Bilateral representation in the deep cerebellar nuclei

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The cerebellum is normally assumed to represent ipsilateral movements. We tested this by making microelectrode penetrations into the deep cerebellar nuclei (mainly nucleus interpositus) of monkeys trained to perform a reach and grasp task with either hand. Following weak single electrical stimuli, many sites produced clear bilateral facilitation of multiple forelimb muscles. The short onset latencies, which were similar for each side, suggested that at least some of the muscle responses were mediated by descending tracts originating in the brainstem, rather than via the cerebral cortex. Additionally, cerebellar neurones modulated their discharge with both ipsilateral and contralateral movements. This was so, even when we carefully excluded contralateral trials with evidence of electromyogram modulation on the ipsilateral side. We conclude that the deep cerebellar nuclei have a bilateral movement representation, and relatively direct, powerful access to limb muscles on both sides of the body. This places the cerebellum in an ideal position to coordinate bilateral movements.

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Elucidating the functional role of the cerebellum in movement control remains an important challenge in neuroscience. It is usually taken for granted that one cerebellar hemisphere controls movements of the ipsilateral side of the body. Cerebellar units modulate their activity with ipsilateral movements (Thach, 1978; Harvey et al. 1979; Smith & Bourbonnais, 1981; MacKay, 1988a,b; Fortier et al. 1989), and focal lesions produce ipsilateral deficits (Mason et al. 1998; Martin et al. 2000; Goodkin & Thach, 2003; Monzee et al. 2004). This is in contrast to the contralateral preference of the primary motor cortex (Evarts, 1966; Muir & Lemon, 1983; Porter & Lemon, 1993).

An ipsilateral cerebellar organization is also supported by the anatomical connectivity. All deep cerebellar output nuclei project to contralateral cortex via contralateral ventrolateral thalamus (Chan-Palay, 1977; Asanuma et al. 1983b; Hoover & Strick, 1999; Kelly & Strick, 2003). Both dentate and interpositus project to contralateral red nucleus (Massion, 1967; Stanton, 1980; Mewes & Cheney, 1991; Horn et al. 2002; Pong et al. 2002). Since both motor cortex and red nucleus are crossed structures, this should lead to an ipsilateral relationship between the cerebellum and limb movement.

Several recent reports have hinted that cerebellar organization may be more complex. Using non-invasive imaging, bilateral cerebellar activation can be seen with a variety of purely unilateral movements (Kawashima et al. 1998; Cui et al. 2000; Kinoshita et al. 2000; Indovina & Sanes, 2001; Ramnani et al. 2001; Ehrsson et al. 2002; Nair et al. 2003). Cerebellar lesions produce measurable deficits in the contralateral arm (Immisch et al. 2003). Finally, some single units in monkey lateral cerebellar cortex appear to modulate with movements of either hand (Greger et al. 2004).

In this study, we have re-examined the laterality of cerebellar organization using two electrophysiological methods in awake behaving monkeys. Firstly, we show that single weak electrical stimuli in the deep cerebellar nuclei can produce facilitation of contralateral, as well as ipsilateral, muscles. Secondly, single unit activity in the interpositus and dentate modulates with contralateral movements. Our results open up new possibilities for the function of the cerebellum in motor control. Rather than narrowly dedicated to the details of a given movement, the cerebellum may play a more integrative role in placing a movement into its wider context, which can include coordination between the two sides of the body.

Methods

Experiments were carried out on two female Macaca mulatta monkeys (E and T), weighing 5–6 kg.

Behavioural task

The behavioural task was the same as used in Soteropoulos & Baker (2006, 2007) and Wetmore & Baker (2004).
The monkey was presented with two precision grip manipulanda, one for the left and one for the right hand. The precision grip task required the monkey to reach out and squeeze two levers with finger and thumb, hold for 1 s and then release. Access to the levers of these manipulanda was initially obstructed by two clear plastic barriers; these were attached to servo motors which could retract the barriers to allow lever access. The animal initiated a trial by placing both hands on home pads, located just in front of the barriers. Following a delay (\(\sim 1\) s), one or both of the barriers vibrated, and LEDs near the barriers flashed. This was a cue to the animal as to whether a left, right or bimanual movement was required. Figure 1 shows an excerpt from a recording session including left, right and bimanual trials showing lever signals (Fig. 1A), muscle activity (Fig. 1B) and cell activity (Fig. 1C and D). The cue was followed by an instructed delay period (\(\sim 1\) s), when the hands remained on the home pads. Both barriers then went down, and the animal moved the cued hand or hands to grip the levers of the precision grip manipulandum (Fig. 1A), and squeeze them above a criterion level. Crossing this level was indicated by a tone. The levers were connected to torque motors and optical encoders, which were controlled by a computer programmed to simulate a spring-like load. After the levers had been held in the target zone for 1 s, a different tone indicated that the animal could release and obtain a food reward. The onset of this tone was used as a behavioural event for analysis of neural activity ('End Hold' event). Premature release of the home pads, or movement of

![Figure 1. Bimanual precision grip task](image)

A, lever position signals and home pad (HP; bar indicates pad depressed) status for left (top traces) and right (bottom traces) hands. B, rectified EMG signals from four muscles recorded bilaterally during the task. C, spike train for an interpositus neuron. D, instantaneous firing rate of neuron in C. This was estimated by convolving the spike train with a Gaussian kernel (50 ms width parameter).
a hand which had not been cued, led to a failure tone and termination of that trial. The trial type (left, right or bimanual) was chosen at random. Only spike responses recorded during unimanual trials are reported here; trials are referred to as ‘ipsilateral’ or ‘contralateral’ depending on the location of the moving hand relative to the recording site.

**Surgical preparation**

After behavioural training was complete, animals were implanted with subcutaneous patch electrodes allowing muscle activity (electromyogram, EMG) to be recorded from seven muscles in each forelimb (Microprobe Inc., Potomac, MD, USA). Wires from the electrodes were tunnelled subcutaneously to a connector on the back (Miller & Houk, 1995). The muscles implanted were triceps (Tri), biceps (Bic), flexor digitorum superficialis (FDS), extensor digitorum communis (EDC), abductor pollicis longus (AbPL), abductor pollicis brevis (AbPB) and first dorsal interosseus (1DI). Following recovery from this surgery, a headpiece was implanted to allow atraumatic head fixation (Lemon, 1984), together with a recording chamber allowing access to the deep cerebellar nuclei (centred at stereotaxic co-ordinates P8.5, L4). The chamber incorporated a small mark, the stereotaxic location of which was measured while the monkey was in the stereotaxic frame. All surgical operations were performed under deep general anaesthesia (2–2.5% isoflurane in 50 : 50 O₂ : N₂O) and were followed by a full course of antibiotics (coamoxyclav 140/35, 1.75 mg kg⁻¹ clavulanic acid, 7 mg kg⁻¹ amoxycillin, Synulox, Pfizer Ltd) and analgesic (buprenorphine; Vetregesic, 10 μg kg⁻¹, Reckitt & Coleman, Hull, UK) treatment (see Wetmore & Baker, 2004). All procedures were carried out under appropriate licences from the UK Home Office.

**Recordings**

Microelectrode penetrations were made in the deep cerebellar nuclei using an Eckhorn multiple electrode microdrive (Eckhorn & Thomas, 1993) loaded with tetrodes (Thomas Recording, Giessen, Germany). Up to six electrodes passed through sharpened guide tubes (30G, one electrode per guide tube), which were inserted into the brain for a few millimetres beyond the cortical dura at the start of each penetration to avoid deviation. Each electrode could be moved independently. For each penetration, the location of the electrodes relative to the chamber mark was noted. As the stereotaxic location of the chamber mark was known, this allowed the location of each electrode for a given penetration to be estimated.

The electrical transient as the electrodes made contact with the tentorium, followed by a burst of cell activity, was used as a landmark for entry into the cerebellum. The descent through layers of Purkinje cells was followed by an area without either cell activity or stimulation effects (lasting typically 1–3 mm). Subsequently, re-entry into cellular activity marked the start of the nucleus. This was verified by the effects of multiple pulse stimulation (see below) and the lack of complex spikes.

Prior to cerebellar recordings, chronic stimulating electrodes (LF501G, Microprobe Inc.) were implanted in the pyramidal tract (PT) bilaterally at the level of the medulla, to allow antidromic identification of corticospinal cells in M1 for a different series of experiments. The EMG responses to contralateral PT stimulation (single biphasic pulse 0.2 ms width per phase, ~1 Hz repetition rate, 200–500 μA intensity, mean number of stimuli 58, range: 12–112) were recorded for both monkeys and the onset latency of the averaged response in each muscle was noted.

Extracellular spiking activity was filtered (300 Hz to 10 kHz) and continuously sampled at 25 kHz, together with EMG activity (bandpass 30 Hz to 2 kHz, gain 500–5K, 5 kHz sampling rate) and task and stimulus markers. Spike waveform files were discriminated off-line into the occurrence times of single unit action potentials using custom-written cluster-cutting software (Getspike; S. N. Baker; Spikelab; Dyball & Bhumbra, 2003). This software parameterized spike waveforms and allowed them to be separated into clusters corresponding to spikes from a single neuron. Only units with consistent spike waveforms and no interspike intervals < 1 ms were used for subsequent analysis.

**Stimulation**

For multiple-pulse stimulation, 18 biphasic electrical stimuli (0.2 ms per phase) were given at 3.3 ms interpulse intervals, 1 Hz repetition rate. Visual inspection of the monkey was used to localize the effects, as well as a stimulus-triggered display of the recorded forearm EMGs. Single pulse stimulation used similar biphasic stimuli, at a repetition rate of 3.3 Hz for monkey T, 6.6 Hz for monkey E. All stimuli were given continuously during task performance to ensure an active background level of EMG. The current intensity during multiple pulse stimulation was incremented slowly up to, but never beyond, 60 μA, until an effect was seen. Most commonly (95% of sites) currents less than or equal to 40 μA were sufficient (mean: 37 μA, median: 40 μA); in two cases effects were seen with currents as low as 10 μA. The current intensity used for single pulse stimulation was the lowest which produced effects with multiple pulse stimulation.

**Analysis of responses to single pulse stimulation**

Stimulus-triggered averages of rectified EMG were compiled for 100 ms either side of the stimulus. The
average was expressed as a percentage of the mean pre-stimulus level. To quantify a response a putative response region was selected, guided by where the pre-stimulus average exceeded a level equal to the baseline plus two standard deviations (see Fig. 2A). The pre-stimulus region was then divided into sections, each as wide as the selected response (Fig. 2A); the number of sample points crossing twice the upper standard deviation level for each pre-stimulus section was then noted (Fig. 2B). This was repeated for the pre-stimulus regions of responses to stimulation at all recording sites for this particular muscle and animal, regardless of whether a significant effect was present in those sessions or not (Fig. 2C, D, E and F). This resulted in a large number of counts (> 200) when collated across the recording sessions (the actual value varied depending on the width of the response, Fig. 2G). If the width of the response was larger than 99.5% of the values determined from the baseline sections then the response was considered to be significant ($P < 0.005$, Fig. 2G). This conservative statistical threshold was used since the response region was freely chosen; multiple tests were therefore implicitly being carried out (though only the most promising region was actually tested). For significant responses, the onset latency and height of the response above baseline were then noted.

Single unit analysis

The activity of single units was analysed by creating peri-event time histograms (PeTHs) aligned to the End Hold task marker (3 s before to 2 s after) for ipsilateral and contralateral trials, using 0.1 s-wide bins. The peak modulation was measured as the difference between the largest and smallest bin. A shuffling method determined whether this modulation was significantly different from zero. Interspike intervals for each trial were shuffled randomly, the PeTH was recalculated, and the peak modulation measured. This was repeated 500 times, with different random shuffles. If the modulation of the unshuffled PeTH was larger than 475 of the modulations after shuffling, the cell was assumed to be significantly modulated ($P < 0.05$).

Histology

Following the end of recordings, the monkeys were deeply anaesthetized and perfused through the heart with phosphate-buffered saline (pH 7.2) followed by fixative (4% formal saline). The brain was removed, sectioned and stained with cresyl violet. The individual microelectrode tracks were not clearly visible due to the small size of the electrodes used (Mountcastle et al. 1991; Swadlow et al. 2005). Microscopic examination revealed some glial scars (Fig. 3), whose trajectory in successive sections was used.

Figure 2. Stimulation response significance testing

A, stimulus-triggered average of EMG from FDS muscle in monkey T. Dotted lines are the 2 standard deviations (SD) level, based on the prestimulus region. User-selected response region was 3.8 ms (19 bins) wide. Pre-stimulus region was divided into 25 3.8 ms sections. The numbers above each section correspond to the number of sample points which crossed the 2 SD line. B, histogram of the number of bins in the pre-stimulus section which crossed the 2 SD line, for the stimulus-triggered average shown in A. C and E, stimulus-triggered average for the same muscle following stimulation at different sites. The pre-stimulus region was also divided into 25 3.8 ms sections. D and F, histogram of the number of bins in the pre-stimulus section which crossed the 2 SD line, for the stimulus-triggered average shown in C and E. G, cumulative histogram across all recording sessions and stimulus-triggered averages for FDS muscle in monkey T. The arrow marks the number of bins which crossed the 2 SD line in the response region shown in A.
to determine the likely target nucleus of the penetrations. This analysis suggested that the bulk of the penetrations were to nucleus interpositus in both monkeys, while in T there were also some tracks towards medial dentate.

Given the small number of the visible gliosis tracks and the fact that in certain anterio-posterior locations in monkey, dentate and interposed nuclei are in very close proximity, it is not possible to exclude the possibility that

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Figure 3. Histological reconstruction of penetrations
Histological tracing of gliosis scars from penetrations (red dots) superimposed on the outline of the cerebellar cortex and deep cerebellar nuclei, in the two monkeys used in this experiment. Numbers beside each section indicate approximate anterior–posterior (AP) location relative to the interaural line. The majority of the tracks headed for nucleus interpositus in both monkeys, although in T there is a minority headed for medial dentate. Yellow corresponds to fastigial nucleus, grey to interpositus, and cyan to dentate nucleus.

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some sites were in the dentate nucleus. Tracks heading for the fastigial nucleus were rare. Additionally, stimulation of the fastigial nucleus frequently elicits saccades (at current intensities as low as $\sim 10 \, \mu A$, Noda et al. 1988), which were never observed in response to multiple-pulse stimulation. It is therefore unlikely that tracks targeted this nucleus. Accordingly, in this paper we will interpret our results by considering the possibility that both dentate and interpositus contributed to the recording and stimulation sites.

**Results**

**Responses to stimulation**

We carried out stimulation at 54 penetration sites within the interpositus and dentate nuclei of two monkeys (24 in monkey E, 30 in monkey T). There was a response to a train of stimuli (18 shocks) for 42 of those sites in at least one ipsilateral muscle. In addition, 26 sites showed a response in at least one contralateral muscle. There was a difference in the frequency with which contralateral responses were encountered in the two animals: 19/30 sites (63%) in monkey T, compared with 7/24 sites (29%) in monkey E. This most likely reflects minor differences in the location of the recordings, which appeared from postmortem histology to be slightly more anterior in E than in T (Fig. 3). It may also be related to some penetrations in monkey T targeting the medial dentate nucleus. Contralateral responses to multiple pulse stimulation often led to visible twitches but could also be seen in individual EMG sweeps (Fig. 4A). For the site illustrated in Fig. 4B, there were responses in averages of rectified EMG for nearly all ipsilateral and contralateral muscles from which we recorded. Pairs of EMGs were tested for electrical cross-talk (Kilner et al. 2002) but this was not found to be substantial (cross-correlations < 0.1).

The use of multiple stimulus pulses at high frequency is likely to cause excitation to spread over several synapses, and makes latency estimation difficult. Single pulse
microstimulation is a more precise way to assess connectivity, but requires thousands of stimuli to produce clear averaged responses (Cheney & Fetz, 1985). All sites with single pulse stimulation analysed here had more than 1800 stimuli available (mean 4732, range 1800–12 690).

Example results from single pulse stimulation in each monkey are shown in Fig. 5A. For each muscle illustrated, there was a significant response in both ipsilateral and contralateral EMG. Figure 5B demonstrates that the effects were repeatable. Stimulus-triggered averages of a single selected EMG using the first 2000 and last 2000 stimuli available for each recording have been overlain. The similar appearance of responses in each epoch indicates that they are not due to, for example, abnormal synaptic facilitation following the prolonged stimulation.

Across the whole dataset, there were 58 (12 in monkey E, 46 in T) contralateral muscle recordings showing significant facilitation, whereas significant facilitation was seen in 126 ipsilateral muscle recordings (51 in E, 75 in T). There were six instances where the primary effect (ipsilateral or contralateral) was a depression of EMG activity, but due to the small number these were not quantified any further. We also noticed that stimulation at some sites produced responses in leg muscles, sometimes bilaterally; occasionally stimulation at one site could produce twitches simultaneously in all four limbs. However, as no EMG electrodes were implanted in the legs further quantification of these effects was not possible. Figure 6 shows the estimated stereotaxic location of the sites where single pulse stimulation was carried out. In the monkey, the majority of the dentate nucleus is more lateral than 5 mm from the midline. It is clear that the majority of the bilateral stimulation effects were more medial than this, implying involvement of the interpositus nucleus.

A single site was often capable of activating multiple muscles. Figure 7A shows the patterns of co-facilitation that were observed. Each row of this figure relates to sites where the muscle labelled was facilitated – the numbers on the diagonal show the number of such sites encountered. The rows grouped at the top relate to muscles ipsilateral to the stimulation site; those below to contralateral muscles. The black squares represent the fraction of instances when the muscle labelled at the bottom of the panel was also facilitated. Where a black square is the same size as the white diagonal squares, this indicates that activation of the reference muscle was always accompanied by activation of the other muscle. Thus, for example, the top row relates to the 26 sites which produced facilitation in the ipsilateral 1DI muscle. Almost all of these sites also facilitated the ipsilateral AbPL muscle, whilst facilitation of the contralateral AbPB muscle from these sites was rare.

The activation seen was clearly highly divergent, but showed no obvious pattern. There was no apparent grouping of responses by flexors or extensors, or by proximo-distal location along the arm. Whilst it was often the case that the same muscle was activated on both sides, this was not always so. Table 1 compresses
the data of Fig. 6A into a simple contingency table, examining the association between ipsilateral and contralateral responses. A $\chi^2$ test on this table revealed a significant association between rows and columns ($P < 0.03$), showing that a muscle was coactivated on both sides more frequently than expected by chance.

Figure 7B shows how frequently each recorded muscle was activated on the ipsilateral and contralateral sides. With the exception of triceps, ipsilateral muscles were more often activated than their contralateral homologues, while for either laterality 1DI and AbPL were the two most frequently activated muscles. Figure 7C provides details of the degree of divergence; it shows the distribution of the number of muscles with a significant response per stimulation site. Whilst 35% (19/54) sites had responses in four or more ipsilateral muscles, this degree of divergence was rare for contralateral responses (only 3.7% (2/54) sites with four or more responding muscles).

Figure 8A compares the magnitudes of all facilitations seen in ipsilateral and contralateral muscles. Magnitude was measured as the peak height above baseline in the averages of rectified EMG, expressed as a percentage of the baseline. Thus a response of 50% had a peak 150% of the baseline level. The response distribution had a long tail for ipsilateral muscles, and ipsilateral responses were significantly larger than contralateral responses ($P < 0.05$, Wilcoxon test; median response size 23% (interquartile range 16–44%) for ipsilateral, 12% (9–18%) for contralateral responses). Figure 8B presents the paired comparison of response magnitude for the 30 instances where a muscle was facilitated on both sides by a single site. Once again, ipsilateral responses were often larger (21/30) than those in contralateral muscles (ipsilateral: median 19%, interquartile range 11–28%; contralateral: 13%, 9–19%, $P < 0.05$, Mann–Whitney $U$ test).

Figure 8C presents a similar comparison of the onset latencies for all responses observed. Most latencies were shorter than 10 ms, although there appears to be a second, smaller peak in the distribution around 20 ms. There was no significant difference between the latencies of ipsilateral and contralateral responses ($P > 0.1$, $t$ test). Figure 8D shows a pairwise comparison of onset latency for the instances where a site facilitated homologous muscles in each arm. The points appear to cluster around the identity line, and there was no significant difference between the two sides ($P > 0.05$, Mann–Whitney $U$ test).

One possible pathway by which the dentate or interpositus could influence muscles is via the primary motor cortex and pyramidal tract. In this case, the response latencies in a particular muscle after cerebellar stimulation should be longer than those expected from the cortex. Fortunately, as part of a related experiment in these animals, we also carried out pyramidal tract stimulation. This provided a direct measurement of the latency of contralateral muscle responses following corticospinal activation. Figure 8E plots the latency following cerebellar stimulation at a particular site, versus the latency following PT stimulation for the same muscle in that animal. Responses in triceps were omitted from this plot, as

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**Figure 6. Map of single pulse stimulation effects**

*Panel A,* histogram of medio-lateral location of stimulation sites. Black bars represent bilateral effects, grey bars ipsilateral only, and open bars represent no effects. *Panel B,* estimated stereotaxic location of the electrode tips in the single pulse stimulation part of the study, for both monkeys. Filled circles represent locations where both ipsilateral and contralateral effects were seen, while open circles are when only ipsilateral effects were observed. Crosses mark sites where no effects were seen.
Table 1. Contingency table showing the relationship between responses in each arm, combined across muscles

<table>
<thead>
<tr>
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<th>Ipsilateral muscle responds</th>
<th>Ipsilateral muscle does not respond</th>
<th>Totals</th>
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<tbody>
<tr>
<td>Contralateral muscle respond</td>
<td>30</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>Contralateral muscle does not respond</td>
<td>96</td>
<td>224</td>
<td>320</td>
</tr>
<tr>
<td>Totals</td>
<td>126</td>
<td>252</td>
<td>378</td>
</tr>
</tbody>
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no PT-to-EMG latency values for this muscle were available.

If we are to compare these two latencies to determine whether the responses to interpositus stimulation pass via the cortex, we must take into account two factors. The first is conduction via cerebello-thalamic and thalamo-cortical pathways. The literature suggests that the earliest effects in M1 following cerebellar nuclei stimulation occur with a 2 ms latency, although 3–4 ms is a more commonly reported value (Shinoda et al. 1985a; Holdefer et al. 2000; Yamamoto et al. 2004). Secondly, we must allow for conduction from M1 to the pyramidal tract at the

Figure 7. Co-facilitation muscle patterns during stimulation

A, each row relates to sites where the muscle labelled on the left was activated. The white squares on the diagonal show the number of sites available for analysis. The area of the black squares represents the fraction of instances when the muscle labelled at the bottom of the panel was also facilitated. A black square the same size as the white diagonal square indicates 100% co-facilitation. B, percentage of sites showing responses in each recorded muscle. C, percentage of sites activating different numbers of ipsilateral and contralateral muscles. Bar labelled ‘1’ relates to sites with no divergence; bar labelled ‘7’ relates to sites facilitating all recorded muscles on that side.
medulla, where our PT-stimulating electrodes were placed. In the monkey, the shortest such delay is 0.7 ms (Lemon et al. 1986). Accordingly, we would expect cerebellar latencies to be at least 2.7 ms longer than PT latencies. This relationship is shown by the line on Fig. 8E. Note that this is a highly conservative estimate; it is likely that responses mediated via M1 would have somewhat longer latencies than indicated by this line.

More than half of the points in Fig. 8E (101/184, 55%) are to the right of the line, indicating that the cerebellar responses were at too short a latency to pass via M1. This was the case both for ipsilateral and contralateral responses (open versus filled circles, Fig. 8E, 72/126 and 29/58, respectively), and for responses in all muscles regardless of their proximo-distal location along the forelimb. The data are re-plotted in Fig. 8F as a histogram showing the difference between the cerebellar and PT latencies.

**Single unit recordings**

A total of 82 single units were recorded from the deep cerebellar nuclei during the performance of a minimum of five ipsilateral and five contralateral trials. When the task dependence of this discharge was examined using PeTHs, it was clear that many units modulated their firing during trials performed with both the ipsilateral and contralateral hands. Figure 9 shows examples of four such units. It is striking that a clear rate modulation was seen in all of these cells regardless of which hand was moving, although
Figure 9. Rate modulation with ipsilateral and contralateral trials

A, B, C and D, PeTHs and dot rasters of different cerebellar units that modulated their firing rate with ipsilateral (Aa, Ba, Ca, Da) and contralateral (Ab, Bb, Cb, Db) trials. Numbers on the right of the PeTHs correspond to trial number for the rasters. E, upper traces are histograms showing the distribution of homepad and lever release events, for a single representative session, compiled relative to the End Hold marker used to align (A–D). Lower four traces provide representative lever displacement signals, also aligned to the End Hold marker. F, rectified and smoothed EMG for 3 distal muscles during trial performance, aligned to End Hold.
the size of the rate changes may differ. Using the shuffle test described in Methods, we found that 90% of cells modulated significantly with ipsilateral, and 83% with contralateral trials; 79% modulated with both.

These results appear to support the findings from stimulation, and to show that the cerebellum has an important role in the control of both ipsilateral and contralateral movements. However, there is a possible confounding factor. Although the task required the animal to maintain the hand which was not moving on the home pad, it is possible that some small muscle activation was nevertheless generated on the side contralateral to the cued movement. Since we made bilateral EMG recordings in every recording session, we were able to examine this possibility in detail. At the bottom of Fig. 9 are averages of rectified EMG for ipsilateral and contralateral trials. It is clear that there is indeed an EMG modulation in some muscles of the non-moving limb, although this is very small compared to the activation seen during movement.

Figure 10A presents the PeTH of a single unit constructed using contralateral trials. This unit appears to pause its discharge as the levers are squeezed. Figure 10B shows averages of rectified EMG from muscles ipsilateral to the recording site. This arm was not permitted to move during these trials. However, a clear modulation was visible in activity from several muscles. This modulation occurred at a similar time to the change in the single unit discharge. Whilst the muscle activation was not sufficient to move the hand from the home pad and cause a trial failure, it obviously confounds any interpretation of the simultaneously recorded single unit activity as relating to contralateral movements.

Fortunately, not all trials appeared to be accompanied by such unwanted muscle activation. We used a program which allowed interactive exclusion of trials based on the level of EMG in selected muscles over defined windows. Of the 45 contralateral trials which had been recorded for this unit, only six survived this selection process. Figure 10D presents the average of rectified EMG for these trials. There was no change in the activation of any of the recorded ipsilateral muscles prior to the end of the hold period.

Figure 10C presents the PeTH of the cerebellar unit using only these trials. The smaller number of trials obviously degraded the signal : noise ratio of this PeTH compared to Fig. 10A; however, the modulation in rate was strikingly similar both in magnitude and time course.

A similar analysis for three further units is illustrated in Fig. 10E and F. Figure 10E presents the PeTH compiled using all available contralateral trials; Fig. 10F shows the PeTH for trials selected to show no changes in ipsilateral EMGs prior to the end of the task hold phase. In each case, the modulation in unit firing rate was very similar.

For each recording, we performed the trial selection to exclude contralateral trials with ipsilateral muscle activation. After this selection process, PeTHs of unit activity were recompiled. Thirteen neurons had at least five trials which survived the rigorous trial selection process. Of these, 6/13 showed a significant modulation in rate during contralateral trials after trial selection, and 11/13 prior to trial selection. However, we deliberately imposed especially stringent selection criteria, to be sure that there was no modulation of ipsilateral EMG: on average, 86% (range: 61–97%) of trials were rejected. This resulted in low numbers of trials that contributed to each PeTH, meaning that only large rate modulations were able to reach significance. The peak modulation in rate for the 7/13 cells that showed no significant modulation after trial selection was 22.9 ± 15.4 Hz when using all available trials. For the other 6/13 cells this was 32.3 ± 12 Hz. It is likely therefore that some of the remaining cells (7/13) actually modulated their discharge in the trial-selected PeTHs, but this was too small to be detected given the lower signal : noise ratio. However, for these cells we cannot exclude the possibility that rate modulated because of the confounding ipsilateral EMG changes.

The mean rate of these 13 cells before trial selection was slightly higher than after selection (56 versus 51 Hz, respectively, \( P < 0.05 \) Wilcoxon signed rank test). The peak modulation in activity was higher after trial selection (27 versus 35 Hz, \( P < 0.05 \) Wilcoxon signed rank test). The mean rates for only the six contralateral modulating cells were not different before and after trial selection (49 versus 49 Hz, \( P > 0.5 \), Wilcoxon signed rank test), while peak rates were significantly greater after trial selection (32 versus 45 Hz, \( P < 0.05 \) Wilcoxon signed rank test).

If the modulation in activity in these cells was due to the ipsilateral EMG modulation, removing trials with the greatest EMG modulation should decrease the modulation amplitude. Instead, the selected trials have a greater peak modulation, suggesting that this activity is genuinely related to the contralateral hand.

**Discussion**

Our results show that stimulating the interpositus and dentate nuclei can result in significant activation of the contralateral forelimb, while single units in the same nuclei can modulate their activity during contralateral hand movements. These are surprising findings. Stimulation of dentate and interpositus has been performed by many groups previously (Schultz et al. 1976, 1979; Giuffrida et al. 1980, 1982; Ekerot et al. 1995; Holdefer et al. 2000; Aumann & Fetz, 2004), but reports of bilateral effects are rare (but see Rispal-Padel et al. 1982 for fastigial nucleus). This is probably due mainly to methodological constraints. Monkeys are usually confined to a primate chair during experiments, which partially obscures the limbs. Given the
expectation of ipsilateral outputs, most previous studies recorded only unilateral EMG. Recognizing the bilateral nature of cerebellar organization is likely to be essential in understanding the contribution made by this enigmatic structure to motor control. We consider below a number of possible routes through which the bilateral effects may be mediated, and the functional implications of these results.

Figure 10. EMG trial selection process
A, PeTH and overlain raster compiled from all contralateral trials performed whilst recording this single unit. B, average rectified and smoothed (Gaussian kernel, 15 ms width parameter) ipsilateral EMGs corresponding to all contralateral trials performed in this recording session. C, PeTH of same cell after exclusion of trials with modulating ipsilateral EMGs. Only six trials survived the selection process in this example. D, averaged ipsilateral EMGs, compiled from the same subset of trials used in C. E–c, three further units that show modulation in rate during contralateral trials; analysis has used all available trials. Fa–c, same cells as in E, using only trials selected to exclude those with ipsilateral EMG modulation. Note similar modulation between E and F.
Possible pathways mediating bilateral outputs

Although it is tempting to interpret the stimulus effects which we have seen as indicating the output organization of the interpositus and dentate, it is possible that stimulation activated afferent axons (e.g. spinocerebellar or vestibulocerebellar, or climbing fibres from inferior olive). In that case, the muscle responses observed would be an axon reflex and not related to cerebellar output (Ito et al. 1969). Another possibility is that stimulation activated efferent fibres from the fastigial nuclei, which pass around the interpositus (Batton et al. 1977). However, we found overt responses to multiple pulse stimulation only occurred ∼300 μm after entry into the nuclei. In penetrations where the nuclei were missed, when electrodes often progressed several millimetres below the dorso-ventral location of the deep cerebellar nuclei (DCN), no effects were seen (crosses in Fig. 6B). Responses were also very sensitive to electrode location, and could disappear following electrode movements of only a few hundred micrometres. The low currents used (over 95% were at or below 40 μA) make stimulus spread to nearby fibre bundles unlikely (Stoney et al. 1968).

Whilst the majority of our penetrations targeted the nucleus interpositus, histological analysis indicated that a minority of tracks may have included the medial dentate nucleus. In the following, we therefore consider possible bilateral pathways from both interpositus and dentate and will refer to both as DCN.

Cortical routes

Contralateral M1 is a major target of both interpositus and dentate via contralateral thalamus (Chan-Palay, 1977; Shinoda et al. 1982, 1985b; Asanuma et al. 1983b; Futami et al. 1986; Aumann et al. 1994; Hoover & Strick, 1999; Kelly & Strick, 2003). There is some evidence to suggest that there is a weak projection to ipsilateral thalamus via the superior cerebellar peduncle (Niiim et al. 1962; Flood & Jansen, 1966; Li & Tew, 1966; McCance et al. 1968; Aumann & Horne, 1996) which could explain the contralateral results obtained here, although other studies have failed to find this (Carpenter & Stevens, 1957; Combs & Dennery, 1960; Asanuma et al. 1983b). It is unlikely that the effects are mediated transcortically from contralateral to ipsilateral M1; there should be a consistent 2–3 ms difference between ipsilateral and contralateral latencies, corresponding to the transcortical delay in monkey (Matsunami & Hamada, 1984; Soteropoulos & Baker, 2007) which was not found. One other possibility is the corticospinal tract, which sends ∼10% of its fibres to the ipsilateral side of the spinal cord (Lacroix et al. 2004). However, M1 stimulation leads to exclusively contralateral muscle responses (Cheney & Fetz, 1985; Day et al. 1989; Rothwell, 1997; Widener & Cheney, 1997; Baker et al. 1998; Alagona et al. 2001), implying that these corticospinal fibres do not form ipsilateral corticomotoneuronal connections. Although the effects of low intensity single pulse stimulation in M1 on ipsilateral muscles have not been systematically studied, the ipsilateral corticospinal tract is thus unlikely to underlie the responses reported here.

Given the short latencies of the effects from DCN to EMG, the bilateral muscle responses are likely to be generated partly via subcortical targets of the DCN. Based on existing knowledge, there are several possibilities. These are discussed below, but the results of this study cannot determine which pathway is responsible.

Subcortical routes

One obvious candidate for mediating the effects reported here is the reticular formation (RF). It is well documented that the reticular formation has bilateral outputs to muscles (Zemlan et al. 1984; Matsuyama et al. 1997, 1999; Davidson et al. 2007); these are often mediated via spinal commissural interneurons (Jankowska et al. 2003). All three cerebellar nuclei project extensively to the reticular formation in rat, cat and monkey (Cohen et al. 1958; Bantli & Bloedel, 1975b, 1976; Chan-Palay, 1977; Faull, 1978; Bentivoglio & Kuypers, 1982; Woodson & Angaut, 1984; Gonzalo-Ruiz & Leichnetz, 1987). In the primate, there is evidence that the dentate can have short latency access to spinal cord, probably via reticulospinal neurons (Bantli & Bloedel, 1975a). The projection patterns from interpositus to the reticulospinal areas in RF, however, have not been as well studied in the monkey although in the cat (Cohen et al. 1958) there are small but definite projections to medullary RF. Interestingly, Schultz et al. (1979) demonstrated that stimulation in either dentate or interpositus can elicit effects in muscles even after decerebration at the level of the colliculi in monkey. As this disconnects the DCN from both M1 and red nucleus, such effects are most likely to be mediated via the reticulospinal tract. Although the reticulospinal tract is often associated with the control of proximal and trunk muscles, it also provides inputs to motoneurons controlling more distal musculature (Davidson & Buford, 2006; Baker & Riddle, 2007), so that the bias towards effects in distal muscles reported here is not incompatible with a pathway via the RF.

One other possible candidate structure for providing the DCN with direct spinal access is the red nucleus. The interpositus projects heavily to the magnocellular regions of the contralateral red nucleus (Massion, 1967; Stanton, 1980; Dekker, 1981), the site of origin of most rubrospinal fibres. The dentate projects mainly to the parvocellular areas of the red nucleus, which have a much weaker but still present spinal projection (Pong et al. 2002).
In monkeys, the rubrospinal tract makes strong mono-synaptic connections to motoneurons. Conduction delays from the red nucleus to forearm muscles are on average comparable to those from the cortex. The shortest delays reported by Belhaj-Saïf et al. (1998) could be compatible with the red nucleus mediating the responses which we report here, although it would be necessary to measure red nucleus and interpositional latencies in the same animal (as done for corticospinal tract and interpositus in Fig. 7E and F) to be sure on this point. However, rubromotoneuronal connections show a marked extensor preference (Belhaj-Saïf et al. 1998), which was not apparent in our data (Fig. 6B). Additionally, anatomical and stimulation studies also show that the rubrospinal system is a highly lateralized one (Ghez, 1975; Cheney, 1980; Cheney et al. 1991; Belhaj-Saïf et al. 1998) and there are no reports of ipsilateral effects following rubral stimulation. However, a recent study reported single unit activity in the red nucleus that was related to movements of either ipsilateral or contralateral limb (Lavoie & Drew, 2002); the role, if any, of the red nucleus in mediating the bilateral effects reported here therefore remains unclear.

One final possible pathway to mediate contralateral effects should be considered: there is a direct projection from cerebellar nuclei to spinal cord. Although these connections are most common in the fastigial nucleus, they also occur in the interpositus (Fukushima et al. 1977; Matsushita & Hosoya, 1978; Wilson et al. 1978; Asanuma et al. 1980, 1983a; Bharos et al. 1981). Asanuma et al. (1980, 1983a) showed that the interposito-spinal tract projects to the contralateral spinal cord. There are dense terminations around intermediate-zone interneurones in the C3 segment (the most caudal segment analysed by Asanuma et al. 1983a); if this pattern also occurs in the cervical enlargement, it would provide an ideal substrate for the divergent activation of multiple muscles from a single site which we observed. To date, the electrophysiological significance of the interposito-spinal tract has not been studied. In particular, it would be important to know the conduction velocity of the fibres, and the synaptic delays introduced by the spinal circuitry to which they connect, before this tract’s possible contribution to the responses which we observed could be assessed.

**Rate modulation of single units with contralateral movements**

Careful examination of our recordings indicated that apparently ‘unilateral’ movements were often accompanied by EMG activation in the stationary limb. Such mirroring is a normal part of motor performance (Mayston et al. 1999), but it represents a confound in the interpretation of central activity related to ipsilateral or contralateral movements. Greger et al. (2004) reported that cerebellar cortical units modulated their firing when movements were made by either limb, but noted that small EMG changes were seen on the stationary side.

In this study, we attempted to exploit the trial-to-trial variability seen in the extent of mirror activation. Trials were selected to exclude those with EMG changes in the stationary limb. Once averages of EMG confirmed the success of this procedure (Fig. 10D), PeTHs of cerebellar unit activity were recalculated using only these trials. The patterns of rate modulation in this subset of trials generally followed closely that seen when using all trials (Fig. 10A, C, E and F). The peak modulation was actually significantly larger in the selected subset.

It is possible that the observed modulation in cerebellar activity was due to changes in the activity of ipsilateral muscles whose EMG was not recorded. Reaching movements are normally associated with anticipatory postural adjustments which are bilaterally organized (Scheepens & Drew, 2003, 2004, 2006). A unimanual reach could therefore recruit bilateral axial muscles to stabilize the posture. It is unlikely, however, that anticipatory postural adjustments are responsible for the changes in cerebellar activity seen. Firstly, our animals were seated and head fixed to permit stable single unit recordings. Unlike the situation in free sitting animals, reaches should not destabilize posture under these conditions. The reaching hand also moved only a short distance (~0.1 m) from the home pad to the precision grip manipulandum. Secondly, units were recorded during the same penetrations as those used in the stimulation part of the experiment. As shown in Fig. 6, stimulation at these sites produced activation in both distal and proximal forelimb muscles. Associated trunk movements were rarely seen. These sites appear more suited to guiding the primary movement itself, rather than shaping its postural context.

It is also possible that more distal ipsilateral arm muscles, not part of the recorded subset, modulated their activity even during the selected contralateral trials where other muscles were inactive. We naturally cannot exclude this possibility. However, muscles are rarely recruited singly, but instead in functionally meaningful synergistic groups (Schieber, 1995). In the present dataset, activity in the recorded muscles co-modulated strongly from trial to trial. By selecting trials without modulation in the recorded muscles, it is thus likely that we also excluded trials with activation of other muscles not recorded from. As the cell responses remained essentially unchanged in the selected compared with the unselected trials, it seems most likely that the activity was genuinely modulated with contralateral movements rather than covert ipsilateral muscle activity.

We conclude that some cerebellar output neurones modulate their activity in relation to movements of the contralateral limb, as well as to ipsilateral movements. A similar conclusion was drawn by Greger et al. (2004),
who observed lateral cerebellar cortex unit firing correlated with the parameters of movement of either limb.

**Functional implications**

There is a growing realization that the cerebellum is involved in bimanual coordination. Serrien & Wiesendanger (2000) showed that patients with cerebellar lesions had impaired intermanual coupling on a task requiring opening a drawer with one hand, and retrieving its contents with the other. Spencer et al. (2003) tested a bimanual finger-tapping task, and again found impairments in cerebellar patients, some of whom had unilateral cerebellar lesions. The observation that the cerebellum has bilateral access to arm muscles may provide a direct substrate for bilateral coordination by the cerebellum.

The comparison of our findings with the literature on motor cortex is instructive. Although M1 is normally assumed to represent contralateral movements, a significant number of neurones modulate their activity with ipsilateral movements (Matsunami & Hamada, 1978, 1980, 1981; Donchin et al. 1998). However, multiple pulse stimulation of M1 produces solely contralateral movements (Cheney & Fetz, 1985; Day et al. 1989; Rothwell, 1997; Widener & Cheney, 1997; Baker et al. 1998; Alagona et al. 2001), although to the best of our knowledge no report searched explicitly for bilateral effects from single pulse stimulation. The cerebellum, by contrast, appears to have rather direct (short latency) access to the peripheral musculature bilaterally. Thus whilst both regions may be carrying out a computation within the bilateral context of a movement, the cerebellum can apply the results of this processing directly to coordinate movements between left and right limbs.

Most everyday movements require a complex synergy of movement across joints, and the cerebellum is often ascribed a role in ‘composing’ such multi-joint movements. Although cerebellar damage does affect movement across single joints (Flament & Hore, 1986; Hore et al. 1991), multi-joint movements are much more severely impaired (Goodkin & Thach, 2003). When joints move they create dynamic interaction torques in adjacent joints, which must be accounted for to produce smooth and accurate movements (Topka et al. 1998a,b). The cerebellum may be involved in integrating information across modalities (proprrioceptive, visual and vestibular) to scale muscle activity to account for these dynamic torques (Bastian et al. 1996).

Interaction torques are usually discussed in terms of proximal muscles, and are mainly confined to one side of the body. For example, flexing the elbow will cause effects on the ipsilateral shoulder unless this is compensated for. In agreement with this focus, Monzee et al. (2004) showed that inactivating DCN impairs reaching and grasping but had little effect on isolated distal movements. However, the concept may need to be extended when considering bimanual movements. If the two hands manipulate an object, the actions of one hand will produce interaction forces and torques on the contralateral hand. Compensation for these will require the recruitment of both distal and proximal musculature, in a wide ranging, bilaterally organized synergy. The cerebellar organization described here provides short-latency access to small groups of muscles bilaterally, and could form the perfect substrate for such coordination.

**References**


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