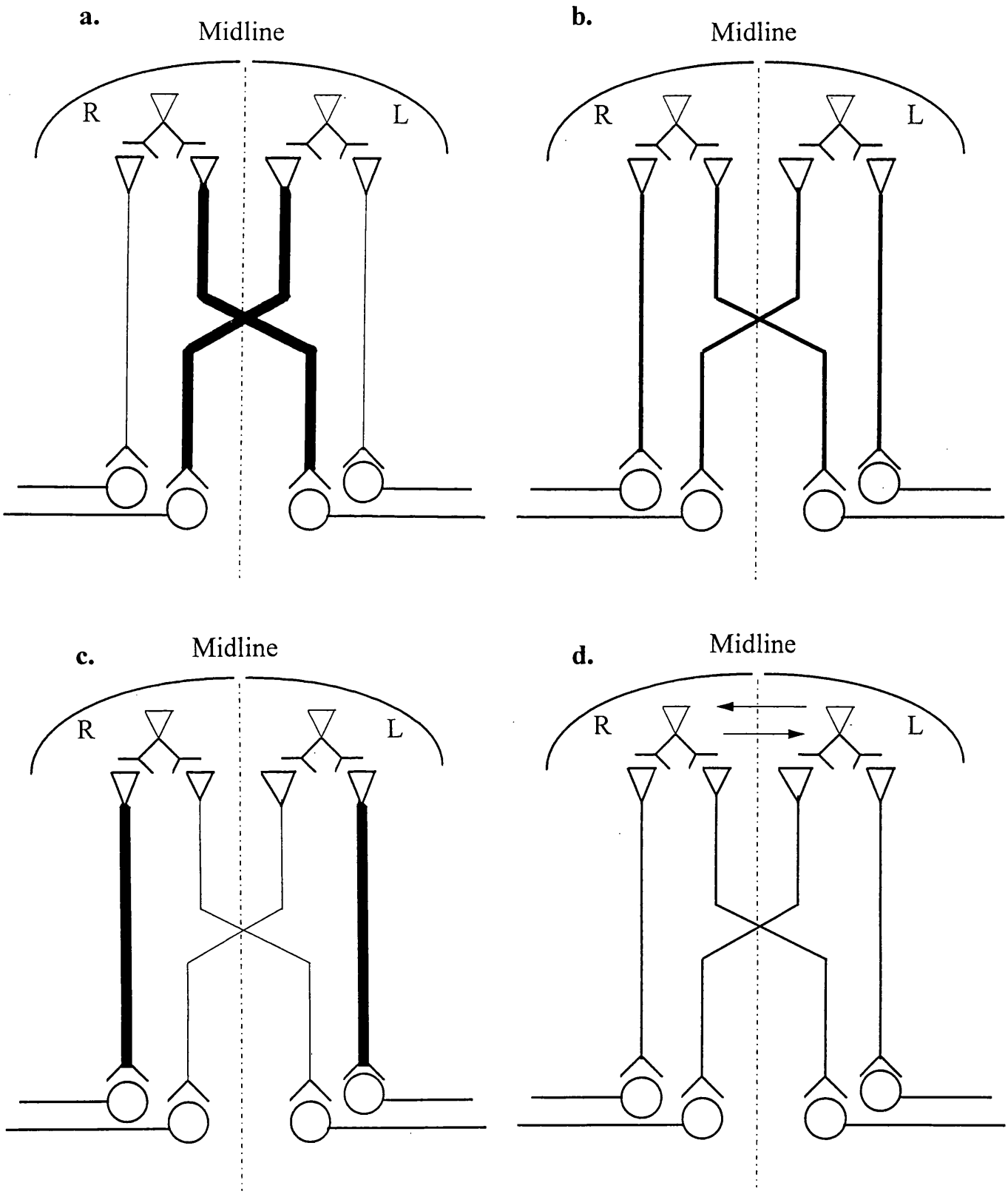


Figure 7: a. Shows the possible scheme of cortical synchronisation of predominantly contralateral corticospinal projecting axons and ipsilateral corticopsinal axons. **b.** Subjects in the middle of the table had a more evenly distributed contralateral and ipsilateral projection while those at the top of the table (**c.**) had a predominantly ipsilateral projection. **d.** Both synchronisation of cortical cells and bilateral cortical acivation maybe responsible for mirror movements in XKS.

pairs.

It is well known that normally unilateral movements are controlled by the contralateral cortex. However, taking subjects K1-3, one might hypothesise that the cortex ipsilateral to the voluntary hand would control that hand as shown in fig. 7c. Taking K1 as an example, when he voluntarily abducts the right index finger, the involuntary activity recorded in the left hand is 10% of that recorded in the right 1DI. If as in normal individuals he was using his left motor cortex, the Magstim data from stimulation of the left motor cortex indicates that the left 1DI EMG recorded should be 34 times greater than that recorded on the right. This is not the case. Alternatively, if he were to use the right cortex (as shown in fig. 7c), then the EMG activity recorded on the left would be expected to be less than that recorded on the right which is the case. This leads us to think that he controls the right hand with the ipsilateral motor cortex. Whilst it is likely that Magstim stimulates the corticospinal neurones directly using the latero-medial coil configuration used in the present study (Roth *et al,* 1991), it is possible that a different population of corticospinal neurones are activated using Magstim than when a voluntary movement is made. Not only this but other supraspinal areas maybe involved. However, considering the size of the ipsilateral projection as revealed by Magstim it is difficult to imagine that this is significantly different. This subject also had a high threshold to Magstim which is known to activate the fast conducting axons associated with RIFM, which could be commensurate with the difficulty this patient had in making selective finger movements.

Using a similar argument, for subjects K11-13, the most appropriate cortex to control distal hand movements would be the contralateral motor cortex shown in fig. 7a, as in normal individuals. When considering the other subjects placed in the middle of the table, K4-K10, the same argument is not so readily applied and these subjects could use either cortex to control distal hand movements as the ipsilateral and contralateral projection is more evenly

distributed to both sides of the body, as shown in fig.7b.

Further evidence for a considerable ipsilateral corticospinal projection in subjects K1-3 is suggested from the results of testing of cutaneomuscular reflexes in which the long latency components were only recorded contralateral to the stimulated side. This suggests that the motor cortex contralateral to the stimulated side primarily projects ipsilaterally while the contralateral projection to the stimulated side is weak and does not produce a visible response to the cutaneous input.

Taking all the data together, it is possible that there maybe more than one mechanism which could account for the mirror movements observed in the subjects with XKS. The peaks in the cross correlograms most likely result from synchronisation of the ipsilateral and contralateral corticospinal projections at the cortex, but in addition to this mechanism, bilateral cortical activation by a command from the premotor area may also be responsible for the mirroring (fig.7d).

Further discussion regarding the choice of motor cortex and voluntary movements is found in chapter 3 of this section.

The association between mirror movements, neurophysiology and phenotype.

This study has used neurophysiological techniques to examine the mechanism underlying the mirror movements which are present in 13 of the 14 subjects with XKS. The intensity of these mirror movements varied from mild (grade1) to marked (grade 3). In general those with the most marked mirror movements had substantial ipsilateral and contralateral corticospinal tract projections when examined using focal magnetic brain stimulation. However, 2 subjects who had significant mirror movements (grade 2), appear at either extreme of table 5, that is the subject with the most predominant ipsilateral projection and the subject with the most contralateral projection. Those subjects with the largest peaks

in the cross-correlograms recorded from voluntarily co-contracting left and right 1DI tended to be in the middle of table 5, and as found using Magstim, there was considerable variation in the size of the peak.

It might be anticipated that the phenotypic anomalies would bear a direct relation to the genotype. However, subjects K11 and K14 possess the same genotype, but K14 has no mirror movements while his brother K11 does. Subjects K9 and K2 are cousins and subject K1 is their uncle; these subjects have the same genotype and share some of the same phenotypic anomalies, and yet the results of the present study indicate that the size of the abnormal ipsilateral projection is different in these 3 related subjects. Other differences have also been found with respect to renal agenesis and morphology of the olfactory bulbs (Duke *et al*, 1995). However, subjects K4 and K5 who were brothers did have similar neurophysiological results.

The mirror movements decreased in intensity from distal to proximal. This was reflected in the neurophysiology in which use of Magstim revealed that the bilaterality of the corticospinal tract projection decreased for the more proximal muscles. In addition the incidence of peaks in the cross-correlogram showed a similar decline. No mirroring was observed in the lower limbs, nor were bilateral MEP's evoked using Magstim, or peaks observed in the cross-correlograms constructed from EMG recorded from co-contracting lower leg and foot muscles. Penfield and Boldrey (1937) observed that of 186 precentral cortical points stimulated, 71 produced hand movements, 67 elbow movements and 23 shoulder movements, suggesting that a larger number of corticomotoneuronal connections target distal muscles. Lawrence & Kuypers, 1968, Lawrence & Hopkins, 1976 and others have shown that in primates there are direct contralateral corticomotoneuronal projections which are responsible for RIFM, whereas proximal movements are also controlled by ipsilateral cortico- and bulbospinal fibres. Thus it has been suggested (Conrad *et al*, 1978),

that the direct monosynaptic connections are more strongly genetically determined and have a more fixed pattern of wiring, and as such maybe more disrupted by the given genetic defect. As previously discussed, there are likely to be homologous proteins to the KAL gene which maybe able to compensate for a lack of KAL, and thus in different individuals the gene deficit maybe manifested in different ways as observed in this and other familial studies. Indeed an XKS pedigree has recently been described in which one apparently unaffected male shares the same mutation as his affected brothers (Parenti *et al*, 1995), and an earlier report identical twins with XKS were described (Hipkin *et al*, 1990). The twins were confirmed identical by genetic fingerprinting: one was hypogonadal and anosmic but although the other twin was hyposmic, he had gone through normal puberty.

Conclusion

Taking all the neurophysiological tests together, there seemed to be no simple relationship between the degree of mirroring and the results obtained, although general trends relating to motor cortical control have been identified. It seems likely that in the subjects with XKS, there is a lack of decussation of the pyramidal tract which results in an abnormal ipsilateral corticospinal tract and underlies the mirror movements associated with this syndrome. But there remains the suggestion that bilateral cortical activation may also be involved. This is discussed in the PET study described in chapter 3 of this section.

Section two; chapter 2. A study of somatosensory evoked potentials in X-linked Kallmann's Syndrome

SUMMARY

1. The subjects with XKS all had shown evidence of an abnormal ipsilateral descending motor pathway in addition to the normal contralateral motor pathway (reported in the previous chapter). Somatosensory evoked potentials (SSEPs) were investigated in a group of 12 normal volunteers and 7 subjects with X-linked Kallmann's Syndrome (XKS) to determine if there was a similar bilaterality of the ascending large diameter sensory pathways to the primary sensory cortex.

2. In all subjects the median nerve was stimulated at the wrist. The configuration and distribution of the SSEP's were examined and size of the resultant SSEP's determined. When a bilateral distribution of the SSEP was found ratios of the size of the ipsilateral to contralateral N20-P25 were calculated to enable a comparison between the normal controls and the subjects with XKS.

3. No evidence was found for an abnormal distribution of the SSEP in the subjects with XKS.

INTRODUCTION

In addition to mirror movements, individuals with X-linked Kallmann's Syndrome are known to have abnormalities other than anosmia and deficiency of gonadotrophin. These include hyposmia, unilateral renal aplasia, nystagmus, cerebellar ataxia, seizures, intellectual impairment, right cerebral hemiatrophy, pes cavus and retinitis pigmentosa (Schwankhaus *et al*, 1989; Duke *et al*, 1995). In the first chapter of this section, it was shown that a likely mechanism underlying the mirror movements investigated in 13 subjects with XKS, is the presence of an abnormal ipsilateral corticospinal tract projection arising from a disorder of pyramidal decussation. In 9 of the subjects reflex EMG responses were recorded contralateral to the side of stimulation, and in theory could result from an abnormal afferent pathway, ie. the ascending volley could project to the ipsilateral cortex instead of, or in addition to the contralateral sensory cortex. In development, the pyramidal decussation occurs at around post-ovulatory (PO) day 57, and the sensory decussation at PO day 52 (O'Rahilly & Muller, 1994), therefore it is possible that an abnormality could also occur in the development of the sensory pathways in patients with XKS, given that it is not known what the time span is for expression of the KAL gene.

The aim of the present study was to investigate the laterality of the afferent pathway to the sensory cortex by the bilateral recording of somatosensory evoked potentials in a group of subjects with XKS studied in chapter one of this section, and a group of normal controls.

METHODS

Subjects

With ethical approval, somatosensory evoked potentials were recorded in 12 normal healthy volunteers aged 22-48 years (5 male) and 7 male subjects with XKS (16-60 years). All of the patients with XKS had mild to marked mirror movements (grade 1-3) and bilateral magnetically evoked potentials (MEP's) from either motor cortex. The neurophysiological tests suggest that in these subjects there is an abnormal ipsilateral corticospinal projection. Although all the subjects had no difficulty in localising the electrical stimulus, more subtle abnormalities of sensory function such as stereognosis, proprioception and two-point discrimination cannot be discounted and therefore formal sensory testing maybe indicated. One subject who was known to be clumsy (K2) also had difficulty with RIFM, but this could be due to a sensory or a motor deficit.

Somatosensory evoked potentials (SSEP)

SSEP's were recorded simultaneously from the left and right sensory cortices with the subjects seated in a warm room in a reclining chair with the neck and head well supported. The subject was instructed to close their eyes and relax. The scalp sites were prepared using a mild abrasive (Omni Skin Prep, Weaver and Co. from Medelec) and silver-silver chloride electrodes (Medelec) were attached to the skin over the left and right sensory cortices using Biotach EEG paste (Medelec). The site for attaching the scalp electrodes was determined using the 10-20 system (Jasper *et al*, 1958). These sites were 2 cm posterior to $C'3$ and $C'4$ which are situated 20% lateral of either side of the vertex. Fz, situated 20% anterior of the vertex was used as a reference site. The impedance of the electrodes was < 4K. The signal was filtered at 10Hz and 2KHz. The right and then the left median nerve were stimulated percutaneously at the wrist at $3sec^{-1}$ using a constant current bipolar stimulator (Medelec

Sapphire), at a stimulus strength sufficient to produce a small twitch of the thumb. The cathode was placed proximally. The cortical potentials were averaged on-line time locked to the stimulus for 500 sweeps. The signals were also stored on magnetic tape for future analysis. The scalp recording showed a prominent negative wave, N20 thought to originate at the primary somatosensory cortex, followed by a prominent positive wave, P25 (Desmedt and Cheron, 1981). The P22 may also be seen, some of which is also seen ipsilaterally. But according to the electrode configuration used in this study, the peaks of interest have been designated as N20 and P25 (Medelec, 1991). For each subject the amplitude of N20-P25 recorded on either side of the cortex was measured and expressed as a percentage of ipsilateral to contralateral. For one subject with XKS (K2) the procedure was repeated on a separate day.

RESULTS

SSEP's in normal healthy adults

Bilateral SSEP's were recorded from all the normal adult volunteers (n=12). The size of the N20-P25 component was measured from the average of 500 sweeps. Figure 1a shows the SSEP's recorded from the left and right sensory cortices following stimulation of the right median nerve in one of the subjects. The amplitude of the SSEP (N20-P25) recorded from the contralateral left cortex was 1.9μV and that recorded from the ipsilateral right cortex was 0.3μV (ratio of ipsilateral to contralateral was 15.1%). Figure 1b shows the SSEP's recorded at the scalp from the same subject when the left median nerve was stimulated. In this case the amplitude of the contralateral cortical potential was 1.8μV and the ipsilateral cortical potential was 0.41μV (ratio of ipsilateral to contralateral was 22.8%). There was considerable variation between subjects and in the results obtained from stimulation of the right and the left median nerve. The ratio of the ipsilateral to contralateral response was independent of whether the left or right median nerve was stimulated (paired *t*-test, P>0.05).When the right median nerve was stimulated, the response recorded over the right sensory cortex ranged from 15.1% to 49.6% (mean 36.5, SEM ± 5.3, n=12) of the response recorded over the left sensory cortex. When the left median nerve was stimulated the response recorded over the left sensory cortex ranged from 11.2% to 69.9% (mean 33.2, SEM ± 5.0, n=12) of the response recorded over the right sensory cortex.

SSEP's in subjects with XKS

Bilateral SSEP's were recorded from all of the subjects with XKS studied. Figure 2a & b shows the SSEP's recorded from subject K1 in whom a predominantly ipsilateral corticospinal tract was revealed using Magstim. The SSEP recorded from the contralateral left cortex was 6.24μV and that recorded from the ipsilateral right cortex was 1.38μV (ratio

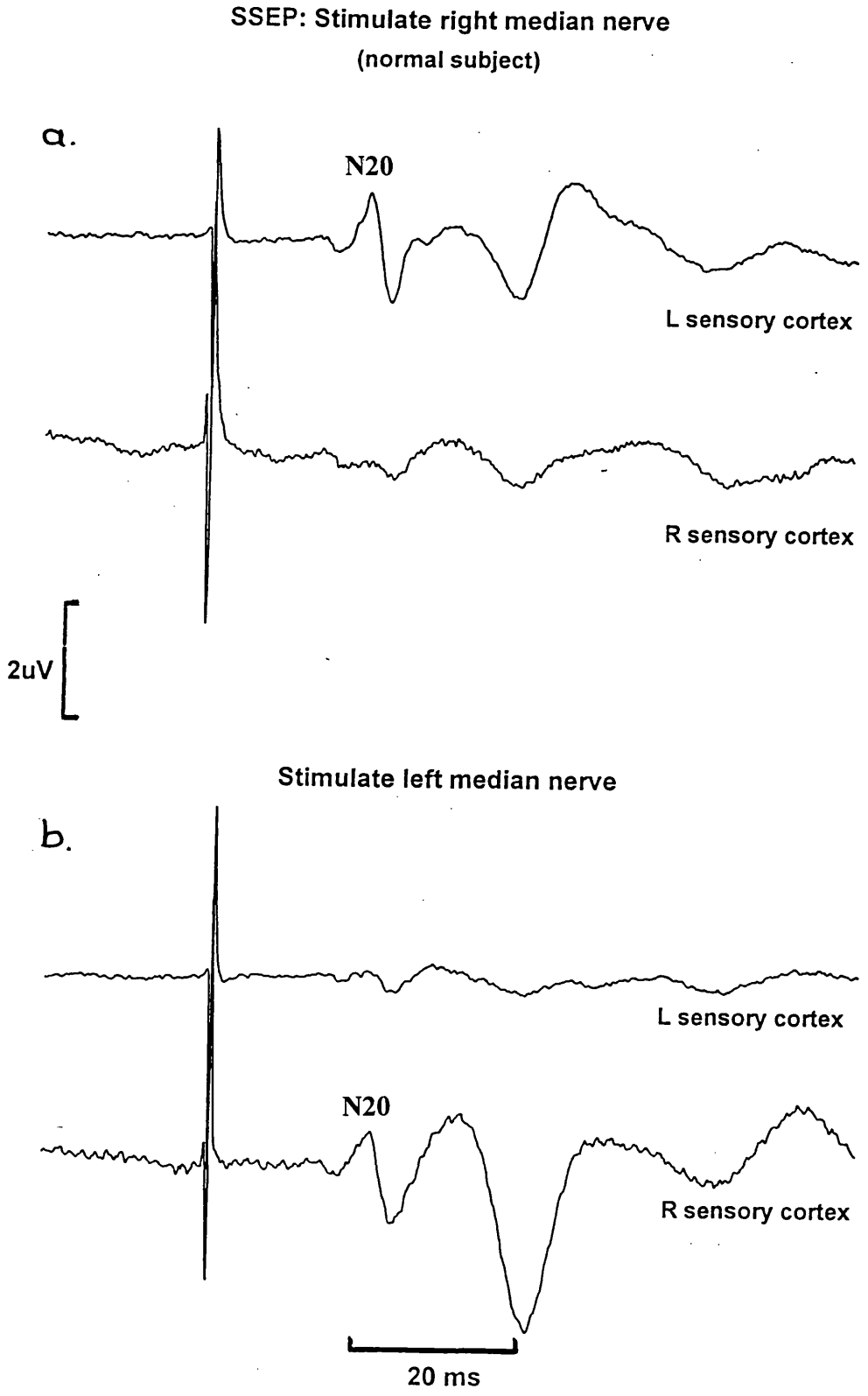


Figure 1: SSEP recorded in a normal subject. The upper panel shows the result of stimulation of the R median nerve. An SSEP was not recorded ipsilateral to the stimulus. But when the L median nerve was stimulated a small ipsilateral response was recorded at the primary sensory cortex. There is a small N20 response at 20.1ms.

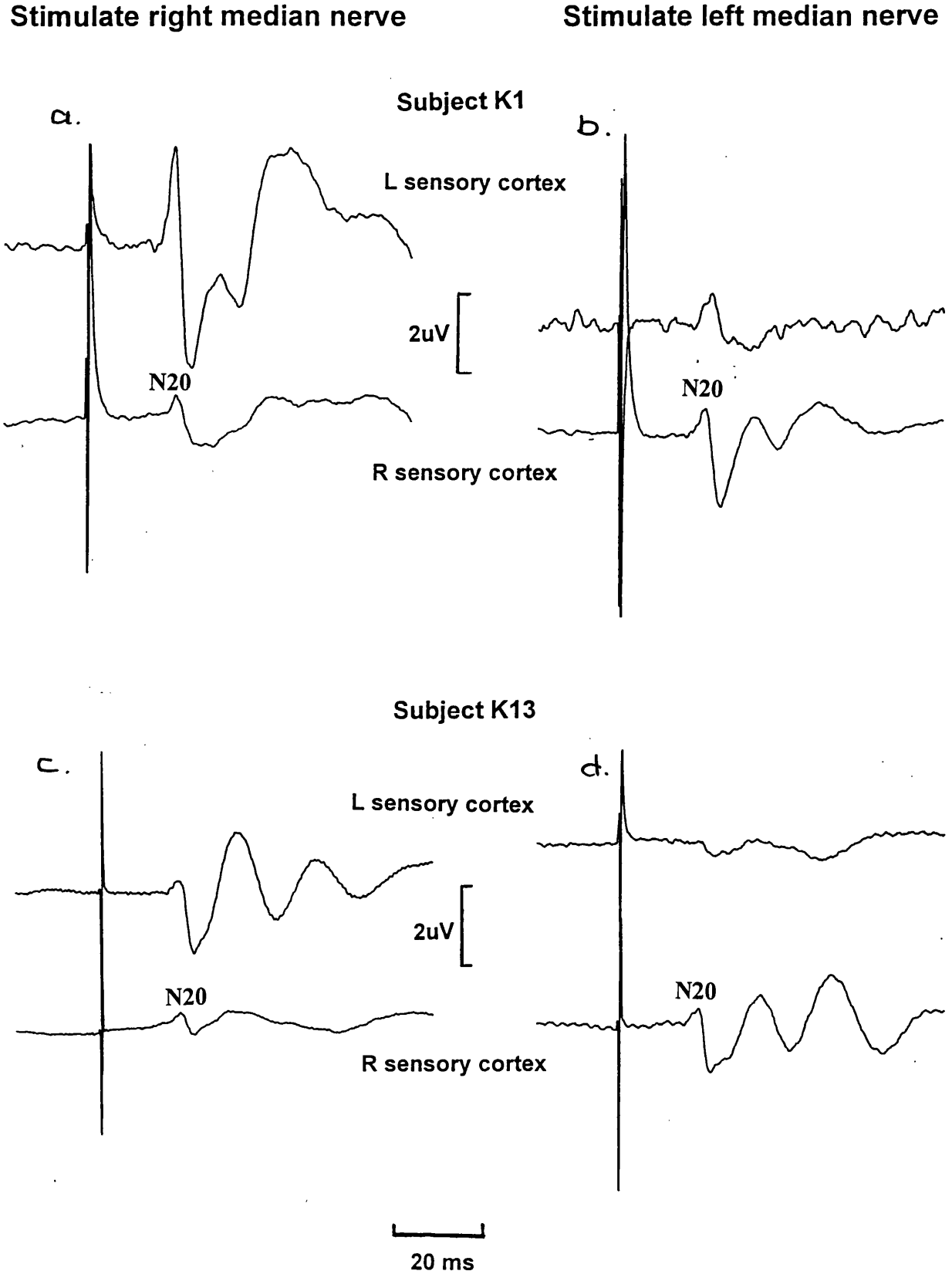


Figure 2: The upper panel shows the SSEP's recorded from K1, in whom Magstim revealed a predominantly ipsilateral projection. When the right and the left median nerve were stimulated a small N20 was recorded ipsilateral to the stimulus at 22.0ms and 23.0ms respectively. In subject K13 in whom Magstim revealed a predominantly contralateral projection, a small ipsilateral SSEP was also recorded at 21.0ms and 22.0 respectively.

of ipsilateral to contralateral was 22.1%) when the right median nerve was stimulated. Fig. 2b shows the SSEP's recorded at the scalp from the same subject when the left median nerve was stimulated. In this case the amplitude of the contralateral cortical potential was 2.74μV and the ipsilateral cortical potential was 1.37μV (ratio of ipsilateral to contralateral was 50.0%). There was considerable variation between subjects and in the results obtained from stimulation of the right and the left median nerve. Figure 2c & d shows the SSEP's recorded from subject K13 in whom Magstim had revealed a predominantly contralateral corticospinal tract projection. When the right median nerve was stimulated the ratio of ipsilateral to contralateral SSEP amplitude was 28.57% and when the left median nerve was stimulated the ratio was 25.29%. There appeared to be little difference between these 2 subjects who were placed at opposite ends of table 5 in the previous chapter (ordered according to degree of ipsilateral projection as revealed using Magstim). The ratio of the ipsilateral to contralateral response was independent of whether the left or right median nerve was stimulated (paired *t*-test, $P>0.05$).When the right median nerve was stimulated, the response recorded over the right sensory cortex ranged from 16.2% to 55.1% (mean 36.5, SEM $\pm$ 5.3, n=7) of the response recorded over the left sensory cortex. When the left median nerve was stimulated the response recorded over the left sensory cortex ranged from 20.3% to 50.8% (mean 32.2, SEM $\pm$ 4.4, n=7) of the response recorded over the right sensory cortex.

In subject K2 in whom recordings were made on two separate days, similar results were obtained. When the right median nerve was stimulated the ipsi- to contralateral ratio was 37.5% and 55.1% respectively, and when the left median nerve was stimulated the ratio was 20.4% and 23.2% respectively).

Comparison of SSEP's in normal volunteers and subjects with XKS

There was no significant difference between the results from the subjects with XKS and those recorded from the normal volunteers (unpaired *t*-test, P>0.05). Both for the normal subjects and for those with XKS, the ratio of the ipsilateral to the contralateral response was independent of whether the right or left median nerve was being stimulated (paired *t*-test, P>0.05).

DISCUSSION

This study has shown that when recording SSEP's bilaterally in normal subjects, that an ipsilateral component can be recorded. The same was found for the group of subjects with XKS. However no significant difference ($P>0.05$) was found between the size of the ipsilateral to contralateral ratio when the two groups were compared, and when left and right were compared. The question arises as to what the origin of this ipsilateral component might be. There appears to be a lack of information in the literature regarding the bilateral recording of SSEP's. Whilst there is general agreement that bilateral recordings in response to unilateral stimulation are the norm by clinical neurophysiologists in this field, little is known as to the exact origin of the ipsilateral component. There are two main possibilities. One possibility is that is due to volume conduction and another might be that there is an ipsilaterally generated potential.

i.) Presence of an ipsilateral sensory pathway

There is a divergence of opinion as to whether there are specific identifiable cortically generated potentials that can be recorded by ipsilateral electrodes. There have been several reports of ipsilateral responses. Tamura *et al*, (1972) recorded ipsilateral SSEP's but these were delayed by 4-8 msec in adults. Salamy (1978), recorded potentials bilaterally in response to a vibratory stimulus and found a similar delay of 4-8 msec in adults, and a longer delay, 20-28 msec in children aged 4-5 years. They attributed the bilaterality of the recording to conduction via the corpus callosum. Further support for this hypothesis is found in the work in monkey by Iwamura *et al*, (1994). In their study they found cells in the cortex which responded bilaterally to unilateral sensory input, and concluded that this was due to conduction via the corpus callosum. Further evidence is found in studies of acallosal subjects and patients with unilateral cerebral lesions, and although these are not in complete

agreement, they are all compatible with the existence of a transcallosal projection from the contralateral hemisphere rather than a separate ipsilateral ascending pathway.

In the present study any ipsilateral response recorded was found to occur at the same latency as the contralateral response (any small latency difference was insignificant), thus it is unlikely that the ipsilateral component is the result of conduction via the corpus callosum. A simpler explanation would be that the ipsilateral component is the result of volume conduction from the contralateral hemisphere. This is likely given the recording arrangement and the close proximity of the cephalic reference electrodes.

ii.) Volume conduction of SSEP's

All ipsilateral responses recorded in the present study were of the same polarity and similar latency to those recorded contralaterally. This is what would be expected if they were volume conducted. In a study by Kakigi (1986), a similar result was obtained. SSEP's were recorded bilaterally in response to stimulation of the median nerve. He found that some components showed a phase reversal (P19 and N25), but all other potentials were of the same polarity and latency and were found to diminish progressively with distance from the contralateral hemisphere. Kakigi concluded that these non-reversing signals were volume conducted from the contralateral hemisphere rather than originating in the ipsilateral hemisphere through uncrossed ascending pathways, or via interhemispheric connections.

Conclusion

With this in mind, the most likely explanation for the bilateral recordings found in the present study is that the ipsilateral recordings are due to volume conduction. Although there is a suggestion of bilaterality of the SSEP's in the XKS group, no significant difference was found in the ipsilateral to contralateral ratios for the subjects with XKS and the normal controls. Although the results suggest that the ascending pathways are of normal laterality and project to the contralateral sensory cortex in the subjects with XKS tested in this study, further investigation of sensory function seems indicated.

Section 2; Chapter 3: A PET study of mirror movements in X-linked Kallmann's Syndrome

SUMMARY

1. Changes in regional cerebral blood flow (rCBF) were measured using H_20^{15} Positron Emission Tomography (PET) during an externally paced finger thumb opposition task in seven subjects with X-linked Kallmann's Syndrome (XKS) and six normal healthy volunteers. The PET scans were analysed on both a group and individual basis. Simultaneous surface EMG recordings were made. Electrodes were attached to the skin overlying the left and right first dorsal interosseous muscles. Any involuntary EMG recorded was rectified and averaged time locked to the start of the voluntary phasic burst and expressed as a ratio of the EMG recorded from the voluntary side.

2. All subjects had an MRI scan which was coregistered with the PET scan to enable accurate localisation of changes in rCBF. Any abnormalities of brain morphology, in particular the corpus callosum and anterior/posterior commissures were noted.

3. It was found that the group of subjects with XKS showed a strong ipsilateral M1 activation when compared with the M1 activation contralateral to the voluntarily moved hand. But the M1 activation contralateral to the voluntary hand was greater. In contrast, taken as a group, the control subjects showed only M1 activation contralateral to the voluntarily moved hand. Single subject analysis revealed ipsilateral activation in 2/6 normal controls, but this was very small.

4. Passive movements in 2 normal subjects produced changes in rCBF which were similar to the changes produced by active movements. Thus the ipsilateral activation measured in the subjects with XKS could be generated by sensory feedback from the mirroring hand.

INTRODUCTION

A number of different mechanisms have been proposed to account for the production of mirror movements in different pathologies. Some authors favour an abnormal corticospinal tract projection (Conrad *et al*, 1978; Britton *et al*, 1991; van der Linden & Bruggeman, 1991; Carr *et al*, 1993), while others suggest bilateral cortical activation occurring as a result of a lack of transcallosal inhibition (Forget *et al*, 1986; Danek *et al*, 1992).

Cohen *et al*, (1991) studied two patients with mirror movements of unknown aetiology. They performed both electrophysiological tests and Positron Emission Tomography (PET) and found abnormalities in maps of motor and premovement potentials and in the PET scans which were consistent with a bilateral representation of hand muscles in the motor cortex. They concluded that there were physiologically active ipsilateral as well as contralateral motor pathways, and that both motor cortices were active during voluntary movement. A similar conclusion was reached by Shibasaki and Nagae (1984) who recorded movement related cortical potentials in a subject with X-linked Kallmann's Syndrome (XKS) and mirror movements. In that study they found a premovement negative slope bilaterally to accompany an intended unilateral hand movement, and suggested that the mirror movements were generated by unintended excitation of the primary motor cortex opposite to the mirroring hand.

It has also been suggested by Danek *et al*, (1992) that the mirror movements observed in XKS are due to bilateral cortical activation resulting from a lack of inhibition across the corpus callosum. This lack of interhemispheric inhibition has also been suggested as the mechanism to account for the mirror movements observed in young normal children during development (Nass, 1985). In the study of such movements described in Section 2, chapter 2, the involuntary EMG recorded in the children studied was found, on average, to commence at the same time as the voluntary EMG, as found in the subjects with XKS. But in those

children, and in contrast to the findings in the subjects with XKS, no central features were observed in the cross-correlogram, and no bilateral responses of similar latency were recorded using Magstim. Thus, there are most likely different mechanisms underlying the mirror movements observed in young children and in pathologies such as XKS, Klippel-Feil Syndrome and congenital hemiplegia.

The findings of the neurophysiological tests reported in the previous chapter suggest that the mirror movements present in subjects with XKS, may result from an abnormal ipsilateral corticospinal tract whose cell bodies are situated close to those of the axons which project to motoneurones innervating the contralateral distal upper limb muscles, and therefore bilateral homologous motoneurone pools may share common synaptic input. In all subjects with mirror movements bilateral magnetically evoked potentials were recorded in the left and right 1DI, and in 12/13 of the group of subjects with XKS and mirror movements a short duration central peak was found in the cross-correlogram constructed from the EMG recorded from left and right 1DI. In addition, the I1 and E2 components of the cutaneomuscular reflex were recorded contralaterally in 8/12 of the subjects, further suggesting the presence of an abnormal corticospinal tract projection.

The present experiment used PET to measure changes in regional cerebral blood flow in seven of the 14 subjects with XKS who had been investigated electrophysiologically. The subjects were scanned while performing a simple finger-thumb opposition task with the left and the right hand. The results of the electrophysiological tests suggested that in the subjects with XKS both the voluntary and involuntary mirror movements may originate from the same cortex. Thus it might be anticipated that those XKS subjects with a predominantly contralateral corticospinal tract projection might control their hand movements with the cortex opposite to that of the voluntarily moved hand. In this case contralateral M1 activation would be expected using PET. On the other hand in those patients with a predominantly

ipsilateral projection, it might be anticipated that movements of the voluntarily activated hand would be controlled by the ipsilateral motor cortex. In this case no contralateral M1 activation, but rather ipsilateral M1 activation would be seen.

Evidence from other human studies using PET show areas of ipsilateral activation during unilateral hand movements in normal subjects (Shibasaki *et al*, 1993; Matsumura *et al*, 1995). But, evidence from experiments in monkeys have shown that the activity of cells in M1 can be modulated by passive movement of the contralateral hand (Lemon, 1981; Cheney & Fetz, 1984). This raises the possibility that any ipsilateral M1 activation could be the result of sensory feedback from the mirroring hand in the subjects with XKS.

The present study has used PET to compare the rCBF in a group of subjects with XKS and a group of normal controls to determine similarities and differences in their cortical control of a simple finger-thumb opposition task. In addition, two subjects were studied to determine if any bilateral activation recorded could be the result of sensory feedback from the mirroring hand.

METHODS

Subjects

Six males with XKS (aged 20-48 years) with mirror movements (grade 1-3) were studied. The subjects had participated in the neurophysiological tests reported in previously in chapter 2.1 (K2, K6, K7, K9, K10 and K11). A seventh male with XKS (K14), who is the brother of K11, also participated in both studies. Six normal male subjects (N1-N6, age 23-47 years) were also studied to act as controls. To compare active and passive movements, a further 2 normal male subjects were studied (N7, N8, aged 34 and 49 years). All subjects were tested for hand dominance using the Edinburgh Handedness Inventory (Oldfield, 1971). All were right handed.

The study involved the administration of 4.8mSv effective dose equivalent of radioactivity per subject, and was approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health of the United Kingdom. The subjects gave informed written consent, and the study was approved by the joint research ethics committee of the Royal Postgraduate Medical School, Hammersmith Hospital, London, and by the Ethics Committees of the Royal Free Hospital School of Medicine and University College London.

Neurological examination

All subjects were examined by a neurologist to exclude any relevant neurological signs other than mirroring in the group with XKS, and to exclude unknown signs in the group of normal volunteers. The degree of mirroring was assessed according to the criteria of Woods and Teuber (1978) as described in section one, chapter one of this thesis.

EMG recordings

Surface EMG was recorded using pre-gelled electrodes as described in previous experiments. EMG was recorded simultaneously from left and right 1DI and left and right index finger flexors in all subjects except N1-N3. The subjects could not be provided with visual or auditory feedback of their performance because all the scans were performed with the subjects lying in supine with their eyes closed in a dimly lit room (see figure 1). The EMG signal was filtered at 100Hz and 5 Khz and stored on magnetic tape for future analysis. In the normal subjects the sensitivity of the channels recording from the inactive muscles was set high (20-50 μV) to ensure that any involuntary EMG would be detected. The EMG was rectified and averaged, time locked to the onset of the phasic burst, and a ratio of involuntary to voluntary EMG was calculated in the subjects with mirrored activity. An average of the 4 trials for each condition was calculated.

i.) active movements:

All subjects were instructed to perform short and sharp phasic finger thumb opposition externally paced by an electronic metronome at one per second (1 Hz). During condition A subjects voluntarily performed right index finger-thumb opposition, and voluntarily performed the same movement with the left hand in condition B. Condition C indicates no voluntary activity and the subject was instructed to relax and listen to the tone. The three conditions were randomised over twelve runs in the order ABCCBAACBBCA. A simple movement paradigm was chosen rather than a complex movement sequence which is known to involve bilateral activation of supplementary motor area (SMA) (Roland *et* al, 1980), as the area of activation of primary interest was M1.

ii.Active versus passive movements:

In 2 subjects, passive movements were carried out. In this case condition A was active right index finger thumb opposition; during condition B the investigator held the thumb and index finger at either side of the distal interphalangeal joint to mimic the subjects active movements. In condition C the subject was instructed to relax. This protocol was used for subject N8, but for N7, only two conditions were given: passive movement (B) and rest (C). As in the previous paradigm, the movements were externally paced using an electronic metronome at 1Hz. All trials whether active, passive or relaxed were monitored on video during all trials. All recordings were examined to look for involuntary EMG in the contralateral limb of the non-mirroring subjects as well as looking for spontaneous EMG activity during rest and passive movements in all subjects.

MRI scans

All subjects had cranial MRI using a Picker 1 Tesla machine. A 3-D reconstruction and scalp editing of the MRI data were performed using ANALYZE (Robb & Hanson, 1991). The scans were also examined to look for any structural abnormalities of the brain such as abnormality of the corpus callosum, significant cerebral hemisphere asymmetries and abnormalities of ventricle size. It had been noted during the neurophysiological study that some subjects (particularly K1, K2, K9 and K10) had an abnormally shaped skull which may reflect abnormal brain morphology.

PET scanning

Scans were obtained using a CTI model 953B-PET scanner (CTI Inc., Knoxville, Tennessee, USA) with collimating septa retracted to increase the acceptance angle and the number of photons recorded (Grootoonk, 1995). Regional cerebral blood flow (rCBF) was measured by

recording the regional distribution of cerebral radioactivity following a 20 second intravenous bolus of $H_2{}^{15}O$ at a concentration of 55 mBq/ml at a flow rate of 10 ml/min through a cubital fossa vein of the left arm. ^{15}O is a radionuclide with a half life of 2.0 minutes and is produced by bombarding stable elements with high energy beams of ionic particles accelerated in a cyclotron, leaving them with an additional proton. Twelve consecutive PET scans were collected at 10 minute intervals, each over a period of 2 minutes. There was an initial 5 minute scan to ensure optimal head position (all scans are realigned to the first emission scan to correct for head movement). The head position was checked before each run and all scans began with a 30 second background scan before delivery of the bolus. The integrated radioactivity counts accumulated over the 90 second acquisition period, were corrected for background and used as an index of rCBF. With a field view of 10.8 cm in the z-plane (vertical axis, above and below the anterior-posterior commissure line), the subjects were positioned so as to include the top of the brain, including all of the supplementary motor area (SMA) and much of the cerebellum.

Data analysis

Image analysis was performed using statistical parametric mapping (SPM 95, Wellcome Department of Cognitive Neurology, London, UK), (Friston *et al*, 1995a&b) on a SPARC 20 workstation (Sun Microsystems Inc. London UK). Calculations and image matrix manipulations were performed in PRO MATLAB (Mathworks Inc., New York, USA).

Each rCBF scan was reconstructed as 31 axial planes and all 12 scans for each subject realigned. A mean image of these 12 scans was then used to coregister individual PET-data onto the individual MRI data set. The MRI scan of each subject was aligned directly to a line linking the anterior commissure (AC) and the posterior commissure (PC). This is known as the standard AC-PC reference line,(Talairach and Tournoux, 1988). After normalisation, the

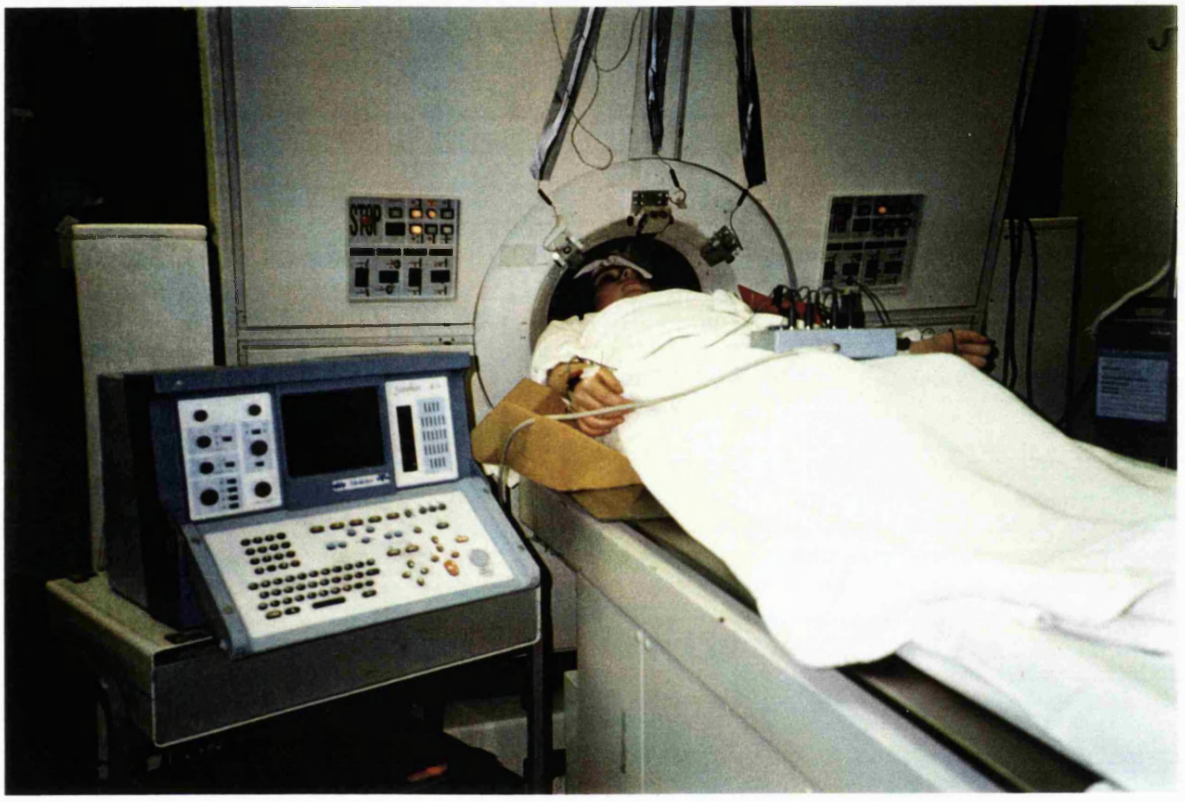


Figure 1. The photograph shows one of the subjects in the PET scanner. The body and limbs are fully supported. Surface EMG electrodes are attached to the skin overlying the left and right 1DI and the index finger flexors.

PET data set extended from -32mm below the AC-PC line to +72mm above it. A smoothing filter of 12mm was used to accommodate intersubject differences in gyral anatomy and to optimise the signal to noise ratio. Differences in global activity within and between subjects were removed by analysis of covariance (ANCOVA) on a pixel by pixel basis. For each pixel in stereotactic space, the ANCOVA generated a condition specific adjusted mean rCBF value. This allowed comparisons of the mean blood flow distributions across all sets of conditions using the t-statistic. The resulting sets of t-values constituted the statistical parametric maps.

Various comparisons were made:

1. Activation for each group

For both the group of subjects with XKS and the normal volunteers, a comparison was made during movement and at rest for each hand.

2. Intergroup comparison

A comparison was made between the activation (movement versus rest) for the group of subjects with XKS and the activation (movement versus rest) for the controls for voluntary movement of each hand.

3. Activations contralateral to voluntarily moving hand and mirroring hand

A comparison was made of the rCBF values in each hemisphere when moving either hand. The size of activation contralateral to the voluntarily moved hand was compared directly to the activation contralateral to the mirror movements. This comparison enabled a determination of which hemisphere was more strongly activated in the case of bilateral activation of homologous cortical areas.

4. Single subject analysis

Whilst it is generally believed that group analysis is more powerful when using PET (Friston *et al*, 1990), because of the intersubject variability of brain anatomy in the subjects with

XKS, single subject analysis was carried out. Recently Rajkowska & Goldman-Rakic (1995) suggested that there are individual differences in cytoarchitecture of the human cortex. They compared their data on the variable morphology of areas 9 and 46 with the Talairach and Tournoux (1988) stereotaxic atlas which is used to localise cortical areas in mapping changes in rCBF using PET. They explained that the Talairach right hemisphere is based on Brodmann's map of the left hemisphere, and that this might introduce some error in the analysis of cortical localisation. High spatial resolution can be obtained by co-registering rCBF with the same level slice of the individual's own MRI thus enabling more accurate localisation of the cortical areas in which significant changes in rCBF are recorded.

5. Active vs passive activation of M1

For the two normal subjects a comparison was made between passive movements and rest, and in one a comparison was also made between active and passive movements.

RESULTS

EMG recordings

i.Normal controls

Table 1 summarises the occurrence of involuntary EMG in the normal volunteers

Examination of the EMG recordings revealed the presence of low amplitude EMG in the resting right hand in subjects N4 and N6 during voluntary movement of the left hand. An example of such activity is shown in figure 2. In this subject (N6), activity of the left 1DI and index flexor is accompanied by involuntary EMG in the right 1DI (observed in all frames) and to a lesser degree in the right index flexor (observed in 39% of the frames). In subject N6 low amplitude ongoing EMG was observed in the resting hand when either hand was voluntarily active.

No involuntary EMG was observed in subjects N7 and N8 who participated in the active/passive movement study.

Table 1

Subject	Tap left	Tap right	Rest
N4	3/4	1/4	1/4
N5	0/4	0/4	0/4
N6	1/4	3/4	4/4

Table 1: Low amplitude EMG was recorded during at least one trial in all conditions in 2/3 normal control subjects in whom simultaneous EMG and PET recordings were made. No involuntary EMG was recorded in subject N5.

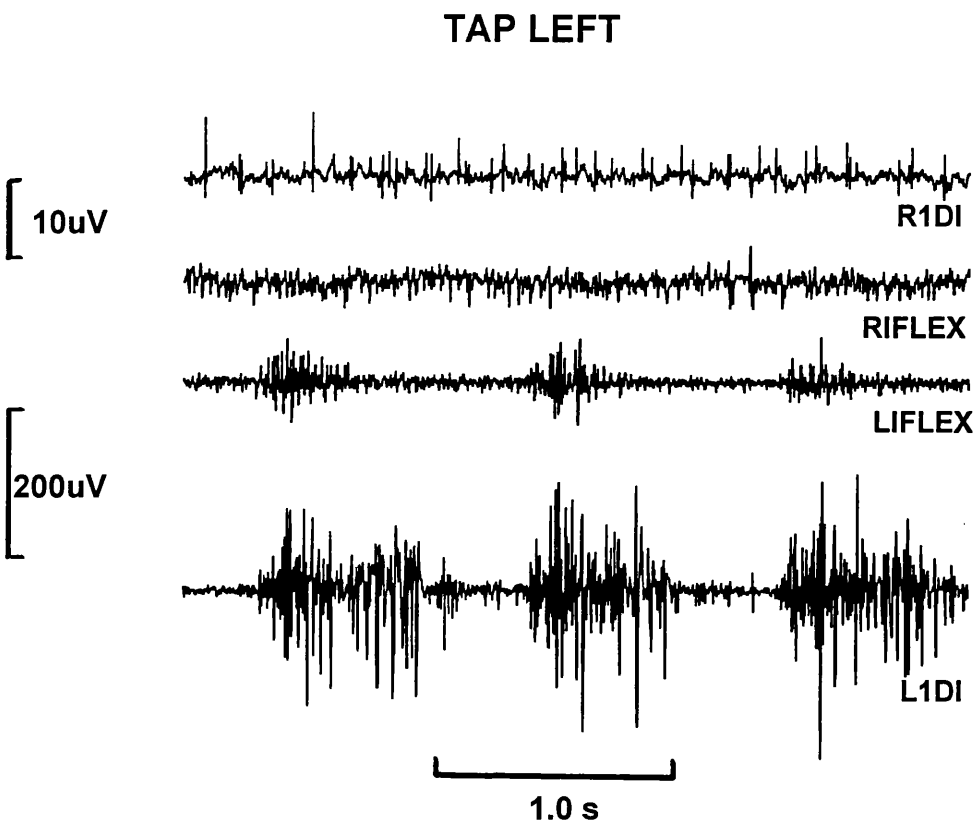


Figure 2. This figure shows the raw EMG recorded from subject N6 during tap left. The upper two traces show the involuntary EMG recorded from right 1DI and index flexors respectively. The lower two traces show the EMG recorded from left index flexors and 1DI respectively during tap left.

ii.Subjects with XKS

Table 2 shows the ratio of involuntary to voluntary EMG in left and right 1DI during the active movement trials. Column 6 of the table also indicates the occurrence of EMG during the rest condition. In all subjects the involuntary EMG recorded was always less than the EMG recorded from the voluntary hand, except for K6 during tap right. In one of the four trials more involuntary EMG was recorded in the mirroring hand than the voluntarily active hand (invol/vol = 2.58), resulting in a mean of 1.18 ±0.47 (n=4, ± SEM).

Table 2

Subject	Right hand active I/V (n=4)	±SEM	Left hand active I/V (n=4)	±SEM	EMG during rest (n=4)
K2	0.07	0.002	0.09	0.002	0/4
K6	1.18	0.47	0.60	0.15	2/4 (bilateral)
K7	0.34	0.06	0.53	0.19	3/4 (mostly L)
K9	0.38	0.04	0.21	0.06	4/4 (bilateral)
K10	0.55	0.10	0.45	0.13	3/4 (bilateral)
K11	0.02	0.01	0.03	0.01	1/4(mostly R)

Table 2: Involuntary EMG was recorded in all of the subjects with XKS. The ratio of involuntary/voluntary EMG ranged from 0.02-1.18 when the right hand was voluntarily active and from 0.03-0.60 when the left hand was voluntarily active. Involuntary EMG was recorded during some of the trials in all subjects except K2.

MRI

No abnormalities were detected in the MRI scans of the normal subjects, but in the subjects with XKS abnormalities including hemispheric asymmetry and dilatation of the ventricles was noted. Fig.3 shows the MRI scan of subject K9 in whom the left hemisphere is anteriorly displaced. In this subject no other abnormalities were detected.

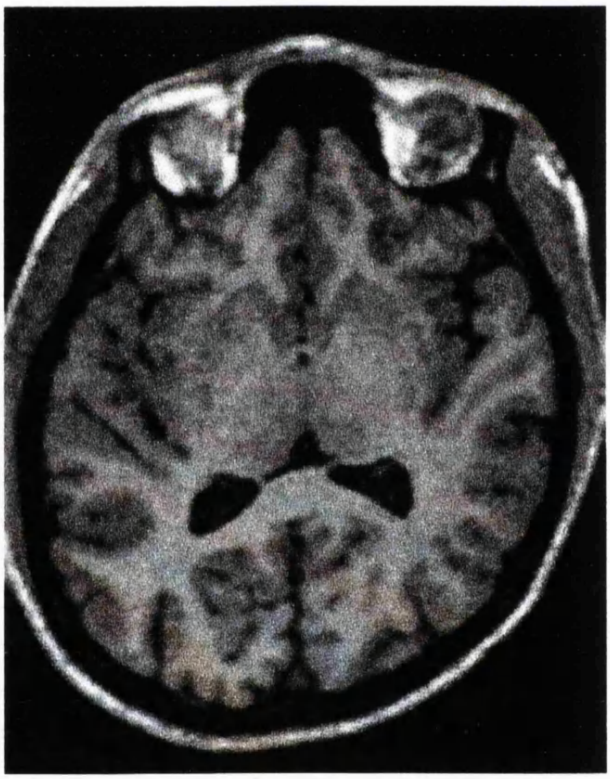


Figure 3: Subject K9 whose MRI scan revealed anterior displacement of the left cerebral hemisphere with corresponding malformation of the skull.

But in subject K2, the ventricles were dilated, right more than left, particularly in the posterior horn region. In subject K7 hemispheric asymmetry was noted and in this case the right hemisphere was slightly anteriorly displaced. Subject K11 also showed hemispheric asymmetry with the right occipital and temporal cortices anteriorly displaced. Ventricular dilatation, left more than right was also noted. No abnormalities were detected in subject K6 and K10, and in all of the subjects the corpus callosum and the anterior and posterior commissures were present and morphologically normal.

PET

i.Activations for each group

Table 3 summarises the areas of activation for the two groups of subjects. These data represent the pooled data in each group of subjects The capital letters represent which cortex is active, and the lower case letters denote which is the voluntarily active hand. An area of activation which did not show a significant change in rCBF is shown as n.s. Foci of significant activation during voluntary movement of the right hand for example are shown in column *Lr* (activation contralateral to the moving hand) and *Rr* indicates activation ipsilateral to the moving hand. In the same way column *Rl* shows significant activation contralateral to the voluntarily moved left hand, and *Ll* represents significant activation of the area ipsilateral to the voluntarily active hand.

Columns 7 and 9 of the table show that there is significantly more ipsilateral activation of M1 and S1 in the subjects with XKS than in the normals during voluntary movement of either hand. This ipsilateral activation can also be seen as being contralateral to the mirroring hand.

	NORMALS				SUBJECTS WITH XKS			
Area	*Lr*	*Rr*	*Rl*	*Ll*	*Lr*	*Rr*	*Rl*	*Ll*
M1	✓	n.s.	✓	n.s.	✓	✓	✓	✓
S1	✓	n.s.	✓	n.s.	✓	✓	✓	✓
SMA	✓	n.s.	✓	n.s.	✓	n.s.	✓	✓

Table 3: Areas of activation comparing movement and rest in normal subjects and those with XKS are indicated with ✓ if significant ΔrCBF and n.s. if no significant change measured.

Individual subject activations

Each activation was checked for its anatomical location by coregistering the individual PET image with the individual MRI. This allowed a distinction to be drawn between activations in M1 and S1. For this individual data the significance level was set at $P<0.01$. This level was chosen to maximise the opportunity of detecting an ipsilateral M1 activation in the normal subjects.

M1

Fig.4 shows the activations for the individuals in the group with XKS when voluntarily moving their right or left hand. The PET images for the individuals are coregistered onto their MRI scans. The co-ordinates given are in stereotaxic space in millimetres (Talairach & Tournaux, 1988) for the maximally significant pixel in each area in the order x (lateral displacement from midline, - for left hemisphere); y (antero-posterior displacement relative to the anterior commissure, - for positions posterior to this) and z (the vertical position relative to the AC-PC line, - if below this line). Only the z position is used in the figures.

In 4/6 subjects with XKS and mirror movements (K2, K7, K10 and K11), the predominant activation was in M1 contralateral to the voluntarily moved hand. This was also the case for

K14 who had no mirror movements. Taken as a group, all of the subjects with XKS who had mirror movements, there was activation of M1 ipsilateral to the voluntarily moved hand, that is contralateral to the mirroring hand. This degree of activation varied between subjects. For K6 there was right M1 activation irrespective of which hand was moved voluntarily.

For subject K9 both the left and the right M1 were strongly activated whichever hand was voluntarily moved. In subject K14, the subject with XKS and no mirror movements, there was a small activation in the left M1 when voluntarily moving the left hand. In the normal subjects, ipsilateral activation of M1 was seen in 1/6 subjects (N2) while moving the right hand and in 2/6 subjects (N2, N6) when moving the left hand.

S1

In all of the subjects with XKS and mirror movements there were distinct foci of activation in M1 and S1. All subjects showed strong S1 activation contralateral to the voluntarily moved hand. In addition all but one subject with XKS with mirror movements (K6), showed S1 activation ipsilateral to the moving hand (contralateral to the mirroring hand). The degree of ipsilateral activation varied between the subjects. There was no ipsilateral activation in K14 (XKS but no mirror movements) or in any of the individuals in the group of normal subjects.

M1 activation during active vs. passive movements

Figure 5 a and b shows the activation for N8 when actively moving the right hand and when the right hand was moved passively. There were significant activations of M1 and S1 of the left hemisphere during passive movements compared with rest of the right hand. This was found in both the subjects studied. In N8 the contralateral M1 activation which resulted from movement of the active hand was not significantly different from the activation resulting from passive movement of the hand ($P < 0.01$). In N8 there was a small but significant

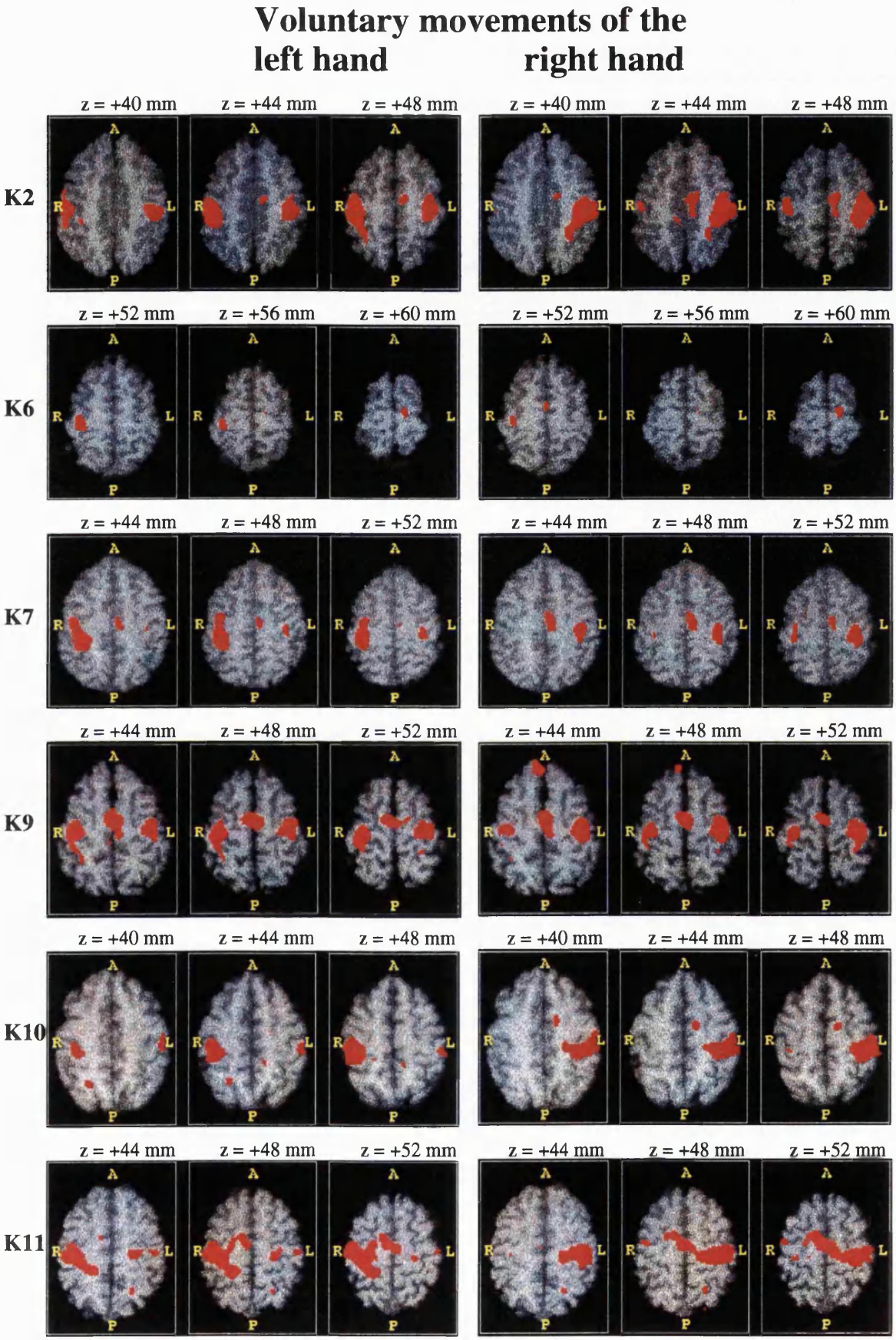


Figure 4: The PET scans for each subject with XKS are shown. Transverse sections have been cut through M1. All show bilateral M1/S1 activation except for K6, in whom only right activation was seen when either hand was moved.

activation in the ipsilateral premotor area or possibly M1 which was only present during active hand movements. This focus of activity was found at 42mm above the AC-PC line (z= +42).

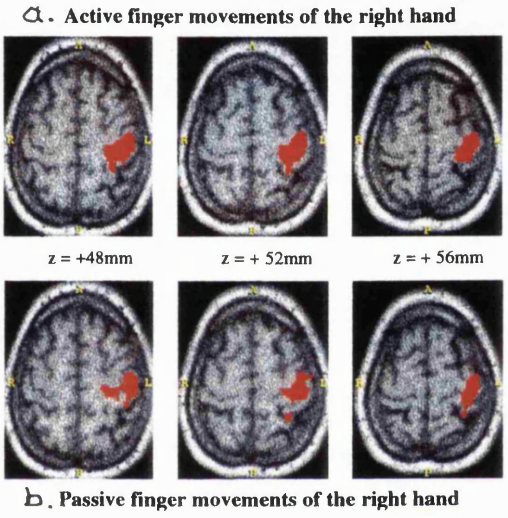


Figure5: This shows the activation of M1/S1 in N8 during active right (upper panel) and passive movement of the right hand (lower panel). No significant difference was found in the area and amount of activation associated with active and passive movements.

DISCUSSION

This study has compared patterns of ΔrCBF during a simple finger tapping task in a group of subjects with XKS and a group of healthy volunteers. The results indicate that the group of subjects with XKS have significant activation of M1 bilaterally, thus significant changes in rCBF were recorded in the hemisphere ipsilateral to the moving hand and opposite to the mirroring hand. Very small but significant ipsilateral M1 activation was also found in K14 (the subject with XKS and no mirror movements) and in 2/6 normal controls when the left hand was active. In one of these two subjects ipsilateral activation was also recorded during movement of the right hand. When compared with the group of normal subjects, in the group of subjects with XKS, there was a significant difference in M1/S1 activation ipsilateral to the voluntarily moved hand ($P< 0.001$). In both the normal volunteers and the group of subjects with XKS the activation of M1 was greater contralateral to the voluntarily moved hand than contralateral to the mirroring hand.

Single subject analysis of the group with XKS also showed greater contralateral activation in 4/6 subjects. In subject (K9), this was only the case for the left hemisphere if a lower significance level was accepted ($P< 0.05$). In addition, in 2/2 normal subjects, passive movements of the right hand resulted in activation of the contralateral M1. In one of these subjects a comparison of M1 activation during active and passive movements revealed no significant difference in the two conditions.

M1 activation ipsilateral to the moving hand

i.normal volunteers

Ipsilateral activation of M1 has been reported in other studies using PET (Cohen *et al*, 1991; Shibasaki *et al*, 1993; Matsumura *et al*, 1995; Stephan *et al*, 1995), *f*MRI (Kim *et al*, 1993a; Rao *et al*, 1993) and magnetoencephalography (MEG) (Salmelin *et al*, 1995).

However, in the study by Shibasaki *et al*, (1993) and Stephan *et al*, (1995), this ipsilateral activation was seen only with complex movement tasks. Unlike in the present study, simultaneous EMG recordings during the tasks were not carried out in those studies. In the current study, involuntary EMG was recorded contralateral to the moving hand and/or during rest periods in 2/3 subjects. It is not known how such activity relates to changes in rCBF. For example, in subject N4 low amplitude involuntary EMG was recorded in the right 1DI and index flexors during tap left, but this was not associated with significant changes in rCBF in the contralateral cortex. However in subject N6 involuntary EMG was recorded in the left 1DI and index flexor in 3/4 tap right tasks and significant changes in rCBF were detected. It is therefore not possible to be certain whether this involuntary activity was associated with ipsilateral M1 activation in the normal volunteers. In addition, in some subjects EMG was recorded during rest conditions, and it is not known how this may affect the recorded rCBF in this condition which is then used as a comparison for the active movement tasks.

In 2/6 normal volunteers, the ipsilateral activation accompanied left, but not right-handed activity. A study by Kim *et al*, (1993b) using *f*MRI suggested that there is a hemispheric asymmetry in the functional activation of the human motor cortex during contralateral and ipsilateral finger movements. In their study, they found predominantly right motor cortical activation during left finger movements, but in addition found that there was substantial activation of the left motor cortex. Indeed this ipsilateral activation was stronger in the right handed subjects (C/I = 1.3) than the left handed subjects (C/I = 5.4). As previously discussed, ipsilateral motor cortical activation has also been linked to task complexity (Shibasaki *et al*, 1993; Stephan *et al*, 1995). It is possible that for these 2 subjects the given task was not usual for them and thus cannot be considered to be simple in their case. For example subject N4 had practised this task many times prior to being a subject for this study and did not show any significant ipsilateral M1 activation when either hand was

used. Although the ipsilateral activation recorded in the present study was significant, the size of this ipsilateral activation was very small and not as substantial as that reported in the study by Kim *et al*, (1993b), and therefore could be commensurate with the task being considered novel for the naive subjects.

ii.Subjects with XKS

a. Consideration as a group

Taken as a group, in the subjects with XKS and mirror movements, significant ipsilateral M1/S1 activation was recorded during the PET scans during tap left and tap right. In their PET study of 2 subjects with congenital mirror movements, Cohen *et al*, (1991) also reported bilateral activation of the sensori-motor cortex. However, in that study it was not possible to distinguish between M1 and S1 activation due to poor spatial resolution of PET at that time, nor were the PET images co-registered with MRI scans to enable accurate localisation of the area of activation. In their 2 patients with mirror movements, bilateral premovement potentials were recorded as well as bilateral responses to magnetic stimulation. They concluded that in these patients that both motor cortices were active during voluntary hand movements, but in addition the results of cortical stimulation showed that there were physiologically active contralateral and ipsilateral pathways. Recently Mayer *et al*, (1995) also reported that they had recorded bilateral movement related potentials in a group of subjects with congenital mirror movements. The results from these studies seem to agree with the data from Shibasaki & Nagae (1984). They reported that they recorded bilateral premovement cortical potentials during unilateral voluntary movement in a subject with XKS. However, the neurophysiological tests reported in this section (chapter 1), indicate that there is a spectrum of abnormal cortical organisation within a group of subjects with XKS, thus the result from one subject with XKS can differ greatly from another. We therefore need

to be cautious in interpreting the findings from this one subject. Furthermore, the mechanism responsible for mirror movements in XKS may differ from that responsible for mirror movements in other pathologies such as congenital mirror movements.

In the present study, comparison of M1/S1 activation contralateral to the voluntarily moved hand with M1/S1 activation contralateral to the mirroring hand showed that the activation is significantly stronger in the cortex contralateral to the voluntarily moved hand. This could be argued to be consistent with the mirror movements being usually of smaller amplitude than the voluntary movements. Danek *et al*, (1992) in their study of 3 brothers with XKS, concluded that a lack of transcallosal inhibition resulting in bilateral cortical activation was responsible for the mirror movements, a finding supported by the present PET study, but unlikely in light of the findings of the electrophysiological study reported in this thesis.

Another possibility that could account for the ipsilateral M1 activation reported in the current study in the subjects with XKS, could be that it results from sensory feedback from the mirroring hand. The present study has shown that contralateral M1 activation can occur as a result of passive movement in the hand. No involuntary EMG was recorded that could be said to be associated with the significant M1 activation recorded. However in the one subject (N8) no significant difference was found in M1 activation during active movement when compared with passive movement. This is confusing at first, but it has been suggested that changes in rCBF measured using PET reflect changes in the activity in cell terminations (Jueptner & Weiller, 1995). If this is the case, the M1 activation seen in this study could represent the sum of the afferent activity from those regions which project to M1, including S1, parietal area 5, premotor cortex, SMA and ventral thalamus (Ghosh *et al*, 1987; Muakkassa & Strick, 1979). Thus, M1 activation could be associated with both active and passive movements. Further support for M1 activation as a result of sensory feedback during passive movement is found in other studies in primates. Lemon, 1981 in monkey; Cheney

and Fetz in the rhesus monkey (1984) and Andersson in anaesthetised cats (1995). In their study of responses of corticomotoneuronal cells to passive movements in the monkey, Cheney and Fetz (1984) found that 14 of the 17 cells (82%) tested responded at short latency to passive wrist movements which stretched their target muscles. Lemon, (1981) in his study recording the response to afferent input from a larger number of neurones (216) found that 38% of neurones responded to movement of one or more joints in the relaxed monkey.

b. Consideration of individual subjects

The data from the neurophysiological tests and the PET study do not seem to be in agreement with each other. The main findings from both the PET and the neurophysiological studies are summarised in tables 4a & b.

For example, take the data for "tap right" in K2 in the summary table below for the subjects with XKS. Motor evoked potentials using Magstim only produced ipsilateral responses in 1DI when relaxed, but bilaterally in pre-activated 1DI, which were considerably larger ipsilaterally when the left motor cortex was stimulated. Stimulation of the digital nerves produced a short latency reflex component ipsilaterally, but the longer latency components were only recorded contralaterally. In addition, the EMG recorded from the active hand during tap right was ten times that recorded in the mirroring hand. Taken together this data suggested that the right hand is most likely controlled by the right hemisphere. But, the PET data suggests that in K2 the right hand is controlled by the left motor cortex which is more active than the right motor cortex (13% and 7% increase respectively compared with rest). A similar finding was found for the contralateral and ipsilateral sensory cortices. The activity recorded in M1/S1 maybe the sum of the activity associated with the mirroring hand. But in the case of K2 the mirror movements were weak and it might be expected than the sensory feedback from the mirroring hand would be associated with less cortical activation than that

from the moving hand and thus could be associated with activity in right M1/S1. An alternative possibility might be that K2 is indeed controlling the movements of the right hand with the left cortex. In this case however, one might expect that the EMG from the mirroring hand would be far greater than that recorded in the voluntarily active hand. However this was not the case. Yet another possibility maybe that right handed movements are controlled by the left motor cortex, and that the large ipsilateral projection revealed by Magstim is not used. That is, the premotor area directs the left cortex to move the right hand, but only the contralaterally projecting axons are activated. This may account for the lack of RIFM observed in this subject, but with the presence of such a large ipsilateral projection revealed

RIFM: 0=absent; 1=almost absent; 2=some; 3=normal **MM**: mirror movements
Mag I/C: Magstim ratio of size of ipsilateral to contralateral MEP areas (n=10)
EMG: involuntary to voluntary ratio during abduction or finger/thumb opposition
M1 = primary motor area **S1** = primary sensory area

Tap right	K2	K6	K7	K9	K10	K11
RIFM	1	3	3	3	1	3
MM	2	3	3	2-3	2-3	1
Mag I/C (Stim left)	10.0*	3.1	2.5	0.7	0.1	0.01**
EMG I/V (L/R)abd	0.5	3.3	0.8	1.0	0.2	0.004•
EMG I/V opposition	0.1	1.2	0.3	0.4	0.6	0.02
Contra M1	13%	8%	7%	15%	7%	9%
Ipsi M1	7%	12%	5%	12%	5%	4%
Contra S1	13%	n.s.	10%	7%	15%	7%
Ipsi S1	7%	11%	7%	5%	10%	4%

Table 4a: Tap right. Rows 3 & 4 show the Magstim data and invol/vol EMG ratios for L:R 1DI for the neurophysiology tests; rows 5-9 show the invol/vol EMG recorded during PET data collection as well as the percentage changes in rCBF (compared with rest) for contra and ipsilateral M1/S1. • = not all sweeps recorded involuntary EMG; * = with contralateral background EMG; ** = with ipsilateral background EMG.

Tap left	K2	K6	K7	K9	K10	K11
RIFM	1	3	3	3	1	3
MM	2	3	3	2-3	2-3	1
Mag I/C (Stim left)	3.6	0.3	0.6	0.8	0.3	0.2
EMG I/V (R/L) abd	0.01•	0.6	0.8	0.1	0.3	0.2
EMG I/V opposition	0.10	0.6	0.6	0.2	0.5	0.03
Contra M1	14%	17%	6%	13%	6%	6%
Ipsi M1	10%	6%	9%	12%	4%	4%
Contra S1	8%	13%	16%	7%	10%	10%
Ipsi S1	10%	n.s.	8%	5%	4%	4%

Table 4b: The neurophysiology data is shown in rows 3 & 4; the PET data in rows 5-9 as in table 4. The rCBF is given as percentage change compared with rest. • = not all sweeps recorded involuntary EMG.

by Magstim testing, it is hard to envisage how it would not be involved in the control of hand movements in this subject. Yet another confounding factor is the data from the SSEP's which ha. shown that the sensory afferents are apparently normal and project from the hand to the contralateral sensory cortex. This suggests that the sensory feedback from the hand is directed to the contralateral cortex. Any deficit in sensory perception would most likely be reflected in clumsiness of hand function and difficulty in identification of sensory stimuli if movements are controlled by the ipsilateral motor cortex. None of the subjects had difficulty in identifying the sensation of digital nerve stimulation, although K2 did have difficulty with RIFM. But his mother also had difficulty with RIFM and had a high threshold to stimulation using Magstim (chapter one, this section) and so this might be explained in terms of heredity.

Although the SSEP's recorded from the subjects with XKS were in the same range as

the normal controls, bilaterality of the sensory afferents cannot be ruled out. More specific sensory testing maybe indicated in addition to testing of the functional use of cutaneous sensory inputs as described by Gordon *et al*, (1994). Alternatively, the appropriate conduction of the sensory afferents to the contralateral cortex may result in that cortex showing significant activity which is associated with sensory feedback from the active hand, with the ipsilateral cortical activation being associated with the motor activity of the active hand. It has been shown that there was no significant difference in M1 activation associated with active or passive movement. It has also been suggested that changes in rCBF measured by PET reflect pre-synaptic activity and thus reflect activity in several neuronal populations which project to M1/S1 (Jueptner & Weiller, 1995). No conclusion can be made about how this subject controls his right hand or his left hand when one considers the PET data alone, although the neurophysiological data strongly suggests the presence of a predominantly ipsilateral corticospinal tract projection which is responsible for the control of finger movements.

At the other extreme we can consider K11, in whom a predominantly contralateral corticospinal tract projection was revealed using Magstim and reflex testing. In his case the PET data suggested that the contralateral cortex was primarily active during movements of either hand and thus as in normal subjects, controls the opposite hand.

In subject K7, testing with Magstim revealed that the corticospinal projection from the right motor cortex was more evenly distributed to the left and right 1DI (ipsi/contra=0.6). Similarly from the left motor cortex a substantial ipsilateral projection was recorded using Magstim (ipsi/contra=2.5). When the digital nerves of the index finger were stimulated the short latency component was recorded ipsilaterally as in normal subjects, but in contrast to normals the longer latency component was recorded bilaterally. In addition a large short duration central peak was present in the cross-correlogram constructed from the EMG

recorded from left and right 1DI. The PET data from K7 revealed bilateral cortical activity with predominant contralateral activation suggesting that the hand is controlled by the contralateral motor cortex. In K7 the ipsilateral activity could be associated with sensory feedback from the mirroring hand. This could also be the case for K9 in whom the PET data showed little difference in the degree of activation of left and right motor cortices when either hand was moved. He had strong mirror movements which could be compatible with significant M1/S1 activation resulting from sensory feedback contralateral to the mirroring hand. Such activity can be as great as the M1/S1 activation associated with active movements as shown in this study. In his case, either cortex could control the movements of the hand.

Subject K6 was different from all other subjects with XKS. When he performed a voluntary abduction with the right index finger, the EMG recorded from the mirroring hand was greater than that recorded from the active hand (I/V=3.3 index abduction; 1.18 $\pm$ 0.45 finger-thumb opposition). Consonant with this finding, the testing with Magstim revealed a larger ipsilateral projection from the left motor cortex (ipsi/contra=3.1), suggesting that the left cortex controls the right hand. But the PET data ha. shown that only the right cortex shows significant activity during movements of the left and the right hand. If this is the case, then the PET findings are consistent with the neurophysiology if for some reason only the contralaterally projecting axons are activated during a voluntary command. This is difficult to envisage given the presence of a significant ipsilateral projection which shares common drive with the contralateral projection as evidenced by the cross-correlogram peaks. Magstim revealed that there is a considerable contralateral projection (3:1) from the right cortex which could control the left hand, but there is a weak projection from the left cortex which may not be strong enough to be shown to be active at the chosen level of significance (P= 0.001) when the right hand is moved. The right cortical activation observed on move right could be associated with sensory feedback from the contralateral mirroring hand.

Whilst the neurophysiological and PET data are consistent with each other for those subjects with a predominantly contralateral or more equal corticospinal tract projection, there appears to be some difficulty in interpreting the data from the subject with a predominantly ipsilateral projection. Another question that arises from this discussion, is the different way in which the data are collected. For example, in contrast to the data from voluntary movements as in PET, the data from the stimulation studies, such as Magstim and CMR's do not require involvement of premotor or association areas as in a voluntary movement (Brooks, 1986). It might be possible that pathways that can be shown to be present for example using Magstim, might not be used when a voluntary movement is produced. Furthermore, it has been suggested that PET represents the sum of activation of other cortical and supraspinal areas (Jueptner & Weiller, 1995), and as such may result in different conclusions than those derived from stimulation studies. Another problem of interpretation is that the rCBF measured using PET cannot distinguish between excitatory and inhibitory activity in the regions studied. It is possible that areas of activity revealed using PET may represent an inhibitory process in an attempt to suppress the involuntary mirrored activity. Therefore the PET data alone cannot give the exact location of the source or type of motor activity associated with the mirror movements investigated in the present study, and the neurophysiological data would appear to be more robust.

Conclusion

In the current study bilateral cortical activation of the motor cortex was found in the patients with XKS. Taken as a group, this activation was greater in the cortex contralateral to the voluntarily moved hand than in the cortex contralateral to the mirroring hand. It is possible that the M1 activation contralateral to the mirroring hand could result from sensory feedback from that hand, as the results have shown that significant M1 activation can result

from passive movements of the contralateral hand. However, bilateral cortical activation cannot be excluded, and the mirror movements in XKS may be produced by bilateral cortical activity in addition to activity in fast conducting corticospinal axons innervating homologous bilateral motoneurone pools and which share common synaptic drive.

The results of this study alone do not enable definite conclusions to be made as to the exact mechanism underlying the mirror movements in XKS. Further sensori-motor studies of the patients with a predominantly ipsilateral corticospinal projection in addition to further studies of active and passive movements in normal controls and a group of subjects with XKS may help to provide a clearer picture.

SECTION THREE

A neurophysiological study of associated reactions and associated movements in man.

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Section three: A neurophysiological study of associated reactions and associated movements in man.

SUMMARY

1. EMG recordings were made in the upper limbs of two children with idiopathic mirror movements and five children with spastic cerebral palsy (spastic quadriplegia) and associated reactions during phasic unilateral hand or finger movements. These results were considered alongside those already obtained from the group of subjects with X-linked Kallmann's Syndrome (XKS) and mirror movements studied in section two. In all of the subjects, voluntary activity was accompanied by involuntary activity in the contralateral homologous muscles.

2. Focal magnetic brain stimulation was used to assess the distribution and integrity of the corticospinal tract. In all of the subjects with XKS and pronounced mirror movements stimulation of either cortex evoked bilateral responses in left and right 1DI; the ipsilateral response was larger in 8 out of the 13 subjects when the right cortex was stimulated, and in 6 out of 13 subjects on stimulation of the left motor cortex. In the subjects with idiopathic mirror movements, stimulation of either motor cortex resulted in motor evoked responses in left and right 1DI, but in one subject the ipsilateral response was larger. In the children with cerebral palsy the results were variable, but in 3/5 bilateral motor responses were evoked from the less damaged cortex.

3. Cross-correlograms were constructed from motor-unit spike trains recorded simultaneously from hand or forearm homologous muscle pairs during steady voluntary co-contraction of the muscle pair (left and right 1DI and/or left and right forearm extensors). As in the subjects with XKS, a short duration central peak was found in the cross-correlogram constructed from

the EMG recorded from the co-contracting bilateral homologous muscle pairs in the two subjects with idiopathic mirror movements. No central features were observed in the cross-correlograms constructed from the EMG recorded from the children with spastic cerebral palsy.

4. Cutaneous reflex responses have been recorded in 1DI following electrical stimulation of the digital nerves of the index finger. Unlike in normal subjects where the reflex is only recorded on the stimulated side, in 8/12 of the subjects with XKS and one of the subjects with idiopathic mirror movements, the long latency component of the reflex was recorded bilaterally. The reflex was only recorded on the stimulated side in the children with spastic cerebral palsy.

5. The study provides evidence to suggest that there are differences in the mechanisms underlying associated m*ovements* and associated *reactions*. Furthermore, the results suggest that there are different mechanisms underlying the associated mirror movements observed in different pathologies.

INTRODUCTION

Associated *movements* maybe defined as unintentional movements that accompany an intended action. Examples of these involuntary movements include mouthing associated with writing, and grimacing associated with a difficult hand task, but when such movements are observed contralateral to an intended action and are similar in appearance, they are known as mirror movements. Mirror movements have been described in detail in the first two sections of this thesis. Such movements can occur normally during development or may accompany forceful activities in an adult. They are also associated with various pathologies.

Associated *reactions* occur under similar circumstances and are always associated with spasticity (Bobath, 1990). They do not mirror the intended movement, but rather are seen as an exaggeration of the pattern of spasticity already present or emerging. For example, an associated reaction maybe seen when a patient with a spastic hemiplegia attempts to use the unaffected hand to perform a task which for him/her maybe difficult. Performance of the task results in an involuntary movement into flexion of the opposite elbow, wrist and fingers, pronation of the forearm and depression of the shoulder girdle (typical pattern of spasticity), irrespective of the direction of the voluntary movement. Thus the involuntary movement does not mirror the intended action.

Walshe (1923) described the involuntary movements observed in patients with spasticity as the "associated movements of hemiplegia" and suggested that they would best be referred to as associated reactions to distinguish them from the associated movements which occur in the presence of normal tone. Earlier Marie & Foix (1916) had described various categories of synkinesis subdividing associated movements into three sub-groups. They referred to the associated reactions as "syncinesies globales". The mirror type of movements were referred to as "syncinesies d'imitation" and the third type which were

involuntary movements synergic to voluntary movements, were referred to as "co-ordinated syncinesies."

Zulch and Muller (1969) attempted to classify associated movements as physiological or pathological, the latter occurring in response to a neurological lesion. They provided a comprehensive review of reports of associated movements in the literature since 1840. But since most of these reports made reference to associated movements in spastic pathologies, generally spastic cerebral palsy, it is possible that the movements which they described were actually associated reactions. The subject is further complicated by the simultaneous occurrence of associated *movements* (mirror movements) and associated *reactions* in some patients with spasticity (clinical observation).

Obligatory mirror movements have been described in association with various pathologies including Klippel-Feil syndrome (Farmer *et al*, 1990), children with congenital hemiplegia (Green, 1967; Carr *et al*, 1993) and Kallmann's syndrome (Danek *et al*, 1992; section 2 of this thesis). They maybe familial (Regli *et al*, 1967) or idiopathic (Cohen *et al*, 1991; Harrison *et al*, 1993), but can be seen during normal development in young children (Connolly & Stratton, 1967; Lazarus & Todor, 1987) and maybe observed in adults performing complex finger movement sequences (Krams, personal communication) or forceful finger movements (Durwen & Herzog, 1992; Armatas *et al*, 1994). In the latter two cases when associated movements are observed in normal individuals, such movements are not obligatory. Thus it is likely that there are various mechanisms that can account for the presence of associated (mirror) movements, and that these mechanisms are different from those underlying the associated reactions which accompany intended movements in patients with spasticity.

These two categories of involuntary movement accompanying an intended skill have often been confused when described in the literature. For example, van Sant and Williams (1986) described a study of associated reactions in normal boys, but used the term interchangeably with associated movements. Cohen *et al*, (1991) and Mayer *et al*, (1995) in their study of congenital mirror movements were careful to make a distinction between the "associated movements" occurring with spasticity and the associated movements seen as mirror movements. Furthermore, the terms associated movements and mirror movements are often used in the same context. Clearly, mirror movements are a sub-classification of associated movements and therefore it would be helpful to clarify the terminology relating to such movements. In addition it would be helpful if it were possible to make a clear distinction between associated reactions and associated movements to enable correct identification and communication of clinical signs.

In a previous section of this thesis the pathophysiology underlying mirror movements in X-linked Kallmann's syndrome has been described (section 2). In the following section these results are summarised again alongside the results obtained in cases of idiopathic mirroring and those in children with CP and associated *reactions*, in order that the different underlying mechanisms producing these different abnormal movements maybe compared.

Some of these experiments have been presented in poster form at the 23rd and 25th Annual meetings for the Society for Neuroscience (Harrison *et al*, 1993; Mayston *et al*, 1995b).

METHODS

Subjects

Thirteen male subjects with X-linked Kallmann's syndrome and mirror movements investigated in chapter 2 (aged 16-60 years), two subjects with idiopathic mirror movements (one male; age 8 &14 years) and five children with spastic cerebral palsy (CP) aged 5-13 years (3 male) were studied with ethical approval and parental consent. The details of the subjects with XKS are given in appendix C. Both of the subjects with idiopathic mirroring had no known neurological signs, but subject I2 was noted to have some difficulty with relatively independent finger movements (RIFM) and the parents had expressed some concern about her hand function generally as well as her performance at school. Subsequently MRI did not reveal any abnormalities: in particular the corpus callosum was noted to be present and morphologically normal. The children with CP all had an asymmetrical spastic quadriplegia, two were severely affected and dependent for most activities of daily life, and the remaining three had a moderately severe quadriplegia (clinical details in appendix D).

Methods

The presence and degree of associated movements and reactions were assessed in each subject in addition to their ability to make RIFM. The associated *movements* were scored according to the criteria of Woods & Teuber (1978) (0-4) and the associated *reactions* were scored by assigning grades to a clinical scale (0 = none; 1 = mild and only present during intended action, 2 = moderate, present during and for a short time after the intended action, 3 = pattern of activity sustained and persists after intended action over).

All of the neurophysiological tests used have been described in detail in section one. Surface electromyography was used to record activity in homologous muscle pairs in the upper limbs;

cutaneomuscular reflexes were recorded during stimulation of the digital nerves of the index finger and transcranial focal magnetic brain stimulation was used to assess the laterality of the corticospinal tract. Cross correlation analysis was applied to determine if any bilateral activity was due to the presence of a common synaptic drive to the left and right homologous motoneurone pools.

RESULTS

Mirror movements and associated reactions

All of the subjects with XKS and the two subjects with idiopathic mirror movements studied had marked mirror movements (grade 1-3) which were generally more marked when the left hand was used. All of the children with CP had associated reactions, although 2/5 showed mirror movements during the first 1-2 repetitions of finger movements, after which they were masked by the increased spasticity as a result of the associated reactions.

Three of the thirteen subjects with XKS had some difficulty with RIFM, but the remaining 10 subjects were all able to make normal independent finger movements. One of the children with idiopathic mirroring could not perform RIFM (I2), was not able to selectively abduct her index finger and had some difficulty with fine hand skills such as managing buttons. None of the children with CP had normal RIFM and showed marked asymmetry of both associated reactions and their ability to make selective finger and hand movements.

EMG recordings

In all of the subjects studied voluntary activity in one hand was accompanied by involuntary activity in the opposite hand. In the children with CP, recordings of phasic grasping were made in 2 of the 5 children. Two children did not have independent finger or hand movements, and one child refused, but in all of the children with spasticity, bilateral EMG was recorded on any attempt to perform a unilateral task. Fig. 1a shows the EMG recorded during three consecutive phasic grasping movements. In addition to the ongoing EMG there is an increase in activity which accompanies each voluntary burst of the right hand. This is shown in a single burst in fig.1b. The EMG was rectified and averaged for 45 sweeps as shown in fig.1c resulting in a ratio of involuntary to voluntary activity of 0.47. The time of

onset of the involuntary activity in this case was 3.0ms before the voluntary burst. The size and the time of onset of the involuntary activity varied within and between the subject groups. The results of the involuntary/ voluntary ratios and times of onset of involuntary EMG are given below for the subjects with idiopathic mirror movements and the children with CP, and summarised for the subjects with XKS. The data from the subjects with XKS are described in detail in Section 2, chapter one. The EMG was rectified and averaged, time locked to the start of the voluntary burst as described in section 1.

EMG was recorded contralateral to the voluntarily moved hand in all of the subjects with XKS. The amount of involuntary activity was always less than the voluntary activity except for subject K6 during right index finger abduction, subject K8 during left index abduction and subject S2 during left finger thumb opposition. The involuntary activity was less in the idiopathic mirroring subjects.

Table 1:

Subject		**Phasic left**		**Phasic right**	
	Number	Invol/vol ratio	Range:time of onset invol (ms)	Invol/vol ratio (range)	Range:time of onset invol (ms)
XKS	13/13	0.01-1.2	-33.0 - 21.0	0.006-3.3	-38.0 - 34.0
Idiopathic	2/2				
I1		0.42	-1.0	1.21	0.0
I2		0.88	+20.0	0.50	-8.0
Spastic	2/5				
S2		1.81	+10.0	0.47	-3.0
S3		n/a		0.01	+36.0ms

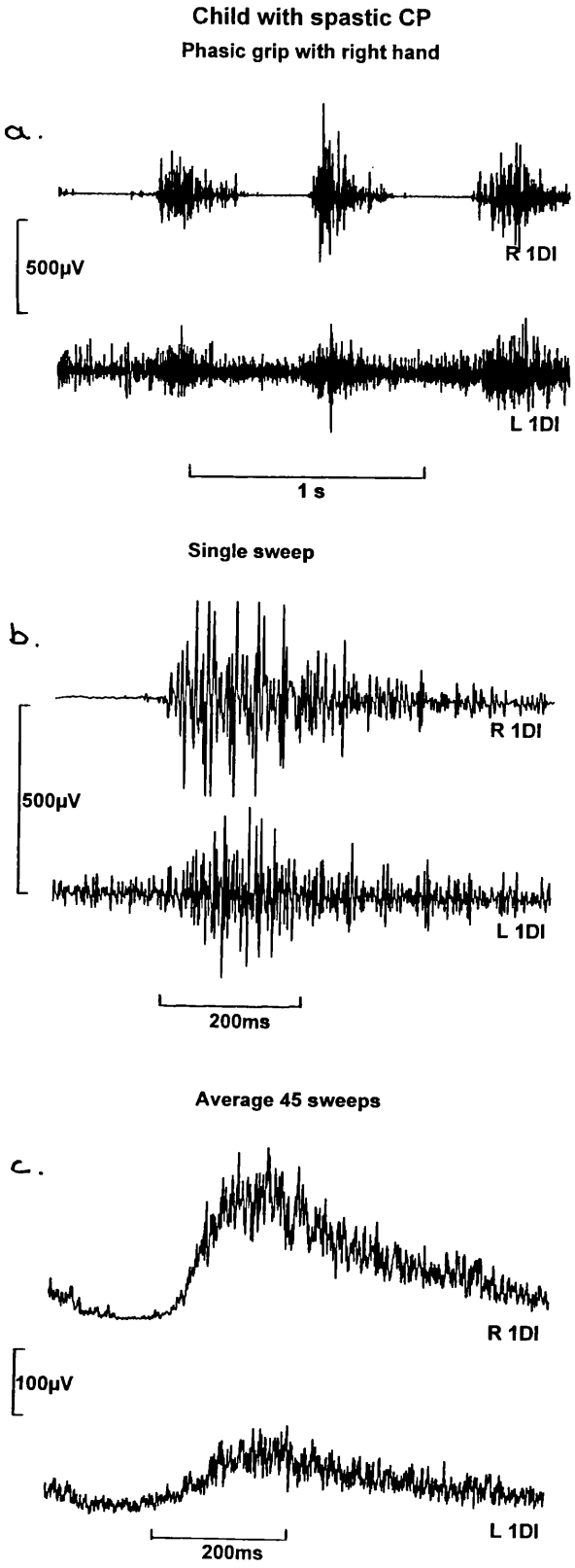


Figure 1: a. Three consecutive sweeps of EMG recorded during phasic hand grasp are shown. Each voluntary burst is accompanied by an involuntary EMG burst which can be seen above the ongoing EMG. The same can be seen in a single sweep (**b.**). The EMG was rectified and averaged for 45 sweeps time locked to the start of the voluntary burst. The Involuntary to voluntary ratio was calculated and was 0.47.

Focal magnetic brain stimulation

Subjects with XKS and mirror movements

The results of focal magnetic brain stimulation are described in detail in section 2, chapter 1. All of the subjects with XKS and mirror movements had bilateral responses in left and right 1DI to unilateral stimulation using Magstim. In 6/13 subjects the ipsilateral projection was equal to or larger than the contralateral projection when either cortex was stimulated; in 2/13 subjects the ipsilateral projection was larger than the contralateral projection when the left motor cortex was stimulated, but the ipsilateral projection was smaller when the right cortex was stimulated. In the remaining 5 subjects, the contralateral projection was larger than the ipsilateral projection when either cortex was stimulated. The ratios are summarised in the following table:

Table 2:

Subject	Number	Stimulate R (L:R 1DI)			Stimulate L (L:R 1DI)		
		number bilateral MEP's	Ipsi/contra (range)	% output (range)	number bilateral MEP's	Ipsi/contra (range)	% output (range)
XKS	13	13/13	0.05-256.7	45-75	13/13	0.004-34.0	45-85
Idiopathic	2	2/2	0.37-1.29	45-80	2/2	0.29-1.89	45-80
Spastic CP	5	1/5	0-0.61	50-90	2/5	0-0.89	60-90

Table 2: Bilateral motor evoked potentials (MEP's) were recorded in all of the subjects with XKS and both of the subjects with idiopathic mirror movements (row 2 & 3). When the right motor cortex was stimulated bilateral MEP's were recorded in 1/5 of the children with spastic CP; when the right motor cortex was stimulated bilateral MEP's were recorded in 2/5 (row 5). In all of the children with spastic CP the bilateral MEP's were recorded in response to stimulation of the less damaged motor cortex.

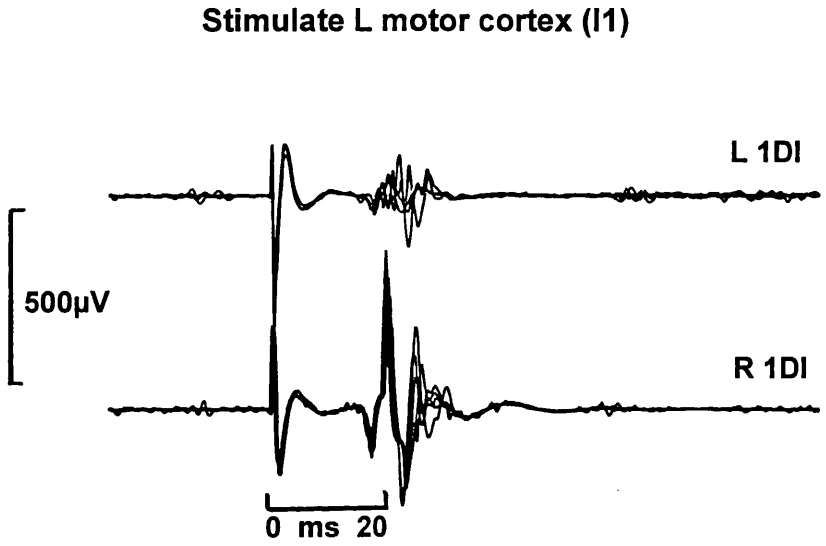
Subjects with idiopathic mirroring

Figure 2 shows 5 consecutive sweeps of EMG recorded from subject I2 when the left motor cortex was stimulated using Magstim. Bilateral EMG responses were recorded in left and right 1DI. As the threshold to stimulation was high (80%), background EMG was required to record the responses. The ipsilateral response was always larger than the contralateral response. Similar to some of the subjects with XKS, subject I1 had bilateral responses to Magstim, but the ipsilateral response was always smaller than the contralateral response when either motor cortex was stimulated. Five consecutive motor evoked responses recorded in left and right 1DI to stimulation of the right motor cortex are shown in fig. 2a. No preactivation was required in I1.

Subjects with CP

In this group of subjects there was a great amount of inter- and intra subject variability. The average of 10 responses using Magstim of the left and right motor cortex in subject S3 is shown in Fig. 3 a&b. When the right more damaged cortex was stimulated only contralateral responses were recorded, but when the less damaged left motor cortex was stimulated bilateral motor responses were recorded in left and right 1DI. Figure 4 shows five consecutive responses to Magstim when the less damaged left motor cortex was stimulated. The results of focal magnetic brain stimulation of the five children with CP are summarised in the following table, which also shows the output of the Magstim 200 used for each subject. In subject S1 who had some independent finger movements (better on the right side) only contralateral responses were recorded in left and right 1DI when either motor cortex was stimulated. In this case 70% output of the stimulator was required to evoke these responses from either cortex. In subject S2, S3 & S4 only a contralateral response was recorded in left and right 1DI when the more damaged cortex was stimulated, but bilateral responses were recorded

a..



b.

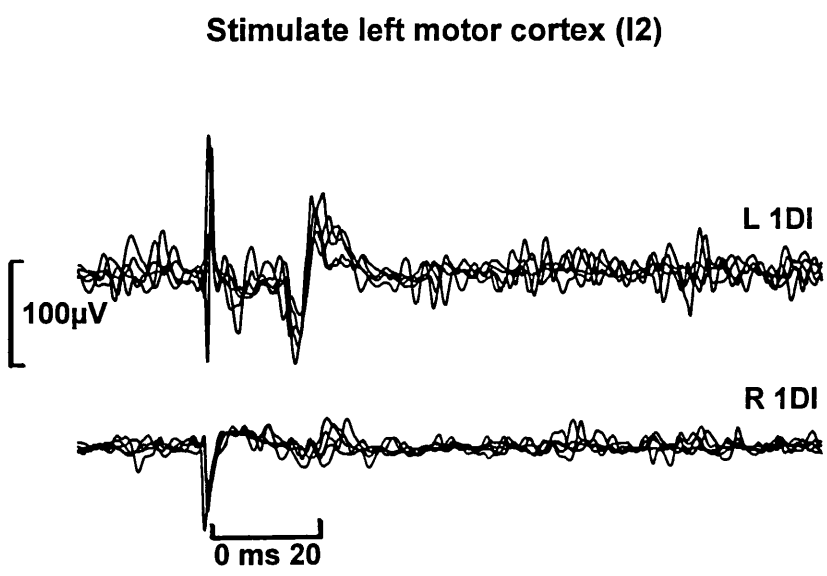


Figure 2: Surface EMG recorded simultaneously from co-contracting left and right 1DI during focal magnetic brain stimulation over the hand area of the motor cortex. **a.** When the left motor cortex was stimulated in I1, bilateral motor evoked responses were recorded in left and right 1DI; the contralateral response was larger. but in I2 **b.** the ipsilateral motor evoked response was larger than the contralateral response. 5 consecutive sweeps are shown in each.

Focal Magnetic Stimulation of right (a) and left (b) motor cortex (S3)

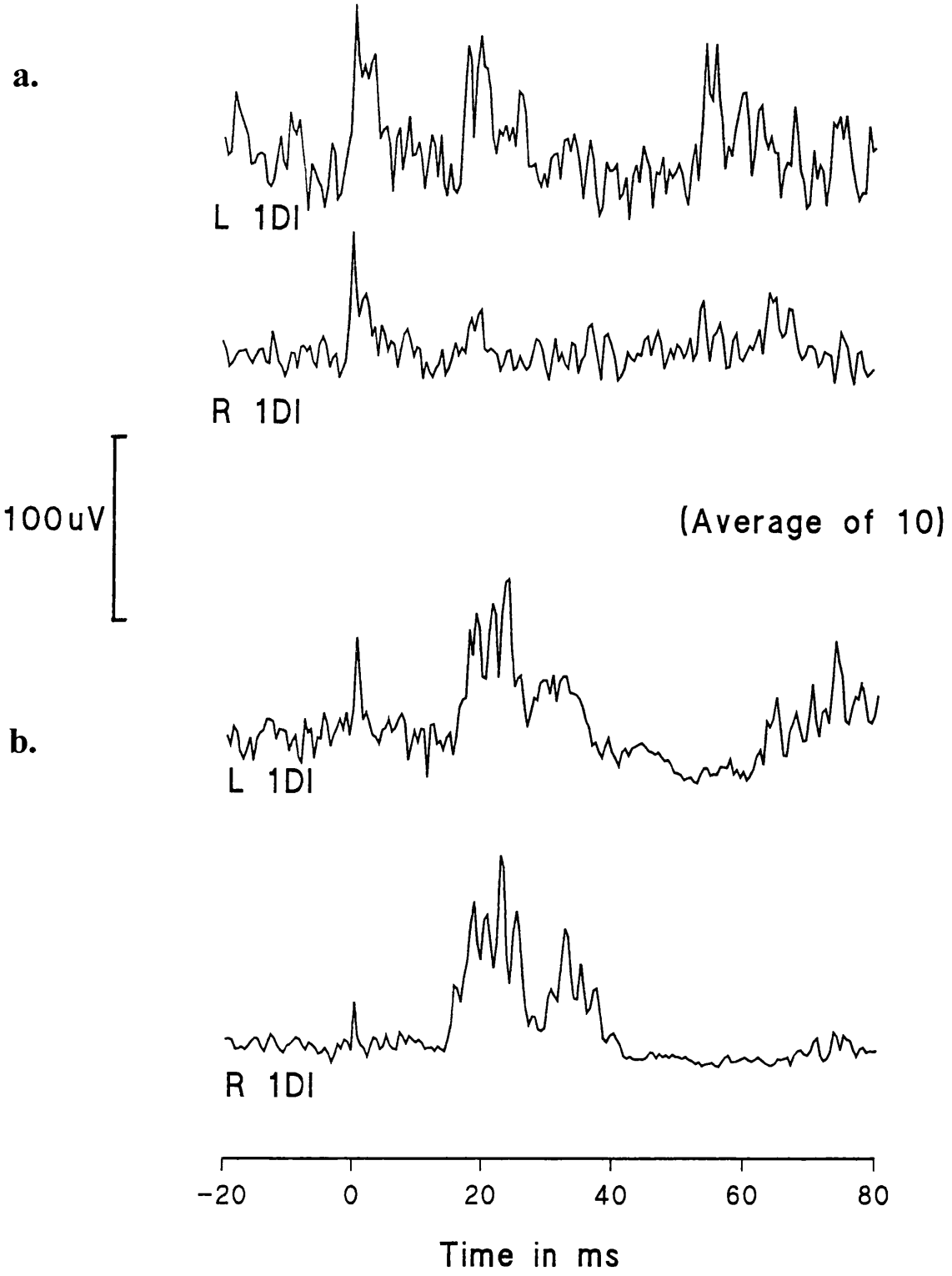


Figure 3: Surface EMG recorded simultaneously from co-contracting left and right 1DI during focal magnetic brain stimulation of the hand area of the motor cortex in subject S3 with associated reactions. When the right more damaged motor cortex was stimulated in subject S3 only contralateral motor responses were evoked (**a**). But when the less damaged left motor cortex was stimulated bilateral motor responses were recorded in left and right 1DI (**b**). The contralateral response is larger than the ipsilateral response (ipsi/contra=0.89).

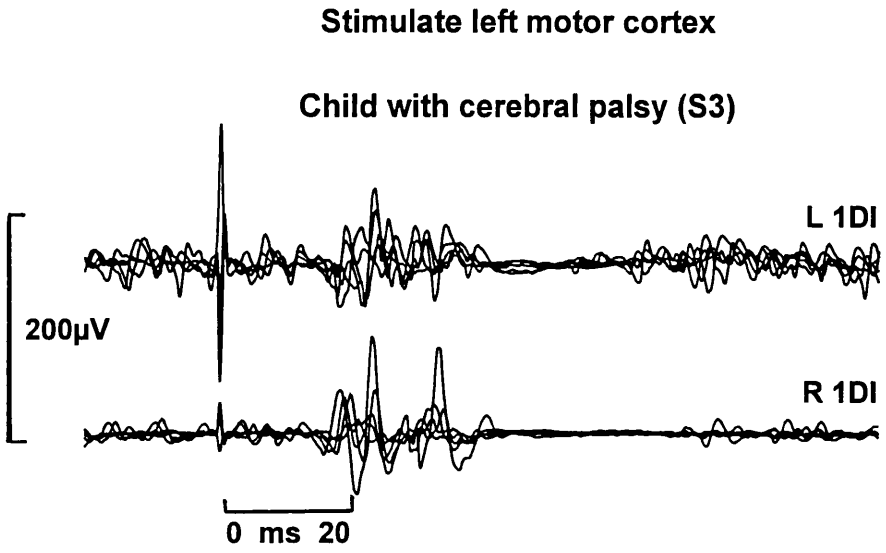


Figure 4: Surface EMG recorded simultaneously from co-contracting left and right 1DI during focal magnetic brain stimulation of the left motor cortex resulted in bilateral short latency motor evoked responses of similar size in left and right 1DI in subject S3. Five consecutive sweeps of EMG are shown.

when the less damaged cortex was stimulated. The recordings from subjects S2 & S3 are shown in figure 3. The latency of the responses was the same. In subject S3 and the remaining two (S4 & S5) high output of the stimulator was required (80 & 90 % respectively). In subject S5 in whom RIFM were absent on the right and difficult in the left hand, no response to Magstim was recorded in left and right 1DI when the figure-of-eight coil or the circular coil were used at the maximum tolerable output (90%).

Subject	Focal Magstim		RIFM		Associated reactions	
N=5	Stim L	Stim R	L	R	L-R	R-L
S1	C (70%)	C (70%)	1	1+	1	2
S2	B (60%)	*C (50%)	0	1+	1	3
S3	B (80%)	*C (90%)	0	1+	2	3
S4	*C (80%)	B (80%)	0	1	2	2
S5	0 (90%)	0 (90%)	1	0	3	1

C = contralateral responses

B = bilateral responses

*** = more damaged cortex**

Table 3: The five children with CP showed variable responses to Magstim, usually high output of the stimulator was required to evoke a response in L & R 1DI. Only contralateral responses to focal Magstim were recorded in the least affected subject S1 as in normal subjects, but bilateral responses were recorded in S2-4. It was not possible to evoke a response in S5 who was the most severely affected child.

Cross correlation analysis

Subjects with XKS and mirror movements

The results of cross-correlation analysis have been described in detail in section two, chapter1 of this thesis. In twelve of the thirteen subjects studied a short duration central peak was observed in the cross-correlograms constructed from the EMG recorded simultaneously from co-contracting left and right 1DI. An example of a cross-correlogram constructed from the EMG recorded from the left and right 1DI is shown in figure 5a (subject K7; see appendix). A short duration central peak of 14.0ms duration (E/M=17.9) is seen around time zero. The mean duration was 16.5 ± 1.3ms (± SEM, n=12), and ranged from 12.0-28.0 ms. The size of the peak (expressed as E/M, the number of extra counts in the peak more than expected by

Left 1DI : Right 1DI (Left Fext : Right Fext*)

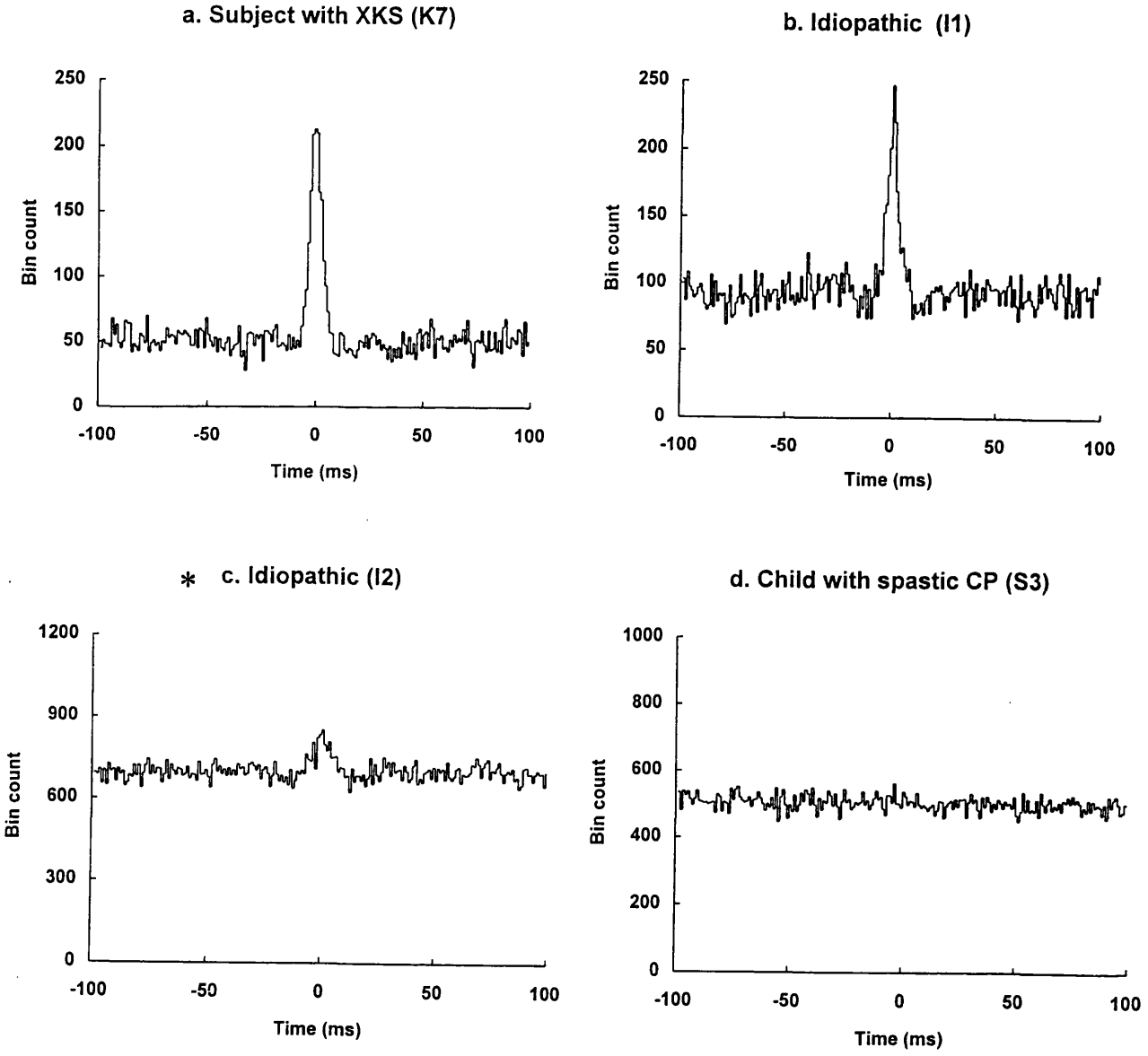


Figure 5: Cross-correlograms constructed from multi-unit surface EMG recorded during voluntary sustained left and right index finger abduction (*left and right wrist extension). Each correlogram was constructed from at least 5000 trigger spikes (left) and 5000 event spikes (right). Subject K7 with XKS and mirror movements; there is a short duration central peak around time zero. **b.** Subject I1 with idiopathic mirror movements; there is also a large short duration central peak. **c.** Subject I2 in whom no peak was found in the cross-correlogram constructed from left and right 1DI, but for the correlogram constructed from the EMG recorded from left and right Fext there is short duration central peak. **d.** Subject S3 with CP and associated *reactions*; the correlogram is flat.

chance; M denotes the mean bin count in an area away from the central feature), ranged from 3.1- 17.9, mean 5.6 ± 1.3 (± SEM, n=12). A short duration central peak was seen in the cross-correlogram constructed from the EMG recorded from co-contracting left and right wrist extensors in subject K13, in whom no synchrony was found between left and right 1DI. In 10/12 subjects a short duration peak was found in the cross-correlograms constructed from left and right Fext, the duration ranging from 10.0-19.0ms (14.4, ± 0.8); E/M 1.2-6.7, (3.2 ±0.7), (mean ± SEM, n=10). A distal to proximal gradient in the size and occurrence of the cross-correlogram peaks was found.

Subjects with idiopathic mirroring

In I1 a short duration central peak was found in the cross correlogram constructed from the EMG recorded simultaneously from co-contracting left and right 1DI shown in fig.5b. A short duration central trough is observed around time zero (duration 15.0ms, E/M 8.2). For the left and right Fext, the duration was 10.0ms and E/M was 2.1. A distal to proximal gradient was found in the size of the peak, being greatest in the distal muscle pair. In contrast in subject I2 the cross-correlogram constructed from the EMG recorded from left and right 1DI was flat. But as shown in fig.5c, the cross-correlogram constructed from the EMG recorded from the left and right forearm extensors (Fext) contained a small short duration central peak, duration 18.0 ms; E/M = 1.9.

Subjects with CP

In all of the subjects with CP, the cross-correlograms constructed from the simultaneous EMG recordings from left and right co-contracting 1DI were all flat (5/5). This is indicated in the summary table (table 5). An example of such a cross-correlogram constructed from the EMG recorded from left and right 1DI is shown in fig.5d. No central feature can be seen.

Cutaneomuscular reflexes

Subjects with XKS and mirror movements

See section 2, chapter 1 (page 97) for details of the results of CMR's. In 3/12 subjects the reflex recorded showed the E1 component only on the stimulated side (thought to be a spinal response) and the I1 and E2 components which are thought to be of supraspinal origin were only recorded contralateral to the stimulated side. In one subject a reflex could not be recorded, 8/12 had an E1, I1 and E2 on the stimulated side but an I1 and E2 were also recorded contralaterally. In one subject an E1 was recorded bilaterally.

Subjects with idiopathic mirroring

The CMR recorded from subject I1 is shown in fig.6a. Figure 6a shows the CMR recorded from stimulation of the digital nerves of the right index finger in which the E1 (thought to be of spinal origin), I1 and E2 (believed to involve supraspinal pathways) were recorded on the stimulated side as observed in normal subjects. The I1 and E2 were also recorded on the opposite side. But in the second subject with idiopathic mirroring subject (I2), only a small E1 was recorded ipsilaterally when the digital nerves of either index finger were stimulated.

Subjects with CP

In all the subjects with CP, the cutaneomuscular reflex was only recorded on the stimulated side, but the E1, I1 and E2 components were not all always present. An example of a CMR recorded from left and right 1DI is shown in fig.6b. An E1 was observed in all subjects when either index finger was stimulated, but the E2 was never recorded when the right side was stimulated and in 2/5 subjects when the left side was stimulated.

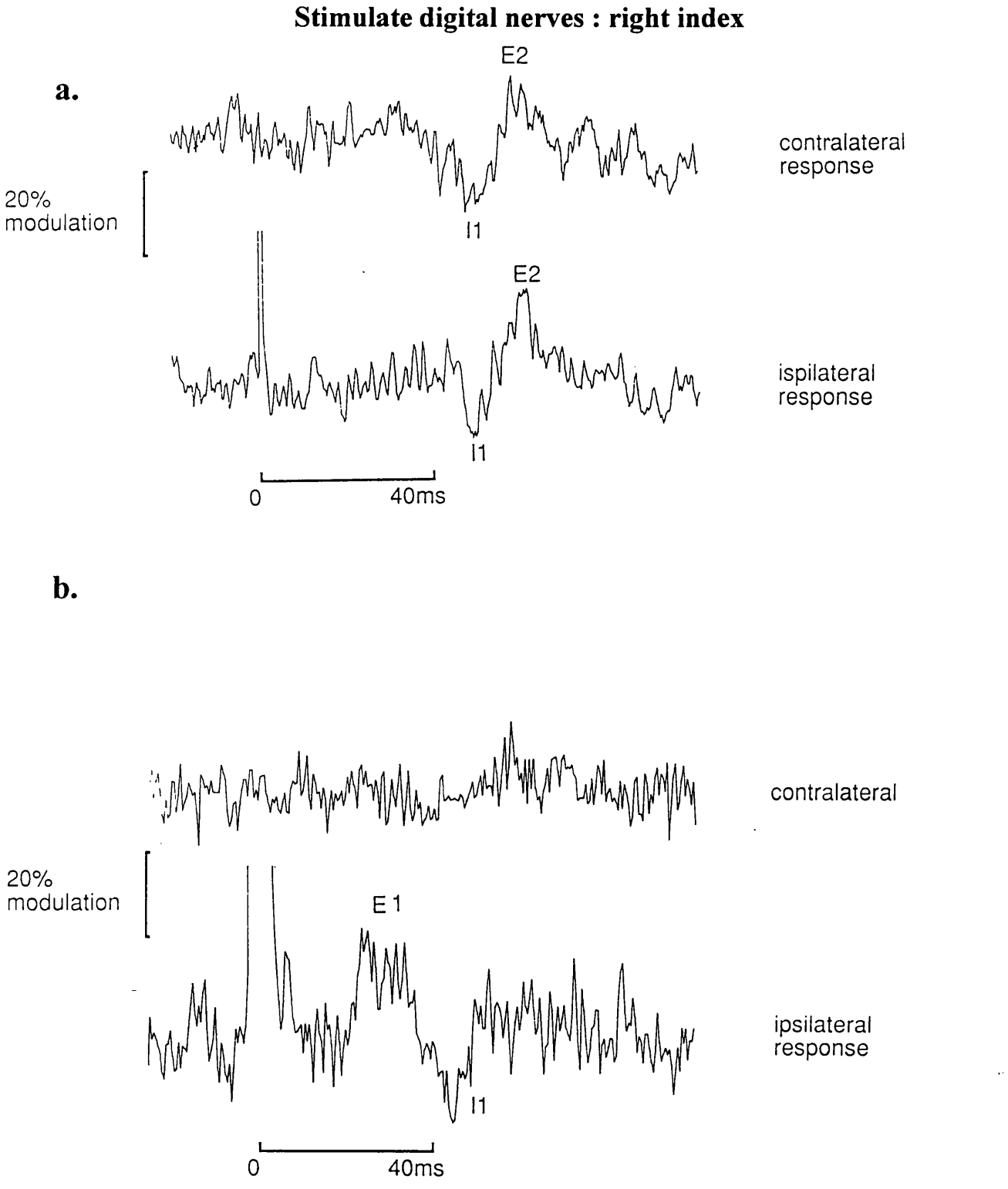


Figure 6: Cutaneomuscular reflexes (CMR) recorded from left and right 1DI following stimulation of the digital nerves of the right index finger at 3^{sec-1} at a stimulus strength twice threshold for perception during sustained voluntary isometric abduction of both index fingers. Surface EMG rectified and averaged for 500 sweeps time locked to the stimulus. **a.** CMR recorded from I1 with associated mirror movements. The I1 & E2 components were recorded ipsilateral and contralateral to the stimulus; **b.** CMR was only recorded on the stimulated side in S3, spastic CP with associated reactions. The E1 of spinal origin and the I1 which is possibly of supraspinal origin.

DISCUSSION

All of the subjects with pathological associated movements in this study have bilateral pyramidal tract projections as evidenced using Magstim. In 5/13 subjects with XKS, and 1/2 subjects with idiopathic mirror movements, the ipsilateral projection was larger than the contralateral projection. Three of the five subjects with CP also had bilateral projections, but the contralateral projection was always larger. In all of the subjects with obligatory associated movements a central peak was found in the cross-correlograms constructed from the EMG recorded simultaneously from co-contracting left and right 1DI and/or left and right Fext. Thus all of the subjects with obligatory mirror movements demonstrated the presence of a common synaptic drive to bilateral homologous motoneurone pools that could underlie the bilateral EMG recorded during unilateral hand movements. These obligatory associated movements are different from those observed in normal adults performing complex tasks and in young children during development. In these cases, no peaks have been observed in the cross-correlograms, and bilateral responses at similar latency have never been recorded using Magstim (Section 1, chapters 1&2). Despite the presence of bilateral pyramidal projections as revealed using Magstim, the cross-correlograms constructed from the EMG recorded from the children with CP were all flat, indicating that there was no common drive to left and right homologous motoneurone pools which could account for the bilateral EMG recorded during unilateral tasks.

Whilst Zulch & Muller (1969) classify associated movements as physiological and pathological with further subdivisions, their paper only discusses the latter, leaving the reader unclear about the exact nature of physiological associated movements. But this classification does not make a helpful distinction between the involuntary movements occurring in patterns of spasticity (referred to here as associated *reactions*) and those which occur in the absence of

spasticity. It maybe useful to introduce a new classification that takes into account the associated movements or associated mirrored activity which can be observed in normal children during development and in adults performing difficult or forceful activities.

CLASSIFICATION OF ASSOCIATED MOVEMENTS AND REACTIONS

Associated movements:

1. Normal

a. mirror movements/mirrored activity observed in children during development and during forceful unilateral hand tasks in adults

b. unintentional movements accompanying a task, such as mouthing accompanying writing

2. Pathological (mirror movements)

a. Some cases of congenital hemiplegia; adult hemiplegia

b. Various syndromes e.g. XKS; Klippel-Feil

c. Hereditary

d. Clinical sign of mild neurological damage; immature CNS

e. Some cases of agenesis of the corpus callosum

Associated reactions:

Only observed in patients with spasticity (adults with spastic hemiplegia and children with spastic CP), and occur in response to voluntary activity, effort and emotion, resulting in an exaggeration of the pattern of spasticity irrespective of the direction of the voluntary movement. The duration of these reactions can be useful to enable a grade of spasticity (mild, moderate and severe) to be determined and to identify the emergence of spasticity post-lesion.

Using this classification, it is possible to relate the results of the neurophysiological tests to the clinical signs produced by these different types of involuntary movements. The results from the experiments with the adults and children (section 1) and the subjects with XKS (section 2) along with the results from the children with spastic CP in the present study are shown in table 4.

Table 4

Subject group	N	Presence of bilateral EMG	Bilateral response to Magstim	Correlogram peak present	Crossed CMR
Associated movements					
1. Normal					
Children	44	27/44	0/10	0/5	0/5
Adults	7	0/7	0/7	0/7	0/7
2.Pathological					
XKS	13	13/13	13/13	13/13	11/13
Idiopathic	2	2/2	2/2	2/2	1/2
Associated reactions					
Spastic quadriplegia	5	5/5	3/5	0/5	0/5

***i)* Associated movements observed in normal children and adults.**

Bilateral EMG was recorded during a unilateral hand task in 27/44 of the normal children and decreased with increasing age (section 1, chapter 2). No bilateral responses to Magstim at the same latency were recorded and no peaks were observed in the cross-correlograms constructed from the EMG recorded from left and right co-contracting homologous muscle pairs. No crossed reflexes were recorded. The same was true for the normal adults when a forceful activity resulted in bilateral EMG, but this only occurred if background EMG was present on the side opposite to the voluntary movement. Thus bilateral pyramidal tract projections are not responsible for the bilateral EMG recorded in normal

children and adults. The bilateral activity is most likely produced by bilateral cortical activation as discussed in section one.

ii) Associated (mirror) movements in pathology

Only two pathological conditions were studied, XKS and subjects with idiopathic mirror movements. In both of these conditions evidence of abnormal ipsilateral pyramidal projections was found. Firstly, bilateral responses to Magstim were recorded in all of the subjects with XKS, in 2/2 of the idiopathic mirrorers. There was a wide range of ratios in the ipsilateral to contralateral responses in the subjects with XKS and in 1/2 of the subjects with idiopathic mirror movements (I2), the ipsilateral response was larger when either motor cortex was stimulated. There was also inter subject variability in the reflex responses in both groups of subjects with pathological mirror movements. But, in contrast to the normal adults and children, in all of these subjects a short duration central peak was observed in the cross-correlograms constructed from the EMG recorded from co-contracting left and right homologous muscle pairs (left and right 1DI &/or forearm extensors). Taken together, the results show that common synaptic input shared bilaterally and produced by bilaterally projecting pyramidal tract neurones from the same cortex maybe responsible for the pathological associated movements observed in subjects with XKS and in the subjects with idiopathic mirror movements. In the subjects with XKS, the bilateral pyramidal projections are probably due to a lack of decussation of the pyramid in response to a lack of KAL, the Kallmann gene product thought to be involved in neuronal migration (Franco *et al*, 1991). This abnormal organisation has resulted in some corticospinal fibres reaching their correct target (the contralateral motoneurone pool), but some fibres have remained ipsilateral. Thus there are fast conducting corticospinal fibres projecting to the contralateral and ipsilateral

homologous motoneurone pools. These fibres also share common synaptic input at the cortex. But the possibility that both cortices are active in addition to activity in these bilaterally projecting pathways cannot be ruled out if the PET data from the XKS subjects is considered. This data provided some evidence for bilateral cortical activation during a unilateral task. But it is not possible to distinguish between activity resulting from sensory feedback or that measured as a result of motor output.

The two subjects with idiopathic mirroring were different from each other. I1 was in many respects similar to the subjects with XKS in the middle of the table (K6-K12). Subject I2 was similar to subject K1 in that she only had a predominantly ipsilateral response to Magstim, and her threshold to stimulation was very high. But in other respects she was like the children with CP. It was difficult to access her corticospinal tract and she could not make RIFM. Taken together, it appears that she may not have a significant corticopsinal tract projection to the distal hand muscles as it is known that direct cortico-motoneuronal connections via this pathway are essential for the performance of selective movements of the fingers. This is supported by work in monkeys (Lawrence & Hopkins, 1976; Bortoff & Strick, 1993; Galea & Darian-Smith 1995) and it is thought to be the same for man, although the maturation time is longer (Forssberg *et al*, 1991; Eyre *et al*, 1991). Nevertheless, in both the children with idiopathic mirror movements, the presence of an abnormal ipsilateral projection has been demonstrated and could at least in part be responsible for the mirror movements observed.

iii) Children with associated reactions

Bilateral EMG was recorded during unilateral hand tasks in all of the children. In the more severe children (2/5) EMG was also recorded during rest, but increased on activity.

There were variable responses to Magstim, and in 3/5 children bilateral responses were recorded in left and right 1DI. In all cases the latency of these responses was the same. In all of the children with CP and associated reactions studied, no peaks were observed in the cross-correlograms constructed from the EMG recorded form co-contracting left and right homologous muscle pairs. Thus the bilateral responses recorded using Magstim did not occur as a result of common synaptic input to homologous motoneurone pools and shared bilaterally. Two of the three children with bilateral responses to Magstim were also the children who showed associated mirror movements before the increase in spasticity producing associated reactions masked these movements. Interestingly, the three children with bilateral responses to Magstim were damaged early in utero,** suggesting that some cortical reorganisation may have occurred as a result of this. Carr *et al* (1993), in their study of children with congenital hemiplegia, showed a relationship between early damage and the presence of an abnormal corticospinal tract projection. In that study 4 groups (A,B,C & D) emerged from the results of the neurophysiological tests. Taking into account the responses to Magstim and the findings from cross-correlation analysis in group A, it was concluded that corticospinal axons from the surviving cortex had branched to innervate bilateral homologous motoneurone pools, resulting in obligatory mirror movements. In group B bilateral responses were recorded in left and right 1DI using focal magnetic brain stimulation, but the cross-correlograms were flat thus there was no evidence for shared synaptic input that could account for the bilateral responses to Magstim. Unlike in the present study in which the latency of ipsilateral and contralateral responses were the same, in the group B subjects the ipsilateral responses were significantly later. Carr *et al*, (1993) argued that the ipsilateral responses could be due to an abnormal ipsilateral projection from the unaffected cortex, recrossing of axons which have normally decussated, enhancement of the normally occurring

** see appendix for details of timing of lesion

ipsilateral tract or enhancement of cortical connections to brainstem pathways. The latency of the ipsilateral response recorded in the children with spastic CP in the present study suggests that it is transmitted via a fast conducting pathway, thus it is unlikely that brainstem pathways such as the reticulospinal tract, or the normally projecting ipsilateral tract could be responsible for this activity. The latter is thought to be slow conducting, mostly influences proximal muscles and generally these fibres do not make direct cortico-motoneuronal connections (Kuypers, 1973), and the brainstem pathway would require at least a disynaptic pathway resulting in a longer latency than that observed in the present study. Adults with an acquired hemiplegia have been reported to show distal muscle weakness (Colebatch & Gandevia, 1989) thus it is possible that ipsilateral corticopsinal projections may influence distal muscles. All the children with ipsilateral responses to Magstim in the present study were damaged early in utero, thus it is possible that there is enhancement of the normally occurring ipsilateral corticospinal projection to the distal muscles. Transient ipsilateral projections have been described in the opossum and cat during development (Cabana & Martin 1985; Alisky *et al*, 1992); such connections may have stabilised and thus could account for the ipsilateral responses recorded in the present study. A similar finding has not been found in monkey (Armand *et* al, 1994) thus this explanation is unlikely.

Timing of CNS damage

Unlike the children with hemiplegia in the study of Carr *et al*, (1993) in whom one motor cortex was unaffected, both cortices were significantly damaged in the children who participated in the present study and in whom bilateral responses to Magstim were recorded. It seems probable that a different kind of reorganisation could occur in response to early damage when both motor cortices are affected. There is no evidence for shared synaptic input in subjects S2-4 as found in the group A children with early damage. Group B children in

whom bilateral responses to Magstim were recorded, were damaged later and presumably there was no opportunity for a similar reorganisation of the unaffected motor cortex. Subject S1 in the present study in whom only contralateral responses were recorded was born full term after an uneventful pregnancy, thus it is likely the critical period for reorganisation had passed. Three of the children with CP in the present study have demonstrated the presence of bilateral pyramidal projections, but there is no evidence for shared synaptic input that could be responsible in this case. It seems likely that in children with early damage but involvement of both motor cortices, a different reorganisation than that accompanying an early lesion observed with congenital hemiplegia results. It is suggested that both the timing and extent of the lesion may dictate what type of reorganisation if any can occur.

In the XKS subjects with an aberrant pyramidal decussation, it is likely that the effect of KAL is manifested early in development, possibly as early as 57 days gestational age (see section 2, chapter 1) and unlike children with CP there is no known cortical damage. In subjects with XKS common synaptic input at the cortex may develop in response to the bilateral projections reaching their spinal targets, albeit on opposite sides of the spinal cord. But the study of quadriplegia and hemiplegia is different. Not only has the neural damage occurred later than in XKS, but rather than a problem of neuronal migration, the children with CP have cortical damage which having occurred early could provoke a reorganisation of the corticospinal projections to the motoneurones innervating the distal muscles. Futhermore, unlike the children with hemiplegia and early damage, both motor cortices are affected in spastic quadriplegia. This may result in a different response to early damage such that a novel ipsilateral projection may develop or become unmasked in these children. A study of a larger group of children with this class of CP (spastic quadriplegia) and in whom the timing of the

lesion is known is required to learn more about the reorganisation observed in the present study.

Taken together results of this study show that there are different mechanisms underlying associated *movements* and the associated *reactions* which are observed in patients with spasticity. Furthermore, there would appear to be different mechanisms underlying the associated *movements* observed during development and those found accompanying different pathologies.

Clinical implications

This study has revealed different mechanisms underlying associated movements and associated reactions. These findings have relevance to the clinician when managing patients who have either of these involuntary movements. The results suggest that in those patients with bilateral responses in homologous distal muscle pairs revealed by Magstim in addition to short duration peaks in the cross-correlograms constructed from the EMG recorded from those muscle pairs, that there is common synaptic input to the motoneurone pools innervating those muscles, and it is this which underlies the associated mirror movements. In this case the most useful intervention by the clinician is to teach the patient compensatory strategies. On the other hand, if there is no peak in the cross-correlogram as found in the children with spastic CP, then the synaptic input to the bilateral motoneurone pools arrives independently from a separate source. In this case the clinician may have the opportunity to apply known techniques to treat the spasticity and thus reduce the intensity of the associated reactions enabling greater functional possibilities for the patient.

SECTION 4

Co-contraction of antagonistic muscle pairs in the lower limb during development and in children with cerebral palsy.

Page 195-231

SUMMARY

1. Surface EMG recordings have been made from co-contracting antagonistic muscle pairs (tibialis anterior and soleus and quadriceps and hamstrings) in a group of normal adults, school aged children, infants and children with spastic cerebral palsy. In the adults concentric needle recordings were made simultaneously during the co-contraction of tibialis anterior and soleus.

2. In the adults co-contraction of the antagonistic muscles was produced by performing a postural task combined with a voluntary task, but in the infants, young children and those with cerebral palsy spontaneous co-contraction of antagonistic muscles was recorded during standing.

3. Cross-correlation analysis of motor unit spike trains recorded from the co-contracting muscle pairs was performed to determine if the co-contraction was produced by a common synaptic drive to the motoneurone pools innervating the antagonistic muscle pair.

4. In all of the adults and 7 of the school aged children, a central trough was found in the cross correlogram indicating the presence of shared reciprocal input to the motoneurone pools innervating the antagonistic muscle pair. In all of the infants, young children and those with cerebral palsy the correlogram was flat indicating that there was no detectable common synaptic drive, either excitatory or reciprocal.

INTRODUCTION

The repertoire of postural and voluntary motor skills increases progressively during childhood. An important feature of this process is the development of reciprocal muscle control. In the adult this allows antagonistic muscles either to contract together, as occurs for example when a joint is stiffened or stabilised, or to act reciprocally as occurs when a limb moves or exerts a force about a joint. In young children, and those with cerebral palsy (CP), muscle activity is less selective and antagonistic muscles characteristically contract together. In children with CP, this co-contraction causes excessive joint stiffness. The purpose of the experiments to be described below was to investigate mechanisms that may underlie this abnormal pattern of activity.

The co-contraction of antagonistic muscle pairs has been reported in a number of studies which have described the development of postural control and locomotion in infants and children. (Forssberg 1985; Berger *et al*, 1985; Leonard *et al*, 1988). A similar pattern of activity has also been described during stance in children with cerebral palsy (Berger *et al*, 1985; Leonard *et al*, 1991), and in many respects the pattern of activation of antagonistic muscle pairs in children with cerebral palsy is the same as the immature pattern of activity in infants and children around 4 years of age and younger (Berger *et al*, 1985).

The mechanisms underlying co-contraction are unknown. There are two likely explanations. One possibility is that it results from activity in excitatory pathways shared in common between the antagonistic motoneurone pools. Such a common excitatory drive could be the result of activity in branches of last order common stem presynaptic fibres making excitatory connections to the two pools. Alternatively the common drive may reach the two pools from separate last order presynaptic input fibres which themselves receive a shared excitatory input.

Another mechanism to account for the co-contraction observed in infancy and in children with cerebral palsy maybe that it is the result of lack of supraspinal facilitation of spinal reflex circuits which are responsible for producing patterns of reciprocal muscle activation in the adult. One possible spinal pathway which receives descending input from supraspinal areas is the "Ia inhibitory interneurone" by which the reciprocal stretch reflex is mediated in the spinal cord. This hypothesis would be compatible with the development of control via the descending tract, in this case the corticospinal tract which is known to reach its spinal target after birth in the monkey (Armand *et al*, 1994) and myelinates later (Yakovlev and Lecours, 1967; Gilles *et al*, 1983).

The H-reflex has been used as a tool to investigate the Ia mediated reciprocal inhibition in normal subjects and children with cerebral palsy (Crone *et al*, 1987; Leonard *et al*, 1990). The results from the H-reflex study by Leonard *et al* (1990) showed that in contrast to normal children, there was no decrease of gastrocnemius-soleus (GS) H-reflex amplitude during active ankle dorsiflexion. The GS H-reflex was also tested using vibration, and a similar trend of modulation of the reflex was found in the normal controls and the children with CP. The authors concluded that the pathways mediating reciprocal inhibition are present in the children with CP but were impaired because of a lack of control from supraspinal centres. The authors do not rule out however changes at the spinal level, such as exuberant common stem excitatory synaptic connections via which agonist and antagonist might be activated simultaneously.

The presence of shared excitatory drive can be detected using cross-correlation analysis. In their paper, Moore *et al*, (1970) described the occurrence of short duration central peaks in the cross-correlogram constructed from two spike trains using a computer model based on a simulation of interactions between Aplysia neurones. These peaks resulted

from shared excitatory input as shown in figure 1a. They also demonstrated the occurrence of central troughs in the cross-correlogram which resulted from shared reciprocal input as shown in figure 1b. This was followed by a series of studies by Kirkwood and Sears (1978, 1991), Kirkwood et al, (1982), and Sears and Stagg (1976) using the cross-correlation technique to study the presynaptic control of respiratory motoneurones.

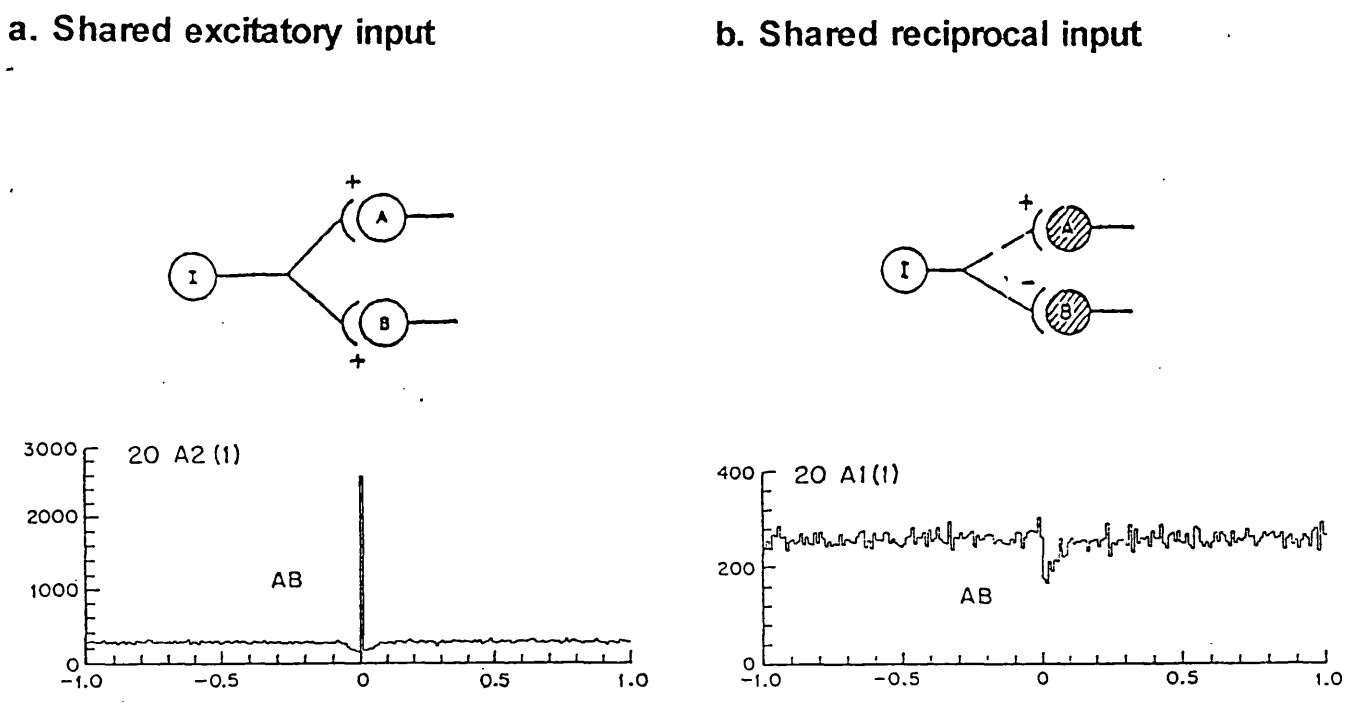


Figure 1: a. A short duration central peak is found around time zero in the cross correlogram constructed from neurones in aplysia which share a common excitatory drive, but, **b.** in the case of shared reciprocal input a short duration central trough is found in the cross correlogram around time zero (from Moore *et al*, 1970) . In this diagram, the cell (I) in **b.** is shown to make an excitatory connection with target cell A and a direct inhibitory connection with target cell B. In the context of the present study it is envisaged that the inhibitory limb of this circuit is not direct but via an inhibitory interneurone which is excited by input I but which inhibits cell B. Such an arrangement would also result in a correlogram trough.

The method has been employed to determine the presence of shared excitatory input to the motoneurone pools of the muscles concerned with posture and movement in health and disease (Bremner *et al*, 1991a&b; Farmer *et al*, 1990 & 1991; Carr *et al*, 1993 & 1994.). The technique has also been applied by Gibbs *et al*, (1994) and Nielsen & Kagamihara, (1994) to study the control of antagonistic muscles.

Using this approach it has been shown that there is common drive to co-contracting muscles which share the same action or which share a common joint or joint complex (Bremner *et al*, 1991 a&b; Gibbs *et al*, 1995a): cross-correlation analysis of multi unit data from co-contracting muscle pairs sharing the same action revealed a short duration central peak indicating the presence of common drive. This type of analysis has also been applied to co-contracting antagonistic muscle pairs (Gibbs *et al*, 1994) which revealed a short duration central trough in the cross-correlogram constructed from the motor-unit activity recorded in these muscle pairs. In the present study, this approach has been used to study the mechanism that may underlie the co-contraction of antagonistic muscle pairs during stance in development and in children with cerebral palsy. If shared excitatory inputs to the motoneurone pools of antagonistic muscles are responsible for the co-contraction of the muscle pair, then a short duration central peak will be observed in the cross-correlogram. Alternatively, if activity in shared reciprocal inhibitory inputs is present, short duration central troughs would be apparent. A flat correlogram would indicate no detectable shared synaptic input.

A preliminary account of this work will be presented to the Physiological Society, April 1996 (Mayston *et al*, 1996).

METHODS

Subjects

Four groups of subjects aged between 35 weeks gestational age (GA) and 46 years were studied with informed consent, written parental consent in the case of the children, and local ethical committee approval. When appropriate the children also gave verbal consent. Five healthy adults aged between 20 and 46 years (2 male); 44 children recruited from a local school aged between 3 and 11 years of age (21 male); 6 infants aged between 35 weeks GA and 12 months of age (2 male) and 7 children with cerebral palsy aged between 5 and 11 years of age (5 male) participated in the study.

EMG recording

EMG was recorded using pre-gelled surface electrodes (see section one, chapter one) attached to the skin overlying the tibialis anterior (TA), the soleus (SOL) and/or the quadriceps (QUADS) and hamstring (HAMS) muscles (over the belly of rectus femoris and biceps femoris respectively). In addition, in 4 of the adult subjects, simultaneous recordings were made using monopolar concentric needle electrodes (DFC25 Medelec, Old Woking , Surrey, UK). The EMG was amplified and filtered (-3dB at 20Hz and 5kHz for the surface recordings; 2kHz and 5kHz for the needle recordings) using a Medelec Sapphire clinical EMG machine and stored on magnetic tape as previously described.

Recordings from the adults

The recordings were made with the subjects standing. The subjects were instructed to lean forwards which activated the soleus muscle posturally, and then at the same time to lift the toes with some eversion of the foot to voluntarily activate the tibialis anterior. Most of the

subjects found this manoeuvre difficult; it is an unusual movement but does result in the co-contraction of these antagonistic muscles. The subjects were instructed to make weak contractions in such a way that activity from 2-3 motor units was recorded by each electrode, and to keep their contractions as steady as possible. Rests were given to minimise fatigue. An attempt was made to record from quadriceps and hamstrings. Subjects were instructed to lean forwards to activate the hamstrings posturally and to tighten the knee to activate the quadriceps; all but one of the subjects found this task too difficult.

Recordings from the children

The children were asked to stand freely in order to ascertain if there was any spontaneous co-contraction of TA and SOL. They were then asked to lean forward, lean backwards and then to attempt the same manoeuvre as the adults, combining a postural and voluntary command. Most of the children found this task very difficult and required a lot of encouragement (see figure 2). In addition, they were asked to reach up onto their toes for a ball as the increased joint stiffness required to balance might facilitate co-contraction of TA and SOL. This enabled recordings of the activity in this muscle pair to be made if co-contraction was not obtained in free standing or through voluntary effort.

Recordings from the infants

In the infants recordings were usually made between quadriceps and hamstrings as the small diameter of the distal part of the lower limbs usually resulted in volume conduction of EMG signals from agonist to antagonist and vice-versa. However in 3 infants recordings were possible from SOL and TA. The EMG signals were filtered at -3dB at 150Hz and 5 KHz.

Each infant less than 3 months of age was held in standing supported around the

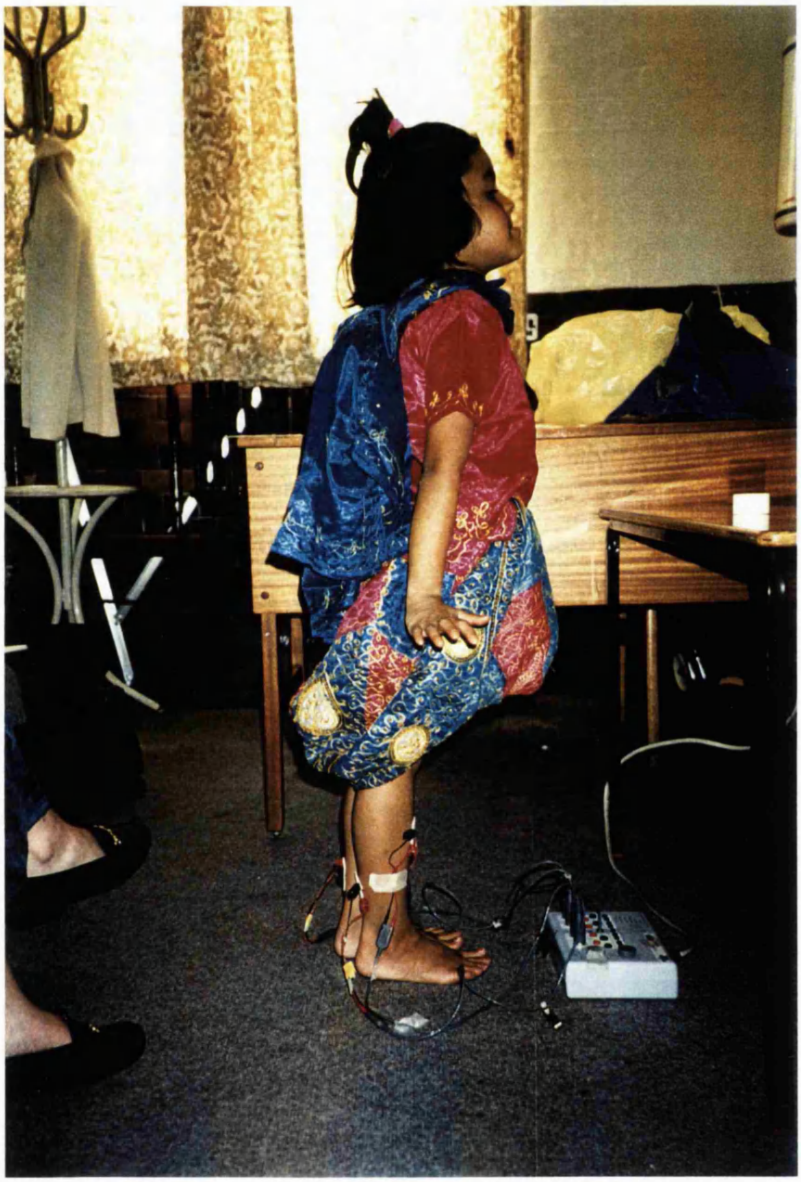


Figure 2: This child has been instructed to perform the postural/voluntary task to facilitate co-contraction in the lower limbs. She is leaning forwards and lifting her toes. The effort required to produce this manoeuvre is reflected in the facial expression and posture of the upper limbs.

trunk and the automatic standing reaction elicited. The infants automatically extended their legs in this position and intermittently pushed more into extension. The testing was best done before a feed when the infant was alert and active (state 5, Brazelton & Nugent, 1995). The infants able to stand with support were held in standing, or if able, played standing at a table during the time of recording.

Recordings from the children with cerebral palsy

The children stood at a table, using it as a support if necessary. Recordings were made from both QUADS/HAMS and TA/SOL when possible.

Cross-correlation analysis

Medium and large amplitude spikes were selected for analysis and cross correlograms constructed as previously described (section one, chapter one). The pre- and post-trigger sweep time was varied in some cases. Cross-correlograms were constructed with a minimum of 3000 spikes from each train. The SOL was active most of the time as it acted posturally and was designated the trigger spike, while the TA was considered as the event spike. A cumulative sum (cusum) of the difference between the number of counts in each bin and the mean bin count was calculated to determine the limits of any central feature in the cross-correlogram (Ellaway, 1978; Davey *et al*, 1986). An estimation of the strength of shared reciprocal input can be gained by determining the size of the trough. Several indices have been used to determine the strength of synchrony in the case of shared excitation (Harrison *et al*, 1991). The most commonly used index is *k*, which is calculated by dividing the highest bin count by the mean bin count, calculated in an area away from the peak. In the case of the presence of a trough, it was decided to estimate the lowest bin count (except in the case of a

single bin with a very low count) and divide this by the mean bin count to provide an estimate of the degree of anti-synchronisation caused by shared reciprocal input to the motoneurone pools of the antagonistic muscle pair. The auto-correlation was also constructed using the spike trains generated from the EMG of the muscles under study. The presence of volume conduction can be detected by the presence of a single bin which has an extremely high count at or near time zero in the cross-correlogram constructed from the two EMG signals. In the case of such a feature, the co-contraction could not be considered to be real and the record was not included in the analysis. In addition, recordings in which there was suspected volume conduction were subjected to spike triggered averaging, to ascertain how much of the signal was volume conducted through the tissues. The signal was first averaged to itself and then to the signal from the antagonist muscle and the relative sizes and shapes of the compound action potentials compared.

RESULTS

EMG recordings

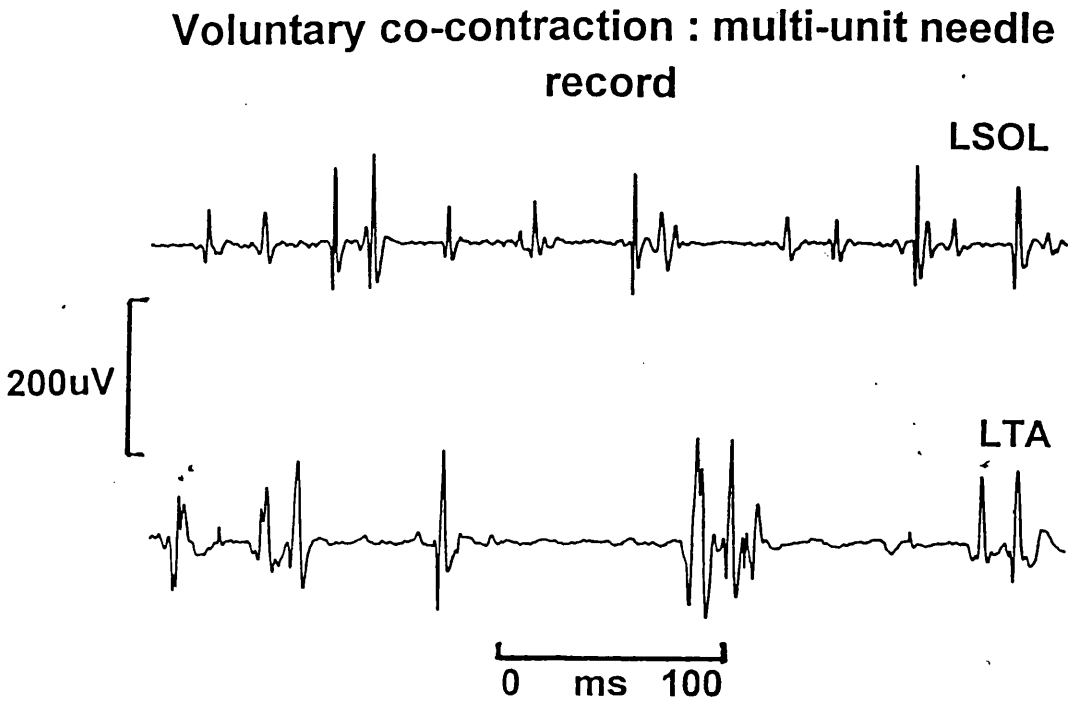
Recordings from the adults

Table 1 summarises the data from the four groups of subjects. None of the EMG recordings from the adults showed the presence of co-contraction of the SOL and TA during normal standing. However, all 5 subjects were able to co-contract this muscle pair by activating the SOL posturally and the TA voluntarily. An example of the needle and surface EMG recordings obtained for 1 subject are shown in fig.3a & b respectively.

Table 1

AGE (years)	Number of subjects	PRESENCE OF CO-CONTRACTION OF SOL & TA OR QUADS & HAMSTRINGS★	
Healthy volunteers		**STANDING**	**VOLUNTARY**
0-1	6	3/3 6/6★	n/a
3-5	7	3/7	0/7
6-8	23	0/23	5/23
9-11	14	0/14	7/14
21-46	5	0/5	5/5
Children with spastic cerebral palsy			
4-11	7	5/7 7/7★	n/a

Table 1: Spontaneous co-contraction was recorded in all of the infants, the children *less* than 5 years of age and in all of the children with CP. Voluntary co-contraction of SOL and TA was produced by all of the adults and 12 of the children aged between 6 and 11 years of age.



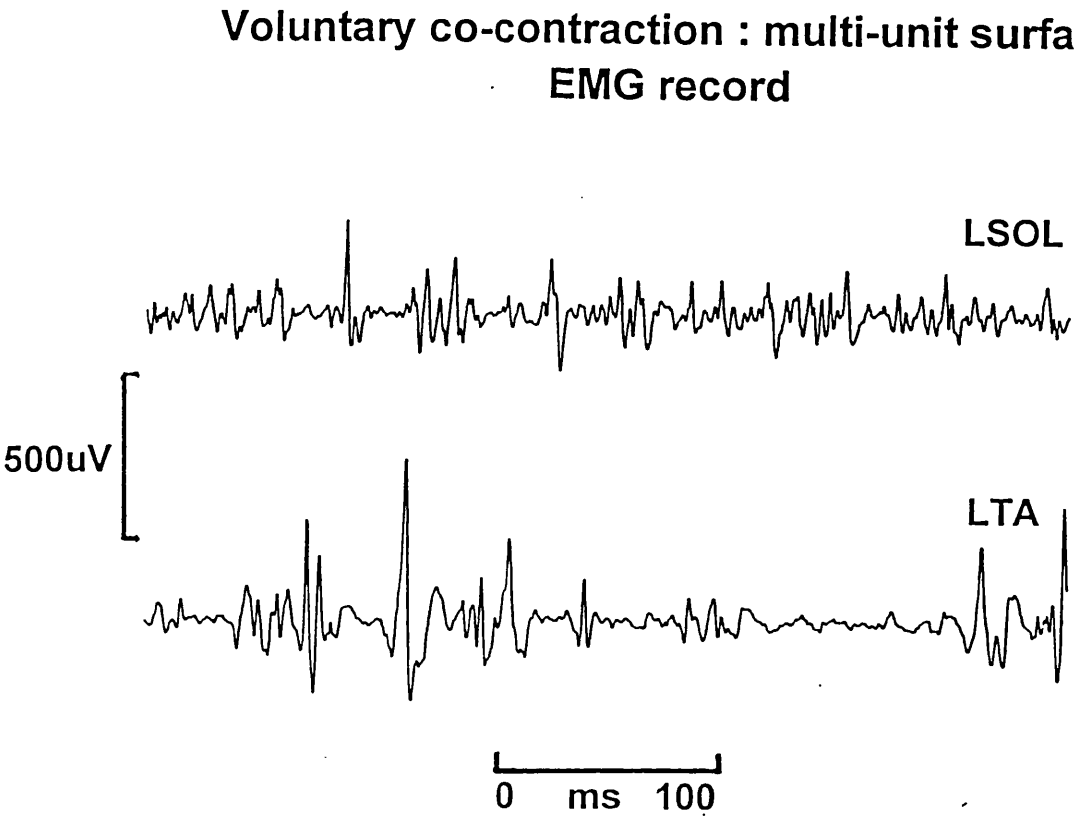


Figure 3: Simultaneous concentric needle and surface EMG recordings taken from a normal adult during co-contraction of SOL (postural command of lean forwards; upper trace in each panel) and TA which was voluntarily activated (lower trace in each panel). **a.)** The multi-unit EMG recorded using concentric needle electrodes and **b.)** Multi-unit EMG recorded from surface electrodes placed next to the needle electrode inserted into each muscle.

Recordings from the normal children

The summary in table 1, shows that 3 of the 7 children in the 3-5 year age group demonstrated co-contraction of SOL and TA in free standing. No evidence of co-contraction of this muscle pair during normal standing was found in the EMG recordings from the children in the older age groups, from 6 to 11 years of age. None of the children in the 3-5 year age group were able to voluntarily co-contract SOL and TA using the same manoeuvre as the adults in which a voluntary command is superimposed onto a postural command. However, 5 of the 23 children in the 6-8 year age group and 7 out of 14 in the 9-11 year age group were successful in this. An example of co-contraction in a 10 year old child is shown in fig. 4a to d. Fig. 4a & b shows that only the agonist is active on leaning forward (SOL) and leaning backwards (TA). However when the subject is instructed to lean forwards and lift his toes, both agonist and antagonist co-contract as shown in 4c. When the child was instructed to reach up onto his toes to retrieve a ball a reciprocal pattern of activation between this antagonistic muscle pair (SOL:TA) was observed as shown in fig. 4d.

Recordings from the infants

Table 1 shows that all of the infants 12 months old and younger demonstrated co-contraction of QUADS:HAMS (n=6/6) and SOL:TA (n=3/3). However as seen in fig.5, which shows the raw EMG recorded from RSOL:RTA in an 8 week old infant, this co-contraction was not obligatory and at times a reciprocal pattern of activation was observed. This figure shows that the first burst of activity in RSOL is not accompanied by a burst from the RTA, whereas the RTA accompanies the second SOL burst. This co-contraction occurred when the infant pushed into more extension which caused greater stiffness of the legs. When the activation of this muscle pair showed a more reciprocal pattern, the infant was less extended and the weight was either more forward (SOL more active) or behind (TA more active).

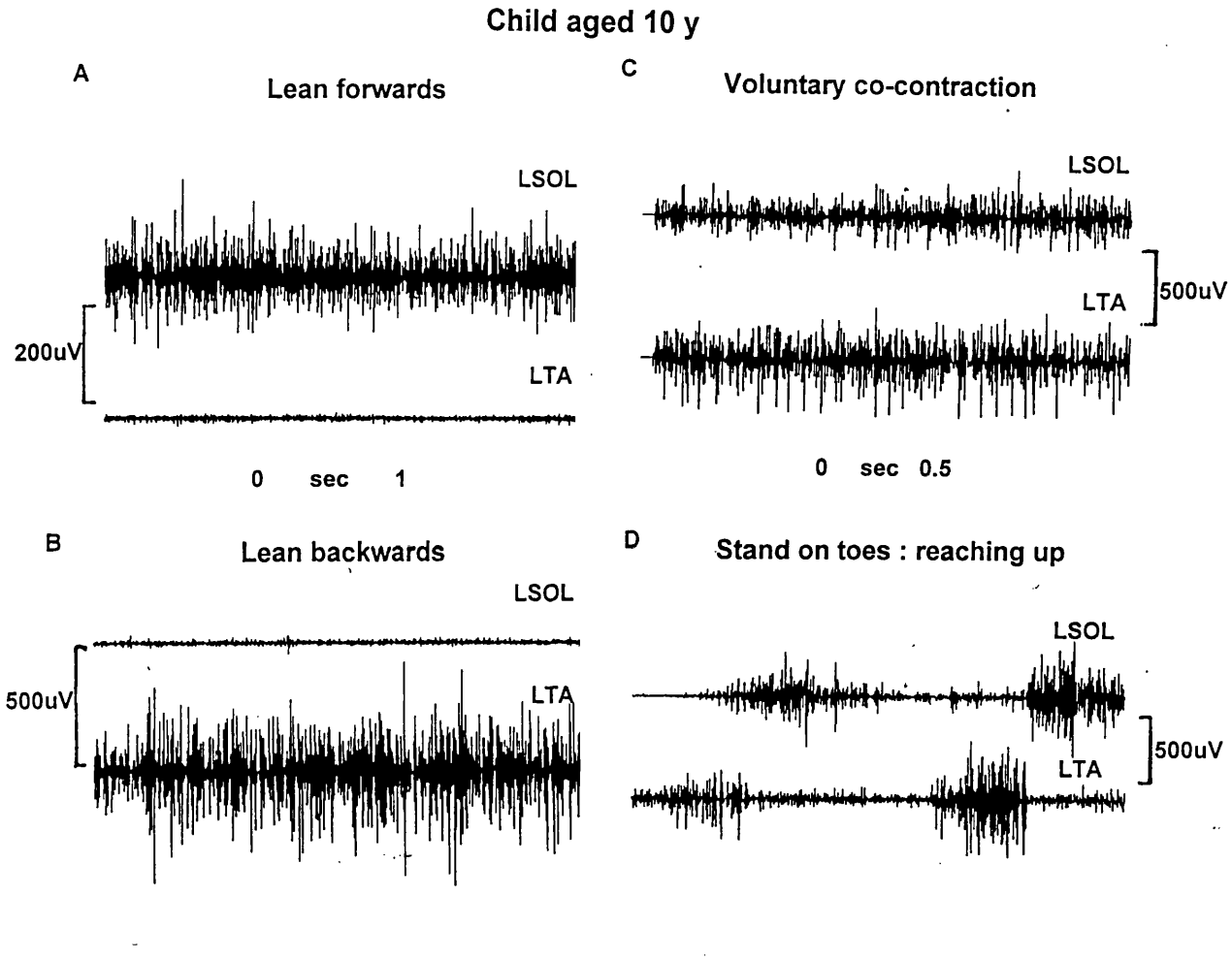


Figure 4: This child aged 10 years showed reciprocal activity of soleus (SOL) and tibialis anterior (TA). **a.** Leaning forwards resulted in activity in SOL (agonist) but not in TA (amtagonist).**b.** Leaning backwards produced activity in TA only to prevent falling backwards. **c.** This child could produce voluntary co-contraction as in the adults by leaning forwards (postural activity) and voluntarily lifting the toes (voluntary activity). **d.** Reaching for a ball resulted in reciprocal activity of the antagonistic muscle pair, SOL and TA.

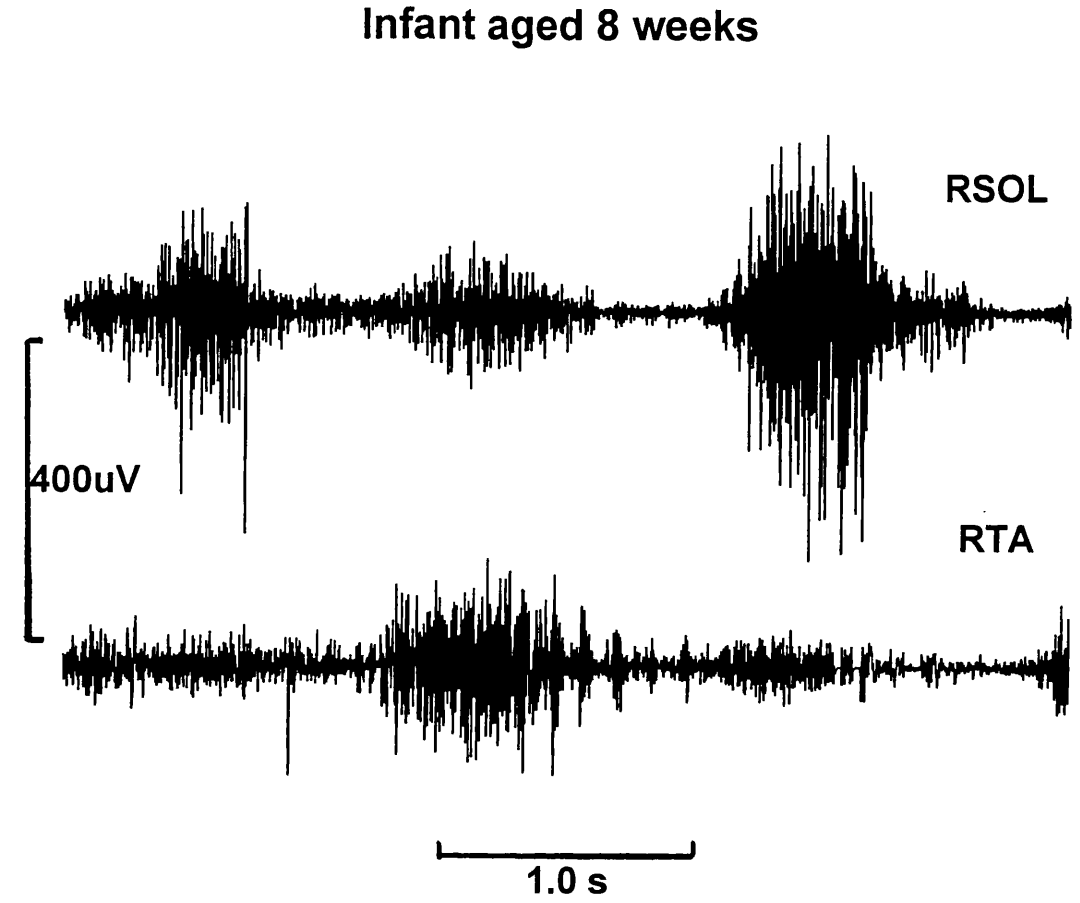


Figure 5: Infant aged 8 weeks. Both patterns of co-contraction and reciprocal activity of antagonistic SOL and TA were observed in the infants. The second burst of SOL activity is accompanied by a burst of activity in TA as a result of the infant pushing into extension of the knees.

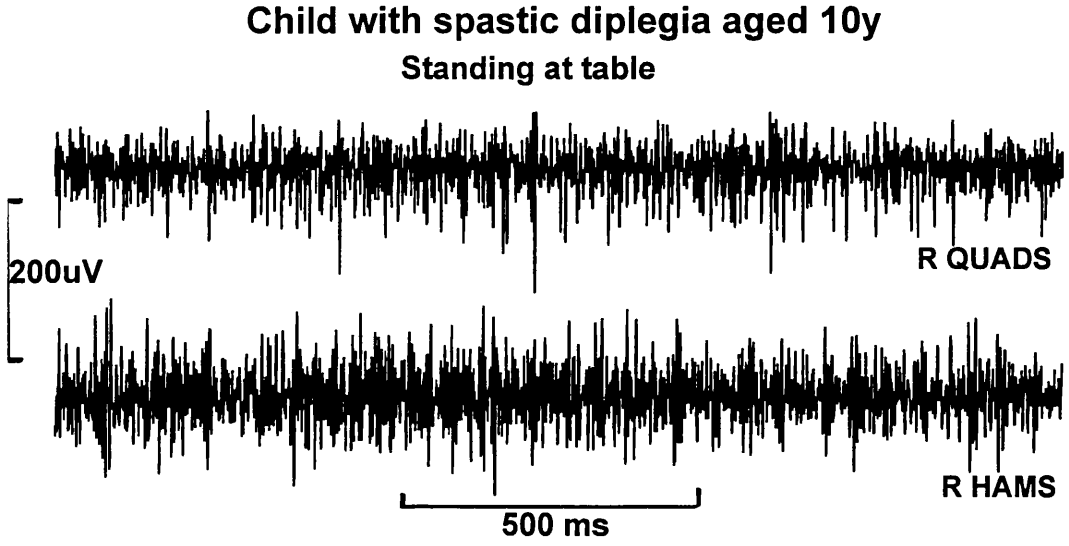


Figure 6: Child with spastic CP. In this child with a severe spastic diplegia, co-contraction of quadriceps and hamstrings was constant in standing. Unlike the normal infants and children no pattern of reciprocal activity was observed.

Recordings from the children with cerebral palsy

As shown in table 1, all of the children with cerebral palsy demonstrated co-contraction of the antagonistic muscle pairs studied in free or supported standing, although some records were rejected due to volume conduction and in two children no co-contraction was found in TA:SOL. One of these children had a mild form of CP and could walk without assistance, the other child was more severe and stood with a very flexed posture. An example of raw EMG recorded from LQUADS:LHAMS in a child with CP is shown in fig.6. This demonstrates the obligatory co-contraction often seen in children with CP. Unlike the spontaneous co-contraction recorded in the normal children and infants, the children with cerebral palsy rarely showed a pattern of reciprocal activity.

Cross-correlation analysis of motor-unit discharge of antagonistic muscle pairs

Adults

Tables 2 & 3 summarise the data obtained from the cross-correlation analysis. Central troughs were present in 8/8 (5 subjects, 8 muscle pairs) of the correlograms constructed from the multi-unit surface and needle recordings obtained from the co-contraction of SOL and TA in all of the subjects, indicating the presence of short-term anti-synchronisation of motor-unit activity in this muscle pair. Examples of this are shown in fig. 7a&b, which shows the cross-correlograms constructed from the raw data shown in fig. 3. The duration of these troughs ranged from 22.0 to 32.0 ms, mean 24.8 $\pm$ 1.4, ($\pm$SEM, n=8). Central peaks were never observed. The size of the troughs ranged from 0.50 - 0.87, mean 0.70 $\pm$ 0.05 ($\pm$ SEM, n=8). In the one subject who was able to co-contract QUADS and HAMS, the duration of the trough was 25ms and the size 0.80.

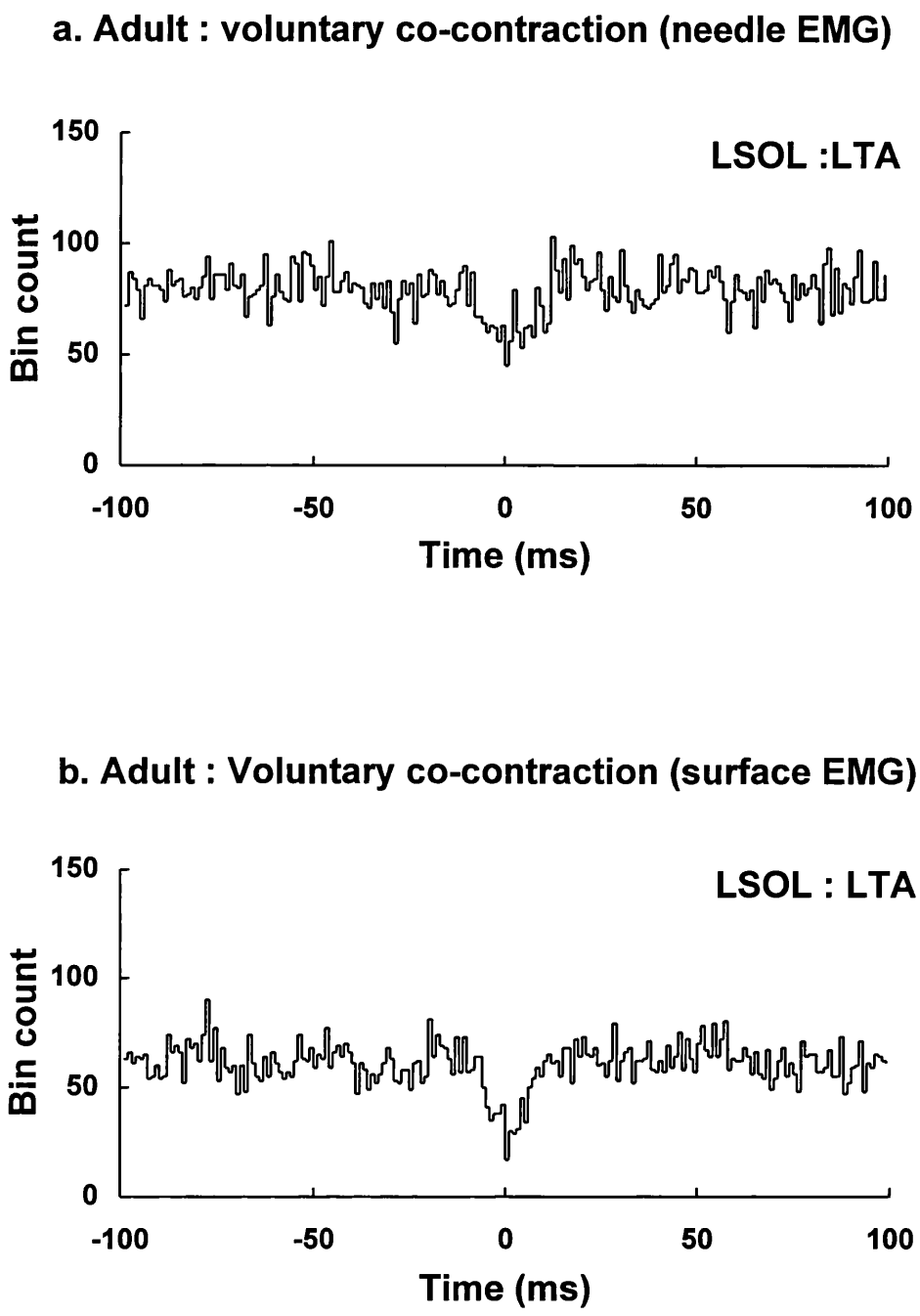


Figure 7: A short duration central trough was found in the cross-correlogram constructed from the spike trains recorded from the co-contracting antagonistic muscle pair soleus and tibialis anterior (approximately 5000 spikes from each muscle). **a.** a short duration trough of 22.0ms ($k = 0.67$) was found in this subject (needle recording) **b.** The central trough found in the cross-correlogram constructed from the simultaneous surface EMG recordings was of similar duration (20.0ms) and size (k=0.50).

Tables 2 & 3

AGE (years)	Number of subjects	PRESENCE OF CO-CONTRACTION OF SOL & TA	
Healthy volunteers		Voluntary co-contraction	Evidence of shared reciprocal input
6-8	23	5/23	3/5
9-11	14	7/14	4/7
21-46	5	5/5	5/5

Table 2: A central trough was found in the cross-correlograms constructed from the EMG recorded from SOL and TA in all of the adults who were able to produce voluntary co-contraction of this antagonistic muscle pair. A similar trough was found in 7 of the 12 children who were able to perform the same manoeuvre.

Age (years)	Number central troughs present	Duration (ms)			*k* lowest bin count / mean count		
	(muscle pairs)	Mean	Range	SEM	Mean	Range	SEM
Normal							
0- 5	0/16						
6-11	12/24	24.0	10.0-34.0	2.2	0.71	0.61-0.87	0.02
21-46	8/8	24.8	22.0-32.0	1.4	0.70	0.50-0.87	0.05
Children with CP							
4-11	0/25						

Table 3: The mean duration of the central trough which ranged from 10.0-34.0ms in the children and from 22.0-32.0ms in the adults is shown in column four, and the mean size which ranged from 0.61-0.87 and 0.50-0.87 in the children and adults respectively in column seven. The mean duration and size of the central trough was similar in the adults and children.

Normal Children

The cross-correlograms constructed from the multi-unit surface EMG recorded from SOL:TA in all of the three children aged between 3 and 5 years who demonstrated co-contraction in free standing were flat, indicating that there was no common drive to this muscle pair. An example of such a correlogram is shown in fig. 8a. This was also the case for 18 of the 23 children aged between 6-8 years and in 7 of the 14 children aged between 9 and 11 years. The presence of a central trough provides evidence of shared reciprocal synaptic input. This was found in 3 of the 5 children aged between 6 and 8 years and in 4 of the 7 subjects in the 9-11 year age group who were able to voluntarily co-contract SOL:TA. An example of a cross-correlogram constructed from the multi-unit EMG of SOL:TA in a 10 year old child is shown in fig.8b. in which there is a central trough of 28ms duration. The central troughs in the correlograms constructed from the children's recordings (aged between 5 and 11 years) were of mean duration 24.0 $\pm$ 2.2 ms ($\pm$SEM, 7 subjects, 12 muscle pairs) and the size was 0.71 $\pm$ 0.02.

Infants

The cross correlograms constructed from the co-contracting muscle pairs were all flat. This indicated that there was no shared excitation or shared reciprocal input to the muscle pairs which might bring about anti-synchronisation as observed in the older children and adults. An example of a typical cross-correlogram constructed from the EMG recorded from LQUADS:LHAMS is shown in fig. 8c, in which no central features are observed.

Children with CP

The results of cross-correlation in this subject group were extremely variable and are summarised in table 3. None of the 7 subjects showed central peaks which would provide evidence of shared excitatory drive to the agonist and antagonist motoneurone pools. In three

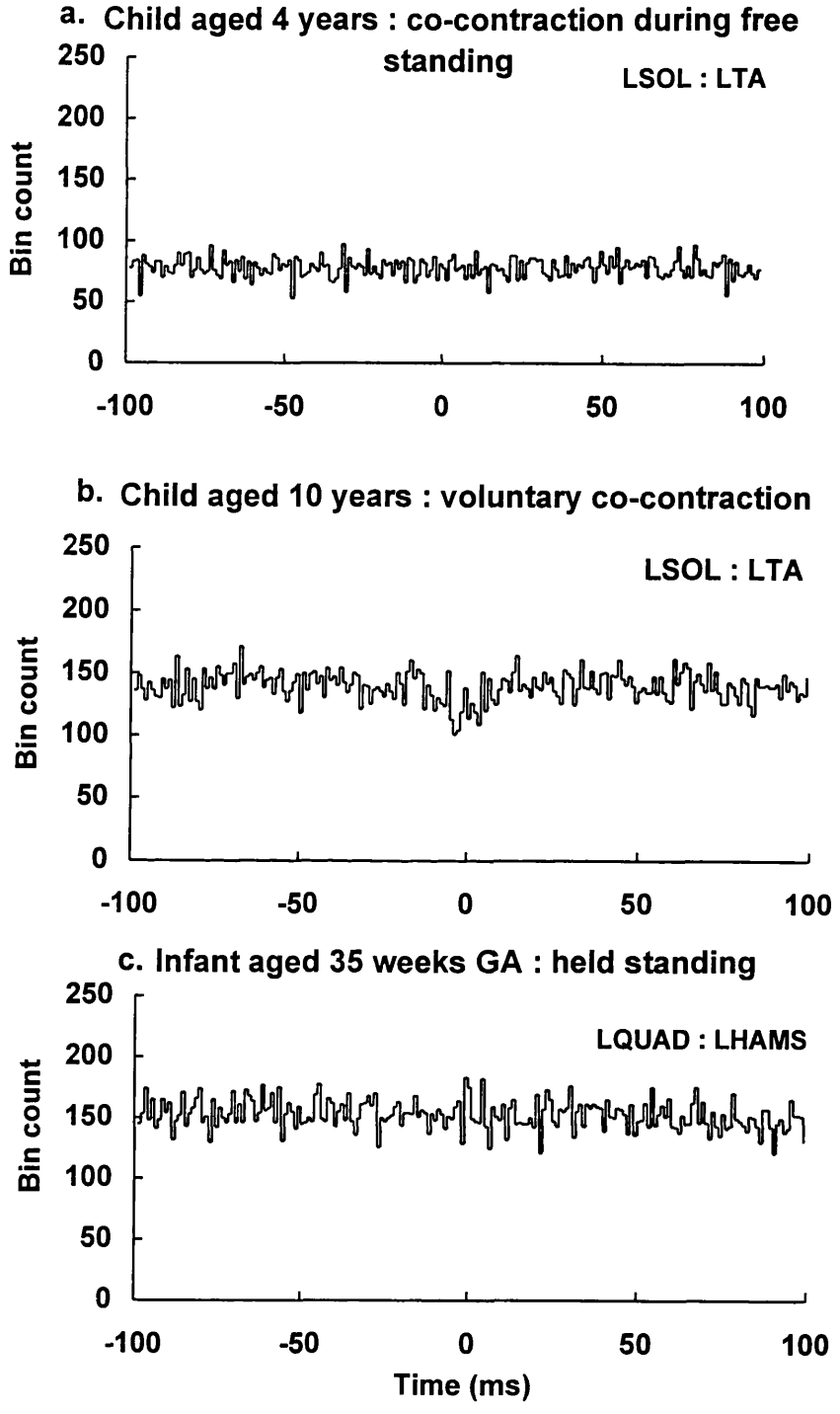


Figure 8: a. The cross-correlogram constructed from the EMG recorded from spontaneously co-contracting SOL and TA in a child aged 4 years (about 2500 spikes from each record). The correlogram is flat. **b.** An older child was able to produce voluntary co-contraction of SOL and TA in the same way as the adults. A short duration central trough (28.0ms) was found. **c.** The cross-correlogram constructed from the EMG recorded from co-contracting QUADS and HAMS in a 35 week gestational age infant was also flat (about 3000 spikes from each record).

cases the presence of volume conduction did not enable an interpretation of the cross-correlogram to be made. A typical cross-correlogram constructed from the multi-unit EMG recorded from the L SOL and LTA is shown in fig.9. The correlogram is flat suggesting that there is no detectable shared reciprocal input to this muscle pair. In no case was a central trough observed.

Table 4

Muscle pair	Co-contraction (number of children)	Cross-correlogram Flat
L SOL/TA	6/7	5/5
R SOL/TA	4/6	4/4
L QUADS/HAMS	6/6	4/6
R QUADS/HAMS	6/6	6/6

Table 4: Co-contraction was recorded in QUADS and HAMS in all of the children with CP and in 6/7 in left SOL and TA and in 4/6 in right SOL and TA. In all cases the correlogram was flat.

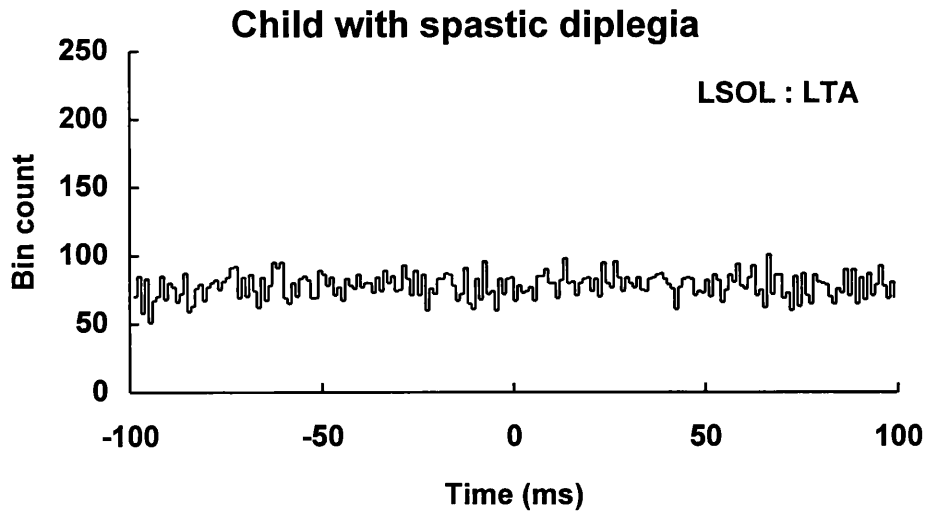


Figure 9: The cross-correlogram constructed from the EMG recorded from the spontaneous co-contraction SOL and TA in this child with CP was flat (about 2500 spikes each train).

DISCUSSION

The occurrence of co-contraction

Simultaneous activation of SOL:TA (and/or QUADS:HAMS in the infants and the children with CP) was found in all infants and children less than five years of age, and in all the children with cerebral palsy. Co-contraction was not found in free standing in the older children or the adults. However, in some of the older children and in all the adults it was possible to record co-contraction by training the subjects to activate one muscle posturally and its antagonist voluntarily.

The finding of co-contraction in the infants and children in the current study is in agreement with previous studies of the development of posture and locomotion in children. The evidence from the studies by Leonard *et al*, (1991), Berger *et al*, (1982) and Forssberg (1985) suggests that there are similarities in the patterns of activity during supported locomotion in infants and children with cerebral palsy. All authors have suggested that children with CP retain characteristics of an immature pattern of activity, which varies greatly between individuals presumably due to differences in lesion size, location and timing of the damage to the brain. One common characteristic reported in these studies is the presence of co-contraction during stance. In his study, Forssberg (1985) commented that there was a high degree of "synchronised" activity of synergistic and antagonistic muscles. In the current study, co-contraction of antagonists occurred when the infants pushed into extension, similar to the stance phase of locomotion which was investigated in the studies previously mentioned. There were times however when reciprocal activity was observed. This occurred when the infant was less extended, and the active muscle was determined by the line of the centre of gravity. That is, when the body inclined forwards, the SOL was more active, but leaning backwards resulted in greater activation of the TA.

Simultaneous activation of agonist and antagonistic muscle pairs during development has been reported in animal studies. Westerga and Gramsbergen, (1993) studied the development of EMG patterns in the quadruped walking of normal rats from post-natal day P10 to P42. Co-contraction was found at times until day P14, but reciprocal activation was also observed. They did not report the circumstances when either pattern of activity occurred, but in the present study of normal infants, both co-contraction and reciprocal activation were found but was dependent on the degree of knee extension. This was also found in the children with CP, but the relative time spent in co-contraction rather than in reciprocal activation appeared to be longer than that seen in the infants; the periods of reciprocal activity were more apparent in the less affected children who were able to walk without assistance.

CROSS-CORRELATION ANALYSIS

Adults

The presence of short duration central peaks in the cross-correlogram of the EMG signals recorded from the co-contracting antagonistic muscles would indicate the presence of shared excitatory input to the two motoneurone pools. No such peaks were found in the present study. Thus it can be concluded that the co-contraction observed was not produced by any detectable shared inputs to the motoneurones supplying the antagonistic muscle pairs studied.

i. The presence of short duration central troughs

The finding of short duration central troughs in the cross-correlogram in the present study, suggests that there is activity in shared reciprocal inputs: some last order input fibres that excite motoneurones innervating one muscle, branch to excite inhibitory interneurones

that innervate motoneurones supplying the antagonistic muscle. This is illustrated in Figure 10a &b.

At least two types of last order excitatory input fibres known to take part in this type of reciprocal circuit are illustrated: Ia afferent fibres from homonymous muscle spindle primary endings, and corticospinal tract fibres. Whilst these two fibre systems share the same inhibitory interneurones, either system alone, or any other pathway having the same type of reciprocal arrangement could produce cross-correlogram troughs. Figure 10a shows the reciprocal circuit for last order input fibres that excite one motoneurone pool and branch to disynaptically inhibit the motoneurone pool of the antagonist muscle. Fig.10b shows the same circuit to both the agonist and antagonist motoneurone pools. The circuit in fig.10a explains the possible mechanism for the occurrence of central troughs in the cross-correlogram. If impulses arrive along a last-order excitatory pre-synaptic input fibre to an agonist motoneurone, occasionally an excitatory post-synaptic potential (epsp) will be generated causing the motoneurone to fire. At the same time, if there is the presence of the branch to innervate an inhibitory interneurone which itself innervates an antagonistic motoneurone (Fig. 10a) then it would be expected that around the time the agonist motoneurone fired, an epsp will be generated in the inhibitory interneurone. This will increase the probability of firing of the inhibitory interneurone which in turn will produce a hyperpolarising effect on the antagonistic motoneurone and a reduction in its firing probability. Thus the firing of an agonist motoneurone is linked to a reduction in the probability of firing of the antagonistic motoneurone as found in the present study. If the arrangement is symmetrical as shown in figure 10b, then an impulse in the antagonist motoneurone can be expected to be associated with a lowered probability of firing of the agonist motoneurone.

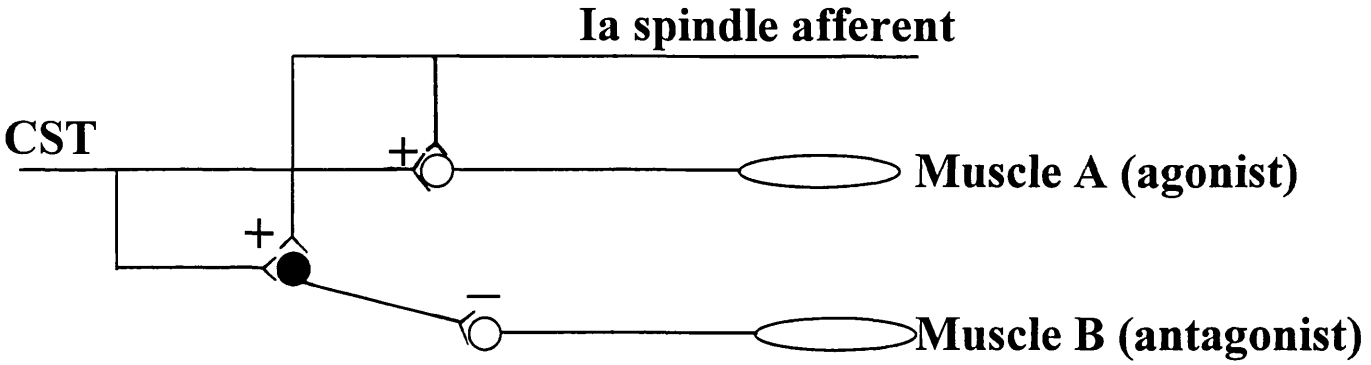


Figure 10: a. The motoneurone pool of the agonist (muscle A) receives excitation from the Ia spindle afferent and the corticospinal tract (CST), which branch to disynaptically inhibit the motoneurone pool of its antagonist (muscle B). This reciprocal arrangement produces activity in muscle A and inhibition of activity in muscle B.

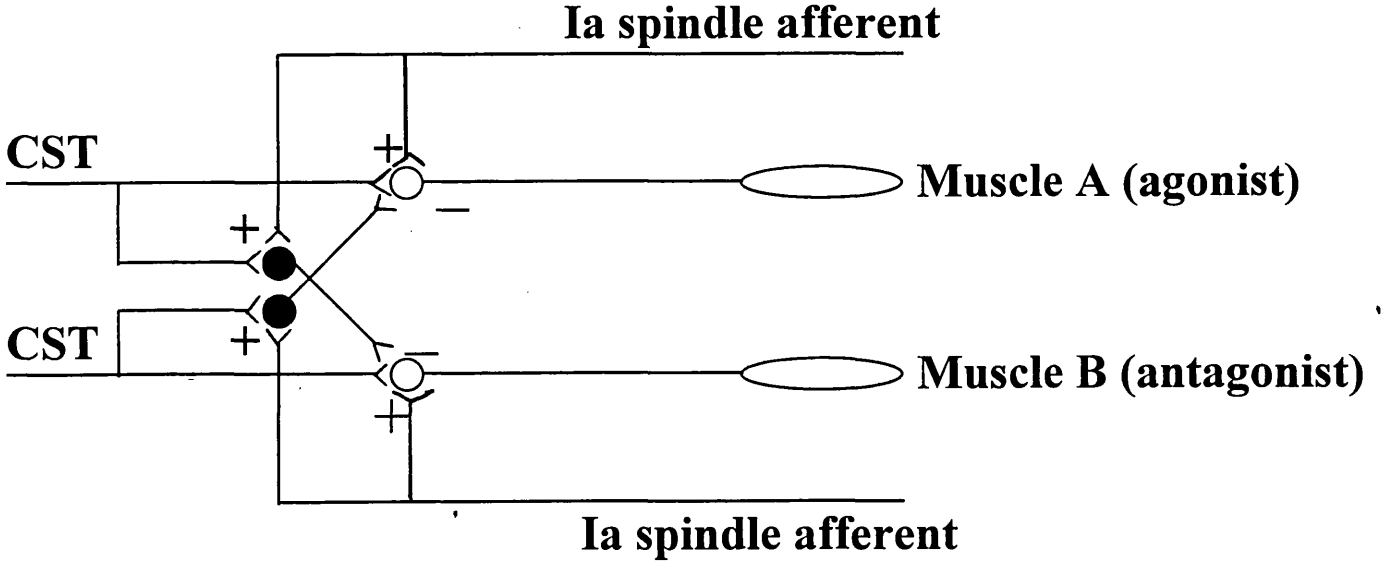


Figure 10b: The same reciprocal circuit shown in figure 10a. is distributed to both the agonist and antagonist motoneurone pools.

The activity in these reciprocal circuits can be observed in the shape of the cross-correlogram trough. Taking 'A' as the trigger and 'B' as the event in fig.10b, and assuming the same peripheral delays, it would be expected that the presence of the reciprocal pathway to the event unit via a branch from the input exciting the trigger unit would result in a trough in the cross-correlogram lying to the *right* of time zero (centre).

On the other hand, if only the reciprocal input to the trigger unit, linked to the firing of the event unit is active, the trough will lie to the *left* of the centre of the cross-correlogram. The latency of the trough from the time of firing will be made up of the time difference in conduction from the branch point to the agonist motoneurone and the inhibitory interneurone, in addition to the conduction time from the inhibitory interneurone to the antagonistic motoneurones. If the reciprocal circuit is active in both directions then the trough will be symmetrical around time zero. In most cases observed in the present study, the trough was symmetrical, indicating that both reciprocal circuits were active.

Further evidence for this proposed reciprocal inhibitory mechanism to account for the central troughs is provided by work in monkey by Kasser and Cheney (1985) who showed that post-spike suppression of antagonistic muscles from corticomotoneuronal cells is detectable with spike triggered averaging of rectified EMG activity. They found that triggering from the same cortical cell could produce post-spike facilitation of the agonist (wrist and finger extensors) as well as post-spike suppression of the antagonist (wrist and finger flexors). The latency of this suppression was about 3ms longer than that of the post-spike facilitation and the magnitude of the suppression about half that of the post-spike facilitation. They concluded that the findings were probably consistent with activity in the collaterals of corticomotoneuronal cells to Ia inhibitory interneurones. They suggest that this activity could be mediated via the corticospinal tract which is known to make connections to

these interneurones (Jankowska & Tanaka 1974; Jankowska *et al* 1976; Schomburg 1990; Iles & Pisini, 1992).

Unlike in the present study, Nielsen and Kagamihara (1994) found short lasting central cross-correlogram peaks or small positive central inflexions in the cusum which were found in 63% (19 out of 30) recordings from SOL:TA muscle pairs. The average duration of these peaks was 13.9ms (range 6-20ms). The time course of these peaks is similar to that recorded from pairs of motor units within the same muscle and indicates the presence of shared last order synaptic input. Thus in their study, the shared reciprocal inputs producing the cross-correlogram troughs in the present study appear either to be inactive or at least hidden by the presence of activity in last order shared excitatory inputs.

It seems likely that this difference in the pattern of shared input maybe related to the task being carried out by the subjects during the recording session. In an earlier H-reflex study, Nielsen and Kagamihara (1992) found a depression of the reciprocal Ia reflex recorded during simultaneous voluntary activation of the SOL:TA antagonistic muscle pair. Subjects performed this same task during the later cross-correlation study, thus we might suppose that under these conditions reciprocal Ia inhibitory interneurones maybe relatively inactive and therefore shared reciprocal inputs between the antagonistic motoneurone pools are weak and unobservable using the cross-correlation technique. In the present experiments, a combination of a voluntary and postural command was used, presumably with each command involving activation of different descending and supraspinal areas. It is known that there are several pathways which converge onto the Ia inhibitory interneurone, including the corticospinal and reticulospinal tracts, therefore different activation of these pathways in the different modes of activation of the muscle pair could account for this difference. It has also been shown that the degree of synchrony varies according to the task. Gibbs *et al*, (1995a)

have shown that the degree of synchrony in lower limb muscles is greater in balancing than standing and lying, thus it is possible that the degree of anti-synchronisation could be influenced in the same way. In the present study the recordings were made in standing whereas in the Nielsen study the recordings were made while sitting.

ii. The duration of the trough

The duration of the central troughs found in the present study for the adult ranged from 22.0-32.0ms (mean 24.8ms, n=8) and for the children 16.0-34.0ms (one value 10.0ms; mean 24.0ms, n=12). In the present discussion it is envisaged that these troughs are produced by activity in last order input fibres that excite motoneurones innervating one muscle that branch to excite inhibitory interneurones that innervate motoneurones supplying the other muscle. By extending the theoretical framework of Kirkwood in Kirkwood and Sears (1978) which considers the case of motoneurones receiving a branched common stem excitatory input, it is possible to include a double reciprocal inhibitory connection and thereby estimate the time course of the reduction in the probability of firing that would be expected in a cross-correlogram constructed from the firing of reciprocally innervated motoneurones. For the case in which the reciprocal inhibitory connections to the two motoneurones are equal in both directions, a trough of approximately twice to three times the width of a cross-correlogram peak produced by activity in branched last order synaptic inputs can be expected (see fig.1 in Gibbs *et al*, 1995b, page 467 in *ALPHA & GAMMA MOTOR SYSTEMS,* Ed. A. Taylor, M.H. Gladden & R. Durbaba). In their single unit study of motor unit synchronisation within individual forearm, hand and finger muscles in normal subjects, Bremner *et al*, (1991 a&b) found central cross correlation peaks ranging from 5.0-31.0ms (90% of values between 8.0 and 18.0ms, mode 13.0ms, mean 14.5ms, n=303), and argued that these values were within the range expected from activity in a branched common stem

pre-synaptic input. The durations of the central troughs found in the present study are approximately twice as long as these values and therefore are within the range expected for the reciprocal arrangement of last order inputs as envisaged above.

iii. The strength of the inhibition

This index ranged from 0.50-0.87 (0.70 ± 0.04, mean ± SEM; n=5 subjects; 8 muscle pairs), that is from 13%- 35% decrease in the probability that B (TA) would discharge at the same time as A (SOL). In the study by Gibbs *et al*, (1994), the mean *k*-value for the troughs found in the cross-correlograms constructed from the EMG recorded from SOL:TA was 0.75 ± 0.04 (mean, ± SEM, n=8), similar to that found in the present study.

Normal children

Cross-correlograms were constructed from the data recorded from the 12 out of 41 children aged between 5 and 11 years who were able to produce co-contraction in the same way as the adults. Seven contained a central trough and five were flat. The duration of these troughs was not significantly longer (unpaired t-test, $P<0.05$) than those found in the adults and were of a similar shape and size.

Only twelve of the older children (aged 8-11 y, n=26) aged between 5 and 11 years of age (n=41) could produce co-contraction in the same way as the adults. Evidence for shared reciprocal input was found in the occurrence of short duration central troughs in the cross-correlograms constructed from the EMG recorded from SOL:TA in 7 of those 12 children. The remaining 5 cross-correlograms were flat indicating no shared reciprocal input, and thus was consistent with individual variations in the development of the CNS, to be discussed in the following section.

Infants, young children and those with CP

All the cross-correlograms constructed from the EMG signals recorded from the infants, young children less than 5 years of age, and children with CP in whom co-contraction was observed were flat. This finding suggests that the co-contraction found was not due to shared excitatory input to the antagonistic motoneurone pools.

No central troughs were found. One possibility that could account for the absence of troughs in the cross-correlogram would be a lack of Ia mediated reciprocal inhibition due to a lack of facilitation of the Ia inhibitory interneurone by the corticospinal tract or the lack of facilitation of the Ia afferent from the muscle spindle. The spinal pathways mediating reciprocal inhibition have been investigated in adults using H-reflex conditioning by electrical stimulation of the nerve to the antagonistic muscle, but such testing has not been reported in the literature in children less than 12 years of age (Leonard *et al*, 1990). Nevertheless, the results from human reflex studies in addition to evidence from animal studies, suggest that there are indeed changes in the reciprocal circuits mediating reciprocal inhibition during development and pathology.

Lack of facilitation of Ia inhibitory interneurone via the muscle spindle afferent

The homonymous Ia afferent excitatory projection is present at birth in man as evidenced by the large tendon jerks that can be recorded in infants and children (Gottlieb *et* al, 1982; Leonard *et al*, 1991; O'Sullivan *et al*, 1991; Myklebust & Gottlieb, 1993; Leonard & Hirschfeld, 1995). These authors also found excitatory responses in more remote muscles, including antagonistic muscles. Arguing on the basis of their latency, the authors suggested that all these responses, including those recorded in antagonistic muscles, were mediated monosynaptically by activity in Ia muscle spindle afferent fibres. The more distant synaptic

connections became less functional with increasing age, so that by five years of age reflex overflow could only be elicited in the directly antagonistic muscle. In contrast, McDonough *et al*, (1993) reported short latency inhibitory responses in biceps following a tap to the triceps tendon in 10/42 neonates, although in the remaining 32 infants reflex radiation was recorded. Thus in the upper limb, if not the lower limb, reciprocal Ia inhibitory connections appear to be functional at birth in some infants.

A number of anatomical (Eccles *et al*, 1956) and electrophysiological studies (Saito, 1979) in the cat and in the rat foetus have indicated that in the new-born animal, Ia afferent fibres project more widely amongst heteronymous motoneurone pools than in the adult. These studies also indicate that these distant synaptic connections become less functional with increasing age. Kellerth *et al* (1971) have also found evidence for a decrease in alpha motoneurone excitability with age, producing a corresponding decrease in the excitability of monosynaptic transmission in the stretch reflex pathway with age.

With this background in mind, a number of authors have suggested that in children with CP, the normal reduction in strength and radiation of the stretch reflex to heteronymous muscles does not take place thus resulting in persistent and exaggerated radiation of reflexes which has been found in these patients (Berger *et al*, 1982; Myklebust *et al*, 1982; Leonard *et al*, 1988, 1991; Leonard & Hirschfeld, 1995). This is supported by the finding of similar patterns of reflex radiation in the lower limbs of infants less that 2 years of age and in children with CP (Leonard *et al*, 1988). This reflex radiation was of larger amplitude in the children with CP, was greater in the more damaged children and unlike the able-bodied children persisted after 2 years of age.

Although these authors suggest that there is radiation to the antagonist via a common inputs, the lack of central peaks in the correlograms from the infants and children in the

present study do not support this conclusion.

Lack of facilitation of the Ia inhibitory interneurone via the corticospinal tract

It has been suggested earlier in this discussion that the corticopsinal tract influences spinal reflex circuits by facilitation of the Ia inhibitory interneurone to bring about reciprocal inhibition of the antagonist muscle. Evidence for the targeting and development of activity in this tract is sparse in the human literature, and there is only limited information in the animal literature.

Evidence from human studies:

Some evidence for activity in the corticospinal tract onto the spinal circuit mediating reciprocal inhibition in infants and children has been provided by the study of Conway *et al*, (1994), who have been able to evoke activity in the descending corticospinal tract and spinal reflex circuits mediating reciprocal Ia inhibition. Their study used magnetic brain stimulation to condition the phasic stretch reflex of biceps in the upper limb of normal adults and neonates. In all of the adults tested and in 14/19 neonates, the cortical conditioning resulted in a rapid brief period of facilitation followed by a significant depression, with delays consistent with a disynaptic linkage. This depression fell lower than the test reflex level in all of the adults but in only 2 of the 14 infants in whom this depression was observed. They concluded that the cortex excites spinal inhibitory interneurones with a disynaptic linkage to motoneurones, but that in some neonates (5 out of 19 in their study), this projection might not be established. These results may also suggest that the supposed reciprocal inhibitory pathways mediating this reflex depression are weak in the neonates although this was not discussed by the authors. However, whilst these pathways can be activated by stimulation

techniques, it is not known whether these pathways are active when called upon functionally.

In a study of normal children and children with CP aged 10-16 years (Leonard *et al*, 1990), the gastrocnemius H-reflex was found to be depressed during voluntary dorsiflexion of the foot brought about by contraction of the tibialis anterior muscle. They attributed this depression of the H-reflex in gastrocnemius to post-synaptic inhibition of gastrocnemius motoneurones brought about by corticospinal tract activity to tibialis anterior motoneurones acting through excitatory collateral connections to the Ia inhibitory interneurone innervating the antagonist gastrocnemius motoneurones. The H-reflex depression was not observed in the age-matched children with CP. On the other hand, in both the normal and the CP group, vibration applied to the TA tendon did depress the gastrocnemius reflex indicating that the Ia inhibitory circuit is active, at least when driven by peripheral Ia input. Thus on this basis it would appear that in their group of children with CP the segmental reciprocal stretch circuitry is functional, but that during voluntary activation the corticospinal projection to antagonistic motoneurones through the Ia inhibitory interneurone is lost.

Further evidence to support this hypothesis is found in the study by Berbrayer & Ashby (1990) who studied the discharge of TA motor units whilst stimulating the posterior tibial nerve in 11 normal subjects and 15 children with CP of mixed classification. They constructed peristimulus time histograms of the discharge rate of TA motor units and found a short latency inhibition of the TA motor unit in all successful recordings from 11 out of 15 the patients with CP who were studied. The corticospinal projection to the lower limbs in children with CP has been studied by Brouwer & Ashby (1991). In the 11 normal subjects, stimulation over the cortex using Magstim produced a significantly greater excitatory effect on SOL rather than TA motoneurones. But in the 10 children with CP, 7 of whom had a spastic diplegia, both SOL and TA motoneurones were similarly facilitated. This was

suggested to be the consequence of abnormal development following damage to the corticospinal system resulting in projections directed equally to the antagonistic motoneurone pools. This proposed explanation, however, is not consonant with the results of the present study in which no evidence of activity in shared excitatory inputs reaching antagonistic motoneurones was found in the children with CP. Alternatively, the result obtained by Brouwer and Ashby (1991) may be the result of a failure of the developing corticospinal axons to make functionally strong connections to segmental interneurones. Thus it may be that the abnormal co-contraction of SOL and TA produced by Magstim in CP may be the result of a loss of the normal reciprocal inhibitory connections made by the corticospinal fibres through the Ia inhibitory interneurone. Such a lack of a functional reciprocal inhibitory mechanism would be consistent with the finding of a flat cross-correlogram constructed from the EMG recorded from SOL and TA in CP found in the present study. This suggests that there are no shared inputs either excitatory or reciprocal. Taking together the two sets of results, it can be concluded that the co-contraction of antagonistic muscles in CP is not the result of a common drive reaching the two motoneurone pools, but rather that the drive is arriving simultaneously from independently driven excitatory inputs without the normal accompanying reciprocal inhibitory drive which is absent in young children, but shown to be present in some children by 8 years of age as indicated by the presence of central troughs in the cross-correlograms constructed from the EMG recorded from co-contracting antagonistic muscle pairs.

Anatomical evidence from animal studies

In their study of the development of the corticospinal projection in monkey, Armand *et al*, (1994), showed that although the projection to the intermediate zone in the spinal cord

was present in newborns, there was only sparse distribution to the motoneurone pools supplying the distal hand muscles. At 5 months of age this distribution was still sparse, and although at 11 months labelling was more dense it was not as heavy as seen in adults. A similar finding was reported by Galea & Darian-Smith (1995) in their study of the macaque monkey. These data suggest that the growth of corticospinal axons and the development of their projection to the motor nuclei is a gradual process which takes some time to mature. It is possible that similarly in man a delay may occur between the arrival of the corticospinal projection at the spinal cord and subsequent innervation of the motoneurone pool. This would result in a delay in the performance of relatively independent finger movements (RIFM) by the children as well as a delay in the influence of the termination of CST axons onto the Ia inhibitory interneurone mediating reciprocal inhibition, as observed in the present study.

Clinical implications

The present study has shown how cross-correlation analysis can be used as a tool to look for the presence of shared reciprocal input to co-contracting muscle pairs. It is easily applied to the clinical setting, requiring only a clinical EMG machine, surface electrodes and the appropriate computer equipment and software. Application of this analysis could enable clinicians to determine if the presence of co-contraction is due to a lack of shared reciprocal input or growth of abnormal branched pathways (as occurs in some children with congenital hemiplegia). This knowledge will assist in clinical decision making about the most appropriate therapeutic intervention.

Future work

It will be worthwhile to study other antagonistic muscle pairs in addition to a greater numbers of subjects, in particular infants of earlier gestational age, and children with CP as well as those at risk for CP. The interpretation of this type of analysis would be improved by knowing better how to interpret the recorded correlograms more quantitatively in terms of the strength of shared inputs. The use of computer modelling to explore the quantitative relationship between the strength and time course of synaptic input and motoneruone output will be of importance in understanding the normal reciprocal inhibitory mechanisms in addition to the development and dysfunction of such pathways.

APPENDICES

APPENDIX A

Modified version of the Edinburgh Handedness Inventory (Oldfield, 1971)
Each item was assigned to either the left or right handed dominance without regard to strength of dominance.

EDINBURGH HANDEDNESS INVENTORY

Surname..... Given names.....

Date of birth..... Sex.....

Please indicate your preferences in the use of hands in the following activities by putting + in the appropriate column. Where the preference is so strong that you would never try to use the other hand unless absolutely forced to, put ++. If in any case you are really indifferent, put + in both columns.

Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in the brackets.

Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking match (match)		
10.Opening box (lid)		
(i) Which foot do you prefer to kick with?		
(ii) Which eye do you use when using only one?		

APPENDIX B

SCHOOL STUDY - Child Details

Child's name _____ Age _____

Date of Birth _____/_____/_____ Class _____

Dominant Side _____

	Left	Right
Use scissors		
Write		
Brush your teeth		
Use a spoon		
Throw a ball		

Grade of mirror movements (0=none, 1=slight, 2=marked, 3=exact mirror image; as determined by sequential finger/thumb opposition).

Left Voluntary _____ Right Voluntary _____

Ability to perform reciprocal pronation/supination
(0=unable, 1=with difficulty, 2=some hesitation, 3=able). _____

Other relevent information (eg clumsiness, learning difficulties, observations).

APPENDIX C

The Kallmann gene has 14 exons which contain the genetic coding material. In some subjects one or all exons maybe deleted. In some cases a defect in the exon results in a point mutation. A defect in a non-coding area results in a lack of expression of the genetic material.

SUBJECT	GENOTYPE	OTHER PHENOTYPIC ANOMOLIES
K1	terminal deletion of Xpter involving entire *STS & KAL* loci	MM, ichthyosis, hypertension, micropenis
K9	terminal deletion of Xpter involving entire *STS & KAL* loci	MM, ichthyosis, L kidney absent, renal impairment, hypertension, proteinuria, hypothyroid, cryptorchid
K2	terminal deletion of Xpter involving entire *STS & KAL* loci	MM, ichthyosis, hypertension, proteinuria, cryptorchid, micropenis, R kidney absent
K12	complete deletion of all *KAL* exons	MM, cryptorchid, R kidney absent
K7	complete deletion of all *KAL* exons	MM, cryptorchid, R kidney absent
K14	point mutation exon 6 G→A substitution at base 260 creating premature stop codon	no MM
K11	point mutation exon 6 G→A substitution at base 260 creating premature stop codon	MM, cryptorchid, L kidney absent
K6	exon 1 deleted	MM, cryptorchid small R kidney (44% total function)
K8	deletion of C_{2458} in exon 12 (frameshift mutation) and premature STOP codon	MM, cryptorchid, R sensorineural deafness
K5	presumed mutation of 5' promotor region	MM, cryptorchid, L kidney absent
K4	no coding sequence mutation	
K13	point mutation exon 5 G→A substitution at base 239 creating premature stop codon	MM, cryptorchid
K10	deletion of steroid sulphatase locus and exons 1 & 2 of Kalig 1 gene	MM, cryptorchid
K3	point mutation exon 5 G→A substitution at base 239 creating premature stop codon	MM, cryptorchid

K9 and K2 are cousins
K13 and K3 are cousins
K14 and K12 are brothers
K1 is uncle to K9 and K2
K12 and K7 are brothers
K4 and K5 are brothers

APPENDIX D

Clinical details of the children with spastic cerebral palsy.

S1
Spastic quadriplegia, left worse than right, but fairly symmetrical; born full-term following an uneventful pregnancy. Normal delivery. Birth weight 7lb 3oz. Described as a "jittery" baby.

S2
Spastic quadriplegia, strong asymmetry, left much worse than right. Born at 35 weeks gestational age but of low birth weight (4lb) and had suffered intra-uterine growth retardation (IUGR). Ultrasound scan did not show any abnormality.

S3
Spastic quadriplegia, one of twins born at 30 weeks gestational age and weighing 3lb 7oz.

S4
Severe spastic quadriplegia born by emergency caesarian section at 26 weeks gestational age with a birth weight of 2lb 2oz. Ultrasound scan revealed periventricular haemorrhage into the white matter.

S5
Severe spastic quadriplegia, right side worse than left. Second twin born by caesarian section at 37 weeks gestational age. An ultrasound scan performed at 20 weeks noted that this child showed signs of microcephaly, a sign which is clearly evident now at 6 years of age. He is also of small stature.

Evidence of early damage:

Two of the subjects were known to have early CNS damage in whom bilateral responses to Magstim were recorded at similar latency (S3 & S4). Subject S4 was born at 26 weeks gestation; S4 at 30 weeks gestation and were diagnosed by ulatrasound scan as having periventricular leucomalacia. Subject S2 in whom bilateral responses to Magstim were also recorded was born at 35 weeks, but was of low birth weight and diagnosed to have intrauterine growth retardation. His mother, who only had one kidney, had difficulties throughout the pregnancy, thus it is possible that he could also have experienced damage early in utero.

APPENDIX E: Copies of publications

Journal of Physiology (1994) 480.P

Bilateral EMG accompanies unilateral tasks in man

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Unilateral tasks may be associated with bilateral activation of the motor cortex (Kristeva *et al.* 1991; Kim *et al.* 1993). In the present study we have looked for the presence of bilateral muscle activity during phasic and maintained unimanual contractions.

Subjects were seated with both arms well supported. With ethical committee approval, EMG was recorded using surface electrodes from left and right first dorsal interossei. Subjects were instructed to maintain a steady voluntary index finger abduction on the non-preferred side (NPS) at 10 % of that achieved during a maximum voluntary contraction (MVC). At the same time subjects were required to make repeated self-paced forceful voluntary index finger abduction movements on the preferred side (PS). The EMG signal from both sides was rectified and averaged for 100 sweeps time-locked to the beginning of the PS EMG.

The average rectified PS EMG signal consisted of an increase in EMG of mean duration 234 ± 9 ms (mean $\pm$ S.E.M.). In all subjects there was also an increase in the average rectified NPS EMG lasting between 76 and 268 ms, mean 191 ± 28 ms ($n = 7$). The time difference between the onset of the EMG bursts on the two sides was not significantly different from zero ($P > 0{\cdot}05$). The mean amplitude of the average rectified NPS EMG signal above the 10 % MVC background was 0·7–2·3 % MVC, mean $1{\cdot}4 \pm 0{\cdot}2$ % MVC. The mean area of the average rectified PS EMG signal was between 22 and 55 times larger than the increase recorded on the non-preferred side, mean $36{\cdot}4 \pm 5{\cdot}2$. The NPS burst was insufficient to cause any visible movement on that side.

Subjects ($n = 8$) were asked to maintain a steady grip force on a hand dynamometer with visual feedback for as long as possible with the PS hand at 20, 50 and 80 % MVC. No instruction was given about the NPS. EMG was recorded from the skin overlying the PS wrist and finger flexor muscles and NPS wrist and finger flexor and extensor muscles. NPS flexor EMG appeared first with a mean latency of 125 ± 52, 33 ± 5 and 22 ± 3 s for the 25, 50 and 80 % MVC PS contractions respectively and then gradually increased until the PS contraction was stopped. At this point the mean integrated EMG of the NPS wrist and finger flexor muscles and wrist and finger extensor muscles had reached $5{\cdot}5 \pm 2{\cdot}3$, $7{\cdot}3 \pm 2{\cdot}3$ and $13{\cdot}1 \pm 4{\cdot}8$ of MVC and $6{\cdot}5 \pm 3{\cdot}7$, $12{\cdot}7 \pm 4{\cdot}2$ and $15{\cdot}9 \pm 3{\cdot}0$ of MVC respectively.

It is concluded that phasic and maintained unilateral voluntary movements in man are accompanied by a small activation of contralateral muscles.

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Journal of Physiology (1995) 483.P

Mirror movements in X-linked Kallmann's syndrome in man

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Kallmann's syndrome is the association of anosmia with hypogonadotrophic hypogonadism (GnRH deficiency). It may occur sporadically but can be of autosomal or X-linked inheritance (XKS). This study investigates mechanisms underlying mirror movements which are found in 90% of the latter group.

With ethical permission, six males aged 16–47 years were investigated. Multi-unit surface EMG was recorded from the left (L) and right (R) first dorsal interossei (1DI), abductor digiti minimi (ADM), forearm extensor compartment (Fext), triceps (Tri) and deltoid (Del). Self-paced phasic abduction of the L and then the R index finger was performed whilst recording bilaterally from 1DI. Bilateral EMG bursts occurred in each case. The time difference between the onset of these bursts was not significantly different from zero ($P > 0{\cdot}05$, paired t test). Cross-correlation analysis of the multi-unit EMG signals was performed during maintained muscle contractions (bin width of 1 ms, 5000 spikes from each spike train). Correlograms constructed from L and R 1DI, ADM and Fext contained a short duration central peak (range 8–17 ms, mean 12·5 ms, $n = 17$). In two subjects Tri and Del correlograms also contained a central peak. There was a distal to proximal gradient in the size of the central peak (estimated as the number of extra spikes in the peak/mean bin count) with larger peaks distally. Focal magnetic brain stimulation produced contralateral muscle responses in 1DI, Fext and Tri. In contrast to normal controls, all subjects also had ipsilateral responses in 1DI and Fext; two had ipsilateral responses in Tri. In three subjects the ipsilateral response was significantly larger than the contralateral in 1DI and Fext muscles (paired t test, $P < 0{\cdot}05$, $n = 30$). Cutaneomuscular reflexes were recorded from L and R 1DI whilst stimulating the digital nerves of the index finger of the L and R respectively. I1 and E2 components of the reflex response were recorded bilaterally in three out of six subjects; the short latency E1 spinal component was only seen ipsilateral to the stimulus.

We conclude that in these patients there is a novel ipsilateral corticospinal projection which accounts for the occurrence of common synaptic drive to L and R homologous motoneurone pools and underlies the mirror movements in XKS.

Central mechanisms underlying task dependence of cutaneous reflexes in man

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Digital nerve stimulation produces a reflex modulation of ongoing EMG recorded from the first dorsal interosseous muscle (1DI) consisting of an increase in EMG (E1), followed by a decrease (I1) followed by a second increase (E2). The E2 component requires the integrity of the dorsal columns, motor cortex and corticospinal tract and its size is task dependent (Evans *et al.* 1989). This task dependency may be related to changes in excitability of the motor cortex and/or to changes in gating of the ascending sensory volley. We have previously investigated motor cortical excitability using a circular coil based on the early design of Merton & Morton (1986) (Datta *et al.* 1989). In the present study, we have have re-examined this using a Magstim 200 stimulator and have recorded sensory evoked potentials (SEP) to investigate any changes in sensory gating.

With ethical permission, the digital nerves of the index finger of the preferred hand were stimulated at 3 s^{-1} at a strength of 2·5 × threshold for perception. Surface EMG was recorded from 1DI and the SEP from the primary sensory cortex (2 cm posterior to C3 using the 10–20 system and with a cephalic reference at Fz). The sensory nerve volley (SNV) was recorded at the wrist. EMG was rectified and both the EMG and SEP were averaged for 500 sweeps time locked to the stimulus. Recordings were made during a sustained isometric abduction of the index finger and during a power grip with EMG maintained at 10 % of that achieved during a maximum voluntary contraction. The area (normalized to background EMG) of the E2 component and the amplitude of the N_{20}–P_{25} component of the SEP were measured for each task. Responses to magnetic brain stimulation (MAGSTIM) were evoked firstly using a circular coil clamped over the vertex and secondly using a focal figure-of-eight coil (Magstim 200) placed over the hand area of the motor cortex. For each coil, the threshold for a response in 1DI with 10 % background level of EMG was found (gain 200 μV division^{-1}). Twenty successive stimuli (12 min^{-1}) were then given at threshold plus 5 % whilst performing each task. Responses were rectified and averaged and the mean area obtained.

For each subject ($n = 8$), both the SEP and the E2 components were smaller during grip than during abduct (18·7 ± 4·7 % and 27·6 ± 4·5 % smaller respectively; means ± S.E.M.; paired t tests, $P < 0·01$; $n = 8$). The SNV was unchanged. The percentage changes for the sizes of the SEP and E2 were not significantly different (paired t test, $P > 0·05$). Using either coil type, the responses to MAGSTIM were not significantly different during the two tasks (paired t tests, $P > 0·05$). This result is different from our previously reported study (Datta *et al.* 1989) and that of Flament *et al.* (1993). It is concluded that sensory gating may be involved in the task dependence of cutaneous reflexes.

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172.6

ASSOCIATED REACTIONS AND ASSOCIATED MOVEMENTS. MJ Mayston, LM Harrison, JA Stephens* Dept. of Physiology, University College London, WC1E 6BT

In certain conditions,contralateral EMG (CEMG) may accompany unilateral movement. In patients with spasticity, the CEMG is generalised and termed an associated reaction (AR). When the CEMG occurs in L and R homologous muscle pairs, as it does during childhood or in pathological conditions such as Kallmann's syndrome (XKS), it is called an associated movement (AM). We have investigated the mechanisms underlying CEMG in the upper limbs of 5 patients with AR's and 8 with AM's, (XKS). Focal magnetic brain stimulation was used to investigate the laterality of pyramidal tract (PT) projections. Contralateral short latency responses were seen in the first dorsal interosseus (1DI) muscle of normal children. Bilateral responses of similar latency were observed in 2/5 with AR's when stimulating the less affected cortex and in 8/8 with XKS when stimulating either cortex. Cross correlation analysis of multiunit EMG recorded from co-contracting L and R 1DI was used to determine whether these bilateral responses resulted from common drive to homologous L and R motoneuron pools. All correlograms from subjects with XKS showed a short duration (mean 13ms, SEM 1.0ms) central peak around time zero. The size of this peak expressed as E/M (E=number of spikes above that expected by chance, M=mean count in a 1ms bin) ranged from 2.2-17.9, mean=6.5, SEM=2.0. No central peak was observed in the AR's group or in normal children with AM's. We conclude that common synaptic input shared bilaterally and produced by bilaterally projecting PT axons from the same cortex are responsible for AM in XKS but not for AM in normal children nor AR in spasticity. The AR's are probably due to a lack of descending PT inhibition.

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Mirror movements in children: a developmental study.

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Unilateral hand movements in young children are often accompanied by unintentional mirroring movements of the opposite hand. With ethical permission, we have studied such mirror movements in children aged 4 to 11 years (n=39). Subjects were instructed to repeatedly sequentially oppose the tip of each finger to the tip of the thumb, from index to little finger and back again, as quickly but as neatly as possible. Surface EMG was recorded from L and R first dorsal interossi (1DI). This task was performed using each hand; the following results combine both hands. Mirror movements were graded on a scale of 0 to 4 where 0 is no observable imitable movement and 4 is a movement equal to that of the intentional hand (Woods & Teuber, 1978). All children aged 4-6 years had mirror movements, 15% with grade 1, 40% with grade 2 and 45% with grade 3. Intensity and frequency of occurrence of mirroring decreased with age until by age 11, 7% had no mirror movements, 79% had grade 1 and 14% grade 2. Involuntary, mirroring EMG accompanied 11 out of 18 hands in the age range 4-6 years and 3 out of 14 hands in the 11 year old children. EMG was rectified and 25 bursts were averaged time-locked to the beginning of the voluntary burst. The ratio of the area of the involuntary burst to the area of the voluntary burst was calculated. This decreased with age from a mean of 0.03 (SEM 0.01) aged 4-6 years to 0.01 (SEM 0.01) at age 11 years. The onset of the burst of involuntary EMG usually occurred after the onset of the burst of voluntary EMG (mean 13ms later, SEM 2ms, n=78) but ranged from 15ms before to 51ms after the start of the voluntary burst.

It has been suggested that the reduction in mirroring with age may be associated with increasing inhibition via activity of callosal fibres (Nass, 1985). To investigate callosal function, we used a condition-test technique; responses evoked in 1DI by focal magnetic stimulation of the contralateral cortex were conditioned by a magnetic stimulus to the ipsilateral cortex . The conditioned response was not significantly smaller than the non-conditioned response (5 children, aged 6 to 10 years; unpaired t-test, $P>0.05$). This contrasts with data obtained from adult subjects where the conditioned response was significantly smaller at condition-test intervals of between 7 and 15ms.

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Co-contraction of antagonistic muscles during development and in children with cerebral palsy.

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In the adult antagonistic muscles can either co-contract to stabilise a joint, or can act reciprocally as occurs when a limb moves or exerts force about a joint. In young children and those with cerebral palsy (CP), agonist and antagonist muscles co-contract and rarely work reciprocally (Berger *et al.* 1985). In previous studies we have found short duration central troughs in cross-correlograms constructed from the occurrence of motor unit spikes recorded from voluntarily co-contracting antagonistic muscle pairs in adults (Gibbs *et al* 1994). These troughs may occur as a result of shared reciprocal input to the motoneurone pools of antagonistic muscle pairs. In the present study we have investigated the occurrence of co-contraction during development and in CP by simultaneously recording from antagonistic muscle pairs in the lower limb. Cross-correlation analysis of multi-unit EMG signals was used to investigate the possible mechanisms underlying this co-contraction. Cross-correlograms were constructed from the times of occurrence of motor unit spikes (typically 3-5000 spikes from each muscle, 1ms bins) recorded during co-contraction of the muscle pairs.

With ethical approval, EMG recordings were made using monopolar concentric needle and surface electrodes in 5 healthy adult subjects (20-48y), and surface electrodes in 50 normal infants and children (35 weeks gestational age to 11 years) and 7 children with CP (aged 5-10y). Recordings were made during standing and/or voluntary co-contraction of antagonistic muscles of one or both lower limbs. Subjects were instructed to lean forwards and lift their toes to facilitate voluntary co-contraction. In all of the children with CP and in 8/9 of the normal infants and children less than 5years old, co-contraction of tibilias anterior(TA)-soleus(SOL) and/or quadriceps-hamstrings was recorded. Correlograms constructed from simultaneous EMG recordings of antagonistic muscle pairs were flat. Co-contraction in normal standing was only recorded in 1/41 children aged 5-11y and never recorded from the adults. All adults were able to voluntarily co-contract TA-SOL and in 8/9 recordings short duration central troughs were found. The mean duration was 25.3 $\pm$1.8 ms ($\pm$SEM, n=8) and the mean size 0.70 $\pm$ 0.05 (expressed as minimum bin count/average bin count $\pm$SEM). Of the 41 children older than 5y, 11 aged 8-11y could voluntarily co-contract TA:SOL. In 4/11 the correlogram was flat but in 7/11 short duration central troughs were found (mean duration 24.0 $\pm$ 2.2ms; size 0.71 $\pm$ 0.02, 7 subjects, 12 muscle pairs).

The occurrence of co-contraction in young children and those with CP may result from a deficit in reciprocal inhibition, caused by a lack of supraspinal facilitation of spinal reflex circuits which are responsible for reciprocal innervation in the adult.

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