

Postnatal Changes of Conduction Velocity of the Fibers in and out of the Mouse Barrel Cortex

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ABSTRACT

There are some conflicts about constancy of conduction velocity (CV) in a given tract of nervous system. By recording excitatory postsynaptic currents (EPSC) in layer IV of the somatosensory cortex we tried to clear changes in CV of thalamocortical tract of mice aged 3 to 50 days old. Field potentials and EPSC were recorded in the layer IV by stimulation of ventrobasal nucleus of thalamus (VB) and white matter (WM). Our results indicate that in mice aged 3 through 17 days old, CV of EPSC evoked by WM and VB stimulation increased up to 2 and 15 times, respectively. Also, the data from field potentials match those from EPSC. CV enhancement of the fibers out of cortex may contribute to myelination as well as increased diameter of neurites. However, it is not the case for WM matter stimulation-evoked responses. *Iran. Biomed. J. 7 (2): 57-63 2003*

Keywords: Barrel cortex, Conduction velocity, Excitatory postsynaptic current, Field potential, Thalamocortical tract

INTRODUCTION

In the earlier studies of central nervous fiber tracts, it was tacitly assumed that individual axons are relatively uniform in the related path. Nevertheless, there are some reports indicating that the conduction velocity (CV) of a given fiber changes along its length. For example, while stimulation of the lateral geniculate nucleus neurons reaches visual cortex typically in 2 ms (range, 1-7 ms) [1, 2] white matter (WM) stimulation produces monosynaptic responses with almost identical latencies in layer IV or II/III neurons [3-5]. Also, Baker and Stryker [6] reported that the CV contributing to the early components of the compound action potential is significantly greater in the optic tract than in the optic nerve.

These controversies, on the one hand, raised a significant question about the developmental mechanisms in the nervous system that relate the axons, their diameters, and the glia with which they are myelinated and, on the other hand, indicate that studies that have relied on the constancy of CV along a fiber may require reappraisal.

Changes of CV have been reported within central nervous system [6], as well as between peripheral nerve roots and the spinal cord [7, 8] but, so far, there is no direct evidence why CV needed to be different in a given fiber. To address this issue, we focused on CV of the neurons transmitting somatic sensations from the thalamus to the related sensory cortex. In addition, it is well known that in different parts of nervous system myelination of neurons alters as a function of age [9]. To test this electrophysiologically, the experiments were performed on the thalamocortical fibers over postnatal development.

MATERIALS AND METHODS

Animals. Male and female mice (C57 BL6) at postnatal age of 3 to 50 days old (P3-P50) were used. For whole cell recording, sixty-four slices were prepared from mice at P3-P17 postnatal age. Also, 42 slices, obtained from mice at P3-P50 postnatal age, were used for field potential recordings. The animals were

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housed in the standard 12 h light/dark cycle at $22 \pm 2^\circ\text{C}$.

Slice preparation. After anesthetizing by florane, mice were decapitated. The brain rapidly removed from cranium emerged in cold and bubbled by oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) consisting of (in mM): NaCl, 124; KCl, 3; KH_2PO_4 , 1.2; MgSO_4 , 1.3; NaHCO_3 , 26; CaCl_2 , 2 and glucose, 10. Slices (500 and 300-400 μm thickness in the young and old animals, respectively) including thalamocortical tract were prepared by a rotoslicer. To preserve thalamocortical fibers through the path, the slices were sectioned with two angles: 10° at a ramp tilt angle and 55° to the right of the posterior-to-anterior axis of the brain [10]. The slices were recovered in the ACSF at the room temperature ($27 \pm 1^\circ\text{C}$) for one hour or more, and perfused by the ACSF at a flow rate of 4 ml/min during experiment.

Electrophysiology:

Stimulation sites. In all experiments, the concentric bipolar stimulating electrodes were placed in ventrobasal nucleus (VB) of thalamus and WM preferentially in a straight line to the recording site (see below). In some experiments, however, another stimulating electrode was placed at a point within the cortex between the WM and the recording electrode (Fig. 1).

Fig. 1. Typical slice illustrating thalamus and somatosensory (barrel) cortex. Abbreviations: IV, layer IV (barrel cortex) where only the recording electrode in all experiments was placed in; WM (white matter); VB (ventrobasal) nucleus of thalamus. Dark points mark the stimulating sites.

Recording site. Regardless of the stimulation sites, in all experiments, the recording site was the layer IV of somatosensory cortex (also named barrel cortex). Responses were pooled by a glass electrode filled by cesium-based solution (consisting of cesium methane sulfonate, 30mM; hepes, 10 mM; cesium chloride, 10 mM and EGTA, 0.5 mM) yielding impedances between $120 - 14$ and $5 \text{ M}\Omega$ for whole cell and field potential recordings, respectively.

Recordings:

Extracellular field potentials. To test connectivity of the thalamus to the cortex, at first we tried to record field potentials by stimulation of the above-mentioned stimulating sites. If the field potential was large enough by a stimulus intensity two times of the threshold (0.5 and 1.5 mV for stimulating WM and VB, respectively) the experiments were continued by whole cell recording.

Whole cell recording. Whole cell patch experiments were done by AXOPATCH 1D. The resting membrane potential of cell was clamped at -70 mV and excitatory postsynaptic currents (EPSC) were evoked by stimulation of the same sites as for field potential experiments. Eight sweeps of EPSC were recorded at 0.2 Hz frequency and 100 μs duration. The data were digitized, sampled (at 10 kHz) and low pass filtered (at 1 kHz) via Clampex 8 software and analyzed by Clampfit 8 software.

Staining, fixation and taking picture. At the end of each experiment, the recording and stimulating sites were stained by pontamine sky blue via iontophoresis, and then the slices were fixed (Fig. 1). The slices were pictured by Fuji photograph software and distances between the stimulating sites and recording site were measured by Corel Draw software. To stain myelin, we used the previously described method [11]. Staining the patched thalamic or cortical cells was performed by neurobiotin, added to the electrode solution, where it did leak from the electrode for about 10minutes .

Data analysis. Conduction velocity (m/s) of thalamocortical fibers was calculated using two parameters: latency (ms) and distance (mm). Latency of the field potentials was measured from stimulus artifact to first negative waveform and the latency of EPSC was measured from stimulus artifact to the beginning of EPSC. For calculating CV of the thalamocortical fibers from WM through barrel cortex (CV_{WM}), the distance

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between stimulating and recording sites was divided to mean latency of eight sweeps of EPSC (or field potentials) evoked by WM stimulation. To exclude synaptic delay during calculation of CV_{WM} , in some experiments, CV of the fibers was calculated within the cortex (CV_{CO}) by stimulation of a point between WM and recording site. CV of the fibers from VB to WM (CV_{VB}) was calculated by subtracting mean latency of EPSC (or field potentials) evoked by the WM and VB stimulation over distance between VB and WM stimulating sites. All the data are expressed as mean \pm SEM. Analysis of variance (ANOVA) was used for comparison of the data in each group.

RESULTS

In the beginning of each experiment we recorded field potentials in the barrel cortex following stimulation of the VB and WM. This was to test 1) whether there is an acceptable connectivity between VB and the barrel cortex and 2) concerning CV, to compare these data to those obtained by whole cell recording.

Characteristics of the field potentials. Stimulating VB and WM, extracellular field potentials were recorded in the somatosensory barrel cortex. Before recording, we determined threshold stimulus intensity for each stimulating site, in which a minimum but obviously distinguishable response was appeared. Then the field potentials were recorded by stimulus intensity almost two times of the threshold. It should be noted that, in general, the threshold was higher in very young animals (P3-P5) than in older ones (P7-P11). The threshold was the highest in aged mice (in particular at P20 and older) and the slices were appeared to represent a rapidly adaptation unless we decreased the stimulus frequency from 0.2 Hz to 0.05 Hz. WM stimulation at younger animals resulted in a response with usually one negative wave, sometimes followed by another small negative wave. Stimulation of VB, at the same ages, elicited field potentials with two volleys. At older animals, the field potentials showed several negative waves, regardless of stimulation site; increasing in number by raising the stimulus intensity replacing by a wide volley at high intensities (Fig. 2).

Characteristics of EPSC. EPSC were evoked by stimulation of the same sites as for field potentials recordings. Resting membrane potential of (usually non-pyramidal) cells was clamped at $-70mV$. To confirm if the cell is health, before recording EPSC, reducing membrane potential to less negative values Na^+ spikes were triggered. If Na^+ spikes were appeared (usually at -50 to $-40mV$), the cell distinguished health and experiment was continued by whole cell recording. At the youngest ages (starting by P3) we observed some failure responses in EPSC (Fig. 3).

Fig. 2. Representative field potentials recorded in the mouse barrel cortex evoked by stimulating the ventrobasal nucleus (VB) of thalamus or white matter (WM). The responses were recorded in slices taken from mice of 3 through 50 days old. This figure shows the records of only four ages; 3, 10, 20 and 40 days old. Each trace indicates an average of 8 consecutive records.

Fig. 3. Representative EPSC recorded in the mouse barrel cortex evoked by stimulating the ventrobasal nucleus (VB) of thalamus or white matter (WM). The responses were recorded in slices taken from mice of 3 through 17 days old. This figure shows the records of only three ages; 3, 9 and 17 days old. Each trace represents an average of 8 consecutive records.



CV of the thalamocortical fibers from VB to WM:

Whole cell recording. Our results showed that CV of thalamocortical fibers from VB to WM increased considerably over postnatal ages (ANOVA: $F_{6,28} = 286.14$; $P < 0.0001$). While CV_{VB} at P3 was 0.259 ± 0.022 m/s, it increased to 0.477 ± 0.083 m/s at P9 and to 2.99 ± 0.356 m/s at P15. Surprisingly, EPSC recorded in the slices from mice P13 up to P17 (5.591 ± 0.137 m/s) showed an abruptly rising (up to 15 times compared to P3) in CV_{VB} (Fig. 4).

Field potential recording. Regarding technical difficulties in patch clamping on slices from old animals (more than 20 days old) we became encouraged to continue the experiments by field potential recording. Concerning CV_{VB} the data from field potentials partly match those from EPSC (Fig. 5). The results from field potentials also showed a significant increase in CV_{VB} from P3 through P50

Fig. 4. CV_{VB} (closed bar) and CV_{WM} (open bar) of thalamocortical fibers calculated using the latency of excitatory postsynaptic currents and the distance (from the stimulus artifact to the volley of EPSC) between VB and white matter (WM) to the recording site in layer IV of the barrel cortex, respectively. While in CV_{VB} shows a slowly but significant increase from P3 through P13 it indicates an abruptly rise in the older animals. CV_{WM} shows only a slight but significant change over the used ages.

Fig. 5. CV_{VB} (closed bar) and CV_{WM} (open bar) of thalamocortical fibers calculated using the latency of extracellular field potentials and the distance (from the stimulus artifact to the first negative wave) between VB and white matter (WM) to the recording site in layer IV of the barrel cortex, respectively. These results obviously match those obtained by whole cell recording; a gradually increase up to end of the second week and then a rapid enhancement.

(ANOVA: $F_{7,32} = 5.88$; $P < 0.0002$). The values are 0.705 ± 0.172 , 2.793 ± 0.269 , 3.054 ± 0.594 , 3.890 ± 0.777 , 5.378 ± 1.546 , 7.405 ± 1.748 and 8.532 ± 2.532 m/s for slices taken from mice P3, P9, P15, P20, P30, P40 and P50, respectively. These results express a 10 times increase in CV_{VB} (Fig. 6).

CV of the thalamocortical fibers from WM to barrel cortex:

Whole cell recording. In contrast to CV_{VB} , CV_{WM} showed a negligible change (at most 100%) during this time. CV_{WM} at P3, P9 and P15 was 0.091 ± 0.008 , 0.107 ± 0.010 and 0.159 ± 0.016 m/s, respectively.

Fig. 6. Latency change of the field potentials over development. Note that latency of the responses taken from both VB (closed square) and WM (open square) stimulation are merged at a point of about 2 ms approximately at the end of second week.

As shown in Figure 4 there is a considerable change in CV_{WM} over these ages (ANOVA: $F_{7,34} = 3.77$; $P < 0.0039$). For calculating a pure CV_{WM} (distinctly from synaptic delay), at some ages, we stimulated a point between the WM and recording site and obtained CV_{CO} (as described before). The results from these

experiments showed that CV_{CO} and CV_{WM} are almost similar at the related ages.

Field potential recording. CV_{WM} calculated from field potentials demonstrated a small change (at most 100%) from P3 through P50 (Fig. 5). The values are 0.183 ± 0.035 , 0.227 ± 0.269 , 0.418 ± 0.061 , 0.423 ± 0.033 , 0.316 ± 0.022 , 0.334 ± 0.020 and 0.363 ± 0.019 m/s for slices taken from P3, P9, P15, P20, P30, P40 and P50, respectively. These data do not match those obtained by patch clamp recording, meaning that in spite of slowly raising in the CV from P3 through P50 the enhancement is not statistically significant.

DISCUSSION

In general, previous studies have pointed out that individual axons are relatively uniform in thickness and myelination. If so, expectable is a constant CV along the neurite. Indeed, such a conception has been accepted as a truth. Nevertheless, albeit scant, there are a few pieces of reports indicating changes in transmitting time of impulse through a single fiber. In the present study we considered the issue of developmental changes of CV of the thalamocortical fibers in somatosensory cortex. The data pooled by whole cell recording indicated that CV_{VB} significantly increased during postnatal ages with a rapid change from P15. However, CV_{WM} elicited a much less change during this time (15 vs. 2 times for CV_{VB} and CV_{WM} , respectively, when we compared the data from P3 and P17). To calculate the CV, we also recorded field potentials from P3 to P50. The field potentials showed a similar trend in rising both CV_{VB} and CV_{WM} , as did EPSC (10 vs. 2 times for CV_{VB} and CV_{WM} , respectively, when the data from P3 and P50 were compared). Taken together, CV_{VB} increases during postnatal ages, in particular after the end of second week of age, CV_{WM} shows only a little change, albeit significant. Regarding some disparity between the data recorded by patch clamp and field potential methods, as it is expectable according to nature of field potential and EPSC, the recruited fibers can be different even in a given slice. Moreover, while determination of the onset latency in EPSC was easy (because the EPSC was a one part current), it was not the case for field potential (in particular in old animals), where there are multiwave responses. However, as a criterion, we focused on the first postsynaptic response, which sometimes followed a presynaptic activity.

We also considered pure conduction latency of the thalamocortical fibers by field potential recording. We found that values for CV_{CO} fell well within a range of CVs reported for intracortical connections [12-14].

For the first two weeks of postnatal age, the latency of the field potentials evoked by VB stimulation was much longer (on average >5 ms) than that of WM stimulation (on average >1 ms). At the older ages, although the responses recorded by both WM and VB stimulation showed shorter latencies, however, such a decrease in latency was more pronounced by VB stimulation, leading to latencies merged at a point of about 2 ms (on average).

It has been reported that the conduction delays in the central components of both motor and somatosensory pathways rapidly decrease after birth. The conduction delayed in the peripheral components of both motor and somatosensory pathways also decreased initially and then progressively increased in proportion to the length. Eyre *et al.* [15] believe that central conduction delay remains constant at adult age, however, Muller *et al.* [16] observed no evidence for a constancy of central conduction delays.

It is generally accepted that conduction time of impulses along an axon depends upon two parameters: diameter and extent of myelination of the fiber. It has been reported that, due to myelination of corticospinal axons, CV of the fastest corticospinal neurons over their cranial course would reach adult values much sooner than do corticospinal neurons in the spinal cord, which increased with age [17-18]. Also, maximum fiber diameter in both motor and somatosensory central pathways increases in proportion to height, leading to constant central conduction delays with growth [15].

We tried to determine probable causes of different changes in CV of the thalamocortical fibers in and out of the somatosensory cortex. Myelin staining showed that the fibers were stained faintly before the first two weeks postnatal age that confirms previous studies [9]. At the older animals, myelination developed more rapidly in the extra than intracortical fibers. There was little variation in myelination between P30 and P50. Intracortical fibers stained heavier with age but it never match those from VB to WM. Regarding the role of myelination, in some extent, our CV data were similar to those over postnatal development. Also, intracellular staining by neurobiotin demonstrated the change of fiber diameter over development matches that of myelination (unpublished data).

Therefore, these results show that although latency of the responses in the thalamocortical fibers declines

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over two weeks after birth, the CV_{WM} shows little increase. On the other hand, opposite is recordings of older slices where the latency remains roughly unchanged, but CV_{VB} enhanced robustly. We suggest such a difference in the CV might be attributed to variations in myelination and/or diameter of the fibers passing WM to cortex. We concluded that at early age, in spite of rising the transmitting time of the impulses along the fibers, size of the brain is also increases accordantly. In older animals, rising in transmitting time precedes the increase in fiber length resulting in CV enhancement.

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Archive of SID

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