Role of Myelination in the Development of a Uniform Olivocerebellar Conduction Time

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Lang, Eric J. and Jack Rosenbluth. Role of myelination in the development of a uniform olivocerebellar conduction time. J Physiol 89: 2259–2270, 2003. First published December 18, 2002; 10.1152/jphysiol.00922.2002. Purkinje cells generate simultaneous complex spikes as a result of olivocerebellar activity. This synchronization (to within 1 ms) is thought to result from electrotonic coupling of inferior olivary neurons. However, the distance from the inferior olive (IO) varies across the cerebellar cortex. Thus signals generated simultaneously at the IO should arrive asynchronously across the cerebellar cortex, unless the length differences are compensated for. Previously, it was shown that the conduction time from the IO to the cerebellar cortex remains nearly constant at ~4 ms in the rat, implying the existence of such compensatory mechanisms. Here, we examined the role of myelination in generating a constant olivocerebellar conduction time by investigating the latency of complex spikes evoked by IO stimulation during development in normal rats and myelin-deficient mutants. In normal rats, myelination not only reduced overall olivocerebellar conduction time, but also disproportionately reduced the conduction time to vermal lobules, which had the longest response latencies prior to myelination. The net result was a nearly uniform conduction time. In contrast, in myelin-deficient rats, conduction time differences to different parts of the cerebellum remained during the same developmental period. Thus myelination is the primary factor in generating a uniform olivocerebellar conduction time. To test the importance of a uniform conduction time for generating synchronous complex spike activity, multiple electrode recordings were obtained from normal and myelin-deficient rats. Average synchrony levels were higher in normal rats than mutants. Thus the uniform conduction time achieved through myelination of olivocerebellar fibers appears to be essential for the normal expression of complex spike synchrony.

INTRODUCTION

Inferior olivary (IO) neurons are electrotonically coupled via numerous gap junctions (Llinás and Yarom 1981; Llinás et al. 1974; Sotelo et al. 1974). Indeed, the density of neuronal gap junctions appears to be higher in the IO than in any other CNS region (Belluardo et al. 2000; Condorelli et al. 1998; De Zeeuw et al. 1995). This coupling allows IO neurons to generate precisely (on a millisecond time scale) synchronized activity that results in simultaneous complex spike (CS) activity in the cerebellum (Lang et al. 1999; Sasaki et al. 1989; Sugihara et al. 1993; Yamamoto et al. 2001).

Maintenance of the synchronization present in IO discharges presents a challenge for the olivocerebellar system because the length of the olivocerebellar pathway to different points on the cerebellar cortex varies. In rats, there can be more than a twofold difference in the length of the olivocerebellar projection to different regions of the cortex (Sugihara et al. 1993). To preserve the precise synchronization present at the IO level, the conduction time to the different parts of the cerebellum must be relatively invariant despite differences in path length. This is indeed the case in the adult rat (Sugihara et al. 1993); however, whether this invariance holds for larger animals has been questioned (Aggelopoulos et al. 1995), although even in the latter study the latency of CS activity evoked by IO stimuli varied by only several milliseconds.

A previous study identified two mechanisms that the olivocerebellar system uses to achieve a uniform conduction time throughout the cerebellar cortex (Sugihara et al. 1993). First, the diameter of an olivocerebellar axon was found to vary with its projection distance. Thus given the relationship of axonal diameter to conduction velocity, olivocerebellar axons projecting to more distant regions of the cerebellar cortex will conduct faster than those terminating in regions closer to the IO. Second, within a single folium, fibers that had closer targets were shown to take more circuitous routes, minimizing the actual difference in fiber length of axons projecting to the base and apex of the folium. While these mechanisms play important roles in producing a uniform olivocerebellar conduction time, other factors, in particular myelination, strongly influence axonal conduction velocity and may also be involved in organizing the timing of CS activity across the cerebellar cortex.

In this study, we sought to determine the role of myelination in the tuning of conduction velocity in the olivocerebellar system. To accomplish this, we measured the conduction time of the olivocerebellar system in young rats [wildtype (wt)] during the period in which myelination of the olivocerebellar pathway occurs and compared these conduction times to those in myelin-deficient (md) mutants, which lack normal myelin throughout their CNS. The results indicate an important role for myelination in the development of a uniform olivocerebellar conduction time. Furthermore, we used multiple electrode recording to investigate the significance of a uniform conduction time for the generation of synchronous CS activity.

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METHODS
Identification of wt and md mutant animals

Extracellular recordings of CS activity were obtained from both wt and md mutant P14- to P41-day-old male Wistar rats. The md rat is a spontaneous X-linked recessive mutation that results in the virtual absence of myelin within the CNS despite normal myelination of peripheral nervous system axons (Denting et al. 1982). What little myelin does form is abnormal, rapidly deteriorates, and at most, covers a few percent of an axon that would normally be ensheathed throughout its entire extent (Rosenbluth 1987). The affected males appear normal at birth, but die by about the end of the fourth postnatal week. Starting at about age P14, md and wt littermates can be distinguished by an action tremor that develops in the mutants. Subsequently the md animals develop generalized tonic seizures at about age 3 wk. At the time of the experiment, wt and md animals were distinguished by these behavioral differences prior to induction of anesthesia (md rats did not display tremors while anesthetized). At the conclusion of the recordings, the animal was perfused and the brain and spinal cord were dissected out and grossly examined. The presence or absence of myelination (“white matter”) in major pathways (e.g., dorsal columns, corpus callosum) was used to verify the presumed classification based on behavioral criteria. In addition, histological sections from the cerebellum, brain stem, and spinal cord were stained for myelin to provide definitive confirmation of wt or md phenotype (Fig. 1, see Histology).

The experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee of New York University, School of Medicine.

Surgical and recording procedures

Rats were initially anesthetized with ketamine (100 mg/kg, ip) and xylazine (8 mg/kg, ip). Supplemental anesthesia was given as 0.1 ml boluses of ketamine (~6 mg/kg, ip) and xylazine (~0.4 mg/kg, ip) once every 0.5 h or as needed to maintain the anesthetic level. The rectal temperature was maintained at 37°C by an electric heating pad. In some experiments, gallamine triethiodide (~20 mg/kg, iv) was given as a paralytic during the recording stage of the experiment to prevent possible respiratory and stimulus-evoked movement artifacts. The paralytic was given only after all surgical preparations for recording were completed and after the level of anesthetic had been adjusted to maintain a deep state of anesthesia with the absence of spontaneous or reflex-elicited movements.

In some experiments, recordings of CS activity evoked by IO stimulation were obtained using a single extracellular electrode. In others, multiple electrode recordings were obtained of spontaneous and/or evoked CS activity from crus 2a of the cerebellum. In both cases, following anesthetization, a tracheal tube for ventilation and delivery of supplemental oxygen was inserted. The animal was then placed in a stereotaxic apparatus, and the occipital bone and dura were removed to expose the dorsal surface of the cerebellum and medulla.

For the single electrode recordings, the exposed surface of the cerebellum was covered with gelfoam soaked in Ringers solution. A bipolar stimulation electrode was then lowered into the brain stem at its midline just caudal to the cerebellum to stimulate the olivocerebellar axons as they exit the IO. A glass microelectrode (tip diam, 2–5 µm, resistance ~ 5 MΩ, filled with a 1:1 solution of glycerol and 2 M saline) was used for recording CS activity. The electrode was attached to a piezoelectric micromanipulator (Inchworm 6000, Burchleigh, Victor, NY), and inserted into the cerebellar cortex through

![FIG. 1. Lack of myelin in fiber tracts of myelin-deficient (md) animals. A and B: portion of 1 folium in the cerebellar cortex of md rat (A) and normal rat (B) showing a core of white matter (W) between 2 granule cell layers (G). The normal white matter is dark because of intense myelin staining. The md white matter, which is devoid of myelin, is pale. Granule cell layers are comparable in density and filled with small pale granule cells. One-micron sections of Araldite embedded tissue stained with toluidine blue. Scale bars = 50 µm. C and D: toluidine blue–stained 1-µm transverse sections of md (C) and normal (D) rat spinal cord lateral column fiber tracts. C: md rat. Dark-stained nuclei and empty capillaries are visible among bundles of faintly stained axons. No dense myelin profiles are present. D: normal rat. Numerous pale axons encircled by black myelin sheaths are visible. Scale bars in C and D = 50 µm. E: cresyl violet stained cross-section through brain stem at mid olivary level showing the location of stimulus electrode (arrow) at midline between the two inferior olivary nuclei (IO). Scale bar = 500 µm.](http://jn.physiology.org/content/89/4/2260/F1)

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small gaps in the gelfoam. Typically, once CS activity was isolated, the responses to 50–150 stimuli (50- to 150-μs pulse of 150–500 μA) delivered at a rate of 0.5 Hz by an isolated pulse stimulator (Model 2100, A-M Systems, Carlsborg, WA) were recorded.

The multiple electrode technique used in the present experiments has been described in detail previously (Sasaki et al. 1989; Sugihara et al. 1993). In brief, following removal of the dura, a silicon-rubber platform was cemented in place over crus 2a. Glass microelectrodes (same as described above for single unit recordings) were individually inserted, using a piezoelectric micromanipulator (Burleigh), through the platform into the molecular layer of the cerebellum until CS activity could be recorded, which was usually at depths of 70–100 μm below the cortical surface. Spontaneous CSs were identified by their typical characteristics: high-frequency (300–500 Hz) bursts of two to three spikes that occur at an average rate of 1 Hz but that also occur as doublets or triplets with an interburst period of ~100 ms, indicative of an ~10-Hz rhythmicity. After isolation of CS activity, the electrode was released from the manipulator and held in place by the platform. Successive electrodes were inserted in this manner until a rectangular array comprised of 8–10 rostro-caudal columns and 4 medio-lateral rows, with an inter-electrode distance of 250 μm, was completed. Following electrode implantation, the threshold for each recording channel was individually set to detect CS activity. Spontaneous CS activity was then typically recorded for 20–40 min. Following this, a stimulation electrode was inserted into the brain stem, and evoked CS activity was recorded as described above. To ensure that only CS activity was detected throughout the recording sessions, the activity of all electrodes was continuously monitored using a light emitting diode (LED) panel (either real or virtual depending on the recording system, see next section) and an oscilloscope. The LED panel consisted of a rectangular array containing one LED for each electrode. Spike activity that crossed the voltage threshold for detection activated the LED corresponding to that electrode. If the LED panel displayed unusual levels of activity for a particular electrode, the analog signal was viewed directly on the oscilloscope to determine the reason for the activity. Electrodes whose CS activity was contaminated by simple spike activity or other noise were eliminated from the data set.

**Multi-channel recording systems**

The multi-channel recording system that was employed for the initial experiments has been described previously (Sugihara et al. 1993). Briefly, CS signals from all recording channels were converted by the amplifier to transistor-transistor-logic (TTL) pulses on the basis of a voltage threshold, stored on VCR tape, and captured onto a Dell Pentium II personal computer using a digital I/O board (National Instruments, Austin, TX) with a 1-ms inter-sampling period for each channel. The TTL signals were also sent to an LED panel for on-line monitoring of activity as described above. Analog records from these experiments were recorded using an A/D converter (InstruNet Model 100B, GW Instruments, Somerville, MA) connected to a Macintosh G3 computer. In about one-half of the experiments, a PC-based multichannel recording system (Multichannel Systems, Reutlingen, Germany) was employed. This system consists of 128 channels (25 kHz per channel sampling rate, gain 1,000×, 0.2- to 8-kHz band-pass filters) with each channel having an independently adjustable single level spike detector. This system records both the time stamps of the CSs and their waveforms, allowing for off-line confirmation of spike waveforms. The complete analog records of stimulus trials could also be recorded. Finally, an oscilloscope and a virtual LED-type display on the computer monitor were used to monitor CS activity in a similar manner to that described above.

**Histology**

On completion of the recording sessions, animals were perfused intracardially with 0.9% saline followed by 10% formalin solution. The dissected brain was immersed in 10% formalin overnight.

To determine the placement of stimulation electrodes, the brain stem was then switched to a 30% sucrose formalin solution for 2–3 days. Frozen 60-μm-thick coronal sections were cut from the brain stem and mounted on gelatin-coated slides. The sections were stained with cresyl-violet (Fig. 1E).

For demonstration of myelin, spinal cords were immersed in 10% formalin, infiltrated with sucrose, cryosectioned transversely at 30–50 μm, and stained by the Weil method. For higher resolution images, 1-mm transverse segments of cervical spinal cord were immersed overnight in 3% glutaraldehyde/2% formaldehyde in cacodylate buffer (pH 7.3–7.4), rinsed, postfixed in 1–2% osmium tetroxide and 1% potassium ferricyanide in the same buffer, and dehydrated and embedded in Araldite. One-micron sections were stained with toluidine blue in borate buffer. Photomicrographs of cortical fiber tracts were taken with a Nikon Coolpix 990 digital camera using 10× and 40× objective lenses.

**Data analysis**

All data analyses were performed within IGOR (WaveMetrics, Lake Oswego, OR) using procedures written by one of the authors for this programming environment. Unless otherwise stated, statistical comparisons were made using two-sided Student’s paired t-test, and mean values are given with their associated SE.

Calculation of correlation coefficients was performed according to previously developed methods (Gerstein and Kiang 1969; Sasaki et al. 1989). The spike train of a cell was defined as a function X(i), where i represents time in steps of some basic intersampling period (usually 1 ms) and runs from 1 to N, the total number of samples in the recording. X(i) = 1 if the onset of a CS occurs in the ith time step, otherwise X(i) = 0. The spike train of a second cell, Y(i), was defined in the same manner as X(i). The correlation coefficient, C(t), was then calculated using the standard formula for determining a correlation coefficient

\[
C(t) = \frac{\sum_{i=1}^{N} X(i) * W(i-t) \sqrt{\sum_{j=1}^{N} V(j)^2 \sum_{j=1}^{N} W(j)^2}}
\]

where

\[
V(i) = X(i) - \frac{\sum_{j=1}^{N} X(j)}{N}
\]

and

\[
W(i) = Y(i) - \frac{\sum_{j=1}^{N} Y(j)}{N}
\]

and t represents the time lag between compared times of the spike trains. The degree of synchronous CS activity was measured by calculating the zero-time cross-correlation coefficient, C(0), using the equation for C(t) with t = 0. A time bin of 1 ms was used for the different analyses. Note that in experiments employing the new recording system, signals were recorded with a resolution of 50 μs, but the data were binned in 1-ms steps prior to performing analyses.

**RESULTS**

Myelination and nonmyelination-related factors reduce olivocerebellar conduction time between ages P14 and P41

To investigate the effect of myelination on olivocerebellar conduction time, the latencies of CS responses evoked by IO stimulation were measured in 351 Purkinje cells (n = 10 animals) from the apices of cerebellar lobules in the posterior lobe of wt rats aged 14–41 days. Measurements were made for vermal lobules 6 (n = 52 cells, 6 animals), 7 (n = 33 cells, 5
animals), 8 \((n = 10\) cells, 4 animals\), and 9 \((n = 8\) cells, 3 animals\), and for hemispheric lobules crus 2a \((n = 209\) cells, 10 animals\), crus 2b \((n = 23\) cells, 4 animals\), and paramedian \((n = 16\) cells, 4 animals\). Responses were evoked by a stimulation electrode placed in the midline of the brain stem between the two IO nuclei (to excite olivocerebellar axons directly as they left the IO; Fig. 1E) and generally consisted of a high-frequency \((200–500\) Hz) burst of two to four spikes that was similar in shape to spontaneously occurring CS activity (Fig. 2A, asterisks). Occasionally IO-evoked responses consisted of only a single spike, as sometimes occurs following direct axonal excitation of olivary axons. However, even in this case, several criteria allowed confirmation of the IO-evoked nature of the response. First, the depth of the microelectrode from the micrometer readings indicated a superficial molecular layer recording site where dendritically generated \((i.e., CSs) but not somatically generated \((i.e., simple spikes) would be detected. Second, spontaneous activity generally consisted of CSs in the absence of simple spikes, confirming the dendritic location of the electrode as well as the fact that the electrode was recording Purkinje cell activity. Third, the evoked spike was relatively wide, similar to the individual Ca-mediated action potentials that comprise spontaneous CSs and distinct from the shorter Na-dependent simple spikes.

For all cerebellar lobules that were investigated, CS latency to IO stimulation dropped monotonically with age from P14 \((youngest animal in which measurements were performed\) to P41 \((Fig. 2B)\). The most rapid decline occurred between days P14 and P25, with a slight further reduction taking place between P25 and P41, at which point values were similar to those in older adults \((Fig. 2B, diamonds\). Evoked CS responses from crus 2a and lobule 6 Purkinje cells from three wt animals from the same litter are shown in Fig. 2A to illustrate this trend. From P14 to P25, the latencies for these cells from crus 2a \((Fig. 2A, a–c) and lobule 6 \((Fig. 2A, d–f) fell from 12.4 to 5.0 ms and from 21.7 to 5.6 ms, respectively.

Myelination of the olivocerebellar axons is a likely explanation for the rapid drop in conduction time between ages P14 and P25, as myelination of fibers in the inferior cerebellar peduncle \(through which the majority of olivocerebellar axons pass\) occurs during this time period \((Hamano et al. 1996; Rozeik and Von Keyserlingk 1987)\). However, other developmental processes, such as changes in axonal diameter and overall growth of the cerebellum, also occur during this time, and thus may influence the overall conduction time. To separate these factors from myelination-related changes, we compared conduction time changes with age in normal rats to those in md rats, in which myelin is virtually absent from the CNS \((Fig. 1, A–D)\).

At P14, the average latency of IO-evoked responses in crus 2a was 15.09 \pm 0.28 ms for md rats \((n = 12\) ; Fig. 3, A and C, unfilled circle at age = 14 days\), which was similar to what was found for normal littermates at this age \((14.19 \pm 0.71\) ms, \(n = 10\) ; Fig. 3, C, filled circle at age = 14 days\). However, unlike the dramatic decrease that develops in normals, the latency of crus 2a responses in md rats exhibited only a slight decline in latency over the next 10 days \(Fig. 3, A vs. B and C, unfilled circles\). This difference between wt rats and md rats was found for each cerebellar lobule that was investigated, as is shown in Fig. 3, C–F, where the conduction times in wt \((filled circles) and md \((unfilled circles) rats are compared for lobules crus 2a, crus 2b, 6, and 7. Thus in the absence of myelination, the net effect of other developmental factors between days P14 and P24 was a modest decrease in olivocerebellar conduction time of 17–25\%. Although modest, the decrease was statistically significant as the slopes of the regression lines in Fig. 4C were different from zero \((vermis, P = 0.002\); hemisphere, \(P = 0.007\)). In contrast, wt rats had a 62–71\% drop over the same time span. Therefore myelination appears to be the predominant, but not only, factor in the reduction of olivocerebellar conduction time during this period.

Two- to three-week-old wt rats have a nonuniform olivocerebellar conduction time

In normal adult rats, there is little variation of olivocerebellar conduction time across the cerebellar cortex \((Sugihara et al. 1993)\).
We examined whether this was the case for young animals, and found that, in fact, the latency of evoked CSs varied significantly between cerebellar lobules. For example, at age P14, the conduction times to crus 2a (14.19 ± 0.71 ms; n = 10) and lobule 6 (20.35 ± 0.88 ms; n = 6) were significantly different (P = 0.002; Fig. 2, Aa vs. Ad and B). Indeed, comparison of latencies for corresponding vermal and hemispheric lobules from the same animal consistently demonstrated significantly longer conduction times to the vermis for animals younger than 25 days. This is shown in Fig. 2B, which plots the conduction times to various cerebellar lobules as a function of animal age. Each data point represents the average latency for all cells of a particular lobule that were measured in an individual animal (average number of cells per lobule = 11.5 ± 2.0, n = 29 lobules). The regression curves are exponential fits to the grouped data from the hemisphere (solid line) and vermis (dashed line), respectively. For animals younger than 25 days, the cells of the vermal lobules (unfilled symbols, dashed line) had significantly longer response latencies than did the cells of hemispheric lobules at each age (filled symbols, solid line). However, by about P25, the vermal/hemisphere differences (and lobular differences in general) in conduction time were minimal, as indicated by the convergence of the two regression curves and the overlapping of the error bars.
Myelination and the development of a uniform olivo cerebellar conduction time

The convergence of conduction times to a uniform value during the same period in which conduction times drop primarily as a result of myelination suggests that myelination could have an important role in the development of a uniform conduction time. To investigate this issue, the latency differences of evoked CSs in crus 2a and vermis lobule 6 were calculated (Fig. 4A). For md rats, the latency difference between these lobules first increased from P14 to P20 and then declined slightly from P20 to P24, and thus remained ≈4 ms. In contrast, in wt animals, the latency difference between crus 2a and vermis lobule 6 showed a rapid monotonic decline between P14 and P24 that was followed by a slight further reduction between P25 and P41. By day P41, the latency difference approached 0 ms (Fig. 4A, filled circles), similar to that in older adult rats. The contrast between the relatively constant latency difference between crus 2a and lobule 6 in md rats and the rapid decline of the latency differential toward zero in wt rats during the same time period implies that myelination of the olivo cerebellar pathway is a major factor in eliminating conduction time differences to different parts of the cerebellum.

Simply by speeding up conduction velocities, myelination would be expected to cause a decline in the absolute differences in conduction time to different cerebellar regions. However, if the conduction velocities of all olivo cerebellar axons were increased proportionally by myelination (e.g., doubled), the ratio of conduction times to different lobules should remain constant (i.e., the relative differences in conduction time to different lobules should not change). Another possibility is that myelination could disproportionately increase the conduction velocities of longer axons, and thus not only decrease the absolute differences in conduction time to different cerebellar regions but also the relative differences. Consistent with the latter possibility, in wt rats, the ratio of the conduction times to crus 2a and lobule 6 rose from ≈70% at P14 to ≈95% by P41 (Fig. 4B, filled circles), whereas in md rats, the conduction time ratio did not similarly increase. In fact, in md rats, the conduction time ratio for crus 2a and lobule 6 actually decreased somewhat from ≈80% at P14–P16 to ≈70% by P20–P24 (Fig. 4B, unfilled circles).

Results similar to those for crus 2a and lobule 6 were found for all lobules tested. The conduction times from all vermal and hemispheric lobules, respectively, were pooled and plotted as a function of age for normals (Fig. 2B) and md rats (Fig. 4C). In md rats, an ≈4-ms latency differential between the hemisphere and vermis remained throughout days P14–P24 despite a small but steady decline in overall conduction time (Fig. 4C). Indeed, the regression lines’ fit to the vermal and hemispheric data have virtually identical slopes (vermis, −0.423 ± 0.095; hemisphere, −0.426 ± 0.128). In contrast, in the wt littersmates, the conduction times to the vermis and hemisphere converged, as did their regression curves (Fig. 2B). Taking the ratio of the hemisphere and vermis regression curves shows that, for the cerebellum generally (at least for lobules of the posterior lobe) in the wt rats, myelination caused a relatively greater decrease in conduction time to the vermis than to the hemisphere (Fig. 4D, solid line). In contrast, md rats showed no evidence of such a disproportionate decrease in conduction time to the vermis (Fig. 4D, dashed line).

**CS latency varies along the folial wall of md, but not wt, rats**

In normal adult rats, the latencies of IO-evoked CSs recorded at different depths along the folial wall are similar, despite differing distances from the IO (Sugihara et al. 1993). To investigate the role of myelination in achieving a uniform conduction time to different parts of an individual folium, cells were recorded along tracks that started at the rostral or caudal borders of the exposed apical portion of either crus 2a or lobule 6 and proceeded down their walls. Evoked responses were recorded from successively deeper points along the tracks to a depth of 1–1.5 mm.

Three tracks (n = 14 cells) were obtained in P22- to P24-old md rats. In each case, the latency of the evoked responses decreased as the recording electrode was lowered. Extracellular traces from one track along the rostral wall of lobule 6 in an md rat are shown in Fig. 5A and demonstrate the systematic decrease in latency with recording depth. When the latencies were plotted versus recording depth, the slope of the regression line was −5.10 ms/mm (Fig. 5C). The average latency of evoked responses recorded from cells at the apex of the lobule 6 (16.95 ± 0.31, n = 11) in the same rat is plotted at 50 μm depth (Fig. 5C, unfilled triangle). Extrapolation of the regression line shows that this value fits with the predicted value from the depth recordings.

Figure 5B shows evoked CS responses of eight cells recorded from a single track down the rostral wall of lobule 6 in a P41 normal rat. Up to a depth of 1,050 μm, there was no systematic variation of response latency with depth; the difference between the latencies of the cells recorded at 70 and 1,050 μm was <200 μs. However, evoked responses at the two deepest points (1,170 and 1,568 μm) did have slightly shorter latencies than the more superficial cells. Nevertheless, the overall slope of the regression line was only −0.348 ms/mm (Fig. 5C), and in the absence of the deepest two points, the slope of the regression line dropped to −0.068 ms/mm. Neither of these slopes was significantly different from zero (P = 0.93 and P = 0.25). Comparison of the latencies of responses along the folial wall to the mean latency of responses recorded from the apex of lobule 6 in the same animal (4.57 ± 0.09 ms, n = 18; Fig. 5C, filled triangle) shows that there was no significant reduction in latency for cells located along the folial wall relative to the cells located on the apex of the folium. A second track in this animal down the rostral wall of crus 2a produced similar results, with a regression line slope of +0.188 ms/mm (P = 0.61).

Tracks in younger wt animals produced more variable results. In a 25-day-old normal animal, one track in crus 2a showed no reduction in conduction time with depth (slope = +0.291 ms/mm), but in a second track, there was a clear shortening of conduction time with depth (slope = −1.15 ms/mm; P = 0.001). In three tracks (n = 8 cells) in lobule 6 of a 14-day-old normal, there was a consistent shortening of conduction time with increasing depth along the folial wall (slopes = −1.79, −1.61, and −1.21 ms/mm). Thus at times before myelination is completed, the evoked response latencies often decreased with recording depth in normal animals, sim-
ilar to what was observed in md animals; however, with increasing age, and therefore the completion of myelination, the variation in response latency with depth was lost in normal animals.

**CS synchrony levels are reduced in md rats relative to wt rats**

The longer response latencies and their greater variability in md rats should lead to decreased synchrony in spontaneous CS activity. To test whether this is in fact the case, multiple electrode recordings of CS activity were obtained from 243 Purkinje cells in 10 wt rats and 127 Purkinje cells in 6 md rats. The animals ranged between P19 and P28 days old, with the same overall age distribution for both groups (wt rats, 23.2 ± 0.9 days; md rats, 23.6 ± 0.4 days). In both wt and md animals, CS activity was isolated at depths of 70–100 μm below the cerebellar cortical surface and was clearly recognizable by its characteristic multiple peaked waveform, which was similar to the responses evoked by IO stimulation (Figs. 2 and 3).

As in adult normal animals, a rostrocaudal banding organization was observed in the wt rats. An example in which 32 cells were simultaneously recorded from a P21 wt rat is shown in Fig. 6. The bubble graphs of Fig. 6, A–C, show the level of synchronous CS activity with respect to three different reference cells (cell M) for all cells in the recording array. The circles’ areas are proportional to the degree of synchronous activity with the reference cell, and their locations reflect the relative positions of the cells in the array. For each of the selected reference cells, the highest levels of synchrony were found among cells neighboring the reference cell and formed a rostrocaudally oriented band (Fig. 6, A–C). Generally cells

![Image](http://jn.physiology.org/doi/fig/10.1152/jn.00008.2002)
with either no separation in the mediolateral direction (i.e., the longitudinal axis of the folium) or only a relatively small separation (≤500 μm) tended to have higher levels of synchronous activity than those that were more widely separated. This is shown in Fig. 6D, in which the average level of synchrony for all cell pairs in this experiment is plotted as a function of mediolateral separation between cells.

CS synchrony displayed a similar spatial organization in md rats to that in wt rats. The results from a P23 md rat in which 19 cells were simultaneously recorded are shown in Fig. 7. The bubble plots show that the highest levels of CS synchrony tended to occur among cells located within 250 μm of each other in the mediolateral direction (Fig. 7, A–C). Plotting the average synchrony as a function of mediolateral separation for all cell pairs in the array (Fig. 7D) confirmed the impression given by the examples shown in Fig. 7, A–C. Thus the shape of the curve for the md rat is similar to that for the wt rat (Fig. 6D vs. Fig. 7D); however, the absolute values of synchrony were lower at all separation distances. In general, the levels of CS synchrony in md rats were found to be lower than those in wt rats, despite the fact that they showed a similar spatial distribution. Indeed, plotting average synchrony as a function of mediolateral separation for all cell pairs in all animals showed that CS synchrony was reduced to 35–65% in the md rats compared with normals (Fig. 8A).

Several factors could underlie the lower levels of CS synchrony in md rats. In particular, slower olivocerebellar conduction velocities in md rats should lead to more cross-correlograms with nonzero time lag peaks because, although the average conduction time is nearly invariant, there is variability in the evoked response latencies of individual cells, and this variability should be increased by slower conduction velocities. When a 1-ms time bin was used and the bins from −10 to +10 ms were searched, ≈50% of the cell pairs (1,520 of 3,033 pairs) recorded from wt rats had cross-correlograms in which the highest bin was 0 ms. In contrast, only ≈33% of cell pairs (419 of 1,277 pairs) in md rats had cross-correlograms with a peak at 0 ms (P < 0.00001, t-test for the difference between 2 proportions). In both wt and md rats, the percentage of pairs with 0-ms peaks increased as the minimum correlation necessary to be included was increased (Fig. 9A). Nevertheless, wt rats always had a higher percentage of cell pairs with peaks at 0 ms. To quantify further the extent to which the correlogram peaks of md rats were dispersed relative to wt rats, the absolute time lag of each cross-correlogram peak was calculated, and the average value of this time lag was plotted for various minimum correlation values (Fig. 9B). md rats had consistently higher average absolute time lags compared with wt rats, even when comparing cell pairs displaying relatively high levels of correlation (Fig. 9B).

The above results suggest that the greater percentage of cross-correlograms with off-center peaks from md rats is responsible for the reduced levels of CS synchrony shown by these animals compared with normals (Fig. 8, A and B). If this is the case, the difference between wt and md rats should be reduced when one compares the maximum correlation over a range of time lags rather than synchrony (0-ms time lag cross-correlation). Thus the maximum correlation for each cell pair was calculated for time lags of ±10 ms surrounding (and including) the 0-ms time bin and then plotted as a function of mediolateral separation (Fig. 8C). In both cases, wt and md, the maximum correlation curves are higher than the corresponding synchrony curves (Fig. 8, A vs. C); however, the increase for the md curve is substantially greater than that of the curve for the wt rats. This can be seen by plotting the wt/md ratios of the synchrony and maximum correlation curves. The wt/md ratio for the maximum correlation curves is closer to one (where a value of 1 indicates no difference between wt and md) than the ratio of the synchrony curves (Fig. 8, B vs. D). This shows that the greater number of nonzero peaks, due to slower conduction velocities in md animals, contributes to the lower synchronization of CS activity in md rats. Also note that the difference

![Image](http://jn.physiology.org/Downloadedfromhttp://jn.physiology.org/)

**Fig. 7.** md rats have a similar CS synchrony distribution to wt rats. A–C: spatial plots of CS synchrony in a 23-day-old md animal. The results are from a 20-min recording of CS activity from 19 cells simultaneously. The distribution of CS synchrony with respect to 3 different reference cells is shown. Orientation of the recording array on crus 2a and scale for synchrony are shown on bottom right. Note the difference in scaling from previous figure. D: plot of average level of synchrony as a function of mediolateral distance between cells for all cell pairs that were recorded. Error bars indicate SE. Time bin for defining synchrony was 1 ms.
between the synchrony ratio and the maximum correlation ratio tends to grow with increasing mediolateral separation (compare Fig. 8, B and D), which is consistent with the greater differences in length of olivocerebellar fibers to more widely separated parts of the cerebellar cortex. Although reduced, compared with the synchrony ratios, the correlation ratios are still above one, suggesting that other factors than reduced conduction velocity in olivocerebellar axons contributed to the lower levels of CS synchrony observed in md rats. One possibility is that IO neurons may be under increased inhibition from GABAergic afferents, because GABAergic activity not only decreases IO activity but also decouples IO neurons (Lang 2002; Lang et al. 1996). Consistent with this possibility, the average CS firing rates of md rats (0.91 ± 0.06 Hz; n = 127) was reduced (17%) compared with the wt rats (1.10 ± 0.05 Hz; n = 243). This difference was statistically significant (P = 0.015) and was consistently found when comparing littermates (Fig. 10).
In this study we investigated the role of myelination in the development of a uniform olivocerebellar conduction time and the importance of this uniformity for generating synchronous CS activity. By comparing normal and md rats during development, we showed that myelination is essential for achieving a uniform olivocerebellar conduction time and that the increased variability in olivocerebellar conduction time in the absence of myelin leads to a decrease in synchronous CS activity.

Both myelin and nonmyelin related factors shape olivocerebellar conduction time

Comparison of evoked CS response latencies between normal and md rats in this study indicates that both myelin and nonmyelin factors play a role in the reduction of olivocerebellar conduction time. That is, during the same developmental period, olivocerebellar conduction time in both normal and md rats dropped. The drop in md rats (25%) was much smaller than that in normals (∼65%) but nonetheless is significant. Processes that might underlie the nonmyelin-related drop include increases in axonal diameter. In addition, changes in ion channel density and total ionic conductance can modify axonal conduction velocity, as shown by theoretical (Adrian 1975; Hines and Shragr 1991; Hodgkin 1975; P. Shragr, personal communication) and experimental (Rosenthal and Bezanilla 2002) studies.

A previous study of olivocerebellar conduction time to the vermis showed that CS response latencies following IO stimulation dropped steadily from a high at P4 until they leveled off around P22 (Crepe 1971). Our results are generally consistent with this prior study in showing a rapid decline in evoked CS latency from P14 to P25. Moreover, they extend the basic findings to include the hemispheric portion of the olivocerebellar pathway. Although we did not measure conduction times in animals younger than P14, our finding that the evoked response latencies in normals and md rats are nearly identical at age P14 suggests that the decline in conduction time between ages P4 and P14 is largely due to intrinsic changes in the olivocerebellar axons themselves (e.g., increasing axonal diameter or ion channel density) rather than myelination. The fact that evoked response latencies did fall, albeit more slowly, in md rats is also consistent with the idea that factors other than myelin underlie the early decrease in conduction time.

To our knowledge, myelination of identified olivocerebellar fibers has not been directly studied; however, the large majority of olivocerebellar fibers enter the cerebellum through the inferior cerebellar peduncle (Sugihara et al. 1993; Voogd and Bigar 1980), and several studies have looked at the time course of myelination of this peduncle in rats (Hamano et al. 1996; Rozeik and Von Keyserlingk 1987). These studies tracked the levels of myelin basic protein (MBP) and found that MBP levels in the inferior cerebellar peduncle and cerebellar white matter begin to rise rapidly at the end of the first postnatal week and then continue their increase through the third and fourth weeks (Hamano et al. 1996; Rozeik and Von Keyserlingk 1987). These results are generally consistent with our physiological measurements of olivocerebellar conduction time showing a rapid drop between days P14 and P25, after which conduction times were close to the adult values. However, the MBP staining results suggest that myelin-related increases in conduction velocity might start as early as the first postnatal week. In contrast, we found that the conduction times of normals and md rats were similar at P14 and diverged subsequently, which suggests that functional increases in axonal conduction velocity due to myelination do not occur until almost the end of the second postnatal week, at least 1 wk after the appearance of MBP. This delay between increased MBP levels and actual changes in myelin-related increases in conduction velocity may not be unreasonable considering that MBP is among the earliest proteins to be synthesized by oligodendrocytes during myelination and is already present when the initial wrappings of myelin are formed around the axon (Cohen and Guarnieri 1976; Tenenko et al. 1980).

Differential myelination underlies uniform olivocerebellar conduction time

Previously two factors were identified as underlying the conduction velocity tuning of olivocerebellar fibers (Sugihara et al. 1993). First, it was found that within a folium the tortuosity of axons that terminated at the base of the folium was greater than that of fibers that projected to the apex. Thus the actual difference in intrafolial axon length was reduced. Second, axonal diameter was found to be correlated with axonal length, implying that longer axons would have faster conduction velocities to compensate for their greater length. However, these factors are not sufficient to account for the near uniformity of the olivocerebellar conduction time to various parts of the cerebellar cortex. The tortuosity differences would help to minimize conduction time differences within individual folia but would not reduce interfolial conduction time differences. On the other hand, variation of axon diameter could potentially compensate for the different path lengths to the various folia; however, the actual variation that was found only accounted for ∼28% of the length differences, assuming that axonal diameter and conduction velocity are linearly related, as is usually the case in myelinated axons (Johnston and Wu 1995).

The present results indicate that differential myelination of axons going to different cerebellar regions is the major factor underlying the uniform olivocerebellar conduction time of normal adult rats. This conclusion is supported by several of our results. First, in normal rats, significant differences in conduction time to different parts of the cerebellum are present in P14 rats, but largely disappear by age P25. Both the overall reduction in conduction time and the fact that the inferior peduncle becomes myelinated between these ages (Hamano et al. 1996; Rozeik and Von Keyserlingk 1987) suggest that myelination is responsible for the reduction of conduction time variations between cerebellar cortical areas. Second, and more convincing, is that significant conduction time differences remain in md rats over the same developmental period, despite the small reduction in absolute conduction time that did occur in these animals. Third, in normal rats, but again not in the md rats, both the absolute and relative conduction time differences to different cerebellar areas were reduced (Fig. 4, B and D). This implies that the degree to which myelination speeds up an axon’s conduction velocity is proportional to its length.

A simple mechanism for achieving this velocity tuning would be to vary the number of nodes of Ranvier per unit length with olivocerebellar axon length. Such a variation could
easily arise from the known relationships between axon diameter, axon length, myelin thickness, and internodal length. Longer olivocerebellar axons tend to have larger diameters, at least within individual folia (Sugihara et al. 1993). Furthermore, a number of studies have demonstrated covariance of axon diameter, myelin thickness, and internodal length (see Peters et al. 1991). Thus the longer olivocerebellar axons should be more heavily myelinated and have longer internodal lengths than the shorter fibers, and thus conduct faster.

In conclusion, myelin plays a significant role in tuning the individual axonal conduction velocities to compensate for variability in olivocerebellar path length and sets the conduction time to each region of the rat cerebellar cortex to \(
35 - 4\) ms, regardless of its distance from the IO.

**Significance of uniform olivocerebellar conduction time for generation of synchronous CS activity**

The olivocerebellar system generates synchronous, on a millisecond time scale, CS activity (Lang et al. 1999; Llinás and Sasaki 1989; Sasaki et al. 1989; Wylie et al. 1995). The ability to generate synchronous activity has long been ascribed to the electrotonic coupling of IO neurons by gap junctions (Llinás 1974; Llinás et al. 1974). Consistent with this idea, morphological and physiological evidence of this coupling has been obtained (Benardo and Foster 1986; Llinás and Yarom 1981, 1986; Sotelo et al. 1974). In fact, the density of neuronal gap junctions in the IO is one of the highest in the CNS (Bellaudo et al. 2000; Condorelli et al. 1998; De Zeeuw et al. 1995). Moreover, synchronous CS activity remains following block of excitatory and/or inhibitory input to the IO (Lang 2001, 2002), which, given the virtual lack of local chemical synaptic interactions between IO neurons, further implicates gap junction coupling as the origin of CS synchrony.

In summary, the morphological and physiological organization of the IO clearly suggests that this system is designed to generate patterns of synchronous CS activity. However, transformation of simultaneous spikes in IO neurons into patterns of synchronous Purkinje cell CS activity presents a challenge because the olivocerebellar path length varies considerably between the different parts of the cerebellar cortex (Sugihara et al. 1993). In the rat, the distance between the IO and the cortex can vary by almost twofold (Sugihara et al. 1993). However, synchronous CS activity does occur (Lang et al. 1999; Llinás and Sasaki 1989; Sasaki et al. 1989; Wylie et al. 1995), even between widely separated regions of the cortex because of mechanisms that allow a uniform conduction time across the cerebellum (De Zeeuw et al. 1996).

The present results indicate that myelination is a major factor in allowing this synchronization to occur. The importance of myelination as a compensatory mechanism was demonstrated by comparing spontaneous CS synchrony levels in normal and md rats. Multiple electrode recordings showed that synchrony levels were significantly reduced in md rats down to 35–65% of levels in normal rats. This reduction in synchrony was observed even though the multiple electrode recordings were all from the surface of crus 2a, where the variation in olivocerebellar pathway length should be relatively small. The loss of synchronization should be even more severe for Purkinje cells located in different cerebellar lobules or along folial walls. This issue is significant because the bands of synchronous CS activity normally are not localized to the apex of a single folium but often extend down folial walls and across multiple lobules (Sugihara et al. 1993; Yamamoto et al. 2001). Thus a uniform conduction time is required for the normal patterns of synchronous CS activity to occur.

**Possible relevance to action tremor**

It has been proposed that synchronous CS activity may facilitate the timing of muscle activation (Llinás 1991), and damage to the cerebellum or IO leads to a variety of motor symptoms, including action tremors, which may be related to the improperly timed activation of muscles (Adams and Victor 1993; Holmes 1939; Hore et al. 1991; Soechting et al. 1976). Moreover, the patterns of synchronous CS activity have been shown to change in relation to voluntary movements (Welsh et al. 1995). The md rat as well as other animals with generalized CNS myelin deficiency and humans with multiple sclerosis also display action or intention tremors (Rosenbluth 1990). While these tremors could arise from damage to a number of motor structures within the CNS, action tremors are often associated with cerebellar damage, and it is interesting to note that lesions of the cerebellum eliminate the tremor in md rats, suggesting that it has a cerebellar origin (Rosenbluth et al. 1994). The present finding of decreased synchrony in the olivocerebellar system is the first description of abnormal cerebellar activity in md animals, and given the normal relation of CS synchrony to movement, it is reasonable to speculate that the loss of CS synchrony in these animals is in part related to the improper timing of muscle activation leading to action tremor. Unfortunately, since md animals did not display tremors under anesthesia, the relationship between CS activity and the tremor could not be directly measured in the present experiments. Nevertheless, consistent with this possibility, the tremor first appears when these animals are about 2 wk old, the same time at which conduction times in the olivocerebellar system of md animals diverge from those in normals.

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**REFERENCES**


