Non-uniform conduction time in the olivocerebellar pathway in the anaesthetized cat

N. C. Aggelopoulos, C. Duke and S. A. Edgley *

Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK

1. It has recently been demonstrated that conduction velocities of cerebellar climbing fibre afferents in the rat are tuned according to fibre length such that conduction time between their origin in the inferior olive and their target cortical Purkinje cells is constant. Here we have examined the situation in the cat, where individual climbing fibres are substantially longer. Complex spike responses of Purkinje cells located at various depths in the vermis (zones a and b) were evoked by electrical stimulation of olivocerebellar fibres close to their origin and were recorded either extra- or intracellularly.

2. The onset latencies of directly evoked complex spikes ranged from 2·6 to 6·9 ms. A consistent trend in each electrode penetration was that the complex spike latencies were longer for the superficially encountered cells (where olivocerebellar fibre length is greatest) and shorter for deeper cells (where olivocerebellar fibre length is shorter).

3. Linear regression analysis suggests that conduction time in olivocerebellar fibres in the cat is not fixed but varies linearly with conduction distance. Our findings would be consistent with a uniform conduction velocity in olivocerebellar fibres of about 6·6 m s^{-1}.

The functional role of the climbing fibre afferent system of the cerebellum has long been controversial. Recent debate has focused largely on climbing fibres as mediators of synaptic modification in the mossy fibre–parallel fibre–Purkinje cell pathway. An alternative view, based on the remarkable properties of the olivocerebellar system, is that the climbing fibres generate timing signals essential to movement (Llinàs, 1988; Llinàs & Welsh, 1993). The neurones of the inferior olive have a set of membrane conductances which confer on the neurones a tendency for oscillation at about 8–10 Hz (Llinàs & Yarom, 1981). In addition, electrical coupling is common between olivary neurones (Llinàs, Baker & Sotelo, 1974; Sotelo, Llinàs & Baker, 1974). As a consequence of these properties, olivary neurones tend to oscillate in synchrony and the timing of spikes is linked to the periodic oscillation. This tendency for synchronous oscillation in olivary neurones is reflected in a high degree of synchrony of complex spike discharges of Purkinje cells within a folium of the cerebellar cortex (Sasaki, Bower & Llinàs, 1989). These properties need to be taken into account in any consideration of the functional role of the climbing fibre system.

Tight synchrony in complex spike discharges throughout much of the cerebellar cortex would require a remarkable organization since the cerebellar cortex is spatially complex and the climbing fibres are relatively slowly conducting. However, Sugihara, Lang & Llinàs (1993) have recently reported that in the olivocerebellar pathway of the rat, conduction velocity is precisely tuned to conduction distance, such that the delay for conduction in the pathway is uniform at about 4 ms (3·98 ± 0·36 ms; mean ± S.D., $n = 660$). This remarkable property means that all parts of the cerebellar cortex are temporally equidistant from the inferior olive. Such an organization would be likely to be fundamental to the function of the climbing fibre system. Working in the rat, Sugihara *et al.* (1993) were unable to determine the basis of the tuning with certainty, but provided evidence that the calibre of climbing fibre axons is likely to be a contributing factor.

In this paper we have examined the conduction delays in the olivocerebellar pathway in the cat, where conduction distances and conduction velocities of the olivocerebellar fibres are greater. The initial impetus for these experiments was that the greater conduction distances would allow examination of the problem of how a uniform conduction time in climbing fibres arises. However, the results suggest that in the cat olivocerebellar conduction times are not constant, but vary in a manner consistent with a uniform conduction velocity.

Some of these results have been presented as an abstract (Aggelopoulos, Duke & Edgley, 1994).

* To whom correspondence should be addressed.

METHODS

The experiments were performed in three female cats weighing 3·0, 4·8 and 5·1 kg. After induction of anaesthesia with ketamine and xylazine (15 mg kg^{-1} and 1 mg kg^{-1} I.M., respectively), deep anaesthesia was maintained with pentobarbitone (20–24 mg kg^{-1} I.V. initially). Limb withdrawal, corneal reflexes and blood pressure were monitored to ensure adequate anaesthesia. Core temperature was maintained at 38 °C by means of a homeothermic blanket. The head was fixed in a stereotaxic frame and part of the occipital bone of the skull removed to expose the cerebellar surface at the mid-line from lobule V rostrally to the caudal pole of the cerebellum posteriorly. To activate the olivocerebellar fibres, we used a bipolar tungsten stimulating electrode (two sharpened 125 μm wires, varnish insulated and with the tips staggered by 500 μm), implanted stereotaxically to the region where fibres from the caudal parts of the medial and dorsal accessory olivary nuclei decussate (Fig. 1). To avoid the cerebellar cortex, an angle of 45 deg (tip rostral) was used for the approach. Electrical stimuli (square pulses, 0·2 ms duration; repetition rate, 1·0–1·3 Hz) at current intensities usually below 100 μA were used. Accurate placement was verified by monitoring evoked climbing fibre field potentials recorded with a silver ball electrode from the cerebellar surface. These and the subsequent microelectrode recordings were made from the mid-line of the cerebellum, the intention being to record from the a-zone (Groenewegen & Voogd, 1977; Oscarsson, 1980), although we cannot exclude a contribution from the b-zone. To minimize movements of the brain, the stereotaxic frame was raised to extend the neck and the exposed cerebellum was covered in agar solution except lobules V–VIII in the mid-line. The exposed part of the cerebellum was kept under a pool of paraffin oil maintained at 37 °C during the experiment to avoid drying.

In all cases we ensured that the complex spikes were evoked by direct activation of the axon by testing whether the complex spike followed a train of three stimuli (10 or 20 ms intervals between stimuli, Fig. 1*B* and *C*). Responses that failed to follow each stimulus in a train were usually of variable onset latency. These

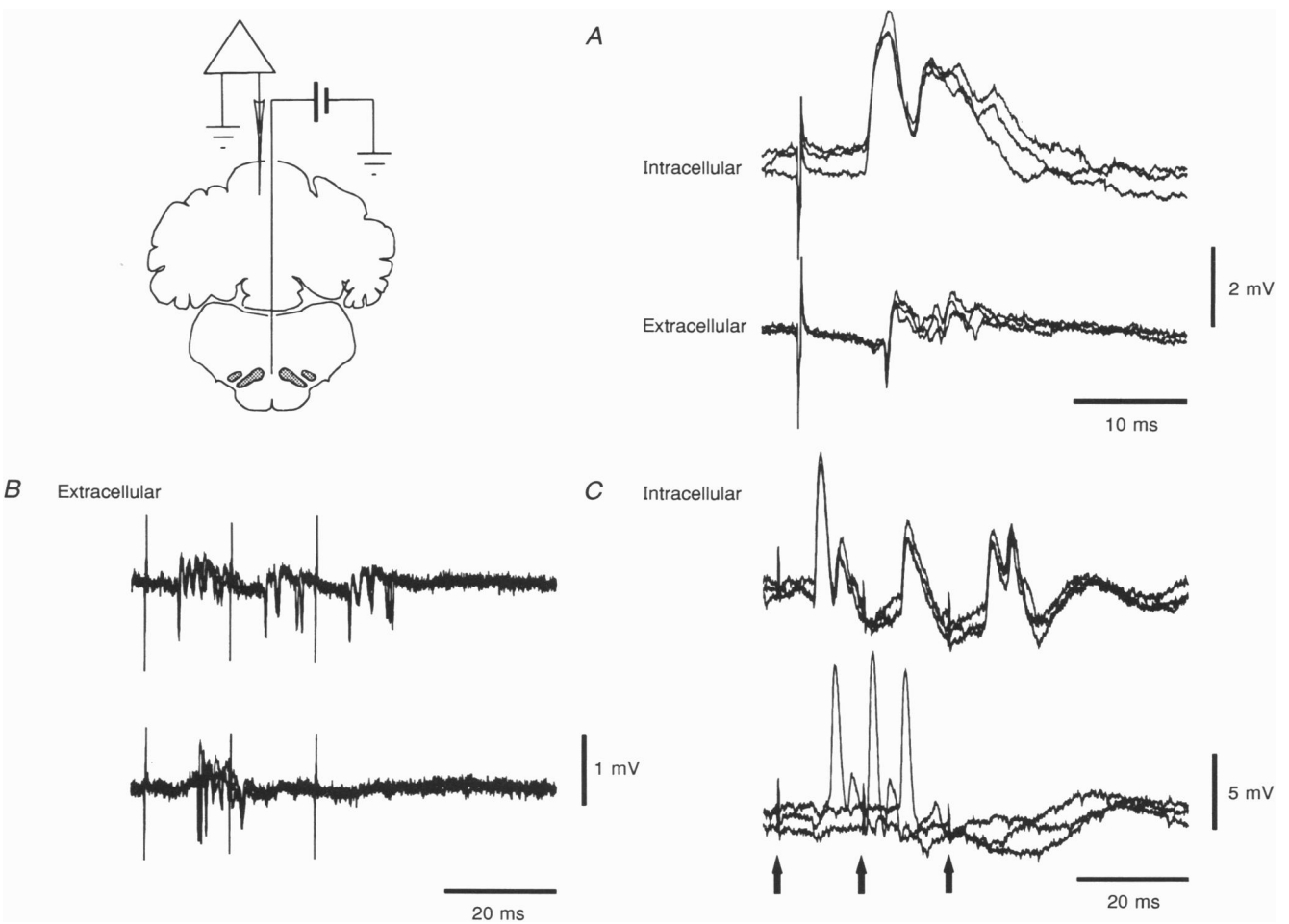

Figure 1. Example recordings from Purkinje cells

The schematic diagram (upper left) illustrates the experimental set-up. *A* shows intracellular and extracellular recordings from the same Purkinje cell. Note that the onset latency of the large EPSP seen in intracellular recordings after spike inactivation is slightly shorter than that of the extracellularly recorded spike. Stimulus intensity was 40 μA. *B* shows extracellular records of directly evoked climbing fibre responses (top trace) with stimuli of 25 μA. Indirect activation of this fibre (illustrated in the lower trace) was seen with weaker stimuli. *C*, as *B* but with intracellular records. Direct activation was achieved with stimuli of 85 μA. The arrows show the timing of the 3 stimuli.

were attributed to indirect (synaptic) activation of the olivary neurones and were rejected.

Extra- and intracellular recordings were obtained from individual Purkinje cells (Fig. 1) in the medial vermis using single glass micropipettes pulled to have a long taper (impedance, 4–13 MΩ). These were filled with 1 M potassium citrate. To allow sampling from deep and superficial cells, the penetrations were made mainly around the primary fissure and the electrode was angled 30 deg tip rostral. All penetrations were made with the same angle and the sites of entry were noted on a diagram to aid reconstruction of recording sites. At the end of each experiment, Pontamine Sky Blue was iontophoretically ejected from a recording electrode to mark the positions of electrode tips. In each experiment, two positions in a single penetration were marked, one close to a Purkinje cell deep in the cerebellum and one 5 mm superficial to this point. These marks allowed determination of the electrode trajectories and correction for any shrinkage in the histological processing. As a further aid to reconstruction, an electrode was placed in the cerebellum at the same angle as the recording electrodes, but in a caudal folium, and was cut off *in situ*. Following transcardiac perfusion with 4% paraformaldehyde, the brain was removed and serial 50–80 μm sections were cut in a vibratome. Mounted sections were counterstained with Neutral Red and the locations of recorded units were estimated from histological reconstruction of the electrode tracks.

Estimating the length of olivocerebellar fibres

Olivocerebellar fibres decussate near their origin, continue laterally and rostrally, pass lateral to the spinal trigeminal nucleus, the external cuneate nucleus and the inferior and lateral vestibular nuclei to enter the cerebellum via the inferior cerebellar peduncle as the 'restiform body' (Voogd & Bigaré, 1980). The length of the olivocerebellar fibres from the medial accessory nucleus of the inferior olive to the end of the restiform body lateral to the anterior division of nucleus interpositus was estimated from the atlas of Berman (1968) to be 15 mm. To this length was added the distance of each recorded unit from this point at the end of the restiform body, which was estimated from the serial sections of the cerebellum, assuming a direct course in the white matter. Tissue shrinkage due to fixation and the histological process was estimated by measuring the distance between Pontamine Sky Blue deposits; all measurements were subsequently corrected for shrinkage by the appropriate factor.

RESULTS

Complex spikes were recorded from a sample of 340 Purkinje cells in the mid-line vermis. Most were extracellular records ($n = 285$) but others were recorded intracellularly ($n = 55$). The form of these potentials was typical of that described previously: in extracellular records the complex spike usually consisted of an initial fast spike followed by a large hump (on which truncated spikes could be seen), but in some cases a full burst of spikes was seen (Fig. 1*A* and *B*). In intracellular recordings the dominant feature was the EPSP from which the first spike took off. On penetration the spikes in most Purkinje cells rapidly inactivated leaving a large underlying EPSP (Fig. 1*A*, top trace). Latencies were measured from the stimulus to the onset of the EPSP (first upward deflection) in intracellular recordings. In extracellular recordings the latencies were taken from the onset of the initial spike. In cases where both extra- and intracellular records were obtained ($n = 17$), the latencies of extracellular spikes were longer than the EPSP onsets. This difference amounted to 0·124 ms on average (±0·03 ms, S.E.M.), but could be as large as 0·5 ms in some cases. The difference was statistically significant ($P < 0·001$, Student's paired t test). Results from the two approaches were therefore kept apart.

All of the recordings accepted for analysis were responses to direct activation of olivary axons, as verified by their fixed shapes and latencies and ability to follow a train of stimuli (Fig. 1*B* and *C*). In some cases indirect activation of olivary neurones, indicated by responses of variable latency which failed to follow trains of stimuli (Fig. 1*B* and *C*), was seen with weak stimuli.

Latencies of complex spikes from the olivary stimulus ranged from 2·7 to 6·9 ms in extracellular records and from 2·6 to 6·5 ms in intracellular records. A consistent finding was that latency varied with the depth of the penetration such that it was shortest in deep cells near the restiform body and longest in cells encountered near to the cerebellar surface. This is illustrated in Fig. 2. Different symbols represent responses with different latency ranges. In each experiment the total number of extracellularly recorded units was rank ordered according to latency, divided into three groups of equal size and the estimated locations of each unit plotted on a sagittal section of the cerebellar cortex. In all three experiments there was a clear trend, in that the units with longest central latencies were located more superficially in the cortex, those with shorter latencies were located deeper in the cortex. These results differ from those of Sugihara *et al.* (1993) and do not support a uniform conduction time in climbing fibres in the cat, but rather suggest that longer conduction distances (superficial units) have longer conduction times, whereas shorter conduction distances (deeper units) have shorter conduction times.

This finding is further illustrated in Fig. 3*A* where the conduction delays in climbing fibres are compared with estimated climbing fibre length. Linear regression analysis of our data indicates that there is a very highly significant variation of conduction time with conduction distance (F test, $P < 0·001$). The upper panel in Fig. 3*A* is a scatter plot for all extracellular units from all three experiments. The conduction velocity expressed as the inverse of the slope of the regression line is 6·6 m s^{-1}. The upper panel in Fig. 3*B* is a similar plot for the sample of intracellular recordings. Although there are fewer observations, the calculated regression line was nevertheless statistically different from the horizontal. The plots in the lower panels in Fig. 3*A* and *B* show the conduction velocities of axons of different estimated lengths. The respective regression lines are not significantly different from the horizontal line, as shown in the figures. These results seem to be consistent with a constant conduction velocity in climbing fibres of about 6–7 m s^{-1}.

DISCUSSION

Our results consistently demonstrate that there is not a strictly uniform conduction time in the olivocerebellar tract in the cat. Rather, the results would be consistent with a constant conduction velocity of 6–7 m s^{-1}. These clearly conflict with the results of Sugihara *et al.* (1993) in the rat. An explanation for this discrepancy is hard to envisage. The observations were made in different species, but *a priori* one would expect that tuning of olivocerebellar conduction velocity to fibre length, if it existed, would be a universal feature rather than a functional specialization of particular species. The recording sites also differ in that Sugihara *et al.* (1993) recorded throughout the cortex whereas we have recorded only from the mid-line vermis, principally the a-zone. Again it is hard to see how this might explain the discrepancy since uniformity of olivocerebellar conduction time within a functional zone would seem to be crucial. A number of observations in the cat cerebellum are consistent with our findings. In the intermediate zone, Garwicz & Ekerot (1994) demonstrated a different latency of climbing fibre field potentials evoked at different depths in the C3 zone of the cerebellar cortex by electrical stimulation within the rostral part of the dorsal accessory olive (see their Fig. 3). Also in the intermediate part of the cerebellar cortex, Armstrong, Harvey & Schild (1973) examined the branching patterns of individual climbing fibres within the cerebellar cortex and found that substantial differences in the conduction time in different terminal branches could be found (1·8 ms in one

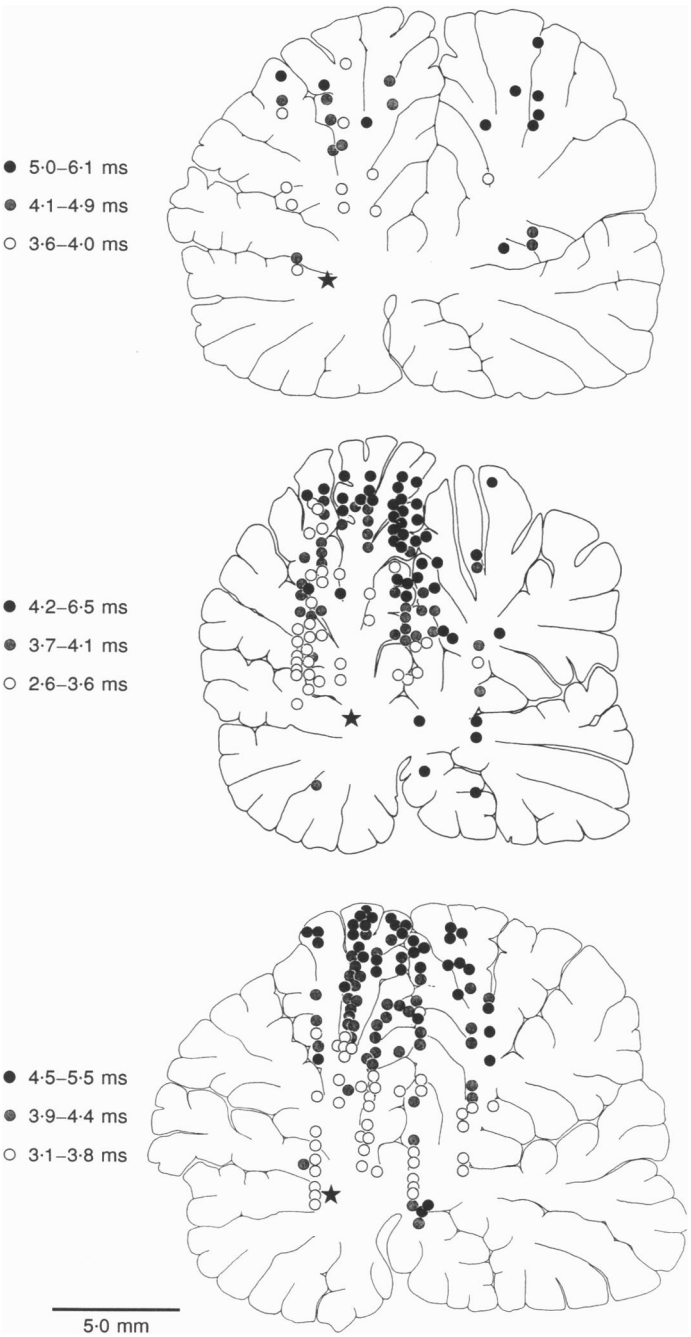

Figure 2. Projection of the position of extracellularly recorded units onto the cerebellar midsagittal plane

For each experiment, the units have been divided into 3 approximately equal sets according to whether they had fast (●), intermediate (◍) or slow (○) responses. The fastest responses were obtained from the units nearest the level of the entry of the inferior cerebellar peduncle containing the olivocerebellar fibres. This area, known as the restiform body, has also been projected to the mid-plane in these diagrams and is marked by ★.

reconstructed climbing fibre arbour). These measurements may be complicated by the fact that antidromic conduction was used to estimate the conduction delays in various segments of the axon and that antidromic invasion of a large proximal segment from a fine distal segment might have a different latency to orthodromic conduction over the same branch point. Nevertheless, these findings together with our observations suggest that a uniform conduction time is not a feature of the cat olivocerebellar system.

The discrepancy between our data and those of Sugihara *et al.* (1993) certainly need not discount the idea that synchronous activity in the inferior olive is a crucial element in cerebellar function. On the basis of our data, synchronous olive activity would produce near synchronous complex spikes in a-zone Purkinje cells. Olivocerebellar fibres cannot be driven at high rates by olivary afferents, and olivary neurones have been shown to oscillate both *in vivo* and *in vitro* at around 8–10 Hz (Armstrong, Eccles, Harvey & Matthews, 1968; Headley & Lodge, 1976; Llinàs & Yarom, 1986). Thus even a difference of 3·9 ms (from our slowest to our fastest recorded response) is small in comparison with the cycle period of the olivary oscillations (about 100 ms). Our data would be consistent with synchrony between complex spike discharges in the a-zone of the cat to within a few milliseconds, rather than to within 1 ms, as reported in the rat (Sasaki *et al.* 1989; Sugihara *et al.* 1993). Intuitively, a jitter of a few milliseconds seems very small for responses which themselves can have a duration of more than 15 ms.

In the event that isochronicity is an essential feature in the operation of the cerebellum, our data would indicate that in the cat, unlike in the rat, function of the mid-line vermis is still compatible with an error of a few milliseconds between Purkinje cell response latencies. Considering the long and

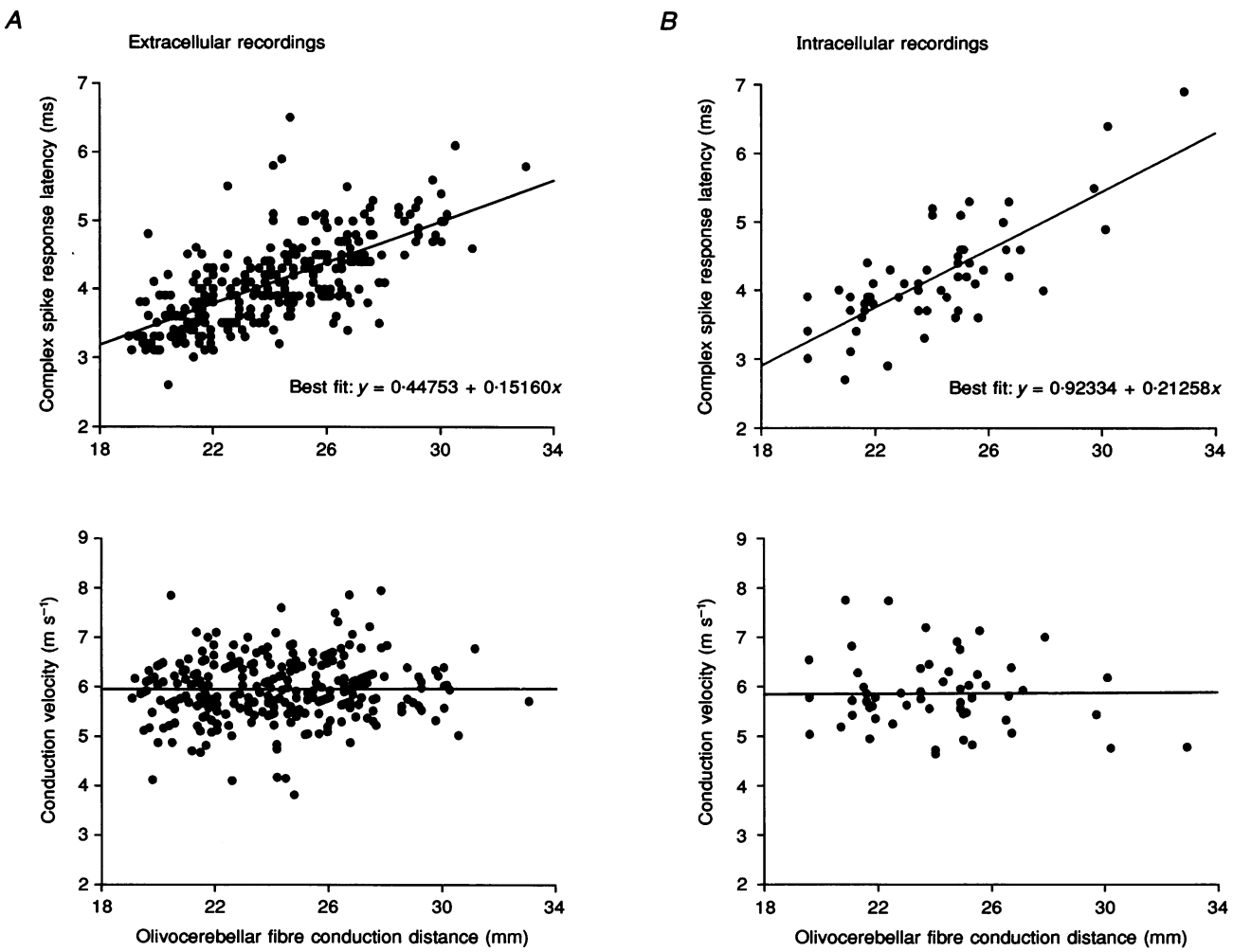

Figure 3

A, a scatter plot of conduction time *vs.* estimated conduction distance at the top and of conduction velocity *vs.* distance at the bottom for the extracellular units ($n = 285$). Conduction velocity has been calculated from the data shown in the top panels by dividing the estimated conduction distance by conduction time for each data point. Regression lines have been fitted. Conduction time increases linearly with conduction distance (top) whereas conduction velocity remains constant (bottom). *B*, as in *A* but for intracellular units ($n = 55$).

complex pathways involved in motor control, the low frequency of olivocerebellar fibre discharge and the long duration of the Purkinje cell response, this small time window in Purkinje cell recruitment may not be critical for movement coordination.

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