

# The Histological Evidences for Developmental Alternations in the Transmitting Time of Impulses along the Thalamocortical Tract

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Received 12 April 2003; revised 15 June 2004; accepted 15 June 2004

## ABSTRACT

Change in transmitting time of impulses along axons is traditionally attributed to two parameters: the myelin formation and the diameter of neurite, both rising during the postnatal development. In the previous study, we showed that conduction velocity of the fibers projecting from the thalamus to the layer IV of the somatosensory (barrel) cortex increases as a function of age. However, the conduction velocity change is not parallel outside and inside of the barrel cortex. Here, we tried to find a probable relationship between disparity of the conduction velocities and the extent of myelination of the thalamocortical pathway. Our results indicate that myelin is evident on the extra-cortical but not intra-cortical fibers at ages >10 days. At the older age, however, myelin wholly covers the fibers both outside and inside of the cortex, more considerably in the former. Our results demonstrate that difference in the conduction velocities of the extra-cortical and intra-cortical axons, at least to more extent, can be attributed to myelination dissimilarity along the thalamocortical fibers. *Iran. Biomed. J. 9 (1): 15-19, 2005*

*Keywords:* Myelination, Postnatal development, Somatosensory cortex, Thalamus, White matter

## INTRODUCTION

The somatosensory cortex of rodents receives its input from large whiskers, or vibrissae, on the rodent whisker pad. The map of the whisker pad is faithfully replicated in layer IV of primary somatosensory cortex, where small neurons aggregate into special structures termed "barrels" [1, 2]. Thalamocortical axons arrive in the developing cortex during embryonic life [3] before the majority of cortical neurons are born [4], but do not form, a topographic pattern of clustered terminals in the barrel field until the first postnatal days [5-7]. The aggregate characteristic of the cells that constitute the barrels begin to form 3 days after postnatal age [8, 9]. Subsequently, oriented growth and regression of dendrites sharpen the boundaries of each barrel [10].

The conduction delays in the central components of both motor and somatosensory pathways decrease rapidly after birth. It has been traditionally assumed that individual axons are relatively

uniform in the related path, however, it has been shown that the conduction velocities of a single fiber change along its length. For example, while stimulation of the lateral geniculate nucleus reaches visual cortex, typically in 2 ms [11, 12], white matter stimulation produces monosynaptic responses with almost identical latencies in layer IV or II/III neurons [13-15]. Reliably, Baker and Stryker [16] reported that the conduction velocities contributing to the early components of the compound action potential are significantly greater in the optic tract than in the optic nerve.

Our electrophysiological findings on the conduction velocity of the neurons transmitting somatic sensations from the thalamus to the somatosensory cortex indicate that the conduction velocity of the thalamocortical fibers outside and inside of the barrel cortex changes differently over postnatal development [17]. The conduction velocity is known to be closely proportional to the diameter as well as myelination of a given fiber. It is well known that in different parts of the nervous

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system, myelination of neurons alters as a function of age [18]. Focusing on the myelin staining method, this study was made to test consistency of our results histologically and electrophysiologically.

## MATERIALS AND METHODS

**Animals and slice preparation.** Electrophysiological experiments were performed on the somatosensory cortex of mice (C57/BL6) aged 3 (P3) to 57 (P57) days old. The animals were housed in the standard 12 h light/dark cycle at  $22 \pm 2^\circ\text{C}$ , deeply anesthetized by isoflurane and then decapitated. The brain was removed rapidly from cranium and emerged into cold ( $2-4^\circ\text{C}$ ) pre-gassed (95%  $\text{O}_2$ -5%  $\text{CO}_2$ ) artificial cerebrospinal fluid. It was to keep viability of the brain during sectioning and experiments, as well. The slices (500 and 300-400  $\mu\text{m}$  thickness in the young and old animals, respectively) including the thalamocortical fibers, transmitting somatic sensations from the thalamus to the layer IV of the barrel cortex, were dissected by a rotoslicer. To preserve thalamocortical fibers through the path, the slices were sectioned with two angles:  $10^\circ$  at a ramp tilt angle and  $55^\circ$  to the right of the posterior-to-anterior axis of brain [19].

**Fixation and sectioning of slices.** At the end of experiments, slices were kept floating freely in the fixative solution (containing of 1.25% glutaraldehyde and 1.0% paraformaldehyde in 0.1 M phosphate buffer) until use. Using freezing microtome, the frozen sections were prepared from each slice. The slices were dissected to very thin sections (40 to 60  $\mu\text{m}$ ) and then the following processes were performed:

**A) Myelin staining.** The processes for myelin staining included two stages [20]:

**1. Initial staining reaction.** The sections were rinsed twice in PBS (0.1 M phosphate buffer, pH 7.4) for 30 min on a shaker. Then, they were placed in 4% normal horse serum and 0.5% Triton-X in PBS at room temperature overnight. The sections were rinsed twice in PBS on the shaker at room temperature for 15 min and were kept in diaminobenzidine (DAB) for 9 min, then, rinsed twice in PBS for 10 min. At this stage, the myelin was only faintly visible. It is important to note that the darkness intensity of the fibers reflects the extent of myelination.

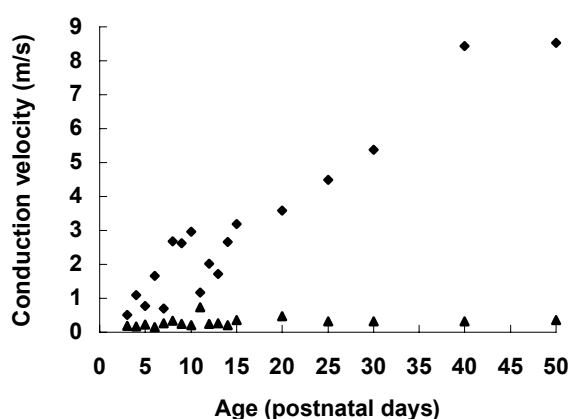
**2. Intensification of the DAB deposit.** The sections were placed in 8% thioglycolic acid in distilled water, either for 4-6 h at room temperature or overnight at  $4^\circ\text{C}$ . Slices were washed 4 times by rotating them in 2% sodium acetate in distilled water for 40 min and placed in the developing solution (5% sodium carbonate, 37% formaldehyde, 2.0 g ammonium nitrate, 2.0 g silver nitrate and 10.0 g tungstosilic acid in 1 liter distilled water) for 8-10 min or more until the desired staining intensity was obtained. Then, the sections were rotated in 1% acetic acid and 0.05% gold chloride for 5-10 and 15 min, respectively. After rinsing in 2% sodium acetate for 30 s, the sections were rotated in 3% sodium thiosulfate for 5 min. Finally, sections were rinsed twice in 0.1 M phosphate buffer at pH 7.4 for 10 min. This stage was repeated for a more intense staining.

**B) Picture preparation.** After staining, the sections were pictured at different zoom using Fuji photograph software and saved on computer for further analysis.

## RESULTS

**Electrophysiological findings.** This study was designed to test if histological findings agreed with the electrophysiological report [17]. Briefly, stimulating the thalamus and the white matter excitatory postsynaptic currents (EPSC) and extracellular field potentials were recorded in the layer IV of the mouse somatosensory cortex. Measuring the response delay and the distance between stimulating and recording electrode conduction velocities were calculated. Conduction velocity from the thalamus to the white matter obtained from EPSC increased rapidly and became more than 20 folds during the first three weeks:  $0.26 \pm 0.02$ ,  $0.47 \pm 0.08$  and  $5.60 \pm 0.13$  (m/s) at P3, P9, and P20, respectively. In contrast, the conduction velocity measured from the part of the fibers within cortex (from the white matter to the layer IV) increased slowly and became only 2 folds:  $0.09 \pm 0.008$ ,  $0.11 \pm 0.01$  and  $0.16 \pm 0.01$  at the same ages as above. The delay of field potentials also matches that of EPSC. In the older animals ( $>3$  weeks), the experiments were continued with only field potential recordings. The conduction velocities increased further parallel to age and reached a plateau ( $8.53 \pm 2.53$  m/s and  $0.36 \pm 0.02$  for extra- and intra-cortical fibers, respectively) at P40. Figure 1 illustrates conduction velocity changes over 50

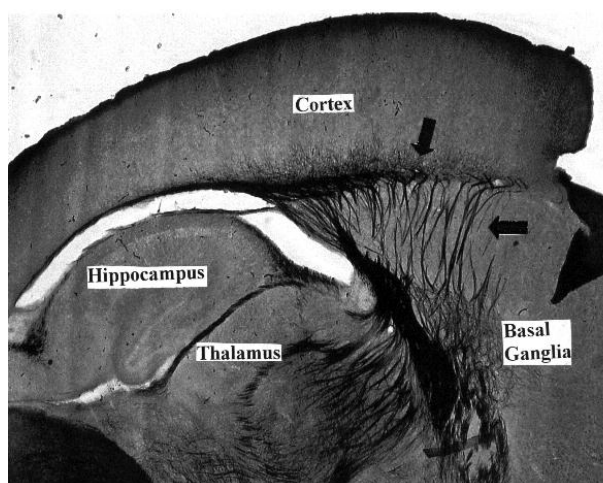
days of the postnatal development in the extra- and intra-cortical fibers. These results demonstrate that the conduction time from the white matter to the layer IV changes slightly during postnatal development, while that of thalamus to the white matter shortens very rapidly, indicating a 10-fold enhancement in the extra- compared to the intra-cortical conduction velocity. Thus, the conduction velocity from the thalamus to the layer IV becomes virtually comparable to the conduction velocity from the white matter to the layer IV about the second postnatal week.



**Fig. 1.** Conduction velocities measured from field potentials recorded in the barrel cortex. The filed potentials triggered by the thalamus and the white matter stimulation were used for calculating the conduction velocities of the fibers outside (square) and inside (triangle) of the cortex, respectively. Conduction velocity was obtained by division of the response delay to the distance between the thalamus to the white matter (for extra-cortical part of the fibers) or between the white matter and the layer IV (for intra-cortical part of the fibers).

**Postnatal formation of myelin.** Theoretically, the conduction velocity is proportional to the diameter of the myelinated axon, and to the square root of the diameter of an unmyelinated axon [21, 22]. Axon diameters can be estimated from the conduction velocity in both myelinated and unmyelinated axons [21, 22]. If the thalamocortical axon was totally unmyelinated and the 10-fold difference entirely was accounted for the diameter difference, a 100-fold difference of diameter would be necessary, which is most unlikely. Thus, we considered the extent of myelination along the thalamocortical fiber to assess any difference of myelination between inside and outside of the barrel cortex. Therefore, we carried out myelin staining in mice at P11, P30 and P57.

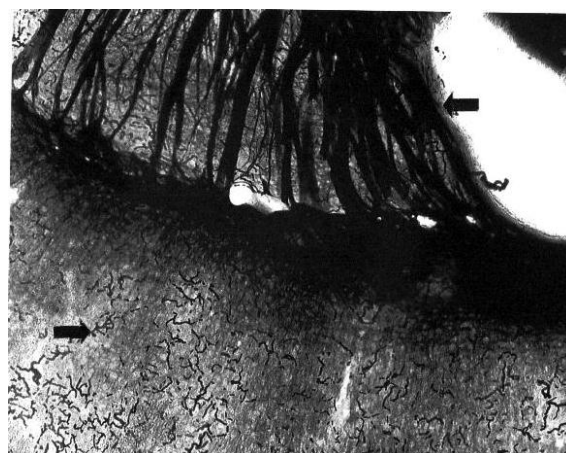
Before P11, we did not observe any stained area in the cortex confirming that the barrel cortex starts myelinating at P10 [18]. However, reaching



**Fig. 2.** A photograph illustrating myelin formation on the thalamocortical fibers at P11. Slice shows myelination markedly on the fibers from the thalamus to the white matter while traces of cortical myelination is evident only near the white matter. Extent of darkness indicates degree of myelination shown by arrows.

P11, faintly stained fibers were evident. Figure 2 illustrates a slice taken from mouse at P11. In this age, the fibers of thalamic cells projecting to the cortex are well stained only out of the cortex. However, the intra-cortical part of the fibers from the same cells shows traces of myelination in the vicinity of the white matter.

At P30, we observed the heavy stained fibers from the thalamus to the white matter. Also, the fibers within cortex developed a substantial myelination showing a gradient increase from the white matter through the surface of the cortex (Fig. 3). Consistently, it has been reported that the



**Fig. 3.** The slice pictured at P30. As is observable the thalamocortical fibers present the myelination entirely from the thalamus through the cortex. The myelination appears more considerable compared to that at P11. The difference is more pronounced between the cortices. Extent of darkness indicates degree of myelination shown by arrows.

thalamocortical fibers projected to parietal cortex start to myelinate at P14 [18]. At P57, although staining demonstrated a small change of myelination in both the extra- and intra-cortical fibers (Fig. 4), comparing the extent of myelination of the fibers at P30 and P57 indicates no pronounced variation. It is believed that the process of myelin formation on bars of axons ends at about P44 and reaches the adult stage by the end of the second month [18].



**Fig. 4.** Severely stained thalamocortical fibers offers a well developed myelination in a mature stage (P57). No detectable difference between extra-cortical myelination at P30 and P57 confirms the electrophysiological findings (refer to the text for more details). Extent of darkness indicates degree of myelination shown by arrows.

## DISCUSSION

The barrel of rodent somatosensory cortex is a special case of the cortical column. The column is the basic functional unit of neocortex and is comprised of a tangentially restricted area that extends through all six cortical layers [23, 24]. Connectivity is enhanced within the column and restricted outside the column. Replication of this basic columnar unit within cortical areas permits the representation of relationships such as topography across sets of columns in an orderly fashion [25].

As described before, the conduction velocity of the thalamocortical fibers rises as a function of age. Our results showed that a considerable difference is evident between the extent of myelination inside and outside of the barrel cortex in the middle of the second week. Consequently, a clear slow conduction velocity of the intra-cortical fiber can be reasonable. At the older ages, generally, the formation of myelin is more observable on the

thalamocortical fibers so that the slices taken from the mice at P30 stained for myelin seem much darker than the younger animals. Conduction velocities of the extra- and intra-cortical increased by a factor of 20 and 2, respectively. These results clearly demonstrate that myelination in the thalamocortical fibers is the main cause for the observed disparity of the conduction velocity between the extra- and intra-cortical fibers. In addition, field potential recordings expressed that the conduction velocity of the extra-cortical fibers continues to increase toward older ages reaching a plateau at about P40. Therefore, it can be said that the myelination and the conduction velocity are increased in parallel. However, this hypothesis is more attributable to the fibers from the thalamus through the white matter rather than those within the barrel cortex. Because the latter is also subjected to increase in both the conduction velocity and the extent of myelination, but the increased conduction velocity outside and inside of the cortex are not comparable. Interestingly, the conduction time from the thalamus to the layer IV becomes virtually comparable to that of white matter to the layer IV by the end of the second postnatal week. It could be important electrophysiologically, since at early age, in spite of the rise of the transmitting time of the impulses along the fibers, the size of the brain increases accordingly. Consequently, the electrical activity triggered in the thalamic cells reaches the layer IV within a constant time.

Studies carried out in other parts of the nervous system also verify our findings. Armand *et al.* [26] and Olivier *et al.* [27] reported that, due to myelination of corticospinal axons, conduction velocity of the fastest corticospinal neurons over their cranial course would reach adult values much sooner than the corticospinal neurons in the spinal cord which increased with age. In addition, changes of conduction velocity along an axon have been reported in other regions of the central nervous system [28].

Altogether, it is expectable that the conduction velocity of thalamocortical fibers increases during postnatal development. However, the enhancement of the conduction velocity is different, in a given fiber, from the thalamus to the barrel cortex leading to a much more increase in the conduction velocity of the extra-cortical compared to intra-cortical fibers. Our results presented here demonstrate that the difference in the conduction velocities might be attributed to diverse postnatal changes in myelination of a given fiber passing the thalamus to the layer IV.

## REFERENCES

1. Welker, C. and Woolsey, A. (1974) Structure of layer IV in the somatosensory neocortex of the rat: description and comparison with the mouse. *J. Comp. Neurol.* 158: 437-453.
2. Woolsey, A. and Van der Loos, H. (1970) The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* 17: 205-242.
3. Catalano, M., Robertson, T. and Killackey, P. (1991) Early ingrowth of thalamocortical afferents to the neocortex of the prenatal rat. *Proc. Natl. Acad. Sci. USA* 88: 2999-3003.
4. Shatz, J. and Luskin, B. (1986) Relationship between the geniculocortical afferents and their cortical target cells during development of the cat's primary visual cortex. *J. Neurosci.* 6: 3655-3668.
5. Blue, E., Erzurumlu, S. and Jhaveri, S. (1991) A comparison of pattern formation by thalamocortical and serotonergic afferents in the rat barrel field cortex. *Cereb. Cortex* 1: 380-389.
6. Erzurumlu, S. and Jhaveri, S. (1990) Thalamic axons confer a blueprint of the sensory periphery onto the developing rat somatosensory cortex. *Dev. Brain Res.* 56: 229-234.
7. Schlaggar, L. and O'Leary, D. (1994) Early development of the somatotopic map and barrel patterning in rat somatosensory cortex. *J. Comp. Neurol.* 346: 80-96.
8. Rice, L., Gomez, C., Barstow, C., Burnet, A. and Sands, P. (1985) A comparative analysis of the development of the primary somatosensory cortex: interspecies similarities during barrel and laminar development. *J. Comp. Neurol.* 236: 477-495.
9. Senft, L. and Woolsey, A. (1991) Growth of thalamic afferents into mouse barrel cortex. *Cereb. Cortex* 1: 308-335.
10. Greenough, T. and Chang, L. (1988) Dendritic pattern formation involves both oriented regression and oriented growth in the barrels of mouse somatosensory cortex. *Brain Res.* 471: 148-152.
11. Toyama, K., Matsunami, K., Ono, T. and Tokashiki, S. (1974) An intracellular study of neuronal organization in the visual cortex. *Exp. Brain Res.* 21: 45-66.
12. Tsumoto, T. (1978) Inhibitory and excitatory binocular convergence to visual cortical neurons of the cat. *Brain Res.* 159: 85-97.
13. Shirokawa, T., Nishigori, A., Kimura, F. and Tsumoto, T. (1989) Actions of excitatory amino acid antagonists on synaptic potentials of layer II/III neurons of the cat's visual cortex. *Exp. Brain Res.* 78: 489-500.
14. Komatsu, Y., Fujii, K., Maeda, J., Sakaguchi, H. and Toyama, K. (1988) Long-term potentiation of synaptic transmission in kitten visual cortex. *J. Neurophysiol.* 59: 124-141.
15. Kimura, F., Nishigori, A., Shirokawa, T. and Tsumoto, T. (1989) Long-term potentiation and N-methyl-D-aspartate receptors in the visual cortex of young rats. *J. Physiol.* 414: 125-144.
16. Baker, G. and Stryker, M. (1990) Retinofugal fibers change conduction velocity and diameter between the optic nerve and tract in ferrets. *Nature* 344: 342-345.
17. Salami, M., Kimura, F. and Tsumoto, T. (2003) Postnatal changes of conduction velocity of the fibers in and out of the mouse barrel cortex. *Iran Biomed. J.* 7: 57-63.
18. Jacobson, S. (1963) Sequence of myelination in the brain of the albino rat, A. cerebral cortex, thalamus and related structures. *J. Comp. Neurol.* 121: 5-29.
19. Agmon, A. and Connors, B. (1991) Thalamocortical responses of mouse somatosensory (barrel) cortex *in vitro*. *Neurosci.* 41: 365-379.
20. McNally, K. and Peters, A. (1998) A new method for intense staining of myelin. *J. Histochem. Cytochem.* 46: 541-545.
21. Waxman, G. and Bennett, V. (1972) Relative conduction velocities of small myelinated and non-myelinated fibres in the central nervous system. *Nat. New Biol.* 238: 217-219.
22. Waxman, G. (1980) Determinants of conduction velocity in myelinated nerve fibers. *Muscle Nerve* 3: 141-150.
23. Mountcastle, B. (1957) Modality and topographic properties of single neurons in cat's somatic sensory cortex. *J. Neurophysiol.* 20: 408-434.
24. Powell, S. and Mountcastle, B. (1959) Some aspects of the functional organization of the cortex of the postcentral gyrus of the monkey. A correlation of findings obtained in a single unit analysis with cytoarchitecture. *Bull. Johns Hopk. Hosp.* 105: 133-162.
25. Wilson, A., Johnston, V., Goldstein, W. and Blue, E. (2000) Neonatal lead exposure impairs development of rodent barrel field cortex. *Proc. Natl. Acad. Sci. USA* 10: 5540-5545.
26. Armand, J., Edgley, S., Lemon, R. and Olivier, E. (1994) Protracted postnatal development of corticospinal projections from the primary motor cortex to hand motoneurons in the macaque monkey. *Exp. Brain Res.* 101: 178-182.
27. Olivier, E., Edgley, S., Armand, J. and Lemon, R. (1997) An electrophysiological study of the postnatal development of the corticospinal system in the macaque monkey. *J. Neurosci.* 17: 267-276.
28. Baker, E. and Stryker, P. (1990) Retinofugal fibres change conduction velocity and diameter between the optic nerve and tract in ferrets. *Nature* 344: 342-345.