Bilaterally synchronous complex spike Purkinje cell activity in the mammalian cerebellum

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Abstract

Complex spike activity was simultaneously recorded from 96 Purkinje cells in the rat cerebellar cortex. Rostrocaudal complex spike synchronicity bands were studied in crus I, IIa and IIb and in vermal lobule 6c. Detailed analysis of complex spike activity was staggered sequentially with a 20–50 cm/sec ‘propagation velocity’ in the mediolateral direction, and that such activity was bilaterally synchronous. The ‘propagation’ of complex spike activity was symmetrical between right and left crus IIa. Temporally, the neurons that aligned in the rostrocaudal direction typically generated complex spikes close to simultaneously. The correlation of complex spike firing was high between crus IIa and crus IIb, moderate between crus IIa and crus IIa. These results indicate that, whilst discrete boarders exist between different isochronicity bands, these bands do communicate with each other in the mediolateral direction via slow ‘propagation waves’ that loosely bind their activity. The results indicate that the olivocerebellar system is organized, bilaterally, to take advantage of the timing signals generated at the inferior olive nucleus.

Introduction

Motor coordination, one of the primary functions of the cerebellum, can be described as consisting of the subtle spatio-temporal control of sequential body movements (Llinaß, 1974). The previous paper described the methods used for the simultaneous recording and analysis of complex spike (CS) activity in up to 96 individual Purkinje cells (Fukuda et al. 2000). The results, confirmed by clustering and principal component analysis, indicate that CS activity is organized in robust and discrete rostrocaudal strips and that isochronicity between strips is not continuous in space. To elucidate further the functional organization of the cerebellar cortex a more detailed analysis in time and space was implemented. In particular, analysis at a high temporal resolution (1 ms) revealed different aspects of the CS firing pattern such as bilateral synchronicity and the mediolateral ‘spreading’ of firing across the cerebellar cortex. Together with previous electrophysiological studies both in vivo and in vitro (Llinás et al., 1974; Llinás & Yarom, 1981a, 1981b, 1986; Benardo & Foster, 1986; Makarenko & Llinás, 1998), demonstrating the presence of electrotonic coupling as well as oscillatory properties of inferior olive (IO) neurons, the present study was designed to investigate whether IO climbing fibre activity affects the timing of motor coordination (Llinás, 1991).

Materials and methods

The experimental procedures and the database for this paper have been described in detail in the accompanying paper (Fukuda et al., 2001). Briefly, 32 or 48 electrodes arranged in four or six rows in the rostrocaudal direction and eight columns in the mediolateral direction, with an interelectrode distance of 166 μm, were individually implanted in the molecular layer of crus I, IIa, IIb, or the vermis 6c of the rat cerebellar cortex (Fig. 1A). Depending on the experiments, a second group of 32 or 48 electrodes was placed in the symmetrical position on the contralateral cortex (Fig. 1B). Assuming that the distance between Purkinje cell somata is 83 μm in the rostrocaudal direction and 55 μm in the mediolateral direction (Ramon y Cajal, 1911) (Fig. 1C), ~23% of the Purkinje cells were sampled by the electrode array when the cells immediately beyond the matrix edge are included.

The amplifier system consisted of three headstage amplifier units and a main amplifier. Signals from each electrode were amplified independently and were transformed into transistor–transistor logic (TTL) levels through window discriminators. All data were stored on VCR tape and were analysed off-line. In the present study, data were sampled at 1-ms intervals, rather than the 10-ms sampling used in the previous analysis (Fukuda et al., 2001). This higher time resolution permitted a more detailed analysis of the spatio-temporal characteristics of CS activity.

Cross-correlation functions were calculated for all data, as described in the accompanying paper (Fukuda et al., 2001) and previous papers (Sasaki et al., 1989; Lang et al., 1999). In the detailed analysis, the peak of the cross-correlation curve was derived from the exact timing t = 0 in some cases, and thus both values of cross-correlation at t = 0 and t = tpeak were calculated to reflect the
exact timing relationship. As a by-product of the cross-correlation, normalized auto-correlation (NAC) values were calculated. The NAC is a measure of the repetitiveness of firing. Peak-to-peak intervals (repetitive time) represent the repetitive frequency.

To analyse the firing pattern in more detail, the absolute time difference (ATD) of firing between neurons was calculated. One of the neurons was designated a master neuron. When this neuron fired at \( t = t_i \), the program determined what other neurons fired within \( \pm 25 \) ms. If other neurons fired at \( t = t_k \), the ATD was calculated as \( |t_i - t_k| \), and the spatial distances between neurons were set to be \( d_{ml} = |x_i - x_k| \) and \( d_{rc} = |y_i - y_k| \) where ‘ml’ indicates mediolateral and ‘rc’, rostrocaudal. The value for each pair was averaged for a period of 7 min with respect to the master neuron. For example, if one neuron, separated 2 divisions (div) along mediolateral direction and 2 div along rostrocaudal direction from a master neuron, fired once 15 ms before the master neuron (\( t = -15 \) ms), the ATD would be +15 ms, and the distance would then be defined as 2 div along the mediolateral direction and 2 div along the rostrocaudal direction. Selecting in turn every neuron as a master element, the ATD, as a function of the distance between neurons, was averaged in the same rostrocaudal and mediolateral distances.

As a variant of the ATD, the relative time difference (RTD) was also determined for all cell combinations and averaged. The only difference between the two was that, unlike the ATD, the RTD evaluated the sign of the time difference \( (t_i - t_k) \). For example, if one neuron consistently fired 15 ms before the master neuron, the RTD would be \( -15 \) ms.

**Results**

In this paper, data listed in Table 1 were used in the analysis of unilateral and bilateral experiments.

**Synchronicity of complex spike firing in unilateral crus IIA**

Because the analysis of multichannel data involves simultaneous changes in the space and time domains for a large number of neurons, it is difficult to represent real-time activity. We chose to use a raster display as illustrated in Fig. 2A, in which the timing of CS activity of 44 Purkinje cells in the right crus IIA is arranged from lateral to medial (top to bottom), as a function of time. This mediolateral variation in the timing of CS activity will be described later. Whilst no characteristic firing patterns were apparent at first glance, careful observation revealed that the Purkinje cells fired synchronously in groups. To estimate the degree of correlation between neurons, a normalized cross-correlation coefficient was calculated for all combinations of 44 neurons. One example of the cross-correlation is shown in Fig. 2B. The dot size represents the degree of cross-
correlation between a given neuron and a master neuron (M). Neurons in the same rostrocaudal column as the master neuron exhibited significantly higher cross-correlation than neurons aligned with the master neuron along the mediolateral axis (P < 0.05, t-test) [note that the ‘t’ of the t-test is different from the ‘t’ used in the cross-correlations]. To represent more clearly the relationship between the cross-correlation coefficient and neuron position, the cross-correlation coefficients for neurons located at a distance of 498–830 μm (3–5 div) from the master neuron was significantly higher in the rostrocaudal direction than in the mediolateral direction (P < 0.05). Tendency of cross-correlation values at t = 0 was similar to those at t = tpeak. Least-square curve fitting revealed that the cross-correlation decreased to 14% for 1 mm in the rostrocaudal direction, and to 25% for 1 mm in the mediolateral direction (Fig. 3Aa and Ab).

Pharmacological studies

The mean ± SD CS firing frequency over a 7-min recording period under control conditions was 1.44 ± 0.64 Hz (n = 44). After i.v. injection of harmaline (5 mg/kg), the mean CS firing frequency increased to 2.75 ± 0.96 Hz. The harmaline effect typically lasted ≈4 h. Its injection remarkably enhanced the synchronous firing of Purkinje cells orientated in the rostrocaudal direction (Sasaki et al., 1989), but only moderately enhanced the synchronous firing of cells orientated in the mediolateral direction, as determined by the cross-correlation coefficients (Figs 2A and B harm, 3Aa and Ab).

Picrotoxin injection (1 mg/kg, i.v.) enhanced synchronous CS firing (Sasaki et al., 1989) in both the rostrocaudal and mediolateral directions (Fig. 2A and B picro, and 3Aa and Ab). At times, all sampled neurons fired simultaneously (Fig. 2A, picro). Although this frequency of firing was not significantly different in the presence of harmaline, the firing patterns following injection of each drug were so clearly different that they could easily be observed in the raster display. CS activity after the picrotoxin injection showed two phases of a characteristic firing pattern: oscillatory firing at 120-ms intervals followed by a low-frequency period lasting several seconds (Fig. 2A, picro).

The first peak of the NAC histogram was at 111 ± 20 ms for spontaneous firing (not shown). After harmaline or picrotoxin injection, it was at 115 ± 21 ms or 106 ± 2.3 ms, respectively. Although the repetitive time after picrotoxin injection showed a very small SD, there was no significant difference in NAC peak time among these three conditions. That is, the oscillatory frequency may be driven by an intrinsic property of the climbing fibre system and may not be influenced by application of drugs.

Time difference in firing between neurons

To analyse the firing pattern in more detail, the ATD of firing between neurons was calculated. An example is shown in Fig. 3Ba and Bb. The ATD for spontaneous CS activity was significantly smaller in the rostrocaudal direction than in the mediolateral direction. After harmaline injection, the ATD in the rostrocaudal direction clearly decreased, whilst the ATD decreased only moderately in neurons in the mediolateral direction. Following picrotoxin injection, the ATD was similar for both directions.

Considering the finding that neurons are highly synchronized in the rostrocaudal direction, ATD values may appear too high, especially for spontaneous activity. However, this might result from the randomness of firing. If the randomness of firing is equal for all combinations of neurons, the influence of randomness on the ATD will be equal. In this case, the gradient of ATD as a function of the distance between neurons is more important than the ATD itself. Between 166 μm (1 div) and 332 μm (2 div) from the master neuron in the mediolateral direction (Fig. 3Ba) the ATD increased sharply, whilst the increase tending to be gradual in the rostrocaudal direction (Fig. 3Bb). This clearly indicates that neurons orientated in the rostrocaudal direction are more synchronized than those orientated in the mediolateral direction.

The RTD was calculated to further clarify the preferred medial-to-lateral order of CS firing. The average RTD in the right hemisphere was negative for neurons in the medial aspect of the cortex for the spontaneous state (Fig. 3C). This indicates that medial neurons fire before lateral neurons, demonstrating a clear preference in firing order. Unlike the ATD results, the RTD values did not increase in proportion to distance. If the firing is completely random, the averaged RTD should theoretically be zero. Whilst all cases of firing are summed in the ATD calculation, most of the RTD cancel each other out, thus leading to an underestimate of RTD.

After harmaline injection, a clear preference in firing order was evident, as shown in Fig. 3C. The RTD was <0.2 ms in the range of 0–332 μm (2 div) from the master cell (Fig. 3C, harm). Neurons within this range fired almost simultaneously. On the other hand, the RTD for Purkinje cells located at a distance of 498–996 μm (3–6 div) demonstrates clear peaks (Fig. 3C, harm). This means that a hypothetical functional unit of synchronously firing neurons may have a width of up to 500 μm in the mediolateral direction, and that harmaline may enhance synchronicity in each such functional unit.

Table 1. Experimental condition and data analysed

<table>
<thead>
<tr>
<th>Expt</th>
<th>Animals (n)</th>
<th>Recording site</th>
<th>Electrodes (n)</th>
<th>Bilateral/Unilateral</th>
<th>Number of recording files</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>Ia</td>
<td>32</td>
<td>Unilateral</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>V</td>
<td>32</td>
<td>Bilateral</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>I + Ia</td>
<td>32</td>
<td>Unilateral</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>I</td>
<td>32 × 2</td>
<td>Bilateral</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>I</td>
<td>32 × 2</td>
<td>Bilateral</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>IIb</td>
<td>32 × 2</td>
<td>Bilateral</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>I + Ia</td>
<td>32 × 2</td>
<td>Bilateral</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>I + IIb</td>
<td>32 × 2</td>
<td>Bilateral</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>IIa + V</td>
<td>32 × 2</td>
<td>Unilateral + (Bilateral)</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>IIa</td>
<td>48 × 2</td>
<td>Bilateral</td>
<td>15</td>
</tr>
</tbody>
</table>

I, Ia, IIb, V, folia of crus I, crus IIa, crus IIb, Vermis in rat cerebellum, respectively.

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but not the connectivity between functional units. After picrotoxin injection, the RTD showed a relatively small monotonic change as a function of distance (Fig. 3C, picro). This result is consistent with the finding of the cross-correlation analysis: most of the neurons in the rostrocaudal and mediolateral directions fired in a time-locked manner following the picrotoxin injection.

**Relationship among different folia in the unilateral side**

CSs from crus I, crus IIa, crus IIb and vermis 6c were recorded to study their correlation among unilaterally different folia. Two arrays of 2 × 8 electrodes were placed on crus IIa and IIb, or crus I and IIa as shown in Figs 4D and 5D, respectively. Two 4 × 8 electrode arrays were placed in the left crus IIa and vermis 6c as shown in Fig. 6D. The mean frequency and mean periodicity for each pair are shown in Table 2 in a series of experiments.

**Correlation between different folia**

The raster display for simultaneous recording between crus IIa and crus IIb in Fig. 4A shows a typical grouped CS firing pattern and synchronicity in the spontaneous state. The cross-correlation of the CS for a master neuron in crus IIa is shown in Fig. 4B. It is obvious that neurons in crus IIb are well correlated to the master neuron in crus IIa and that these values are almost the same between crus IIa and IIb. This suggests that neurons in crus IIb are well organized in band structures along the rostrocaudal direction in similar fashion to those in crus IIa. The averaged cross-correlation of the CS between crus IIa and crus IIb was $0.023 \pm 0.013$.

In crus I recording, the spontaneous CS firing frequency was low compared with crus IIa, although firing synchronicity was clear in both areas (Table 2 and Fig. 5A). When the cross-correlation was
Bilateral synchrony in the cerebellar cortex

Fig. 3. Analysis of time differences among firing. (Aa and b) The cross-correlation averaged for 44 neurons as a function of the distance between neurons for (a) the mediolateral and (b) rostrocaudal directions. The cross-correlation for the distance from 498–996 μm is significantly larger in the rostrocaudal than that in the mediolateral direction. Solid lines (AV), cross-correlation value at the peak of the correlation distribution; broken lines (TOAV), value at time lag 0 in correlation distribution. (Ba and b) Absolute time difference (ATD) for (a) mediolateral and (b) rostrocaudal directions. ATD in the rostrocaudal direction was significantly smaller than that in the mediolateral direction for the spontaneous state. After harmaline injection, ATD decreased sharply in the rostrocaudal direction, whilst ATD decreased slightly in the mediolateral direction. After picrotoxin, ATD for both directions was almost the same. (C) Averaged relative time difference (RTD) for spontaneous, harmaline and picrotoxin recordings. Medial neurons had negative RTD values, whilst lateral neurons had positive values in all cases.

Pharmacological studies

Harmaline injection enhanced the characteristic firing pattern in the crus IIa and vermis 6c, which was similar to that seen in crus IIa (Figs 4A, harm and 6A, harm), that is, synchronized firing of groups of Purkinje cells became more evident. The averaged maximum cross-correlation between crus I and crus IIa was relatively low (0.003 ± 0.003). The vermis 6c neurons showed clear synchronicity with a clear high cross-correlation in the rostrocaudal direction (Fig. 6A and B). However, the correlation between vermis 6c and crus IIa was relatively small with an averaged correlation of 0.009 ± 0.004. It should be noted that neurons in the symmetrical location contralateral to the master neuron show some degree of banding in spite of the low cross-correlation values, showing symmetrical organization (Fig. 6B).

Picrotoxin injection elicited a typical synchronous firing pattern in the vermis 6c were slightly symmetric in the mediolateral direction. This corresponds to the symmetry observed in the cross-correlation
analysis. However, medial neurons in the vermis 6c had higher values than that of the lateral neurons in this case, indicating that in this animal, lateral vermis neurons tended to fire first. The RTD analysis revealed an interesting temporal relationship between vermis 6c and crus IIa. When one of the neurons in the vermis 6c was selected as a master cell, most RTD values in the vermis neurons were positive. On the other hand, RTD values in crus IIa were negative or close to zero. If the time relationship between CSs is random, the RTD is zero. Taking into consideration a relatively small SD of RTD values, this tendency was considered significant. The maximum difference in RTD was 3.67 ms in this case. Considering the surface distance of \( \approx 0.5 \) mm between crus IIa and vermis, the calculated propagation speed was near 14 cm/sec.

**Bilateral recordings in the same folia**

**Bilateral synchrony**

To determine synchronicity of firing in the bilateral crus IIa, 48 electrodes were placed on the right and 48 electrodes on the left crus IIa, in \( 6 \times 8 \) arrays (Fig. 1). The bilateral cross-correlation in the spontaneous state after harmaline and picrotoxin injection is also shown in Fig. 4 of the accompanying paper (Fukuda et al., 2001). Here, as a typical case, the firing pattern after picrotoxin injection is shown in Fig. 7A. A characteristic firing pattern in bilateral recording is bilateral synchrony in the right and left cerebellar cortex. The raster display shows clear alignment of spontaneous firing in the right and left cortex. This bilateral synchrony was also true in the spontaneous state and after harmaline injection.

To determine the nature of bilateral synchronicity, cross-correlation to contralateral site was calculated. Figure 7B shows relationships of cross-correlation averaged on six neurons in the same column as a function of mediolateral distance in the spontaneous state. When a column on the right side of the right cortex was selected as a master column (filled triangle), cross-correlation on the ipsilateral side indicated a sharp band of organization. A symmetrical band of organization (filled triangle, peak position) also appeared on the contralateral side. If a more medial column was selected as a master column, the cross-correlation on the ipsilateral side was negative, indicating an inhibitory relationship. The calculated propagation speed was near 14 cm/sec.
column (open triangle), the contralateral band also shifted medially. This suggests that in the spontaneous state the CS activity defines an organization of bilateral synchronicity. After picrotoxin injection to block inhibition, the bilateral synchronicity became more clear (Fig. 7A).

A group of 64 electrodes was positioned for bilateral crus I recording (Fig. 8D). A raster display of firing and cross-correlation in crus I is shown in Fig. 8A and B, respectively. Neurons tended to fire in bilaterally synchronous groups in similar fashion to that of crus IIa. Indeed, neurons close to the master neuron on the left side demonstrated a higher correlation with cells in the symmetrical location of the contralateral side (Fig. 8B). The frequency of spontaneous CSs in crus I was lower (1.13 ± 0.7 Hz) than the typical frequency of 1.44 ± 0.64 Hz in crus IIa. Harmaline injection enhanced bilateral synchronicity in both areas (Fig. 8A, harm). The firing frequency of crus I Purkinje cells was 2.15 ± 1.14 Hz, which was lower than 2.75 ± 0.96 Hz of typical crus IIa cells. The averaged maximum cross-correlation was 0.040 ± 0.014 for ipsilateral neurons and 0.016 ± 0.007 for contralateral neurons. After picrotoxin injection, the firing frequency (1.50 ± 1.02 Hz) increased above its spontaneous levels, but was still lower than that displayed after harmaline injection. The raster display shows firing in groups and bilateral synchronicity with slight divergence (Fig. 8A, picro). The degree of synchronicity, however, was not as clear as seen in crus IIa. The cross-correlation was enhanced in both the rostrocaudal and the mediolateral directions. The averaged maximum cross-correlation was 0.061 ± 0.026 for ipsilateral neurons and 0.026 ± 0.008 for contralateral neurons.

In another set of experiments two arrays of 4 × 8 electrodes were placed symmetrically in the right and left crus IIb (Fig. 9D). Characteristics of bilateral synchrony in crus IIb were similar to those of crus IIa. Purkinje cells tended to fire in groups and bilateral synchronicity was evident in the spontaneous state and after harmaline injection (Fig. 9A and B). After picrotoxin injection,
Bilateral synchronicity became clear and the repetitive bursts tended to last > 10 s (Fig. 9A, picro). Periods of low frequency firing (≈ 1.5 Hz) lasted for up to 5 s, and then a repetitive firing period (5.3 Hz) followed.

Spatio-temporal timing in bilateral folia

One of the characteristic firing patterns is shown at an expanded time scale on the right side of Fig. 7A. Medial neurons on the bilateral crus

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**TABLE 2.** Mean frequency and periodicity of complex spike activity in various experimental conditions

<table>
<thead>
<tr>
<th>Recording site</th>
<th>Mean frequency (spikes/s)</th>
<th>Periodicity (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spontaneous</td>
<td>Harmaline</td>
</tr>
<tr>
<td>Unilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crus IIa</td>
<td>1.49 ± 0.62</td>
<td>2.62 ± 1.15</td>
</tr>
<tr>
<td>Crus IIb</td>
<td>1.51 ± 0.66</td>
<td>2.56 ± 1.20</td>
</tr>
<tr>
<td>Crus I</td>
<td>1.17 ± 0.81</td>
<td>1.69 ± 0.89</td>
</tr>
<tr>
<td>Crus IIa</td>
<td>1.27 ± 0.29</td>
<td>3.86 ± 1.15</td>
</tr>
<tr>
<td>Crus IIa</td>
<td>1.62 ± 0.77</td>
<td>3.01 ± 1.34</td>
</tr>
<tr>
<td>Vermis V6c</td>
<td>1.73 ± 0.92</td>
<td>2.64 ± 1.56</td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crus IIa</td>
<td>1.44 ± 0.64</td>
<td>2.75 ± 0.96</td>
</tr>
<tr>
<td>Crus I</td>
<td>1.13 ± 0.70</td>
<td>2.15 ± 1.14</td>
</tr>
<tr>
<td>Crus IIb</td>
<td>1.29 ± 0.54</td>
<td>2.79 ± 0.88</td>
</tr>
</tbody>
</table>

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Fig. 6. Simultaneous recordings from crus IIa and vermis 6c. Two arrays of 4 × 8 electrodes were placed in left crus IIa and vermis 6c. (D) Diagram showing position of electrodes. (A) Raster display. This shows clear synchronicity between crus IIa and vermis in all conditions. (B) Dot display of cross-correlation distribution to a master neuron (M) in the vermis. There is a strong correlation in the rostrocaudal direction, whilst the correlation between vermis and crus IIa was small. (C) RTD of the crus IIa and vermis neuron as a master neuron. RTD values in the vermis as a function of mediolateral direction were symmetrical. Other descriptions as for Fig. 4.
IIa fired before the lateral neurons. In order to determine the temporal aspect of this synchronicity and the direction of ‘propagation’ of the CS activity, bilateral RTDs were calculated next (Fig. 7C, similar to Fig. 3C for left–right comparison purposes). As expected, bilateral medial neurons showed negative RTD values, whilst bilateral lateral neurons had positive values. This again demonstrates that medial neurons on both sides of the midline fired first, and the ‘wave’ of CS activity propagated to the lateral region of the folia. In addition, the ATD study clearly showed a symmetrical distribution (Fig. 7D). When a neuron located on the medial aspect of the right hemisphere was chosen as a master neuron (open triangle), there was a clear dip in ATD of the medial neuron on the left side. When the lateral neuron on the right side was selected (filled triangle), there was a clear dip in ATD of the lateral neuron on the left side. These findings indicate that bilateral neurons are synchronized and tend to fire in a symmetrical manner.

The RTD results in bilateral crus I indicate a clear tendency for the medial neuron to exhibit negative RTD values, whilst lateral neurons tended to have positive RTD values (Fig. 8C). The difference of the RTD between medial and lateral neurons was 2 ms. As cells are ≈ 1 mm apart, this indicates a ‘propagation’ velocity close to 50 cm/sec.

For bilateral crus IIb recording, bilateral medial neurons had negative RTD values, whilst lateral neurons had positive RTD values (Fig. 9C), indicating that medial neurons tended to fire before lateral neurons. The difference in the RTD for medial and lateral neurons was ≈ 3 ms, giving an estimated mediolateral ‘conduction velocity’ of 45 cm/sec.

**Discussion**

**Rostrocaudal organization**

Analysis of CS firing in the spatial domain confirmed our previous finding that the CS activity of Purkinje cells orientated in the rostrocaudal direction is more highly correlated than the activity of Purkinje cells orientated in the mediolateral direction (Llinás & Sasaki, 1989; Sasaki et al., 1989; Sugihara et al., 1993; Welsh et al., 1995; Lang et al., 1996; Lang et al., 1999). In addition, recording from a significantly large number of Purkinje cells per unit area (166 μm from each other) has allowed us to visualize more subtle spatio-temporal patterns of Purkinje cell CS activity. Analysis of the cross-correlation between Purkinje cells and the ATD between CSs revealed that a higher correlation of rostrocaudally orientated neurons is produced primarily by two factors. First, CS firing is more synchronous (±25 ms time range). Second, the Purkinje cell synchronized within a narrow time window of a few ms. Moreover, the highly correlated width of the mediolateral Purkinje cells range from 166 to 332 μm (Fig. 3Ba). Anatomical studies have shown that the distributional spans of Purkinje cell somata are ≈ 55 μm in the
mediolateral direction and 83 µm in the rostrocaudal direction (Ramon y Cajal, 1911). This means that the functional unit would encompass 3–7 Purkinje cell columns. Harmaline enhanced the synchrony of CS firing, but was most effective for Purkinje cells in the rostrocaudal direction. Thus, asymmetry in the level of synchronicity between rostrocaudal and mediolateral bands was clear. Because harmaline acts at the level of the IO to enhance T-type calcium current (Llinás & Yarom, 1981a, 1981b), it should not differentially affect the functional organization of the olivocerebellar system. Indeed, it does not, as previously determined (Llinás & Sasaki, 1989; Sasaki et al., 1989). The asymmetry seen in these experiments is probably due to the connectivity between the IO and the cerebellar cortex in that the climbing fibres project to Purkinje cells in a branching, rostrocaudal direction.

In contrast, picrotoxin injection uniformly enhanced the synchrony of CS firing, obliterating the rostrocaudal banding that was so pronounced in the presence of harmaline. Picrotoxin, an antagonist of GABA_¿ receptors, eliminates the decoupling of IO neurons normally provided by activation of GABA receptors located near the gap junctions in the IO glomerulus (Llinás & Yarom, 1981a; Sotelo et al., 1974; Llinás, 1991). It is also possible that the total synchronization produced by picrotoxin may be induced by inferior olivary disinhibition from the other sources besides the cerebellar nuclei. By eliminating this sculpting activity normally provided by the GABAergic system, picrotoxin may enhance functional connectivity in the mediolateral direction in the IO.

Mediolateral 'propagation' wave

Analysis of the timing of spontaneous CS firing revealed that Purkinje cells in the medial aspect of the cortex fired before those placed laterally; thus, CS activity propagated in the medial to lateral direction. RTD analysis showed that Purkinje cells within a rostrocaudal band fire within 0.2 ms of each other. The RTD gradient estimated the medial to lateral 'propagation velocity' at between 20 and 50 cm/sec. This value is within the same range as the conduction velocity of the parallel fibres (Eccles et al., 1966).

It is unlikely that the estimated propagation velocity is related to the parallel fibre input for several reasons: (i) the CS and the simple spike are distinctly different in waveform, making them easily discriminated; (ii) firing frequency of the simple spike is higher than that of the CS; indeed, it is uncommon that frequency of the CS exceeds 10 Hz. In addition, the physical difference of climbing fibre length along the mediolateral direction is unlikely to be the cause of this propagation. The conduction velocity of climbing fibres is ≈ 5 m/s, which is almost 10 times faster than the velocity of propagation described here. Thus, it is reasonable to propose that these time delays arise from the IO nucleus. It is very likely that the speed of propagation is related to the propagation of subthresholds as demonstrated in vitro in the inferior olive (Lampl & Yarom, 1993). The increase of propagation velocity following harmaline and picrotoxin injection is probably due to the increase in effective electrotonic coupling.
In any case, the basic firing pattern is maintained irrespective of drug application: higher correlation in rostrocaudal direction, less time difference in rostrocaudal direction and firing propagation at a specific speed. In addition, an immature form of repetitive firing pattern, which is typically observed after picrotoxin, was sometimes observed even without such a drug. These firing properties were considered to be inherent in the inferior olivary system.

The anatomical and functional properties of IO neurons may play an important role in determining these characteristic firing patterns. All of these firing patterns may reflect the function of IO neurons and their anatomical connection to the cerebellar cortex. The present auto-correlation analysis confirmed the oscillatory behaviour of the CS to be \( \approx 10 \text{ Hz} \) (Llinàs, 1974). This coincides with the oscillatory property of IO neurons (Llinàs & Yarom, 1981a, 1981b, 1986; Makarenko & Llinàs, 1998).

Given this information, the CS firing pattern may be accounted for in the following way. Each IO neuron, having oscillatory properties and being electrically coupled to other IO neurons, may belong to a resonant group – a functional unit. IO axons project as climbing fibres to Purkinje cells and they are distributed in the rostrocaudal plane. IO neurons belonging to a functional unit will be near to each other and thus may be strongly coupled and will tend to spread their axons over the same rostrocaudal cerebellar plane, leading to synchronous CS firing in a rostrocaudal organization. Neurons belonging to different functional units will be less strongly coupled and thus more susceptible to descending or ascending inputs.

The mechanism for dynamic connectivity may lie in modifying the electrotonic coupling between IO neurons (Llinàs, 1974). Indeed, the time period needed for firing propagation between different functional units may be calculated as \(< 1 \text{ ms}\), under the assumption that the dimensions of the cortical functional unit are 166–332 \( \mu \text{m} \) with a propagation speed of 20 cm/sec.

This explanation coincides with the finding that the frequency of physiological tremor is 10 Hz (Llinàs & Volkind, 1973; Brooks & Thach, 1981). The IO may provide a timing clock of 10 Hz, which corresponds with the fact that active movements can occur with a maximum frequency of 10 Hz (Llinàs, 1988).

Although any given functional unit cannot fire faster than 10 Hz, multiple functional units, firing out of phase with a slight delay, would provide motor control with a higher time resolution than 10 Hz. For example, crus IIA has a lateral extension of \( \approx 5 \text{ mm} \) which is equivalent to a time shift of 25 ms, given a propagation velocity of 20 cm/sec. Thus, the relatively slow intrinsic frequency of 10 Hz is but one component of a system with multiple timing clocks that work in parallel with short time delays.

**Interfolial synchronicity**

Purkinje cells in bilaterally symmetrical regions of the cortex fires CSs within short periods of one other. Indeed, the cross-correlation between neurons located in symmetrical locations was, in some cases, higher than that between neurons on the same side. The direction of propagation of CS activity was also symmetrical.

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**FIG. 9.** Bilateral synchronicity in crus Iib. Two 4 × 8 electrode arrays were placed in symmetrical locations in bilateral crus Iib. (D) Diagram showing position of electrodes. (A) Raster display for spontaneous state, harmaline and picrotoxin injection. The firing patterns were similar to those in crus IIA. Neurons tended to fire in groups. Bilateral synchronicity was clearly visible. (B) Relationship between cross-correlation and location of neurons. Neurons closest to the master neuron had relatively high cross-correlation. As a whole, cross-correlations of neurons in the rostrocaudal direction was higher than those of neurons in the mediolateral direction. (C) RTD. Bilateral medial neurons had negative RTD values, whilst lateral neurons had positive RTD values.
It was clear that the firing patterns in crus I, crus IIA, crus IIB and vermis 6c neurons were basically similar. Neurons which fired in groups were usually distributed in rostrocaudal bands and neuronal activity in symmetrical regions on either side of the midline was well correlated in all cases. The degree of bilateral cross-correlation was 0.065 in crus IIB, a comparatively high value.

Although the cross-correlation is a convenient and powerful analytical tool, it is not meaningful for a low number of CS events. The concept of the RTD was introduced to analyse the firing pattern under the latter condition. In crus I, crus IIA and crus IIB, RTD analysis revealed a preferred mediolateral direction of firing propagation. In addition, this preference was bilaterally symmetrical. The firing pattern is not only point-to-point symmetrical, but the macroscopic directionality of firing propagation is also symmetrical.

The degree of synchrony among folia was larger than expected. Although crus I, crus IIA, crus IIB and vermis 6c are neighbouring folia on the surface of the cortex, the anatomical distances among them are relatively large. Crus I receives its main projection from the contralateral medial accessory olive, the dorsal accessory olive and the principle olive (Voogd & Bigaré, 1980; Azizi & Woodward, 1987). This area also receives sensory input, mainly from the head and upper face via the mossy fibre system (Shambles et al., 1978). On the other hand, crus IIA and crus IIB neurons receive projections from the medial accessory olive whilst crus II receives sensory input from the peri-oral area via the mossy fibre system (Voogd & Bigaré, 1980; Azizi & Woodward, 1987). The vermis receives climbing fibres mainly from the medial accessory olive, which may be related to body movement (Azizi & Woodward, 1987; Voogd & Bigaré, 1980).

Irrespective of the origin of climbing fibres within the IO subnuclei, the firing of Purkinje cells in the same rostrocaudal plane is highly correlated. RTD analyses supported this finding in that Purkinje cells in the rostrocaudal plane fired within short times of each other. Anatomical studies have shown that the cerebellar cortex consists of longitudinal parasagittal bands and that each band receives projections from different parts of the IO complex (Azizi & Woodward, 1987).

Our findings provide the functional correlate for the anatomical data with surprising precision, showing that structure and function in the cerebellum are well correlated, as originally proposed almost four decades ago, when electrophysiological studies of the cerebellar cortex began (Eccles et al., 1966).

The finding that the spontaneous CS activity of Purkinje cells over a wide area of the cerebellar cortex is bilaterally synchronized is attractive. The IO neurons may provide CSs as the clock signal to the wide area of the cerebellar cortex. Neurons belonging to the same parasagittal plane are firing in a synchronized manner. Neurons belonging to the neighbouring parasagittal plane have similar but slightly off-phase clock signals from IO neurons.

A few ipsilateral projections from the IO to the cerebellum have been reported in anatomical studies of the rat (Chan-Palay et al., 1977; Brown, 1980). However, the fact that the great majority of IO axons branch at the contralateral IO level before projecting to the contralateral cerebellum provides an anatomical mechanism that may explain the bilateral synchrony. IO axons may send branches or otherwise interact with corresponding contralateral IO cells as they cross the brain stem before ascending in the inferior cerebellar pedunle. Recently, it was demonstrated that olivary dendrites in the dorsomedial cell columns cross from one side to the other and from this to adjacent olivary subdivisions via the T-area (De Zeeuw et al., 1996). Single fibre tracing in the olivocerebellar axon showed bilateral projections crossing the midline within the cerebellum (Sugihara et al., 1999). The bilateral synchrony may be induced by electrotonal coupling within different subdivisions from both sides.

Bilateral synchrony is of fundamental functional significance to motor control and thus must have a robust and stable anatomical and physiological basis. This becomes obvious when considering a few examples. Crus II, which has the largest surface area in the cerebellum, plays a role in coordinating peri-oral and vibrissal muscle activity that must be bilaterally coordinated, even synchronous. Other structures requiring synchronous bilateral control include the midline and antigravissal muscle groups underlying posture and stance. Muscle groups involved in eating, such as the masticatory deglutition and ventilation, will also clearly benefit from synchronous bilateral cerebellar timing control. Further studies will be needed to further clarify the physiological significance of the ‘propagation wave’ in motor control.

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Abbreviations

ATD, absolute time difference; CS, complex spike; harm, harmaline; IO, inferior olive; M, master neuron; NAC, normalized auto-correlation; picro, picrotoxin; RTD, relative time difference; spont, spontaneous activity; TTL, transistor–transistor logic.

References


