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Correlation between Electrophysiological Properties, Morphological Maturation, and Olig Gene Changes during Postnatal Motor Tract Development

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Abstract

Background—Functional maturation of the nervous system in postnatal (PN) animals is a progressive process that may be assessed using evoked potentials of the auditory, visual, or somatosensory systems. This study investigated electrophysiological and histological changes as well as alterations of myelin relevant proteins of descending motor tracts in rat pups. MEP responses were recorded bi-weekly from postnatal (PN) week-1 to week-9 (adult).

Results—MEP latencies in PN week-1 rats averaged 23.7 milliseconds and became shorter during early maturation, stabilizing at 6.6 milliseconds at PN week-4. During maturation there was a rapid increase in the conduction velocity (CV). The CV increased from 2.8 ± 0.2 at PN week-1 to 35.2 ± 3.1 mm/ms at PN week-8 which represented functional maturation. Histology of the spinal cord and sciatic nerves revealed progressive axonal myelination. Expression of the oligodendrocyte precursor markers PDGFR α and NG2 were gradually down-regulated in spinal

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Authors Contributions:

CJ: Involved with the planning and performance of the experiments, data acquisition, writing the manuscript.

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XX: Provided oversight and an advisory role on the molecular aspect of the study.

CS: Involved with the planning protocol and electrophysiology studies.

cords, and myelin-relevant proteins such as GalC, CNP, and MBP were increased during maturation. Oligodendrocyte-lineage markers Olig2 and MOG, specifically expressed in myelinated oligodendrocytes, peaked at approximately PN week-3 and were down-regulated thereafter. A similar expression pattern was also observed in neurofilament M/H subunits (NF-M/H). Noticeably, NF-M/H was extensively phosphorylated in adult spinal cords but not in neonatal spinal cords, suggesting an increase in axon diameter and myelin formation. Ultra-structural morphology of axon and myelin sheaths in the ventrolateral funiculus (VLF) showed axon myelination of the VLF axons (99.3%) at PN week-2, while only 44.6% were sheathed at PN week-1. Furthermore, increased axon diameter and myelin thickness in both the VLF and sciatic nerves were highly correlated to the CV ($r_s > 0.95$).

Conclusions—Results from this study indicate that MEPs may be a predictor for the morphological maturity and integrity of myelinated axons in descending motor tracts.

Keywords

Motor evoked potentials; Conduction velocity; Ventrolateral funiculus; Spinal cord; Sciatic nerve; Maturation

Background

Clinical assessment of CNS maturation may be performed using behavioral tests such as the Moro response as well as placement and stepping reflexes [1]. Electrophysiological examinations, such as evoked potentials, provide objective measurements of nervous system maturation. The somatosensory system undergoes an increase in CV during development reflecting a progressive maturation of myelin of the ascending sensory tracts of the spinal cord [2,3]. An increase in brainstem central CV reflects maturation of auditory pathways [4]. Brainstem auditory evoked potentials (BAEP) are able to assess hearing in infants [5,6], and visual evoked potentials (VEP) can monitor visual development in normal neonates [7–9]. Peripheral nerve conduction studies demonstrate that the nerve CV in infants is one-half that of adults and reaches adult values at 7 months of age [10].

Locomotor development parallels nervous system maturation in humans and rodents, however, rodents mature at a much more rapid pace. In a kinematic study, neonatal rats quickly develop foot positioning during gait and then progress rapidly from uncoordinated to coordinated movements within 4–5 weeks [11]. Electrophysiological tests are able to assess this locomotor development. The motor CV is considered an important index in childhood development [12]. Application of magnetic stimulation in children allows the detection of abnormalities in motor pathways in newborn babies and young children [13]. The correlation between electrophysiological properties and morphological maturation of the motor system during development has not been studied in humans or animals. Such temporal information of stage-relevant maturation of motor tracts clinically and in the laboratory may assist in the developmental evaluation of normal neonates and in the diagnosis of neurological diseases [8,14].

Motor evoked potentials (MEP) characterize the conductive status of motor pathways. The use of transcranial electrical stimulation is painful and requires administration of sedation or

general anesthesia that would suppress or abolish MEP responses [15]. In contrast, the transcranial magnetic motor evoked potentials (tcMMEP) have minimal discomfort and may be used to monitor descending motor tracts in both humans and animals [16–18]. Descending motor signals recorded by the tcMMEP are transmitted through the ventrolateral funiculus (VLF) of the rodent's spinal cord [19,20]. The non-invasive tcMMEP measurement has been applied to monitor the motor pathway conduction during neural development.

The CNS conductivity corresponding to maturation may be correlated to histological examinations [21–23]. During maturation, increased conduction through nerve fibers parallels an increase in axon diameter and myelin thickness [24–27]. Conduction resistance decreases with the square of axon diameter, and the capacitance increases in direct proportion to the axon diameter resulting in a net increase in CV [28]. Myelination and an increase in axon diameters occur during the embryonic stage and continue throughout postnatal development [29]. Myelination plays a greater role in increasing CV compared to an increase in axon diameter. Action potentials spread rapidly between internodes of myelinated axons increasing CV [28]. In this study, we measured the tcMMEP on unanesthetized non-sedated developing rats and investigated the correlation between electrophysiological properties and morphological maturation, including myelin formation, axonal diameters, and thickness of the myelin sheath in the spinal VLF and sciatic nerve of descending motor tracts.

Materials and Methods

Animal care, handling, and surgical procedures were in accordance with guidelines for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996) and with the University of Louisville Institutional Animal Care and Use Committee (IACUC). Rat pups remained with their mothers until weaned and then were housed individually with access to food and water *ad libitum*.

Evoked responses - tcMMEP recording

Maturation of the descending motor pathways in postnatal rats was evaluated electrophysiologically and histologically as well as by Western blot and EM. A total of 34 female Sprague-Dawley rats were studied (electrophysiological: 17; histological: 6; Western blot: 6; and EM: 5.) Rats underwent biweekly electrophysiological testing from PN week-1 until adulthood at PN week-9 (Table 1). During the tcMMEP test, rats were awake without sedation and were immobilized in a soft stockinet restraint that was pinned to a board. [16,30] Magnetic stimulation was induced using a 5 cm magnetic coil positioned over the union of the skull connected to a MES-10 stimulator (Cadwell Laboratories, Kennewick, WA). Maximal output (100%) was 2 Tesla, and each magnetic pulse was 70 μ s. Hindlimbs were exposed through the stockinet, and recording electrodes were inserted into the gastrocnemius muscles. Active recording electrodes were placed in the muscle belly, a reference electrode in the muscle tendon, and a ground electrode in the lumbar paraspinal muscles. Interelectrode impedances remained below 3.5 KOhms. The amplifier gain ranged between 1K and 2K, and the bandpass filter was 10–3000 Hz. Responses were replicated.

Each sweep was 50 ms following the magnetic stimulation, with a 5 ms pre-stimulus interval. ERs were recorded with the Cadwell Excel monitor (Cadwell Laboratories, Kennewick, WA). Onset latency was the interval between the stimulus artifact and the onset of the ER. Body length (mm) was the distance from the inion to the caudal torso. The descending CV was estimated by dividing the body length (distance between nose and anus plus femur length) by the onset latency of the ER (body length [mm]/ER latency [ms]). Peak-to-peak amplitude of the ER was measured from the major peak to the adjacent trough. The threshold stimulus was the minimal magnetic stimulation required to elicit an ER. ER intensity was elicited by delivering the maximum magnetic stimulus (100%) and then lowering the stimulus magnitude by 10% decrements until no ER was elicited.

Histological assessment

Spinal cords were dissected from different postnatal stages between PN week-1 and adult rats. Specimens were perfused with PBS followed by 4% paraformaldehyde (PFA), then fixed in 4% PFA, equilibrated in 30% sucrose, and mounted in O.C.T. (Tissue-Tek). Transverse sections of the spinal cord at T9 (12 μ m) were processed for cryostat-section. Luxol fast blue (LFB) staining is commonly used to observe myelin under light microscopy, which detects lipoproteins in myelin. Briefly, rehydrated tissue sections were immersed in a 0.1% LFB alcoholic solution at 37 °C overnight, then washed by distilled water, and developed with 0.05% lithium carbonate. Single immunofluorescence on cryostat spinal sections was performed as described previously [31]. The images were captured using a Nikon 800 epifluorescence microscope. Antibodies were commercially available. The dilution ratio of antibodies was as follows: rabbit anti-Olig2 (1:800, Millipore), mouse anti-Olig1 (1:100, Millipore), mouse anti-Nkx2.2 (1:50, DSHB), genius pig anti-Nkx6.2 (1:6000, gift from Dr. Johan Ericson), and rabbit anti-MBP (1:6000, Millipore).

Western blotting

Protein samples were prepared from PN week-1, 3, and adult rat spinal cords in CellLytic™ MT Cell Lysis Reagent (Sigma) plus Complete Protease Inhibitors (Roche) at 4°C. Equivalent total protein amounts were loaded onto 7% polyacrylamide gels (Bio-Rad) and then transferred to Protan BA83 Nitrocellulose Membranes (Midwest Scientific). Blots were probed and recognized with the following 1st and 2nd antibodies: rabbit anti-Olig2 (1:3000, Millipore), rat anti-PDGFR α (1:1000, BD), rabbit anti-NG2 (1:1000, Millipore), mouse anti-CNP (1:100, Millipore), mouse anti-GalC (1:500, Millipore), rabbit anti-MOG (1:3000, Abcam), mouse anti-NF200 (1:3000, Abcam), mouse anti-phospho-NF200 (1:1000, Abcam), rabbit anti-Synapsin I (1:1000, Millipore), mouse anti- β -actin (1:5000, Sigma), and HRP-linked goat-anti-mouse or -rabbit (Jackson Immunology). Signals were developed by using chemiluminescence with ECL western blotting detection reagent (Pierce) and exposed to film [32].

Electron microscopy

To assess developmental changes of micromorphology in axon and myelin sheath, the spinal cord and left sciatic nerve were harvested from five rats. EM studies were performed on one rat at each of the following time points: PN week-1, 2, 3, 5, and 9 (adult). Rats were deeply anesthetized (pentobarbital 100 mg/kg, i.p.) followed by cardiac perfusion and fixation with

2% paraformaldehyde and 2.5% glutaraldehyde in a 0.01M PBS solution. The spinal cord and sciatic nerve were dissected and postfixed overnight. The samples were osmicated (1% OsO₄ buffer), dehydrated, and embedded in LX 112 epoxy plastic (Ladd Research Industries, Burlington, VT). Ultrathin cross-sections were obtained from the VLF and the left sciatic nerve. Sections were collected on 200 mesh copper grids and stained with uranyl acetate and lead citrate. Specimens were studied under a Philips CM-10 electron microscope at 60kV (Philips, North America Headquarters, New York, NY). A representative area measuring 984 μm^2 (29.1 $\mu\text{m} \times 33.8 \mu\text{m}$) from the VLF and the sciatic nerve was evaluated at 5,500x. Axon diameter and myelin sheath thickness were measured. The ratio of unmyelinated/myelinated axons in the VLF was determined by an absolute axon count in the same representative area.

Statistical analysis

Data was reported as means $\pm$ standard deviation. The relationship between developmental age and latency, conduction velocity, amplitude, axon diameter, and myelin thickness in the VLF and sciatic nerve was examined with non-linear parametric analysis. The correlation between CV and axon diameter or myelin thickness in the VLF and sciatic nerve was assessed by non-parametric analysis (Spearman's rho correlation, r_s). There were no lateralizing differences at any time point for all hindlimb ER and, therefore, results from the left and right side were combined, averaged, and compared with paired t-tests. When more than four t-tests were required, the significance levels were adjusted using the Bonferroni-Holm correction to decrease the likelihood of a Type I error. After adjusting for the number of t-tests, probability values of 0.05 or less were considered significant.

Results

Threshold of evoked responses, conduction velocity and amplitude of the descending motor pathway

Electrophysiological maturation of the descending motor pathways is represented by decreasing the required stimulation threshold to evoke the response and increasing the CV and amplitude of the ER. At the maximal stimulation (100% output), ER was elicited in only 77.1% of PN week-1 rats which increased to 100% at PN week-2. Reduction of threshold stimulation indicated increased excitability of the animals. The threshold stimulus significantly declined which corresponded to maturation of the motor system especially during the first two weeks after birth: ($70 \pm 5.8\%$ at PN week-1, $43.8 \pm 7.5\%$ at PN week-1½, and $37.5 \pm 6.5\%$ at PN week-2, $p < 0.05$, $n=17$). Higher levels of magnetic stimulation were required to obtain an ER in younger neonates. After PN week-3, the threshold stimulus stabilized at approximately 25% of magnetic output (Fig. 1). The tcMMEP measurement is summarized in Table 1. At PN week-1, the latency of ER was 23.7 ± 2.7 ms which dramatically declined thereafter for every half week prior to PN week-3½ ($p < 0.05$) and reached approximately 6 ms around PN week-5 without a further decrease in older rats (Fig. 2). The body length increased along with somatic growth throughout the study. The decreased ER latency combined with greater body length resulted in a significant increase in the CV for every half week during the first four weeks, with differences observed on weekly intervals until PN week-6½ ($p < 0.05$). The ER peak-to-peak amplitude was more

variable between individual mice but still significantly increased weekly during the first 4 weeks of age ($p < 0.05$). The non-linear regression analysis showed that the CV along descending motor tracts highly correlated with the developmental age ($R^2 = 0.9955$) (Fig. 3).

Changes of oligodendroglial and neurocytoskeletal molecules in postnatal spinal cords

Maturation of axons and myelin sheaths significantly impacted conductive capabilities through the spinal cord. Since the events of oligodendrocyte differentiation, myelination, and axon maturation mainly occur after birth in mice, staged expression of oligodendrocytic markers and neurocytoskeletal proteins in spinal cords were examined corresponding to different stages of maturation. The expression of pan-oligodendroglial marker Olig2 was continuously elevated during the first 3 weeks, suggesting a dramatic increase in oligodendrocytes. In contrast, the PDGFR α and NG2 proteins which are co-localized in oligodendrocyte progenitor cells were down-regulated (Fig. 4a). Meanwhile, the transcription factors that regulate oligodendrocyte differentiation (Nkx2.2 and Olig1) and myelination (Nkx6.2) were predominantly expressed in oligodendroglia-lineage cells (Fig. 4b) in parallel with high syntheses of myelin-relevant proteins such as GalC, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), and myelin/oligodendrocyte glycoprotein (MOG) (Fig. 4a). The synthesis of NF-M/H was robustly increased during the first three postnatal weeks and was highly phosphorylated thereafter. In addition, Synapsin I (indicators of synapse formation) localization in the nerve terminals of axons was significantly boosted (Fig. 5).

Ultra-structural maturation of axon and myelin sheath during neural development

Electron microscope (EM) analyses of descending axons in the developing spinal cord and sciatic nerve were conducted to quantitate the morphological changes of axons and myelin sheaths. At PN week-1, only 44.6% of the axons in the spinal VLF were myelinated. A total of 93.3% of the axons were myelinated at PN wk-2, reflecting a rapid myelin-forming process. A corresponding increase in axon diameter and myelin thickness in the spinal VLF and sciatic nerve was also observed. Following the initial stages of myelination, axon diameters increased during developmental maturation, while myelin sheaths became thicker and more compact (Fig. 6, Table 2). In the PN week-1 rat, 240 axons were counted in the VLF representing a 984 μm^2 observation area, and 128 axons were noted in the peripheral sciatic nerve. The number of axons in the representative area progressively declined due to an increase of axonal diameter. In the PN week-9 adult rat, there were only 23 axons in the VLF and 20 axons in the peripheral nerve. The average axonal diameter in the VLF at PN week-1 ($0.83 \pm 0.53 \mu\text{m}$) had doubled by PN week-5 ($1.73 \pm 1.1 \mu\text{m}$). Similarly, the average diameter of axons in the sciatic nerve was $1.36 \pm 1.02 \mu\text{m}$ at PN week-1 and $2.78 \pm 1.14 \mu\text{m}$ at PN week-5. The average myelin thickness in the VLF was $0.14 \pm 0.09 \mu\text{m}$ at PN week-1 which increased 1.1, 1.6, 5.0, and 6.0 fold at PN week-2, -3, -5, and -9 (adult), respectively. At PN week-5, the myelin sheath thickness in the VLF had almost reached the adult level. Myelination in the sciatic nerve occurred earlier and had a thickness of $0.26 \pm 0.40 \mu\text{m}$ at PN week-1 which increased 1.6, 2.8, 3.2, and 4 fold at PN week -2, -3, -5, and -9, respectively. There was a significant increase of the axonal diameter and myelin thickness in both VLF ($R^2 = 0.998$, $R^2 = 0.901$, respectively) and sciatic nerve ($R^2 = 0.982$, $R^2 = 0.966$, respectively) corresponding to developmental progression (Fig. 7).

Correlation of the electrophysiological motor conductivity with structural maturation of axon and myelin sheath

The conduction properties along the descending motor tracts that innervate muscles in the hind limbs are associated with the maturation of cortical neurons, axons, and myelin sheaths in the brain, spinal cord, and peripheral nerves including sciatic and tibial nerves. Thus, correlation exists between CV and ultra-structural maturation of descending motor tracts (e.g. axonal diameter and myelin thickness). Non-parametric analyses showed a positive Spearman correlation corresponding to an increasing monotonic trend between the CV along descending motor tracts and axonal diameter or myelin thickness in the VLF and sciatic nerve ($r_s=0.971, 0.958, 0.985, \text{ and } 0.979$, respectively).

Discussion

MEP induced by magnetic stimulation in adult rodents is an objective approach to obtain data on motor conduction under a normal physiological status, without using anesthetic agents [18,33,34]. Well-coordinated locomotor function of the hindlimbs develops within a few weeks in the postnatal rat due to the rapid maturation of motor systems. There is a comprehensive morphological change of almost every structure of the motor pathway during development, including elevated excitability of cortical motor neurons [35], increased elongation and density of the brain-spinal projections [36], and establishment and maturation of synapses and nerve-muscle junctions [37–39].

In this study, we successfully recorded electrophysiological properties during maturation of the descending motor tracts in the awake neonatal pup to adult rats. The ER was more easily initiated by lower threshold stimulation in the adult compared to early stage postnatal rats (Fig. 1) indicating that neuronal excitability developed during the first few weeks after birth [40]. The CV through the nervous system mainly relies on axon diameter and degree of myelination and synaptogenesis. Our data confirmed that conduction properties along the descending motor tracts are associated with maturation of axons and myelin sheaths that occur during early postnatal life [41]. The electrophysiological signals are conducted from pyramidal neurons in layer V of the cerebral cortex and descend through the brain stem, spinal cord, and the sciatic nerves to the neuromuscular plates of the target muscles. Since our recording electrode was placed in the gastrocnemius muscles, increases in the CV of the motor evoked potentials resulted from maturation of the motor systems involving both CNS and peripheral nerves. Ultramicroscopically we demonstrated progressive morphological development of axons and myelin sheaths in the spinal VLF and sciatic nerves that accounted for the quantitative evolution of tcMMEP responses. Between PN week-1 and PN week-2, axons in the VLF transformed from being unmyelinated to almost completely myelinated, which occurred one week later than in the sciatic nerve (Fig. 6). The diameter of the axon and thickness of the myelin sheath progressively increased between PN week-1 and PN week-6 in both the VLF and sciatic nerve (Fig. 7).

The temporal profile of myelin-relevant protein expression in the rat sciatic nerve has been reported [42]. Myelin-associated glycoprotein (MAG) was expressed maximally at 13 days of age, approximately one week earlier than MBP and P0 glycoprotein. The proteolipid protein (PLP) and CNP were not expressed developmentally in a manner that correlated with

the myelination of the sciatic nerve. In the rodent spinal cord, the differentiation of oligodendrocytes predominantly occurred after birth (Fig. 4) [43], and the majority of axons were myelinated prior to the PN week-3 (Fig. 6). Phosphorylation of NF-M and NF-H subunits of the neurofilament (NF) triplet not only regulates the axonal diameter but also reflects the myelination condition of the axons [44,45]. The robust synthesis of NF-M/H during the first three weeks and its phosphorylation thereafter may be a molecular sign of maturation of axon myelination in the spinal cord (Fig. 5). Congruously, the MEP latency rapidly declined during the first 4 weeks while the CV consistently increased until 8 weeks which is tightly correlated to the developmental age (Figs. 2 and 3, Table 1).

Measurement of tcMMEP provides a non-invasive approach to assess the electrophysiological function of descending motor tracts. The increase of CV represents a temporal progression of functional maturation. A positive correlation was present between the CV, axonal diameter, and myelin thickness. Developmental evaluation of the nervous system is not novel, however, our study represents the first time that the postnatal rat descending motor conductivity maturation has been correlated with morphological changes of the descending fibers in CNS and PNS and the representative molecular signs of oligodendrocyte maturation.

The brain and spinal cord initiate maturation early and have a tremendous capacity for development within weeks postnatally following which minimal changes occur. Postnatal development is guided by genetic programs and is influenced by environmental factors. For example, the cell adhesion molecule L1 and a profile of neurotrophic factor genes change in the postnatal spinal cord during development [46]. Olig genes, specifically Olig1/2 and Nkx2.2, are important in regulating myelin cell differentiation during spinal cord development [47]. The environment may also affect neural development, such as perinatal asphyxia that induces developmental apoptosis in the spinal cord of the neonatal rat [48]. Any disorder from nature (gene) or nurture (environment) during postnatal development may affect the maturation of descending pathways. Although the development of the nervous system has been studied extensively in many species, limited information pertaining to electrophysiological function is available in postnatal rodents. Thus, characterization of the developmental profile of tcMMEP signals in newborn animals combined with morphological maturation of axon/myelin complexes and olig gene changes in the developing spinal cord may provide a template for studies on both normal and genetically modified animals. This knowledge may contribute to the understanding of various diseases affecting the development of descending pathway.

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Figure 1.

The ability of the brain to generate motor responses by tcMMEP increased with age. In the rat pups, a greater stimulation was needed to induce a MEP response. By PN week-3, the excitability of the motor system had been stabilized to approximately 30% magnetic stimulation output which was similar to the adult.

Figure 2.

TcMMEP recordings of rats from PN week-1 to week-5 rats. The arrowhead (^) indicates the onset latency of the action potentials. There was shortening of the MEP latency and an increase in the tcMMEP amplitudes. After PN week-5, there were no changes in the MEP waveforms.

Figure 3.

Change in latencies of tcMMEP, conductive velocities, and amplitudes in rats over 9 weeks. The non-linear regression analysis showed that the conduction velocity along descending motor tracts highly correlated with the developmental age ($R^2=0.9955$). Latency and amplitude did not correlate with the developmental age.

Figure 4.

Expressions of oligodendrocyte-lineage molecules and oligodendrocyte differentiation in the developing spinal cords. (A) A dramatic increase in the expressions of Olig2, PDGFR α , and NG2 during the first 3 weeks indicated that many oligodendrocytes exist in the spinal cord, especially oligodendrocyte precursor cells. Myelin-relevant proteins GalC, CNP, and MOG were highly expressed after the first postnatal week suggesting that more oligodendrocyte precursor cells were differentiated. (B) Abundant Nkx2.2+ differentiating oligodendrocytes were seen during the first postnatal week while more mature oligodendrocytes (Olig1+/Nkx6.2+) were present after two weeks.

Figure 5.

Expression of neurofilament M/H subunit and their phosphorylated forms as well as Synapsin I in the developing spinal cord. NF-H and -M subunits were synthesized during the first three postnatal weeks and were highly phosphorylated thereafter, indicating a progression of axonal maturation. Enhanced synthesis of Synapsin I suggested that more synapses were formed in the developing spinal cord.

Figure 6.

Ultrastructural analysis of the sciatic nerve (top row) and VLF (bottom row) at different postnatal stages. The formation of the myelin sheath in the sciatic nerve occurred earlier than that in the VLF. The majority of axons in the VLF of the spinal cord were non-myelinated at PN week-1 and almost completely myelinated at PN wk-2. Axon diameters increased during developmental maturation, while myelin sheaths became thicker and more compact. All images are at the same magnification.

Figure 7.

Changes in axon caliber and myelin thickness in the sciatic nerve and VLF. Non-linear regression analysis showed that the axon caliber and myelin thickness in both areas tightly correlated with the developmental age ($R^2 > 0.90$) over 9 weeks. Developmental age was more closely correlated with axon size than myelin thickness.

tcMMEP responses were obtained twice per week. Latency (ms), amplitude (mV), and body length (mm) are listed from PN week-1 to PN week-9 (adult) rats. There was a progressive decrease in the conduction latency, an increase in body length, and an increase in the conduction velocity. The MEP amplitude also increased during the first three weeks. Data represent the mean $\pm$ SD.

Table 1

Postnatal Age (weeks)	Number of Rats	Response Latency (ms)	Body Length (mm)	Conduction Velocity (mm/ms)	tcMMEP Amplitude (mV)
1	17	23.7 $\pm$ 2.7	66.1 $\pm$ 2.5	2.8 $\pm$ 0.2	0.5 $\pm$ 0.4
1½	17	16.2 $\pm$ 2.2	78.9 $\pm$ 2.6	5.0 $\pm$ 0.8	1.1 $\pm$ 1.0
2	17	11.6 $\pm$ 1.6	86.2 $\pm$ 3.1	7.5 $\pm$ 0.9	1.3 $\pm$ 0.8
2½	17	8.4 $\pm$ 1.0	92.5 $\pm$ 3.4	11.2 $\pm$ 1.4	1.8 $\pm$ 1.0
3	9	7.2 $\pm$ 0.5	109.1 $\pm$ 4.5	15.2 $\pm$ 1.2	2.7 $\pm$ 1.8
3½	9	6.6 $\pm$ 0.4	127.8 $\pm$ 4.6	19.4 $\pm$ 1.4	3.9 $\pm$ 2.1
4	9	6.7 $\pm$ 0.5	139.1 $\pm$ 3.8	20.9 $\pm$ 2.1	3.3 $\pm$ 2.4
4½	9	6.6 $\pm$ 0.5	154.1 $\pm$ 5.2	23.4 $\pm$ 1.6	3.7 $\pm$ 2.4
5	9	6.3 $\pm$ 0.5	160.3 $\pm$ 6.2	25.6 $\pm$ 1.6	4.1 $\pm$ 2.3
5½	9	6.2 $\pm$ 0.6	175.9 $\pm$ 6.0	28.6 $\pm$ 1.6	3.1 $\pm$ 1.3
6	9	6.1 $\pm$ 0.4	185.2 $\pm$ 6.2	30.5 $\pm$ 2.5*	2.9 $\pm$ 1.2
6½	9	6.0 $\pm$ 0.6	194.6 $\pm$ 6.3	32.5 $\pm$ 2.6	3.4 $\pm$ 3.2
7	9	6.0 $\pm$ 0.5	197.3 $\pm$ 6.0	33.4 $\pm$ 2.7*	3.5 $\pm$ 2.4
7½	9	5.9 $\pm$ 0.5	200.1 $\pm$ 5.7	34.2 $\pm$ 2.8	3.6 $\pm$ 2.1
8	9	5.8 $\pm$ 0.5*	202.2 $\pm$ 5.3	34.8 $\pm$ 2.6*	3.1 $\pm$ 1.9*
8½	9	5.8 $\pm$ 0.5	204.3 $\pm$ 4.9	35.4 $\pm$ 2.3	2.6 $\pm$ 1.7
9	9	6.1 $\pm$ 0.5	213.8 $\pm$ 4.6	35.2 $\pm$ 3.1	3.6 $\pm$ 3.6

[^] Conduction velocity=body length (mm)/latency (ms)

* Extrapolated

Table 2

Progressive increase in axon diameter and myelin thickness measured from PN week-1 to PN week-9 (adult) in the rat.

Postnatal Age (weeks)	Axon diameter in VLF (μm)	Axon diameter in SN (μm)	Myelin thickness in VLF (μm)	Myelin thickness in SN (μm)
1	0.83 ± 0.53	1.36 ± 1.02	0.14 ± 0.09	0.26 ± 0.40
2	1.11 ± 0.47	1.81 ± 0.49	0.15 ± 0.10	0.41 ± 0.28
3	1.25 ± 0.61	2.44 ± 0.96	0.21 ± 0.16	0.73 ± 0.32
5	1.73 ± 1.10	2.78 ± 1.14	0.68 ± 0.29	0.84 ± 0.10
9 (adult)	2.60 ± 1.00	3.34 ± 0.83	0.81 ± 0.32	1.07 ± 0.22