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Postnatal maturation of the dendritic fields of motoneuron pools supplying flexor and extensor muscles of the distal forelimb in the rat

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SUMMARY

In the rat cervical spinal cord the corticospinal projection on motoneurons either direct or indirect (via interneurons) comes about postnatally making it accessible for experimental research. Therefore, the postnatal developmental changes of motoneurons and in particular their dendritic fields were examined. Motoneurons innervating the two antagonistic muscles in the distal forepaw, the m. flexor digitorum profundus and the m. extensor digitorum communis, were retrogradely labelled by intramuscular injections of cholera toxin subunit B conjugated with horseradish peroxidase in rats of various postnatal ages. Following a 48-72 hour survival period the motoneurons and their dendritic fields were studied in the seventh and eighth cervical spinal cord segments.

Both the number and the position of motoneurons were found to remain constant throughout postnatal development. Extensor motoneurons were positioned dorsolaterally in the ventral horn at the border of grey and white matter, flexor motoneurons were in general medial to extensor motoneurons. The results on the dendritic field demonstrate firstly, that during postnatal development the extension of the dendrites of both flexor and extensor motoneurons changes from spreading out in all directions at postnatal day 2 to spreading in only a few, specific directions from postnatal day 21 onwards, with the restriction that both motoneuron pools follow a different time scale to achieve this. Secondly, both pools have a temporal dendritic component extending into the white matter of the lateral funiculus. Thirdly, the dendritic extension pattern of flexor motoneurons differs from that of extensor motoneurons: the former has a permanent component in the medial part of lamina VI while the latter only has a transient component (from postnatal day 2 to 10) in the lateral part of lamina VI. The functional implications of the different dendritic extension patterns are discussed.

Key words: postnatal development, motoneuron, dendritic field, cervical spinal cord, distal forepaw, CTB-HRP, rat

INTRODUCTION

The rat corticospinal tract (CST) is an important descending pathway from the cortex to the spinal cord and plays a role in the control of voluntary movements through contacts with motoneurons either directly (Liang et al., 1991) or through interneurons. Lesions of the CST result in the fine digital flexion movements being affected (Castro, 1972). Understanding of the development (e.g. outgrowth, target finding, synaptogenesis) of the CST might provide insight into how functional repair can be achieved after damage to the central nervous system. CST outgrowth throughout the spinal cord occurs postnatally (e.g. Schreyer and Jones, 1982; Gribnau et al., 1986) making it accessible for experimental research.

In order to study the synaptogenesis of the rat CST either directly or indirectly with motoneurons (MNs), we first examined the developmental changes in MNs projecting to the lower forepaw with particular attention to their dendritic patterns, comprising most of their receptive fields. Two muscles in the distal forepaw were under investigation: the m. flexor digitorum profundus and its functional antagonist the m. extensor digitorum communis. Since in younger rats the muscles are not as well differentiated as in the older stages, these muscles will be more generally referred to as flexor- (FLEX) or extensor-muscles (EXT) respectively.

The position of MNs in several parts of the spinal cord has received much attention using various species and different techniques (Goering, 1928; Reed, 1940; Romanes, 1951; Sterling and Kuypers, 1967; Cruce, 1974; Baulac and Meininger, 1979; Fritz et al., 1981, 1982, 1986a,b; Jenny and Inukai, 1983; Mutai et al., 1986; Oka et al., 1989; Scarisbrick et al., 1990). It is generally accepted that MNs supplying different muscles are found in separate longitudinal columns (the so-called motor pools; Romanes, 1964), their position depending upon the location of the muscles they project to, i.e. MNs projecting to rostral or, in the case of the limb, proximal musculature are, in general, found in a more rostral position than MNs projecting to caudal or distal musculature. In the transverse plane, MNs projecting

to functionally or ontogenetically related muscles occupy a relatively constant position (e.g. FLEX-MNs in general medial to EXT-MNs).

The position of the FLEX- and EXT-MNs and their dendritic fields in the rat cervical spinal cord have, so far, not been studied. Application of HRP to the cut radial or median nerves that supply, amongst other muscles, EXT and FLEX, respectively, revealed that MNs projecting through the radial nerve are located from the fifth cervical to the first thoracic spinal cord segment in the dorsolateral part of the ventral horn, at the border of grey and white matter. The MNs with their axons in the median nerve are located from the sixth cervical to the first thoracic and also in the dorsolateral part of the ventral horn but in general medial to radial nerve MNs (Baulac and Meininger, 1979; Scarisbrick et al., 1990).

A major disadvantage of the HRP-technique is that the dendritic arbour of a MN is not completely revealed. In the last decade another tracer has been shown to be superior to HRP, which is passively taken up by axon terminals: the enterotoxin cholera-toxin produced by *Vibrio cholerae* (CT, Trojanowski et al., 1982; Wan et al., 1982). CT and its non-toxic subunit B (CTB) are actively taken up. Other advantages of CT or CTB are the complete filling of dendritic structures in a Golgi-like way and the simple detection when conjugated with HRP (CT-HRP: Goldstein et al., 1990; CTB-HRP: Beattie et al., 1990; Mong, 1990; Liang et al., 1991).

The present study reports on the postnatal development of the dendritic fields of MNs projecting to EXT- and FLEX-muscles in the rat cervical spinal cord using small injections of CTB-HRP. Part of these results were published in abstract form elsewhere (Curfs et al., 1992).

MATERIALS AND METHODS

Animals

Postnatal Wistar rats (Central Animal Laboratory, University of Nijmegen) of either sex were used, ranging in age from postnatal day 0 (P0) to young adult (P60); the day of birth was designated P0. At least 4 animals per age group were examined; the ages of the animals given in this paper are the ages at their respective days of killing.

Labelling of MNs using CTB-HRP

After anaesthetizing the animals with sodium pentobarbital (depending on age: 18-60 mg per kg body weight, i.p.), the skin of the forepaw was incised dorsally or ventrally to expose the right EXT- or the left FLEX-muscles respectively; care was taken to avoid damaging the muscle fascies (damage results in extensive diffusion of tracers; Haase and Hrycyshyn, 1986). Using a 5 μl Hamilton syringe fitted with a glass micropipette, 0.5 μl of a 0.1% CTB-HRP solution (List Biological Laboratories, Inc.) was pressure injected into the EXT- or FLEX-muscles. Postinjection survival times were kept constant at 48 hours, except for the P60-animals in which 72 hours yielded optimal results. The animals were reanaesthetized (sodium pentobarbital, 25-90 mg per kg body weight, i.p.) and transcardially perfused with ice-cold 5% sucrose in 0.1 M phosphate buffer (PB, pH 7.2), followed by an ice-cold mixture of 1% paraformaldehyde and 2% glutaraldehyde in the same buffer. After perfusion, the brain and spinal cord were dissected from the skull and spine respectively, postfixed by immersion for 2 hours in the fixative mentioned above, cryoprotected by immersion overnight in 0.1 M PB containing 20% sucrose and embedded in 15% gelatin in the same solution. The material was cut on a freezing microtome into 30 μm sections either in the horizontal or the transverse plane. All horizontal and alternate transverse sections were reacted for HRP histochemistry using tetramethylbenzidine (TMB) as a chromogen (Mesulam, 1978), mounted onto glass slides, counterstained with neutral red, dehydrated and coverslipped with Depex.

Drawings of the labelling in the seventh and eighth cervical spinal cord segments (pilot studies revealed that FLEX- and EXT-MNs were located in these two segments) were made under dark-field illumination using a Zeiss microscope equipped with a drawing tube. Each segment was divided into three parts: a rostral, middle and caudal third and every transverse section was projected onto its respective third. The resulting composite illustration was equally comparable in all animals studied. Two sets of composite illustrations were drawn: one to show the position of the MNs and one to show the dendritic field. Photomicrographs were taken using an automatic Zeiss-photomicroscope II.

RESULTS

CTB-HRP proved to be very suitable for retrograde labelling of MNs since cell bodies were densely filled and cell processes showed granular filling with the TMB-reaction product; dendrites could often be followed up to about 0.5 mm (Fig. 1). Since the main scope of the present study concerned the development of the dendritic field and in particular the radially extending dendrites, only results obtained in transverse sections will be described. It should be mentioned, however, that these data are in accordance with those obtained from horizontal sections.

Position of MNs

MNs projecting to the EXT- or FLEX-muscles were each found in a separate longitudinal column. Throughout postnatal development, labelled MNs of both muscles maintained the same position in the spinal cord (relative to landmarks such as the central canal and the lateral funiculus, as well as to each other) in both the dorsoventral and the lateromedial axis. Since the spinal cord shows growth in all dimensions, the MNs come to lie scattered in their respective columns. EXT-MNs were located in the dorsolateral part of the ventral horn in lamina IX of the grey matter, at the border of spinal white and grey throughout the seventh (C7) and eighth (C8) cervical spinal cord segments (Figs 2, 3). FLEX-MNs were also located in the dorsolateral part of lamina IX, though generally medial to EXT-MNs (Figs 2, 3). This can also clearly be seen in the horizontal sections (Fig. 1B).

Development of the MN dendritic field

The extension pattern of MN dendrites in the grey matter is described according to the laminar scheme of the rat cervical spinal cord presented by Molander et al. (1989).

EXT-MNs

Postnatal day 2 (P2, Fig. 4). Dendrites of MNs labelled after injections of CTB-HRP were found throughout C7-C8. Dendrites stretched out radially in all directions; dor-

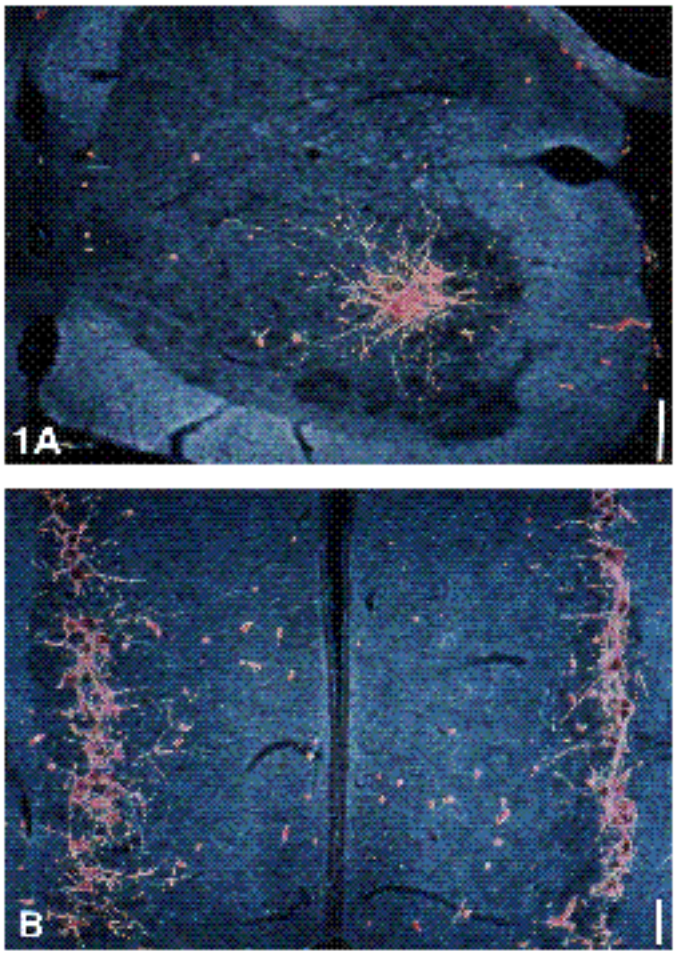

Fig. 1. Photomicrographs of sections of the cervical spinal cord of the rat under dark-field illumination showing the extensive labelling of the motoneurons and their dendritic fields obtained after intramuscular injections of CTB-HRP in the forepaw muscles of the rat. (A) Transverse section of the seventh cervical spinal cord segment at postnatal day 10 after injecting the FLEX-muscle. Lateral is to the right and dorsal to the top. Scale bar, 100 µm. (B) Horizontal section through the seventh and eighth cervical spinal cord segments at the level of the central canal at postnatal day 7. Top is rostral. On the left side FLEX-MNs are labelled and on the right side EXT-MNs. Scale bar, 100 µm.

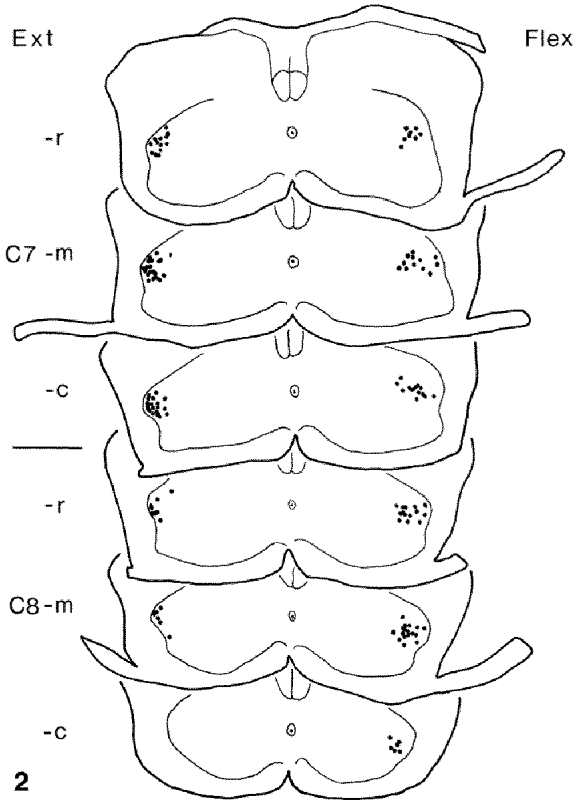

Fig. 2. Composite illustration of the seventh and eighth cervical spinal cord segments at postnatal day 2 showing the position of the EXT- (left side) and FLEX- (right side) MN somata after intramuscular injections of CTB-HRP. Both segments were divided in three equal parts: a rostal (-r), middle (-m) and caudal (-c) third. The data of all sections were pooled for their respective thirds.

sally, dorsolaterally, laterally and ventrolaterally into the lateral funiculus, dorsomedially into the lateral part of lamina VI and medially, ventromedially and ventrally into lamina VII (it should be mentioned however that axons cannot be distinguished from dendrites).

Postnatal day 4 (P4). In comparison with P2 there was an increase both in the amount of label and in dendritic extension. The increase in dendritic extension was particularly due to those dendrites located in the grey matter extending medially and ventromedially further into lamina VII.

Postnatal day 7 (P7, Fig. 5). An increase in the amount of label was observed. Compared to P4 the dendritic extension pattern remained unchanged.

Postnatal day 10 (P10). When compared to P7 a decrease in the amount of label was observed. At this age virtually no dendrites were observed extending laterally and ventrolaterally into the white matter, in all other directions the loss of dendrites was approximately equal.

Postnatal day 14 (P14). The amount of label continued to decrease. Dendrites extending dorsolaterally and dorsally into the lateral funiculus almost completely disappeared and as a result virtually no dendrites reached out into the white matter at this age. In addition, no labelled dendrites were found in lamina VI, consequently almost all labelled dendrites were confined to lamina VII.

Postnatal day 21 (P21, Fig. 6). The amount of label further decreased, in particular in the medial and ventromedial directions. No other changes were observed when compared to P14.

Postnatal day 60 (P60). No changes in relation to P21 were observed. Labelled dendrites were found throughout C7 and C8. Dendrites extended mainly dorsomedially into the dorsal part of lamina VII, ventrally into the ventral part of lamina VII and medially into lamina VII.

FLEX-MNs

Postnatal day 2 (P2, Fig. 4). Dendrites of MNs labelled after injection of CTB-HRP were also found throughout C7 and C8, however they outnumbered those of the EXT-side.

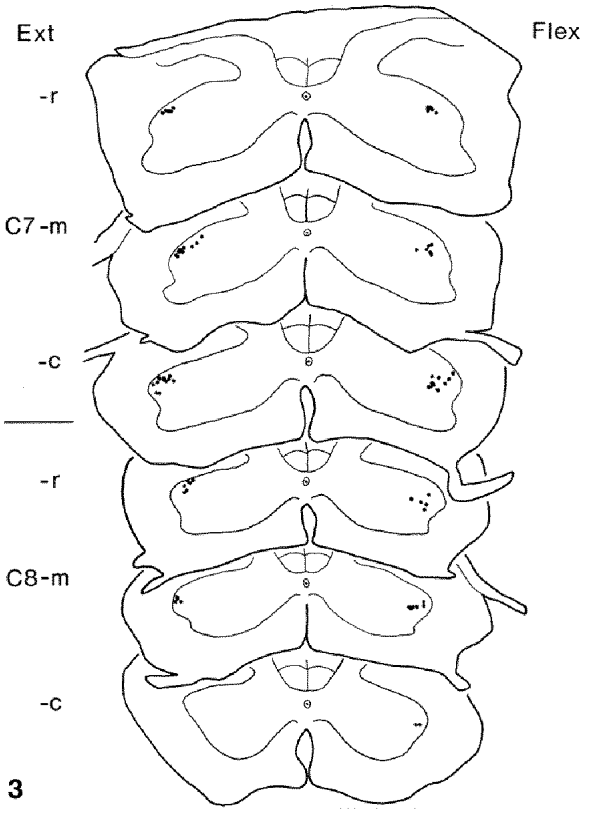

Fig. 3. Composite illustration of the seventh and eighth cervical spinal cord segments at postnatal day 21 showing the position of the EXT- and FLEX-MN somata. See Fig. 2 for full explanation.

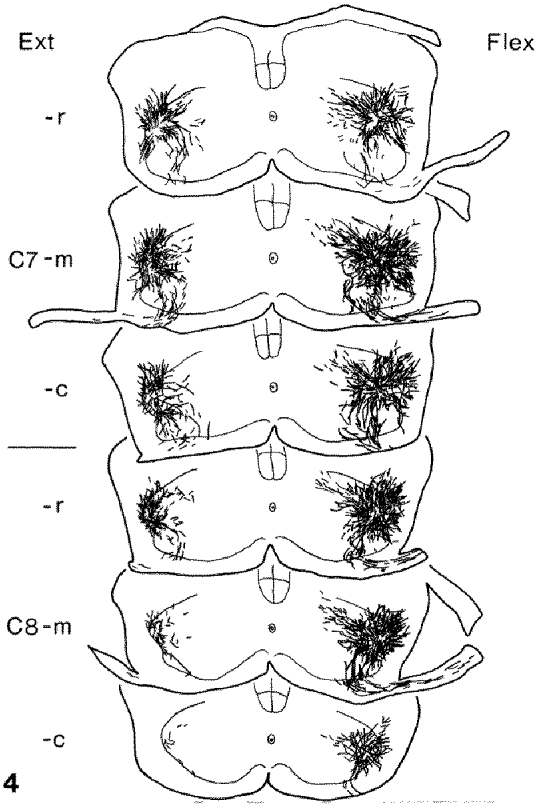

Fig. 4. Composite illustration of the seventh and eighth cervical spinal cord segments at postnatal day 2 showing the dendritic extension pattern of the EXT- (left side) and FLEX- (right side) MNs after intramuscular injections of CTB-HRP. Both segments were divided in three equal parts: a rostal (-r), middle (-m) and caudal (-c) third. The data of all sections were pooled for their respective thirds.

The dendritic extension pattern was comparable to that of the EXT-MNs in that they spread out in all directions, however, they extended further, especially dorsolaterally into the lateral funiculus and dorsomedially into the medial part of lamina VI.

Postnatal day 4 (P4). An increase in the amount of label in all directions and an increase in dendritic extension was observed, especially of those spreading out into lamina VII.

Postnatal day 7 (P7, Fig. 5). A decrease in the amount of label was observed. This decrease was observed in all directions but was mainly due to dendrites no longer extending dorsally towards and into the lateral funiculus, medially and ventromedially into lamina VII and ventrolaterally into the white matter. Relative to the EXT-side less label was observed.

Postnatal day 10 (P10). A decrease in the amount of label was observed, this decrease however was not as large as on the EXT-side. This resulted in an equal amount of labelled dendrites on both the EXT- and the FLEX-side. Almost no dendrites were observed extending into the white matter either dorsally, dorsolaterally, laterally or ventrolaterally. In all other directions dendrites disappeared to about the same degree.

Postnatal day 14 (P14). Only a slight decrease in the amount of label was noted, resulting in a larger amount of label than on the EXT-side. The difference was mainly due to dendrites extending dorsomedially into the medial part of lamina VI on the FLEX-side, which were absent on the EXT-side. No change was observed in the dendritic extension pattern of the FLEX-MNs.

Postnatal day 21 (P21, Fig. 6). The amount of label continued to decrease slightly, mainly due to dendrites no longer extending ventromedially and medially. No further changes were observed when compared to P14.

Postnatal day 60 (P60). No changes were observed when compared to P21. Dendrites were found throughout C7 and C8. Dendrites extended mainly dorsomedially into the medial part of lamina VI, laterally into the lateral part of lamina VII and ventrally into the ventral part of lamina VII. Relatively more label was found than on the EXT-side.

DISCUSSION

Methodological considerations

CTB-HRP provided reproducible results in this study, even though labelled MNs were found in spinal cord segments rostral to C7 and C8. These MNs, however, could be clearly identified as being labelled through diffusion to more proximal muscles and were therefore omitted in the present analysis. Diffusion of the tracer in the distal forepaw

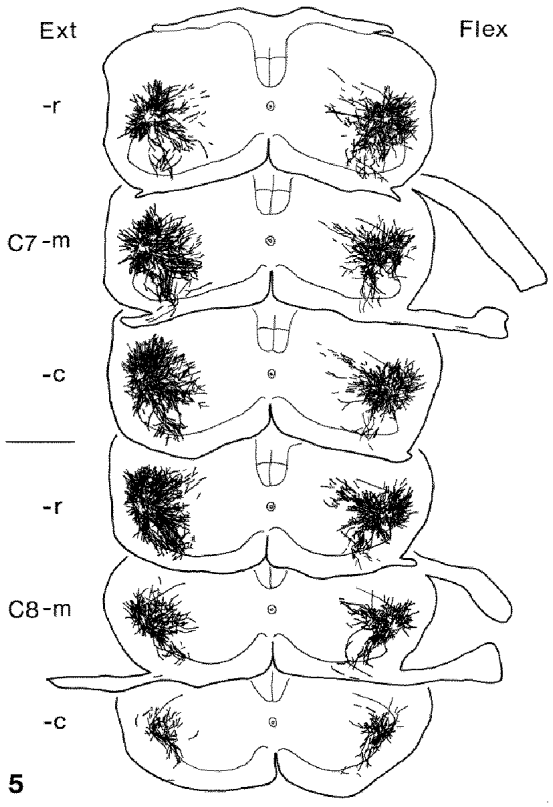

Fig. 5. Composite illustration of the seventh and eighth cervical spinal cord segments at postnatal day 7 showing the dendritic extension pattern of the EXT- (left side) and FLEX- (right side) MNs. See Fig. 4 for full explanation.

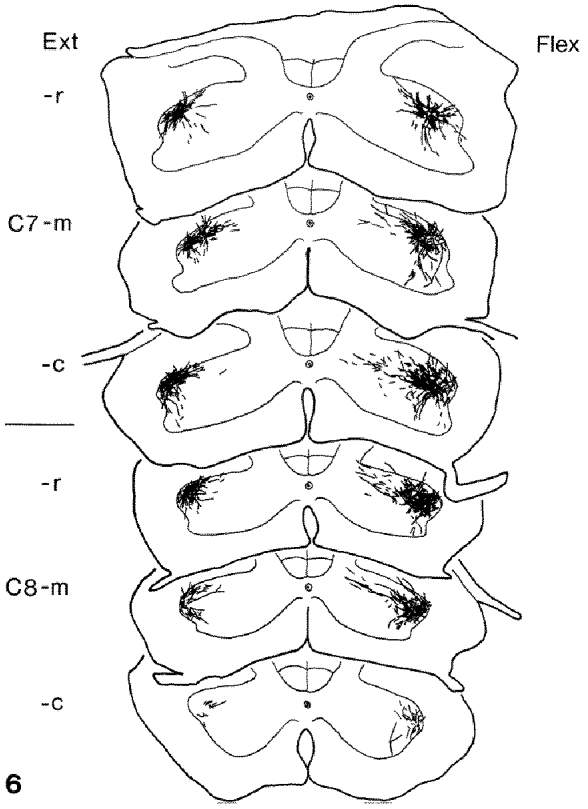

Fig. 6. Composite illustration of the seventh and eighth cervical spinal cord segments at postnatal day 21 showing the dendritic extension pattern of the EXT- (left side) and FLEX- (right side) MNs. See Fig. 4 for full explanation.

muscles was confined to those parts of the muscles bordering on the muscle injected, i.e. either other digital and carpal flexors or extensors respectively as was shown by immunological detection of CTB-HRP in transverse sections of the paw (results not shown).

Both our results and the data presented by other authors (Beattie et al., 1990; Goldstein et al., 1990; Mong, 1990; Liang et al., 1991; Hirakawa et al., 1992; Ritz et al., 1992) show extensive labelling of the dendritic fields of MNs after injecting different variants of CT into several muscles. As was shown by Ritz et al. (1992) the labelling after injection of CT-HRP resembles the patterns after intracellular HRP-injections.

Number of MNs

In the present study a slight, non-significant decrease in postnatal MN numbers was noted (results not shown) which is in line with other studies (Janjua and Leong, 1984; Hardman and Brown, 1985; Bennet et al., 1986; Oppenheim, 1986).

Position of MNs

The position of MNs in the cervical spinal cord projecting to FLEX- or EXT-muscles has not previously been investigated in the rat, as it has been in several other mammals (cat: Fritz et al., 1981, 1982, 1986a,b; monkey: Jenny and Inukai, 1983; dog: Mutai et al., 1986). The relative transverse positions of the two groups of MNs described in the present paper (EXT-MNs against the border between white and grey matter and FLEX-MNs in general medial to EXT-MNs) are in accordance with these data. The longitudinal position however differs. The above mentioned authors found MNs projecting to both FLEX- and EXT-muscles one segment further caudally than we did. This shift of approximately one segment in rat and also mouse, relative to cat, dog and monkey, appears to be structural when corresponding muscles and nerves are compared (Baulac and Meininger, 1979, 1980; Pollin et al., 1990; versus Sterling and Kuypers, 1967; Thomas and Wilson, 1967; Fritz et al., 1981, 1982, 1986a,b; Jenny and Inukai, 1983; Alstermark and Kümmel, 1986; Mutai et al., 1986), a phenomenon also noticed in the lumbar spinal cords of rat and monkey by Janjua and Leong (1984).

Although no information is present to date about the postnatal positional changes of MNs in the cervical spinal cord of the rat, several studies concerning other species and/or other parts of the spinal cord report on the constant relative positions of MNs along both the longitudinal and the transverse axes (Baulac and Meininger, 1983; Smith and Hollyday, 1983; Janjua and Leong, 1984; Ulfhake et al., 1988; Ramírez and Ulfhake, 1991; Tanaka et al., 1992).

Development of the dendritic field

The adult pattern of the dendritic field of MNs develops

predominantly postnatally as can be deduced from studies on longitudinally directed dendritic bundles (Scheibel and Scheibel, 1970, 1971; Bellinger and Anderson, 1987a,b; Cameron et al., 1991; Lindsay et al., 1991; Westerga and Gramsbergen, 1992). It was further shown that in the cat the maturation of the dendritic bundles in the lumbar spinal cord lags approximately two weeks behind that of the cervical spinal cord (Scheibel and Scheibel, 1970, 1971). Such a difference in time-scale was also described by Ulfhake and Cullheim (1988) within the lumbar spinal cord of the cat: maturation of dendrites of intrinsic footsole MNs lags behind that of triceps surae MNs. Therefore, it can be concluded that both a rostrocaudal and a proximodistal gradient exists, which is also present at all stages of prenatal MN differentiation (Altman and Bayer, 1984). In the present study a mediolateral gradient was also found: the postnatal maturation of EXT-MNs lags behind that of FLEX-MNs, which may be attributed to the fact that FLEX-muscles are innervated at an earlier stage than EXT-muscles (Angulo y González, 1940; Altman and Bayer, 1984).

We have noted an increase in the amount of label followed by a decrease later in development. Since the number of MNs remains constant throughout postnatal development it is reasonable to assume that the number of dendrites initially increases and then decreases. The increase is in agreement with the data from other workers who reported that this rise was always due to an increase in the number of second or higher order dendrites and never to the formation of new first order dendrites (Ulfhake et al., 1988; Cameron and Fang, 1989; Cameron et al., 1991; Ramírez and Ulfhake, 1991). We further noted an increase in dendritic extension followed by, in some directions, a decrease. In the literature, the data on dendritic extension during postnatal development vary considerably. It was found to decrease (Ramírez and Ulfhake, 1991), to increase (Cameron et al., 1991), to initially increase and then to remain constant (Ulfhake et al., 1988) or to initially increase and then to decrease (Goldstein et al., 1990).

The dendritic extension patterns of different MN populations show a great diversity depending on muscle function and the synaptic input they receive. When a strong coordination between bilateral muscles is needed, contralaterally extending dendrites were found (bulbocavernosus: Goldstein et al., 1990; phrenicus: Lindsay et al., 1991; the tail: Ritz et al., 1992). In MNs mediating the toe-extension reflex, extensive dorsomedially extending dendrites in the direction of the primary afferents were found (Egger et al., 1980). Up until now, the dendritic extension pattern was never found to change during postnatal development (Goldstein et al., 1990; Cameron et al., 1991; Ramírez and Ulfhake, 1991). Thus, the present study is the first to describe a change in the directions in which dendrites extend in the transverse plane.

In conclusion, we have shown that the MN pools supplying two antagonistic muscles have a different postnatal developmental path to reach their specific adult dendritic extension pattern and, in particular, that the maturation of EXT-MNs lags behind that of FLEX-MNs. Both start at P2 with their dendrites extending in all directions, then follows a period of increase both in dendritic extension (which is more than the growth of the spinal cord itself) and in dendritic number, after which a period of specific dendritic retraction is found in which presumably, dendrites that did not form functionally significant contacts are eliminated. Among the latter are dendrites extending into the white matter of the lateral funiculus. It was further shown that both MN pools exhibit a different extension pattern. Both populations have a ventral component directed towards MNs of axial and more proximal limb muscles, but EXT-MNs have a medially, and FLEX-MNs a laterally directed component, which may point to a reciprocal connection between the two antagonist muscles. From P2 onwards, both have a dorsomedial component directed towards interneurons and primary afferents, however in FLEX-MNs this extension reaches as far as the medial part of lamina VI while EXT-MN dendrites only extend temporarily as far as the lateral part of lamina VI. This distinction may point to a different way of innervation of the MNs, for example by the CST.

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