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Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres

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Summary

From studies of subhuman primates it has been assumed that functional corticospinal innervation occurs post-natally in man. We report a post-mortem morphological study of human spinal cord, and neurophysiological and behavioural studies in preterm and term neonates and infants. From morphological studies it was demonstrated that corticospinal axons reach the lower cervical spinal cord by 24 weeks post-conceptional age (PCA) at the latest. Following a waiting period of up to a few weeks, it appears they progressively innervate the grey matter such that there is extensive innervation of spinal neurons, including motor neurons, prior to birth. Functional monosynaptic corticomotoneuronal projections were demonstrated neurophysiologically from term, but are also likely to be present from as early as 26 weeks PCA. At term, direct corticospinal projections to Group Ia inhibitory interneurons were also confirmed. Independent finger movements developed much later, between 6 and 12 months post-natally. These data do not support the proposal that in man, establishment of functional corticomotoneuronal projections occurs immediately prior to and provides the capacity for the expression of fine finger movement control. We propose instead that such early corticospinal innervation occurs to permit cortical involvement in activity dependent maturation of spinal motor centres during a critical period of perinatal development. Spastic cerebral palsy from perinatal damage to the corticospinal pathway secondarily involves disrupted development of spinal motor centres. Corticospinal axons retain a high degree of plasticity during axon growth and synaptic development. The possibility therefore exists to promote regeneration of disrupted corticospinal projections during the perinatal period with the double benefit of restoring corticospinal connectivity and normal development of spinal motor centres.

Keywords: corticospinal tract; development; human; spinal cord; α-motor neuron; Group Ia inhibitory interneuron

Abbreviations: ADM = abductor digiti minimi; biceps = biceps brachii; CMCD = central motor conduction delay; CV = conduction velocity; EPSP = excitatory post-synaptic potential; GAP43 = growth associated protein 43; NMDA = *N*-methyl-D-aspartate; PCA = post-conceptional age; PMCD = peripheral motor conduction delay; PVL = periventricular leucomalacia; stretch reflex = homonymous phasic stretch reflex; TMCD = total motor conduction delay; TMS = transcranial magnetic stimulation; triceps = triceps brachii

Introduction

Man is unrivalled in achieving complex language, remarkably skilled and flexible manual dexterity and adept bipedal gait, abilities that are dependent upon highly proficient neural control of motor neurons and muscles. The performance of skilled movements of the extremities has been shown to rely upon the integrity of the corticomotoneuronal system and specifically on direct monosynaptic input from the motor cortex to spinal α-motor neurons (Kuypers, 1962, 1981; Lawrence and Hopkins, 1976; Bortoff and Strick, 1993; for reviews, see Porter and Lemon, 1993; Armand *et al*., 1996). In subhuman primates direct corticomotoneuronal projections are largely restricted to the motor neurons of muscles control-

ling movements of the arm, hand, foot and tail and their density is related to the degree of skill achieved (Phillips, 1971; Kuypers, 1981; Heffner and Masterton, 1983; Bortoff and Strick, 1993; Porter and Lemon, 1993). Man is unique in possessing direct corticomotoneuronal projections to all motor nuclei so far investigated (Porter and Lemon, 1993), implying a greatly expanded role for the corticomotoneuronal system. If damage to the corticomotoneuronal system occurs in adulthood, great difficulty follows in learning new or relearning former sequences of skilled movements (Brodal, 1973), emphasizing the role of the corticomotoneuronal system in the acquisition and maintenance of skill. When lesions occur in the perinatal period, not only is learning of skilled movements severely impaired, but the development of α-motor neurons and their afferent segmental reflex control is secondarily disrupted (Myklebust *et al*., 1982; Leonard *et al*., 1991; Berger, 1998; O'Sullivan *et al*., 1998). This implies a further role for the corticomotoneuronal system in man: activity dependent regulation of the development of spinal motor centres. Such a proposal, however, is controversial. Subhuman primates do not establish functional corticospinal projections to α-motor neurons, whether mono- or oligosynaptic, until the first appearance of fine manipulative skills at 3 months post-natal age (Kuypers, 1962; Felix and Wiesendanger, 1971; Lawrence and Hopkins, 1976; Flament *et al*., 1992*a*, *b*; Lemon, 1993; Stanfield and Asanuma, 1993; Armand *et al*., 1994, 1997; Galea and Darian Smith, 1995; Olivier *et al*., 1997; for review see Armand *et al*., 1996), suggesting an absence of effective corticospinal influence on motor neurons over this important period of rapid post-natal development. We have performed anatomical, neurophysiological and functional studies to determine whether in man, corticospinal innervation also occurs as late in post-natal development as 6–12 months of age, when fine manipulative skills first appear.

Method

Anatomical studies (Fig. 1)

Spinal cords taken at post-mortem from eight premature neonates, 24–35 weeks post-conception age (PCA), who died from non-neurological causes, were fixed in buffered 4% paraformaldehyde solution. Immunoreactivity to growth associated protein 43 (GAP43), a protein expressed by growing axons, was selected to identify corticospinal axons in the spinal cord (Kinney *et al*., 1993). Sections were immunostained for GAP43 using a monoclonal primary antibody (Sigma, Poole, UK) and standard techniques.

Neurophysiological studies (Fig. 2)

Subjects

The studies were performed on 413 subjects aged from 26 weeks PCA to 55 years, including 223 preterm or term neonates. Ethical approval from the Joint Ethics Committee Newcastle and North Tyneside Health Authority and University of Newcastle upon Tyne and written, informed consent from subjects or their parent(s) were obtained.

Electromyogram and electroneurogram

The EMG of biceps brachii (biceps), triceps brachii (triceps) and abductor digiti minimi (ADM), and electroneurogram over the spine at C5 were recorded using miniaturized, skin mounted differential amplifiers, to which the inputs were muted for 1 ms during magnetic stimulation to reduce the stimulus artefact (Barker *et al*., 1987). A –3 dB bandpass of 5–1500 Hz was applied for EMG and 1–1000 Hz for electroneurogram; both signals were sampled at 5000 Hz for computer analysis.

Excitation of motor pathways

Transcranial magnetic stimulation (TMS) (Magstim Company Ltd, Whitland, Wales, UK), using a 9 cm circular coil placed tangentially above the motor cortex, was used to excite corticospinal neurons (Eyre *et al*., 1991; Edgley *et al*., 1997). The intensity of TMS was increased until responses were evoked in the contralateral contracting muscle in 50% of trials, defined as threshold. The mean onset latency of at least 20 responses to TMS at 1.1× threshold gave the total motor conduction delay (TMCD). Magnetic stimulation using a 5 cm circular coil placed over the spines of C5–8 excited spinal motor roots (Plassman and Gandevia, 1989) and the longest onset of responses at threshold estimated the peripheral motor conduction delay (PMCD) (Fig. 2B). Subtraction of PMCD from TMCD estimated the central motor conduction delay (CMCD; Fig. 2).

Estimation of maximum corticospinal axon conduction velocity and corticomotoneuronal synaptic delay

Two averaged evoked corticospinal volleys to 100 TMS stimuli at 1.1× threshold for contracting ADM or biceps, whichever was the lower, were obtained by surface recording over the spine of C5 in each subject. The adult subjects lay quietly supine with all muscles relaxed. The neonates were recorded whilst in quiet sleep, again with all muscles relaxed. The conduction delay to C5 was estimated from the latency of the first negative peak of the averaged volley (Burke *et al*., 1993) (Fig. 2C and Table 1). The corticospinal pathway length to C5 was estimated from the distance from the vertex to vertebra prominens, which we have previously demonstrated to be 1.3 times the corticospinal pathway length (Eyre *et al*., 1991). The conduction velocity (CV) was estimated by dividing this distance by the onset latency of the corticospinal volley. The corticospinal synaptic delay was estimated by subtracting the onset of the corticospinal volley from the CMCD to biceps.

Excitation of Group Ia afferents

Small taps to the muscle have been shown to excite almost exclusively Group Ia afferents (Burke *et al*., 1976*a*, *b*; Pierrot–Deseilligny *et al*., 1981). A hand-held electromechanical tapper (model 201; Ling-Altec Ltd, Royston, UK) delivered taps, peak force 0.1–1.0 N, duration 5 ms, rise and decay times of 2.5 ms, to the tendon of biceps or triceps. The force was increased until a homonymous phasic stretch reflex (stretch reflex) was evoked in 50% of trials, defined as threshold (Fig. 2H).

Spatial summation of subthreshold Group Ia and corticospinal volleys at biceps α-motor neurons

CMCD estimated the delay from the onset of TMS to the onset of the corticospinal excitatory post-synaptic potential (EPSP) at biceps α-motor neurons (Fig. 2). The delay from tap to arrival of Group Ia volleys was estimated by subtracting PMCD (Fig. 2B) from the latency of the stretch reflex in biceps (Fig. 2H). Tap to biceps at 0.8× threshold, TMS at 0.8× threshold and the two stimuli together at a defined interstimulus interval were delivered in sequence. Trials comprising five repeats of the sequence were conducted over a range of interstimulus intervals such that the corticospinal EPSP arrived at biceps α-motor neurons from 5 ms before to 5 ms after the estimated arrival of the Group Ia volley (Fig. 2D).

Spatial summation of subthreshold Group Ia and corticospinal volleys at presumed triceps Group Ia interneurons

Similar spatial summation trials were performed for subthreshold tap to triceps while recording the EMG in biceps (Fig. 2E). CMCD to triceps Group Ia inhibitory interneurons was assumed to be the same as CMCD to triceps. The Group Ia afferent delay to triceps Group Ia inhibitory interneurons was also taken to be the same as that to triceps α-motor neurons.

Corticospinal conditioning of biceps stretch reflex

Subthreshold cortical conditioning (c) of TMS at 0.8× threshold, a test stretch reflex (t) in biceps, evoked by a tap at 1.2× threshold and the two stimuli together at defined interstimulus (condition to test, c–t) intervals (Fig. 2I) were delivered in sequence and repeated 20 times. c–t intervals were determined such that the corticospinal EPSP would be evoked in biceps α-motor neurons from 10 ms before to 5 ms after the estimated arrival of the Group Ia volley.

Skill of performance of relatively independent finger movements in relation to CMCD

Tests of skill and CMCD to biceps and ADM were performed at 3 monthly intervals in a longitudinal study of healthy newborns for the first 24 months after birth (Fig. 3). The skill of relatively independent finger movements was measured using a Klüver Board (Lawrence and Hopkins, 1976) comprising wells of progressively smaller diameters, 35–5 mm, into which small chocolate buttons were placed. Infants were tested when hungry and the diameter of the smallest hole from which an infant could retrieve the chocolate was recorded.

Results

Anatomical studies

GAP43 immunoreactivity was widespread in both white and grey matter at 24–25 weeks PCA ($n = 2$; Fig. 1A). By 27 weeks PCA, GAP43 immunoreactivity was greatly reduced in white matter except in the corticospinal tracts ($n = 2$; Fig. 1B) and weaker immunoreactivity extended from these axon tracts into the intermediate grey matter. At 30–33 weeks PCA ($n = 3$), the corticospinal tracts remained the only major axon tracts strongly immunoreactive for GAP43; immunoreactivity was also intense in the intermediate grey matter and was present in the motoneuronal pools and dorsal horn (Fig. 1C). At 35 weeks PCA ($n = 1$), when the great majority of axons expressing GAP43 appeared to derive from the corticospinal tracts, a high power image of a section counterstained with cresyl violet (Fig. 1D) showed Nissl-stained motor neuron cell bodies closely apposed by GAP43 immunoreactive varicose axons.

Neurophysiological studies

Results unless stated otherwise are given as mean ± standard error of the mean.

Excitation of motor pathways

EMG responses in biceps and ADM were evoked in all subjects including preterm neonates following TMS and peripheral motor nerve stimulation. The thresholds for responses to TMS were high in the neonates and fell exponentially with age (term neonates: biceps 95 ± 5%, ADM 81 ± 5%; adults: biceps 35 ± 4.5%, ADM 32 ± 3.7%). The CMCDs to biceps and ADM were significantly longer in neonates than in adults and fell abruptly between 9 and 18 months to achieve adult values (biceps: adults 4.2 ± 0.2 ms, term neonates 18.9 ± 0.4 ms, $P < 0.001$; ADM: adults 5.3 ± 0.3 ms, term neonates 20.6 ± 0.5 ms, $P < 0.001$).

Maximum corticospinal axon conduction velocities and estimated corticomotoneuronal synaptic delays (Table 1 and Fig. 2C)

The onset latency of the corticospinal volleys at C5 was significantly shorter in the adults (median 3.4 ms, range

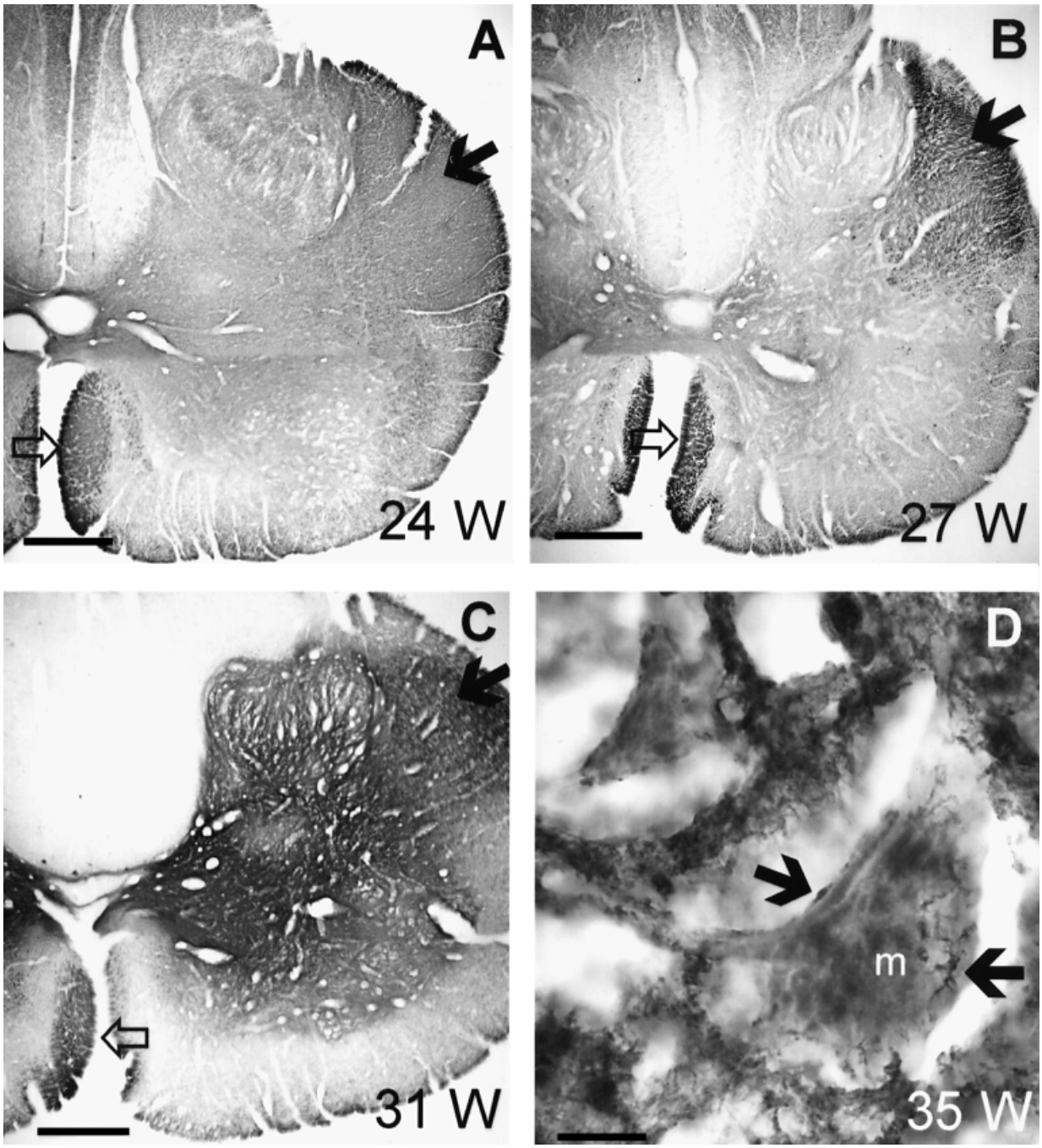

Fig. 1 Anatomical studies of human spinal cord C5–6: (**A**) at 24 weeks PCA, GAP43 immunoreactivity is widespread in white and grey matter; (**B**) at 27 weeks PCA, corticospinal tracts are the only major axon tracts expressing GAP43 from which weaker immunoreactivity extends into the intermediate grey matter; (**C**) at 31 weeks PCA, immunoreactivity is also now intense in the intermediate grey matter and present in motoneuronal pools and dorsal horn; (**D**) at 35 weeks PCA (section counterstained with cresyl violet), motoneuron cell bodies are closely apposed by GAP43 immunoreactive varicose axons. In **A**, **B** and **C,** the solid arrows mark the lateral, and open arrows the anterior corticospinal tracts; in **D** the solid arrows indicate GAP43 expressing varicose axons. M = Nissl stained motoneuronal cell body. Scale bars: in **A**, **B** and **C** = 500 μm**;** in **D** = 20 μm.

3.2–4.8 ms) than in the neonates (median 17.5 ms, range 6.8–18.4 ms, $P < 0.05$; Table 1) despite a significantly longer estimated pathway length in the adults (adults: median 27 cm, range 26.5–27.5 cm; neonates: median 13.5 cm, range 13.1–13.8 cm; $P < 0.001$; Table 1). The estimated maximum axonal conduction velocities were significantly greater in the adults (median 80 m/s, range 56–85 m/s) than in the neonates (median 7.8 m/s, range 7.3–19.3 m/s, $P < 0.001$; Table 1). The duration of the corticospinal volley was measured to estimate the degree of dispersion of the compound volley. The duration was slightly shorter in adults than in neonates suggesting less dispersion but this difference did not reach

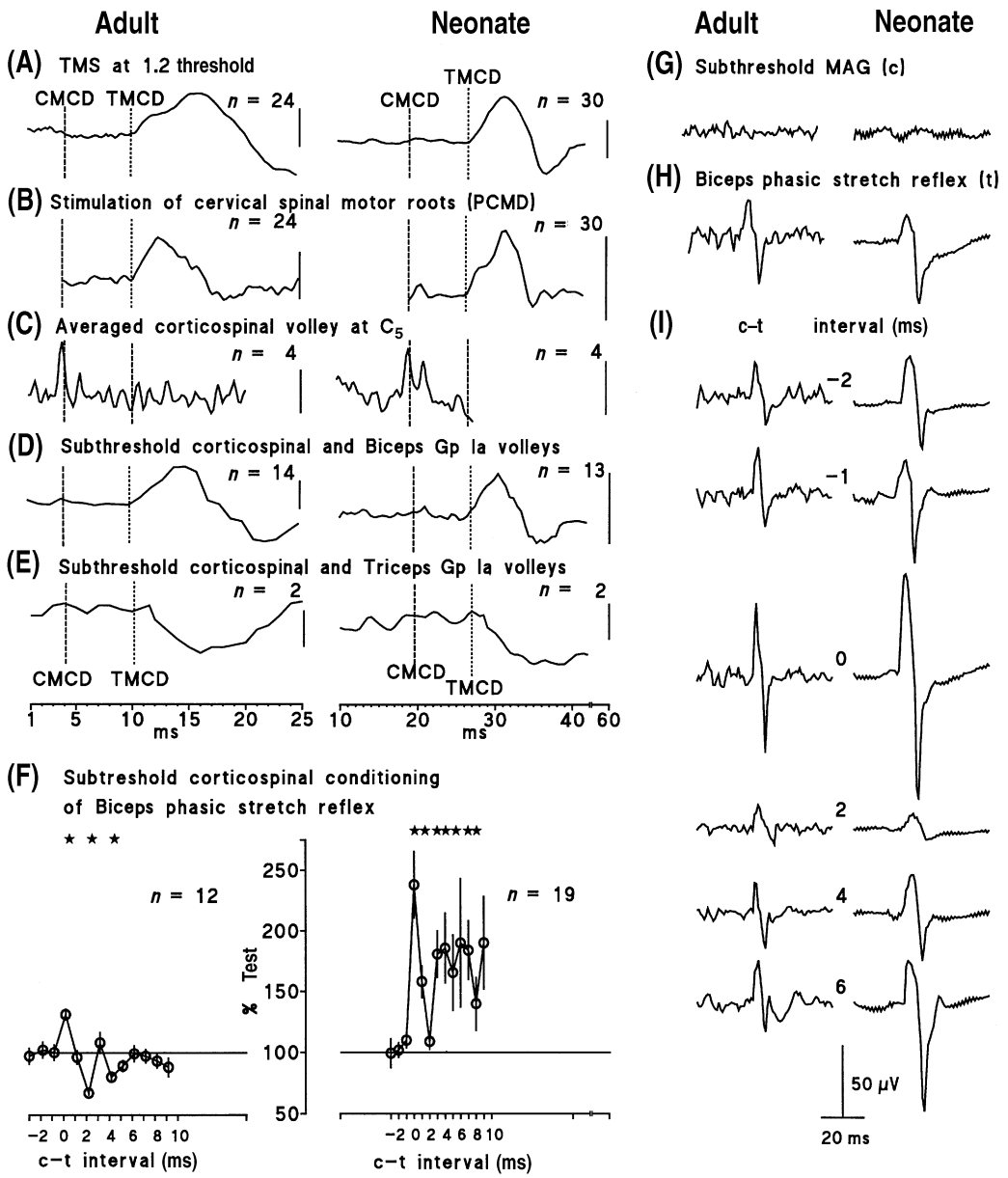

Fig. 2 Neurophysiological studies in an adult and a neonate. All illustrations of EMG and electroneurogram are from the same adult or neonate. *n* = number of subjects. Time base in **A**–**E**: delay from activation of corticospinal neurons by TMS. Dashed vertical lines = CMCD to biceps; dotted vertical lines = TMCD to biceps; vertical calibration lines = 50 μV for rectified EMG of biceps in **A**, **B**, **D** and **E**, and 0.5 μV for electroneurogram in **C**. (**A**) Response in biceps following TMS. (**B**) Response in biceps following stimulation of spinal motor roots. (**C**) Averaged corticospinal volley recorded over C5 spine. (**D**) Excitation of biceps following spatial summation of a subthreshold corticospinal volley and a subthreshold biceps Group Ia afferent volley at their estimated coincidence at biceps α-motor neurons. (**E**) Inhibition of biceps following spatial summation of a subthreshold triceps Group Ia afferent volley and a subthreshold corticospinal volley at their estimated coincidence at triceps Group Ia inhibitory interneurons. (**F**–**I**) Time course and magnitude of subthreshold cortical conditioning of biceps stretch reflex. (**F**) Open circles represent the mean and vertical lines the SEM of the amplitude of the conditioned stretch reflex expressed as a percentage of the test stretch reflex (% test). Time base c–t interval 0: the interstimulus interval of expected spatial convergence of a corticospinal and Group Ia volley at biceps α-motor neurons; negative c–t intervals: Group Ia before corticospinal volley, positive c–t intervals: corticospinal before Group Ia volley. Stars indicate statistically significant differences ($P \leqslant 0.05$) between conditioned and test reflexes determined by analysis of variance. (**G**–**I**) Unrectified EMG of biceps: (**G**) subthreshold TMS, (**H**) suprathreshold tap to biceps, (**I**) the two stimuli together at defined c–t intervals.

statistical significance (adults 1.85 ± 0.06 ms; neonates 2.02 ± 0.04 ms, $P > 0.08$).

We estimated axonal diameters from the conduction velocities using the ratio between the CV of the largest corticospinal axons and their diameter of 5.2 m/s/μm, derived by Olivier and colleagues (Olivier *et al.*, 1997). The estimated largest axonal diameters were significantly greater in the adults (median 15.5 μm, range 10.7–16.3 μm) than in the neonates (median 1.5 μm, range 1.4–3.7 μm, $P < 0.001$; Table 1).

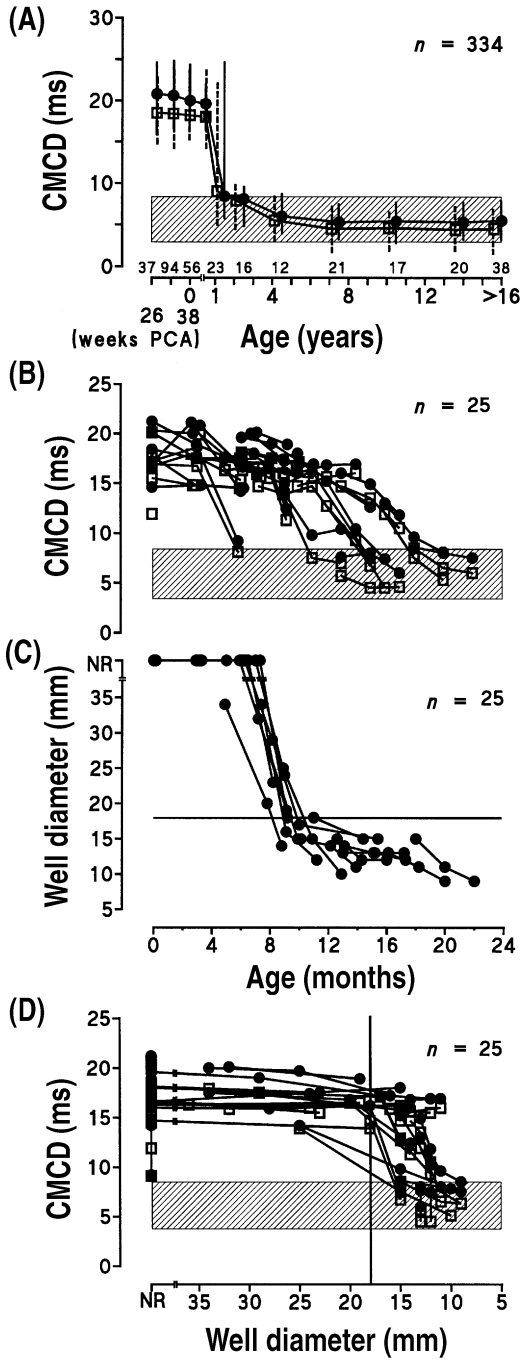

Fig. 3 Functional studies of relatively independent finger movements. n = number of subjects; squares represent data from biceps; circles represent data from ADM. The hatched box defines the 10th–90th centile range for CMCD to ADM in normal adults. The solid horizontal line indicates the well diameter requiring a relatively independent finger movement of the index finger to retrieve a chocolate button. (**A**) CMCD to ADM and biceps. Numbers above the abscissa indicate the numbers of subjects in each age group. The symbols represent the median, the vertical lines the 10th–90th centile range. (**B–D**) Longitudinal study with lines joining results from the same individual for CMCD to ADM and biceps (**B**) and skill of performance of relatively independent finger movements (**C**). The ordinate indicates the diameter of the smallest food well from which a subject successfully retrieved a chocolate button. (**D**) CMCD in relation to the skill of performance of relatively independent finger movements. Increase in skill occurred with little or no change in CMCD.

The CMCDs to biceps were significantly shorter in the adults (median 4.6 ms, range 4.2–5.2 ms) than in the neonates (median 17.7 ms, range 7.8–19.5 ms, $P < 0.05$; Table 1). The onset of the corticospinal volley was subtracted from the CMCD to estimate the corticomotoneuronal synaptic delays. There were no significant differences in estimated synaptic delays between adults and neonates (adults: median 1.0 ms, range 0.4–1.6 ms; neonates: median 0.7 ms, range 0.3–1.1 ms; $P > 0.4$; Table 1).

Spatial summation of subthreshold Group Ia and corticospinal volleys at biceps α-motor neurons and presumed triceps Group Ia inhibitory interneurons

Spatial summation of subthreshold corticospinal volleys and subthreshold biceps Group Ia volleys occurred in both the neonatal and adult subjects at their estimated coincidence at biceps α-motor neurons (Fig. 2D) and resulted in EMG responses in biceps which had the same onset latency as those evoked by suprathreshold TMS (Fig. 2A and D). Spatial summation occurred over a brief range of c–t intervals (adults, 2.6 ± 0.15 ms; neonates, 2.3 ± 0.13 ms). Spatial summation of subthreshold triceps Group Ia volleys and subthreshold corticospinal volleys also occurred at their estimated coincidence at triceps Group Ia inhibitory interneurons and resulted in inhibition of biceps with an onset latency 1–2 ms longer than EMG responses evoked in biceps by suprathreshold TMS (Fig. 2E).

Corticospinal conditioning of biceps stretch reflex

Subthreshold cortical conditioning led initially to facilitation of the test biceps stretch reflex at the time of expected coincidence of corticospinal and Group Ia afferent volleys at biceps α-motor neurons (Fig. 2F–I). The facilitation was significantly greater in neonates than in adult subjects (peak amplitude of conditioned reflex: adults 136.5 ± 7.1% of test reflex; neonates 238 ± 28% of test reflex; $P < 0.02$). For all adult subjects and two neonates the first period of facilitation was followed by a brief period of inhibition, which began 1 ms and was maximal 3 ms after the onset of facilitation (peak amplitude of conditioned reflex: adults 68 ± 2.8% of test reflex; neonates 59% and 62% of test reflex; Fig. 2I) In the remaining neonates the initial period of facilitation was also brief (Fig. 2F; mean duration 3.1 ± 0.3 ms) and was followed by a prolonged second period of facilitation.

CMCD to ADM related to skill of performance of relatively independent finger movements

The CMCD to ADM was longer in the neonates than in adults (38 weeks PCA, mean 20.6 ± 0.5 ms) and fell abruptly sometime between 6 and 18 months post-natal age. For the

Table 1 *Estimated maximum corticospinal axon conduction velocities and corticomotoneuronal synaptic delay in four adults and four neonates*

CMCD (ms)	Onset of corticospinal volley (ms)	Synaptic delay (ms)	Pathway length (cm)*	Conduction velocity (m/s)	Maximum axon diameter (μm)†
Adults					
4.2	3.2	1.0	27.5	85	16.3
4.8	3.2	1.6	26.8	84	16.2
4.4	3.5	0.9	26.5	76	14.6
5.2	4.8	0.4	27.1	56	10.7
Neonates					
18.6	18.4	0.4	13.8	7.5	1.4
16.8	16.5	0.3	13.4	8.1	1.5
7.8	6.8	1.0	13.1	19.3	3.7
19.5	18.4	1.1	13.5	7.3	1.4

*Pathway length = distance vertex to vertebra prominens × 0.75 (Eyre *et al*., 1991); †maximum axon diameter in micrometers = maximum CV/5.2 (Olivier *et al*., 1997).

majority of subjects CMCD was within the adult range by 24 months post-natal age (Fig. 3A and B). Infants were unable to retrieve objects from the Klüver board before 6 months post-natal age. Between 6 and 12 months a rapid increase in the skill of relatively independent finger movements occurred, so that by 12 months all subjects could retrieve objects from food wells with a diameter of ⩽18 mm, which required relatively independent finger movements (Fig. 3C). This increase in skill occurred with little change in CMCD (Fig. 3D).

Discussion

The central and most important findings of this study in human subjects are combined morphological and neurophysiological observations, which together provide strong evidence for the prenatal establishment of functional corticospinal innervation in man. Such corticospinal innervation was not associated with a significant developmental milestone of motor behaviour. These observations differ from the evidence in subhuman primates, where functional corticospinal innervation occurs late in post-natal development and appears to instigate fine control of finger movements (Kuypers, 1962; Felix and Wiesendanger, 1971; Lawrence and Hopkins, 1976; Flament *et al*., 1992*a*, *b*; Lemon, 1993; Stanfield and Asanuma, 1993; Armand *et al*., 1994, 1997; Galea and Darian Smith, 1995; Olivier *et al*., 1997; for review see Armand *et al*., 1996).

Morphological evidence for prenatal corticospinal innervation

The corticospinal tract is the last of the major descending fibre systems to enter the spinal cord. Its entry is antedated by the development and myelination of the major ascending projections from the spinal cord (Tanaka *et al*., 1995; Grever *et al*., 1996; Weidenheim *et al*., 1996). GAP43 is a well-established marker for growing axons, being present in growth cones and fibres (Benowitz and Routtenberg, 1997). In the present study it was found to be localized to both the lateral and anterior corticospinal tracts at all ages studied. This confirms that corticospinal axons reach the spinal cord by 24 weeks PCA, if not earlier, as has been previously described (Humphrey, 1960). By 27 weeks, the corticospinal tracts were the only major tracts expressing GAP43 immunoreactivity and this immunoreactivity had largely disappeared from the grey matter except for an area near to the lateral corticospinal tract in the intermediate grey matter. We interpret this as showing that by this age, corticospinal axons are the only axons still in the growth phase of development but, as yet, have not extensively innervated the grey matter. Such a waiting phase is a characteristic pattern of axon innervation. However, by 33 weeks PCA, GAP43 was greatly increased in the grey matter. Our interpretation is that this largely represents the outgrowth of corticospinal axons both dorsally and ventrally. This progressive outgrowth of axons from the intermediate grey matter towards the dorsal and ventral horns is characteristic of corticospinal tract development in both rodents and monkey (rat: Gribnau *et al*., 1986; Curfs *et al*., 1994; mouse: Gianino *et al*., 1999; hamster: Kuang and Kalil, 1994; macaque: Kuypers, 1962; Galea and Darian-Smith, 1995, Olivier *et al*., 1997) but differs from other descending projections (see below).

In the rat, GAP43 immunoreactivity appears in the corticospinal tract after GAP43 expression is being downregulated by other fibre systems in the spinal cord (Fitzgerald *et al*., 1991) and continues to be expressed by corticospinal axons long after corticospinal synaptogenesis has taken place. This also appears to be the case in human spinal cord development. However, it is possible that aminergic fibres also make some contribution to the GAP43 staining observed; such axons would contain the protein during growth and can also contain amounts detectable by immunocytochemistry in maturity in some studies (Arvidsson *et al*., 1992; Ching *et al*., 1994). Indeed, some GAP43 positive axons could be observed scattered through the ventral funiculus at all stages studied and these are likely to be aminergic fibres. However, it is unlikely that their axonal arbours make a significant contribution to the changing pattern of GAP43 immunoreac-

tivity with development seen in the present study in the grey matter. In the rat, brainstem serotonergic projections first invade the ventral horn around the time of birth (Skagerberg and Björklund, 1985; Bregman, 1987). Innervation then proceeds in a ventral to dorsal direction over the first 3 weeks of life. In the human, serotonergic fibres are believed to innervate the ventral horn first at around 16 weeks post-conception, judging by the presence of nerve fibres containing neuropeptides co-expressed with serotonin (Luo *et al*., 1992). If GAP43 immunoreactivity in the present study was largely derived from aminergic axons, a ventral to dorsal spread of the expression would have occurred. However, GAP43 expression was low at 27 weeks PCA and spread predominantly from the dorsolateral funiculus close to the location of the lateral corticospinal tracts.

In conclusion, in the human, corticospinal axons reach the lower cervical spinal cord by 24 weeks PCA at the latest. Following a waiting period of up to a few weeks, it appears they progressively innervate the grey matter such that there is extensive innervation of spinal neurons, including motor neurons, prior to birth. This being the case, it would differ from the macaque monkey, where the waiting period appears to continue until around the time of birth and innervation proceeds over the next few post-natal months (Armand *et al*., 1997).

Neurophysiological evidence for prenatal establishment of functional connections from the cortex to the spinal cord

Transcranial magnetic stimulation (TMS) evoked responses in upper limb muscles in all subjects studied (Figs 2A and 3A and B). The stimulus intensities used to evoke responses were higher in the neonates and thus raise the possibility of subcortical activation of the corticospinal pathway or of brainstem descending motor pathways in the neonates, particularly in light of the relatively small brain size at birth. Our previous studies of the site of excitation of TMS in macaque monkeys, however, make these possibilities unlikely. From recordings of action potentials in single corticospinal axons evoked by TMS in the adult macaque monkey, we have demonstrated that TMS excites the majority of corticospinal axons within the cortex, even at stimulus intensities as high as 2.5 × threshold. Furthermore, the latency of the volley evoked by TMS (recorded on the surface of the cord) in adult macaque monkeys did not change with increasing stimulus intensity up to 100% power, providing further evidence for little significant centrifugal shift in the site of activation of corticospinal axons with high stimulus intensity. Finally, the spinal cord volley evoked by TMS could be completely collided by appropriately timed microelectrode stimulation of the medullary pyramid, indicating that the volley arose from activation of corticospinal axons and did not involve a significant component from descending brainstem motor pathways (Edgley *et al*., 1990). These observations are particularly relevant to newborn and preterm babies since the size of the adult macaque monkey brain is similar to that of babies of 35–38 weeks PCA. The pathway length from cortex to C5 in the macaque monkey is 12–13 cm (Edgley *et al*., 1990, 1997) and in human newborns at term is slightly longer at 13–14 cm (Eyre *et al*., 1991; see Table 1).

The maximum corticospinal axon diameters in the human newborn are very much smaller (1–1.5 μm) (Verhaart, 1950; see below) than those of the adult macaque monkey, which are likely to be up to 18 μm based on the observations of Olivier and colleagues for a ratio of 5.2 m/s/μm between the CV of the largest corticospinal axons and their diameter in the medullary pyramid (Olivier *et al*., 1997), and our own observations of corticospinal axon conduction velocities varying between 24 and 95 m/s in the adult macaque monkey (Edgley *et al*., 1997). Corticospinal axons in the human neonate therefore have a considerably higher threshold for activation. For example, human neonatal threshold to TMS in ADM in the present study is 81% of that of the adult macaque monkey and 40% of that in the first dorsal interosseus (Olivier *et al*., 1997). The intensity of the magnetic field and therefore the induced electric current in the brain falls rapidly with distance from the coil (Jalinous, 1991); thus, distal activation of the smaller diameter and higher threshold corticospinal axons of the human neonate must be much less likely than in the adult macaque monkey.

Since exactly the same coil and stimulators have been used in our studies of the macaque monkey as in the human subjects in the present paper, and the stimulus intensities were confined to a maximum of 1.1 × threshold, it is concluded that TMS excited corticospinal axons are predominantly within the cortex in the subjects of the present study, even the neonates. This conclusion is reinforced by the observations of Burke and colleagues, who applying even maximal TMS in adult human subjects were unable to demonstrate shortening in the onset of the corticospinal volleys recorded with cervical spinal epidural electrodes (Burke *et al*., 1993).

Conduction velocities in the fastest conducting corticospinal axons

The CMCDs were longer in preterm and term babies than in older children and adult subjects (Figs 2A and 3A and B; Table 1). Such prolonged CMCDs could be consistent with slower conduction velocities in the corticospinal axons than in adults and/or oligosynaptic activation of α-motor neurons, since CMCD includes both the corticospinal axonal conduction delay and the corticomotoneuronal synaptic delay (Plassman and Gandevia, 1989; Eyre *et al*., 1991).

The median CV of the largest corticospinal axons in the adults was 80 m/s with a range of 56–85 m/s (see Table 1). These values are compatible with those estimated previously

in the adult macaque monkey and in adult man, where maximum conduction velocities have varied from 50 to 95 m/s (macaque monkey: Evarts, 1965; Lemon *et al*., 1986; Edgley *et al*., 1997; Olivier *et al*., 1997; Philips and Porter, 1997; man: Boyd *et al*., 1986; Inghilleri *et al*., 1989; Herdmann *et al*., 1991). Although the number of adult subjects in which we were able to estimate conduction velocities was small, it should be noted that the highest conduction velocities of 85 and 84 m/s were observed in the oldest subjects, aged 41 and 59 years, respectively, and the lower conduction velocities of 76 and 56 m/s were observed in younger subjects aged 24 and 22 years, respectively. A similar observation was made by Olivier and colleagues in the macaque monkey (Olivier *et al*., 1997), and our observations would support their proposal that corticospinal axon diameters may continue to increase slightly throughout life, after the established period of growth up until adolescence (Eyre *et al*., 1991; Armand *et al*., 1996).

The corticospinal axons of the neonates conducted slowly with a median CV for the largest axons of 7.8 m/s (Table 1). This value is the same as the mean CV observed in newborn macaque monkeys by Olivier and colleagues (Olivier *et al*., 1997) and indicates that in both the human newborn and the newborn macaque monkey the axons are poorly myelinated. The median diameter of the largest axon can be estimated to be 1.5 μm (see Olivier *et al*., 1997), which is similar to the value obtained by Verhaart (Verhaart, 1950) from direct measurement of corticospinal axon diameters in the pyramid of a newborn baby. However, Olivier and colleagues have demonstrated that although the corticospinal axons of the newborn macaque were slowly conducting, they were capable of conveying impulses at frequencies as high as 250 Hz, comparable to the frequencies conveyed by the larger myelinated axons of adult macaque monkeys (Olivier *et al*., 1997).

One of the four neonates in whom we measured the onset of the corticospinal volley had a maximum corticospinal CV twice that of the others (19.3 m/s) and therefore had a CMCD approaching adult values (7.8 ms). We have previously observed wide individual variability in maturation of CMCD in the first 18 months after birth (Eyre *et al*., 1991) and this is also reflected in the widely varying ages at which the rapid reduction in CMCD occurred in the present longitudinal study (see Fig. 3B). Such variability in an individual's level of maturation may explain why, in the morphological study of a spinal cord at 27 weeks PCA in one baby, there was little evidence for innervation of the ventral horn by corticospinal axons and yet in another it was mature enough to be studied at 26 weeks PCA and we were able to obtain a response in biceps and ADM following TMS.

Edgley and colleagues found that axons with a CV of $<$40 m/s responded to TMS with I waves at threshold (Edgley *et al*., 1997). It is possible, therefore, that the volley recorded at the spinal cord in neonates arose from indirect activation (I wave) of the corticospinal axons. If this is the case, then the CVs and axon diameters in neonates would be marginally greater (CV: median 8.4 m/s, range 8.1–24 m/s; axon diameter: median 1.6 μm, range 1.5–4.7 μm) since the first I wave appears 1.5 ms after a D wave, due to the additional time required for transynaptic activation of the cortical pyramidal neuron.

Prenatal establishment of a monosynaptic corticomotoneuronal projection

In the present study the synaptic delays were estimated in four adult and four neonates by subtracting the onset of the corticospinal volley at C5 from the CMCD to biceps. The synaptic delays obtained (neonates: median 0.7 ms, range 0.3–1.1 ms; adults: median 1.0 ms, range 0.4–1.6 ms) were similar to those we had previously measured invasively in a macaque monkey to plantar motor neurons following TMS (mean 1.0 ms; range 0.6–1.2 ms; Edgley *et al*., 1997). The method of non-invasive estimation of CMCD used in the present study may be subject to error since the motor units responding to TMS near threshold are the small, low threshold units with slow CVs (Hess *et al*., 1987). Those responding to magnetic stimulation of the spinal nerves are large high threshold motor units with fast CVs (Plassman and Gandevia, 1989). This discrepancy will lead to an overestimate of the CMCD since TMCD will include the conduction delay of the slowest conducting peripheral motor nerves and PMCD that of the fastest conducting motor nerves. Since the synaptic delay is calculated by subtracting the onset of the corticospinal volley from the CMCD, the potential error will lead to an overestimate of the synaptic delay. The error is likely to be smaller in neonates than in adults, since newborn spinal motor neurons are smaller and more uniform in size than in adults (kitten: Mellström and Skoglund, 1969; Conradi, 1976; rat: Ramirez and Ulfhake, 1991) and thus will have more homogenous CVs. This may partly explain the shorter synaptic delay found in the neonatal subjects. Given that the synaptic delays obtained in the present study may be overestimated, they can only be consistent in neonates and adults with a monosynaptic projection (Porter and Hore, 1969; Tamarova *et al*., 1972; Jankowska, *et al*., 1975).

In man, Group Ia afferents establish a monosynaptic projection to α-motor neurons early in foetal development (Okado and Kojima, 1984; Konstantinidou *et al*., 1995). Therefore, two other observations in the present study also substantiate a monosynaptic corticomotoneuronal projection in human neonates: (i) the demonstration in neonates (and adults) of spatial summation of a corticospinal volley with a biceps Group Ia volley, beginning at the time of expected coincidence of the volleys at biceps α-motor neurons (Fig. 2D); (ii) the cortical facilitation of biceps stretch reflex beginning at the time of expected coincidence of a corticospinal and Group Ia afferent volley at biceps α-motor neurons (Fig. 2F–I). Surprisingly, this facilitation was significantly greater in neonates, most probably reflecting

the increased α-motor neuron excitability early in development. Excitability of α-motor neurons has been shown to decrease with development (cat: Fulton and Walton, 1986; human: O'Sullivan *et al*., 1991, 1998), reflecting increases in the membrane area of the soma (cat: Mellström and Skoglund, 1969; Conradi, 1976) and of the dendrites (cat: Conradi, 1976; rat: Ramirez and Ulfhake, 1991), and increased negativity of the resting membrane potential (cat: Kellerth *et al*., 1971; rat: Ziskind-Conhaim, 1988). In addition, redistribution of synapses from soma to dendrites occurs with increasing age (rat: Kudo and Yamada, 1987; cat: Conradi and Skoglund, 1969; Conradi, 1976; monkey: Bodian, 1966), which may also involve synapses from the corticospinal projection.

The responses to TMS in preterm babies must also result from monosynaptic rather than oligosynaptic projections, since a change from oligosynaptic to monosynaptic corticomotoneuronal projections in the post-natal period prior to the onset of relatively independent finger movements would be accompanied by a substantial decrease in CMCD and threshold. No such decrease in threshold for TMS occurs before the onset of relatively independent finger movements (Eyre *et al*., 1991). The rapid decline in CMCD observed with development occurs after the expression of relatively independent finger movements (Fig. 3). This rapid reduction in CMCD can be entirely accounted for by the more than fivefold increase in axonal diameter which occurs during this period (Verhaart, 1950).

Prenatal corticospinal projection to Group Ia inhibitory interneurons

Cortically evoked composite excitatory post-synaptic potentials in α-motor neurons, unless followed by inhibitory post-synaptic potentials, have durations of up to 15 ms in the mature macaque monkey (Jankowska *et al*., 1975, 1976). The very brief durations of cortical facilitation of the biceps phasic stretch reflex (Fig. 2F–I) and also of spatial summation of corticospinal volleys with Group Ia volleys at biceps α-motor neurons in both the adults and neonates suggest the subsequent arrival of an inhibitory post-synaptic potential evoked by TMS. Significant cortical inhibition of biceps stretch reflex in adult subjects and two neonatal subjects substantiate this conclusion (Fig. 2I). In neonatal and adult subjects, spatial summation of a subthreshold corticospinal volley and a subthreshold triceps Group Ia afferent volley occurred at their expected coincidence on triceps Group Ia inhibitory interneurons and led to inhibition of biceps EMG (Fig. 2E). Such spatial summation demonstrates that cortical inhibition was also mediated by direct monosynaptic corticospinal projections to Group Ia inhibitory interneurons (Jankowska *et al*., 1976).

Summary

Each of the four studies, namely estimation of the corticospinal synaptic delay, spatial summation of corticospinal volleys and Group Ia volleys from biceps or triceps, and cortical conditioning of the phasic stretch reflex, were conducted independently of each other. When taken together the studies provide compelling evidence for the prenatal establishment of monosynaptic corticospinal projections to α-motor neurons and Group Ia inhibitory interneurons.

Corticomotoneuronal projections precede the onset of relatively independent finger movements

In the longitudinal study of healthy neonates all subjects could retrieve objects from food wells with a diameter of 18 mm or smaller by age 12 months, which required relatively independent finger movements (Lawrence and Hopkins, 1976). Monosynaptic corticomotoneuronal projections therefore preceded the appearance of relatively independent finger movements by at least 12 months.

Corticospinal tract and activity dependent maturation of spinal motor centres

Why are synaptic connections from the motor cortex to spinal α-motor neurons and interneurons important in their actions on α-motor neurons established prenatally in humans (but only post-natally in subhuman primates) born with functionally more mature motor control? We propose that the early corticospinal innervation demonstrated by our study occurs in man so that the cortex can be intimately involved in spinal motor centre development from an early stage, reflecting the uniquely dominant role of the corticomotoneuronal system in human movement control. Existing literature in animals, including monkeys, establishes a critical period in early development in which synaptic inputs from other descending motor pathways and from segmental afferents shape spinal motor centre development. Motoneuronal cell soma size, dendritic morphology and the pattern of synaptic input have been shown to be altered by activity deprivation during critical periods in early development (McCouch *et al*., 1958; Kalb and Hockfield, 1992; Commissiong and Sauve, 1993; Kalb, 1994; Howland *et al*., 1995; Maier *et al*., 1995; O'Hanlon and Lowrie, 1995; Clowry *et al*., 1997; Kalb and Fox, 1997; Dekkers and Navarrete, 1998). In cats, rats and subhuman primates, input from the cortex can be argued to play a far less dominant role in this process, since perinatal lesions of the corticospinal system in these animals do not lead to major disruptions of motor development (e.g. Lawrence and Hopkins, 1976; Leonard and Goldberger, 1987). In contrast, in man, perinatal lesions to the corticospinal system can result in the development of the severe motor impairments of spastic cerebral palsy (Paneth et *al*., 1994). In the immediate period after the lesion, however, babies who will develop spastic cerebral palsy display only subtle, if any, abnormalities of movement control (Nelson and Ellenberg, 1979; Forslund and Bjerre, 1985; Burns *et al*.,

1989; Ferrari, *et al*., 1990; Bouza *et al*., 1994). There follows over many months, even years, the progressive development of a movement disorder, associated with significant secondary disruption of spinal motor centre development (Myklebust *et al.*, 1982; Leonard *et al*., 1991; Berger, 1998; O'Sullivan *et al*., 1998).

The neurotransmitters of the corticospinal tract are glutamate and aspartate (Giuffrida and Rustioni, 1989; Valtschanoff *et al*., 1993). Activity dependent development of spinal motor centres is likely to be contingent upon *N*-methyl-D-aspartate (NMDA) receptor activation in a similar manner to activity dependent development of the vertebrate visual system (Bear *et al*., 1990; Constantine-Paton *et al*., 1990). Molecular markers for neural differentiation indicate that motor neurons in developing kittens and rats undergo activity dependent development during a circumscribed critical period, and this requires activation of NMDA receptors (Kalb and Hockfield, 1992; Kalb, 1994; Maier *et al*., 1995). NMDA receptor blockade in the spinal cord of neonatal rats, for example, inhibits growth of motoneuronal somata, restricts dendritic branching in motor neurons and leads to abnormal maturation of the neural circuits for locomotion (Kalb, 1994). Significant levels of high affinity NMDA receptors are transiently expressed in man in the ventral horn from 24 weeks PCA to 2 months post-natally (Kalb and Fox, 1997) indicating that a critical period for plasticity in α-motor neuron development is also likely to occur in man in the perinatal period.

Spastic cerebral palsy and perinatal lesions of the corticospinal tract

These observations throw new light on 'cerebral palsy', a term originally used by William Osler (Osler, 1889) to highlight the significant consequences on motor development of lesions to the brain which occur in the perinatal period. We propose that the seminal observations of William Osler identify the perinatal period, not only because of the special vulnerability of the motor system to damage at this time (Johnston, 1998), but also because abnormal or decreased input from the corticospinal pathway during this critical perinatal period will secondarily disrupt development of spinal motor centres (Myklebust *et al*., 1982; Leonard *et al*., 1991; Berger, 1998; O'Sullivan *et al*., 1998).

Spastic diplegia, where often a normally intelligent child develops spasticity in all four limbs but most severely in the lower limbs, is the commonest type of congenital motor disorder. Its prevalence is increasing as smaller, more premature infants survive because of better neonatal care (Paneth *et al*., 1994; Johnston, 1998). Spastic diplegia results from injury to the periventricular white matter (periventricular leucomalacia, PVL), which occurs only during a temporal window of development that ends at 30–32 weeks PCA. A characteristic feature of PVL is disruption of corticospinal axons, while the cortical pyramidal projection neurons are left intact and subsequently make aberrant intracortical axonal projections (Marin-Padilla, 1997). The rapidly expanding understanding of CNS axonal regeneration indicates that with early intervention there are realistic prospects of inducing corticospinal axons to regrow through the cystic areas of PVL and to find their appropriate targets (Terashima, 1995). Glial scars forming a barrier to axonal penetration are not formed initially in PVL (Marin-Padilla, 1997). Myelin is inhibitory to axonal growth (Thallmair *et al*., 1998) but this should not pose an encumbrance to axonal regrowth, since the corticospinal tract is poorly myelinated before term (Tanaka *et al*., 1995). Finally, our own observations in this study indicate that corticospinal axons are actively growing, innervating spinal cord and expressing GAP43 during this period and are thus likely to have a high degree of plasticity (Benowitz and Roultenberg, 1997). If corticospinal axons can be induced to grow through the lesions of PVL, they are likely to express the receptor molecules and to encounter the normal developmental cues to enable target finding and establishment of appropriate synapses (Terashima, 1995). Interventions providing early regeneration of corticospinal projections and reinnervation of the spinal cord in preterm babies with PVL would be likely to reduce disability, not only by re-establishing the cortical input to spinal motor centres but also by facilitating their subsequent normal development.

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