Different Contributions of the Corpus Callosum and Cerebellum to Motor Coordination in Monkey

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Soteropoulos DS, Baker SN. Different contributions of the corpus callosum and cerebellum to motor coordination in monkey. J Neurophysiol 98: 2962–2973, 2007. First published August 22, 2007; doi:10.1152/jn.00236.2007. We investigated the different contribution of the corpus callosum (CC) and cerebellum to motor control in two macaque monkeys trained to perform a precision grip task with one or both hands. Recordings were made from antidromically identified CC cells and nearby unidentified neurons (UIDs) in the hand representation of the supplementary motor area (SMA) and compared with cells from the deep cerebellar nuclei (DCN). All cells showed their greatest modulation in activity (rate change locked to particular task event) during the movement epochs of the task (CC, 21.3 ± 22.2; UIDs, 36.2 ± 30.1 spike/s for contralateral trials; DCN, 63 ± 56.4 for ipsilateral trials; mean ± SD). Surprisingly, CC cells fired at very low basal rates compared with UIDs (3.9 ± 4.9 vs. 10 ± 9.1 spike/s) or DCN neurons (50.8 ± 23.8 spike/s). However, SMA cells had the greatest rate modulation to baseline ratio (CC: 12.1 ± 13.7; UID: 5.3 ± 5.4; DCN: 1.7 ± 2.0). This would allow them to code the timing of a behavioral event with better fidelity than DCN cells. A multivariate regression analysis between cell firing and EMG measured cells’ representation of moment-by-moment modulations in muscle activity, CC neurons coded these real-time behavioral parameters significantly less well than the other cell types, using both linear and nonlinear models. Basal firing rate substantially constrains cell function. CC cells with low basal rates have restricted dynamic range for coding continuous parameters, but efficiently code the time of discrete behavioral events. DCN neurons with higher basal rates are better suited to control continuously variable parameters of movement.

INTRODUCTION

In the behaving primate, many everyday actions require bimanual coordination. The underlying neural system is a distributed one with contributions from a wide range of cortical and subcortical areas (Debaere et al. 2003; Donchin et al. 2002; Immisch et al. 2001; Kermadi et al. 2000; Sadato et al. 1997; Serrien et al. 2001; Stephan et al. 1999; Ullen et al. 2003). Of these, two structures with a putative role in bimanual control are the corpus callosum and the cerebellum.

The cerebellum is known to play an important part in sensorimotor processing. Suggested functions range from a role in movement timing (Cooper et al. 2000; Hore and Villis 1984; Ivry et al. 1988; Spencer et al. 2003) to motor learning and calibration of movements and reflexes (Baizer et al. 1999; Hirata and Highstein 2001; Kolb et al. 2007; Martin et al. 2000; Oulad Ben Taib and Manto 2006; Thach and Bastian 2004). Strongly influenced by Holmes’ original reports on effects of cerebellar lesions in humans (Holmes 1939), the majority of research has been on cerebellar activity during ipsilateral arm movements (Fortier et al. 1989; Harvey et al. 1979; MacKay 1988a,b, Smith and Bourbomais 1981; Thach et al. 1978) with few studies (Greger et al. 2004) looking at the activity of cerebellar neurons during movements of the contralateral or both arms. Yet there is recent and accumulating evidence that the cerebellum is involved in control of the contralateral side of the body (Cui et al. 2000; Ehrrson et al. 2002; Immisch et al. 2003; Indovina and Sanes 2001; Kawashima et al. 1998; Nair et al. 2003; Rammani et al. 2001; Kinoshita et al. 2000; Soteropoulos and Baker 2003), although the precise nature of this relationship still remains obscure and controversial.

The corpus callosum contains ~250 million fibers in man and provides reciprocal connectivity mainly between homotopic cortical areas (Liu et al. 2002; Rouiller et al. 1994). Despite its undoubted importance in inter-hemispheric communication, there are few reports of the activity of identified motor callosal neurons in awake behaving animals. Those available, from the rabbit and cat, reveal low spontaneous firing rates (generally <1 spike/s) (Swadlow 1994), and little modulation in activity with either locomotion or a postural stabilization task (Beloozerova et al. 2003a–c). Callosal activity is of interest not just for overt bilateral movements but also in cases where only one hand is used as any firing by these cells will be necessarily transmitted to the other side of the brain.

Recent work has suggested that these two structures may have different roles in motor coordination. Patients with callosal lesions show normal coupling of the hands on a discrete tapping task (Kenmerley et al. 2002) and also when opening a drawer with one hand and retrieving an object inside with the other (Serrien et al. 2001). However, when both hands draw circles continually, callosal patients show impairments in intermanual coupling compared with controls (Kenmerley et al. 2002; Serrien et al. 2001). By contrast, cerebellar patients are impaired on the tapping task (Spencer et al. 2003) and the drawer task (Serrien and Wiesendanger 2000) but show normal left-right temporal correlation when drawing continuous circles (Kenmerley et al. 2002; Serrien et al. 2001).

Based on these results, it has been suggested (Spencer et al. 2003) that the cerebellum mediates bimanual coordination where there is an explicit requirement for temporal coupling—an example is when a subject throws a ball from one hand to the other. Then the side catching must receive information about the moment of ball release from the other hand to will be necessarily transmitted to the other side of the brain.

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trigger an effective interception. By contrast, the callosum was hypothesized to mediate real-time inter-hemispheric communication required for continual task performance. Inter-manual temporal coupling then evolves incidentally as an emergent property of this continual coordination process. An example of such a task would be when we unscrew the top from a bottle: the hand holding the bottle must precisely compensate for the torque applied by the hand gripping the cap.

Anatomical evidence also supports possible distinct functions for the cerebellum and callosum in bimanual control: the main cortical receiving areas for cerebello-thalamic projections are motor and lateral premotor cortex (Middleton and Strick 2000), whereas for the hand representation, the densest callosal connections are made to SMA (Rouiller et al. 1994).

The aim of this study was to record the activity of callosal neurons in monkey SMA and cells in the deep cerebellar nuclei (DCN) during movements using either or both arms. We find that the firing patterns in these two structures are very different, irrespective of which movement laterality is considered. In SMA, the archetypal “bimanual” area and the main source of callosal connections in the primate motor system (Rouiller et al. 1994), callosal firing rates are low. Callosal cells appear to encode continuous movement parameters poorly. Their discharge seems better suited to signal the occurrence time of discrete behavioral events. By contrast, the much higher firing rate of cerebellar neurons can allow for coding of movement parameters with higher fidelity but may be less well suited to signaling discrete event timing.

**METHODS**

*Behavioral paradigm*

Two female rhesus macaques (~6 kg, ~4 yr old) were trained on the precision grip task described in Soteropoulos and Baker (2006). The animal was presented with two precision grip manipulanda for left and right hands. Access to the manipulanda levers was obstructed by plastic flags. The monkey commenced a trial by placing both hands on hompad switches in front of the flags (Fig. 1, A and B). After ~500 ms, a 1-s-long audiovisual cue indicated the required movement (left hand, right hand, or both), chosen at random. After the laterality cue was an instructed delay period (0.7, 1 or 1.3 s randomly chosen, Fig. 1G), during which the animal kept its hands on the switches. Both flags then moved down (the go cue, Fig. 1H), permitting access to the manipulandum. The animal reached out with the correct hand or hands and grasped the manipulandum levers between finger and thumb in a precision grip (see lever traces in Fig. 1, C–F). The levers were held above a criterion displacement for 1 s (hold period, Fig. 1G), before being released to obtain a food reward. Motors opposed lever movement simulating the action of springs (force for initial lever movement, 0.15 N; spring constant, 0.03 N/mm). Incorrect movements or premature switch release resulted in a failure tone and termination of
that trial. We refer to unimanual trials as “contralateral” or “ipsilateral,” indicating whether the hand contralateral or ipsilateral to the recording site moved. After lever release, the animal took a food reward using the hand that had performed the task.

**Recordings**

Forelimb electromyograms (EMGs) were recorded bilaterally using chronically implanted electrodes (Miller and Houk 1995) from seven muscles: first dorsal interosseus, abductor pollicis brevis (AbPB), abductor pollicis longus, (AbPL) flexor digitorum superficialis (FDS), extensor digitorum communis (EDC), biceps, and triceps. The monkey was implanted with a headpiece to allowatraumatic head fixation (Lemon 1984) and a recording chamber over a craniotomy (center A20 ML0) allowing access to SMA-proper bilaterally. The SMA hand representation was identified when microstimulation (intensities: <50 μA, 18 pulses at 300 Hz, 0.2-ms width) produced visible twitches or EMG responses. Cerebellar recordings were made through a separate chamber centered over nucleus interpositus (center P8.5, L4).

Surgical operations were performed under deep general anesthesia and followed by a full course of antibiotic and analgesic treatment (see Wetmore and Baker 2004). All procedures were carried out under license from the UK Home Office.

Cells were recorded with a 16-channel Echhorn microdrive (Thomas Recording, Marburg, Germany), using glass-insulated platinum electrodes or tetrodes. Signals were amplified and filtered (300 Hz to 10 kHz band-pass, gain: 2–10 K) before being digitized for off-line analysis (25-kHz sampling rate). Single units were isolated using custom-written cluster-cutting software (Getspike, S.N. Baker; Spikelab, G. Bhumbra). Units with consistent spike shapes and no interspike intervals 1 ms were accepted for subsequent analysis.

**Callosal unit identification**

Two to four insulated tungsten stimulating electrodes (Microprobe, Potomac, MD) were chronically implanted into the corpus callosum (A21-26, Height 21–26) using a double angle technique (Soteropoulos and Baker 2006). Electrodes were positioned to maximize the antidromic field potential in an epidural recording from SMA. Correct placement was subsequently confirmed by post mortem histology. During recording sessions in the conscious state, callosally projecting cells were identified by constant latency (antidromic) responses to stimulation through these electrodes (Fig. 2A, maximum stimulus intensity: 900-μA, 0.2-ms pulse, 1 Hz). A 0.2-ms jitter in antidromic latency was allowed before a cell was rejected from being considered as a callosal neuron. Callosal cells can show an activity-dependent change in conduction velocity (Swadlow et al. 1978), which is greater for cells with higher firing rates. This could have resulted in cells with a latency jitter >0.2 ms being erroneously rejected as callosal neurons. As we encountered such cells infrequently, we do not believe that this has substantially biased our recordings toward slower firing CC cells.

Identification was confirmed using a collision test (Fig. 2B). The inter-hemispheric conduction distance was estimated by measuring the length of the CC from histological sections in each monkey (AP 20-26). Conduction velocity estimates were based on half this distance because stimulating electrodes were close to the midline.

**Analysis of unit activity**

Only units recorded for at least five trials per movement type were analyzed further. Perievent time histograms (PETH; 100-ms bins, ±1-s window) were generated relative to the following task events: trial start (switch press), laterality cue end, go cue, reach (switch release), squeeze (first movement of manipulandum levers), end of hold period, and lever release (peak lever velocity, see Fig. 1H).

As an indication of the cell’s baseline activity, the mean firing rate was measured over the 1.5 s after trial start across all trials regardless of laterality. During this period, the monkey had both hands on the homopads.

Neuronal responses in SMA and DCN are nonhomogeneous often with brief increases and decreases in rate occurring close together. For such activity, averaging the rate over a window corresponding to a particular behavioral period will underestimate the modulation that occurs. Instead we quantified the modulation of a cell’s firing aligned to a particular event by measuring the difference between the PETH bins with minimal and maximal rate.

Modulation was calculated from PETHs compiled from all the task events described in the preceding text and for left, right and bimanual trials separately. The maximal modulation across all events (found separately for each laterality) was used as an overall measure of the task-related modulation. As some task events were closely related in time (for example go cue, switch release and squeeze, see Figs. 1H and 3E), a cell that modulated its activity with one event would be likely to show a “smeared” response to other temporally close events. However, by using the largest modulation, we ensured that the modulation relating to the event where the neuronal activity was best aligned across the trials was used.

A shuffling method determined whether the modulation was significantly different from zero. Interspike intervals for each trial were shuffled randomly 500 times; for each shuffle, the PETH was recalculated, and the peak modulation measured. If the modulation of the unshuffled PETH was >475 of the modulations after shuffling, the cell was assumed to be significantly modulated by that event ($P < 0.05$).

**Signal to baseline ratio**

For each task modulated cell, we measured the signal-to-baseline ratio (SBR), as the maximum modulation divided by the baseline firing rate. This was used as a measure of how well a unit’s firing conveyed the time of a behavioral event. Even during a period when the underlying firing rate is constant, neurons show irregularity in their interspike intervals. Such irregularity is often well characterized by a coefficient of variation (Davies et al. 2006; Prut and Perlmutter 2003; Stern et al. 1997), indicating that the SD of the interspike intervals scales with the mean firing rate. The baseline rate therefore provides an approximate measure of the “noise” in the background firing. The peak modulation shows how large is the rate change aligned to a behavioral event. Because it is calculated from PETHs, neural spiking with nonconstant timing relative to the behavior will be smeared out, and the estimated peak modulation will be smaller. The SBR is therefore a good approximate measure of how reliably a neuron codes the occurrence of a behavioral event. An SBR much larger than one indicates a cell the rate of which changes greatly compared with baseline during the task; an external observer would be able to detect the occurrence of the behavioral event with high fidelity for such a cell. By contrast, cells with SBR smaller than one have a rate modulation that is comparable to the background firing and likely to be difficult to detect reliably among interspike interval variability. Because we used the maximum modulation found across all task events for calculation of the SBR, this showed the best event representation which that cell was capable of in our task.

**Regression analysis**

Linear regression analysis was employed to assess how well unit discharge encoded muscle activity. EMG signals were rectified, low-pass filtered and down-sampled to 200 Hz. Spike-triggered averages (SpTA) were compiled (±1 s) of each available EMG and smoothed by Gaussian kernel convolution ($\sigma = 50$ ms). Each EMG signal was then convolved with the corresponding SpTA. This had the effect of shifting the EMG in time with different shifts weighted by the extent...
of the EMG-unit correlation at that lag. The instantaneous firing rate (IFR) was estimated by Gaussian kernel smoothing of the spike train \((\sigma = 50 \text{ ms})\). A linear multiple regression model then attempted to predict the cell’s IFR from the 14 processed EMGs. The model yielded an \(r^2\) value, showing the fraction of the cell’s rate variance that was explained linearly by the muscle data.

Although it has been shown that some neurons can code EMG activity reliably in a linear fashion (Morrow and Miller 2003), we subsequently extended the linear analysis by including higher powers of the EMG signals. The regression model thus became

\[
\text{IFR} = b_0 + \sum_{j=1}^{i_{\text{max}}} \sum_{i=1}^{n} b_{ij}(\text{EMG}_i)^j + \epsilon
\]

where the terms \(b\) are regression coefficients, \(i_{\text{max}}\) is the highest power considered, and \(\epsilon\) refers to that part of the IFR unexplained by the regression. Because any nonlinear function may be expressed to arbitrary precision by a Taylor Series expansion, this regression model allowed us to test a general nonlinear model. Powers up to \(i_{\text{max}} = 5\) were tested.

As the number of independent variables increases, \(r^2\) becomes upwardly biased; we therefore used an adjusted \(r^2\), described by

\[
\text{Adj} \quad r^2 = 1 - \frac{(n - 1)}{(n - p)}(1 - r^2)
\]

where \(n\) is the number of data points and \(p\) is the number of independent variables used (Montgomery et al. 2001).

**RESULTS**

We encountered 35 units in SMA that could be antidromically activated from the callosum (example in Fig. 1, A and B); 14 of these were recorded during sufficient trials of the task for full analysis. A further three callosal units had sufficient trials only for unimanual analysis; their behavior was however similar to the main database. We also recorded the activity of 52 unidentified units (UIDs) from SMA and 82 cerebellar cells. Histological analysis revealed that the majority of gliosis tracks in the cerebellum were toward the interpositus with some targeting the medial dentate. Tracks rarely targeted the fastigial, and microstimulation at the recording sites failed to evoke stimulus-locked saccades, confirming that recordings excluded this nucleus.

Although electrodes were in the SMA hand representation and the animal was actively performing the task, we were surprised to encounter two additional corpus callosum (CC) units that fired no spontaneous spikes for the entire recording session (>1000 s, i.e., larger than 1000 seconds). The continuing presence of these callosal cells was verified by intermittent CC stimulation, which yielded constant-latency antidromic activation of the silent neurons (Fig. 2, E and G). Figure 2, C and D, shows two 5-min-long sections at the start of recording and 25 min later, during which CC stimulation was briefly given. No spontaneous spikes were seen, despite the good signal-to-noise ratio of this recording (compare noise level in Fig. 2F with size of antidromic spikes in Figs. E and G). Figure 2, H and I, shows the distribution of antidromic latencies (mean: 1.8 ms) and estimated conduction velocities (mean: 11.2 ms). Our estimate of the length of the CC is likely to be a slight underestimate due to tissue shrinkage following histological preparation. Thus the conduction velocities are also likely to be underestimates.

Most CC neurons recorded were active but at low rates. Some modulated their activity around a behavioral event. For

**FIG. 2.** Antidromic identification of callosal units. A: example antidromic response (13 sweeps overlain); B: successful collision of antidromic with spontaneous spike at trigger-to-stimulus interval of 3.6 ms (top) but not at 3.7 ms (bottom). C and D: recordings from electrode close to a callosal cell taken from the beginning and end of session. Corpus callosum (CC) stimulation was delivered at points shown. E: expanded trace from C showing antidromic spike response to CC stimulation. F: 1,000 overlain sweeps of same length as E to indicate noise level. G: expanded trace from D showing antidromic response. H, latency and I, estimated conduction velocity of CC cells.
the neuron shown in Fig. 3A, basal firing rate was low while the arms were resting on the homepads (4.7 spike/s), but peak modulation rose above 29 spike/s during contralateral and bilateral trials. Similarly, the CC units shown in Fig. 3, B and C, also had low basal rates (1.5 and 0.8 spike/s, respectively). Their peak response rate, however, rose above 45 and 8 spike/s, respectively, for contralateral trials. Some other cells behaved as the unit illustrated in Fig. 3D with a low baseline rate (1.5 spike/s). There was a significant (but low, 3.4 Hz) rate modulation (shuffle test, $P < 0.05$). For comparison, the representative UID cell in Fig. 3E had a baseline rate of 15.3 spike/s and a peak modulation of 31.4 spike/s.

Figure 3F illustrates a representative cerebellar neuron. This fired at a much higher basal rate of 40.2 spike/s with a peak modulation of 82 spike/s. Figure 3G shows the distribution of other task events accumulated over all available recording sessions aligned relative to the squeeze phase.

Figure 4 shows population data for all three cell types. Figure 4A consists of box plots of the basal rates as well as the peak modulations for trials of different lateralities. Figure 4, B–E, shows pairwise comparisons of the rate modulations for different cell classes. As a population, CC neurons had low basal rates of $3.9 \pm 4.9$ spike/s. The peak modulations for trials of all lateralities (Fig. 4A) were greater than the basal rate ($P < 0.01$, paired t-test). The modulation for ipsilateral trials was significantly smaller than for contralateral trials ($13.3 \pm 8.9$ vs. $21.3 \pm 22.2$ spike/s, paired t-test, $P < 0.05$; Fig. 4B) and bilateral trials ($24.8.3 \pm 26.1$ spike/s, paired t-test $P < 0.05$). Peak modulations during bilateral trials were very similar to those during contralateral trials ($P > 0.3$, paired t-test; Fig. 4C).

For SMA UIDs the mean basal rate was $10 \pm 9.1$ spike/s and was significantly greater than that of CC cells (unpaired t-test, $P < 0.01$). Peak modulations were significantly different ($P < 0.05$, paired t-test) from basal rates in SMA UIDS (ipsilateral: $19.1 \pm 12.2$, contralateral: $36.2 \pm 30.1$, bilateral: $34.8 \pm 29.2$ spike/s, for all lateralities $P < 0.05$). Peak rates were not significantly different from those of callosal cells for any

FIG. 3. Activity of representative units during task performance. Activity is aligned to lever squeeze. A–D: callosal units; E: supplementary motor area (SMA) unidentified neuron (UID); F: deep cerebellar nucleus (DCN) cell. G: distribution of other task events accumulated over all available recording sessions.
laterality (unpaired t-test, $P > 0.05$). The peak modulations seen for bilateral trials were not significantly different from contralateral trials (Fig. 4D, $36.2 \pm 30.1$ vs. $34.8 \pm 29.2$ spike/s, paired t-test $P > 0.05$).

In cerebellar neurons, basal rates were greater than for both CC and UID cells ($50.8 \pm 23.8$ spike/s, unpaired t-test, $P < 0.05$) as were the peak modulations (unpaired t-test, $P < 0.05$). Peak modulations were significantly greater during bilateral trials ($67.3 \pm 56.8$ spike/s, $P < 0.05$, paired t-test), than during both types of unimanual trials (Fig. 4E, bilateral vs. ipsilateral), and ipsilateral modulations were greater than contralateral ones ($63 \pm 56.4$ vs. $42 \pm 19.3$ spike/s, $P < 0.05$, paired t-test).

Figure 4F shows the distribution of the events for which cells showed their greatest significant modulation. For all three cell classes, there was a preponderance of cells which modulated best with the ballistic components of the task, namely the
reach and release phases. In the SMA more cells showed a significant modulation for bilateral and contralateral trials than for ipsilateral ones (CC: 11 vs. 11 vs. 8, respectively; UIDs: 51 vs. 45 vs. 38), whereas in the DCN the fraction of cells was similar across lateralities (ipsilateral: 77, contralateral: 76, bilateral: 74).

Why do SMA CC units fire at such low baseline rates compared with other nearby SMA neurons (Fig. 4A)? These low rates will have a marked impact on the ability of callosal cells to code information (Rieke et al. 1997), but one possibility is that the low baseline activity allows more reliable detection of a phasic response. To assess this, we measured a signal to baseline ratio (SBR), as described in METHODS.

The results of this analysis are shown in Fig. 5, as cumulative probability distributions. Figure 5, A–C, compares the SBR between the different cell types during trials of different lateralities. Figure 5A illustrates data using ipsilateral trials for SMA and contralateral trials for the cerebellum; B uses contralateral trials for SMA and ipsilateral trials for the cerebellum. Figure 5C presents results for bilateral trials. CC neurons had the greatest SBR on average (ipsilateral: 7.9 ± 5, contralateral: 12.1 ± 13.7, bilateral: 8.0 ± 6.2) for all lateralities compared with UIDs (ipsilateral: 3.2 ± 3.0, contralateral: 5.3 ± 5.4, bilateral: 5.3 ± 5.0) or DCN cells (ipsilateral: 1.7 ± 2.0, contralateral: 1.0 ± 0.5, bilateral: 1.8 ± 2.0). For all lateralities, the SBR distribution was significantly different between CC and DCN populations (Kolmogorov-Smirnov test, P < 0.05).

The remainder of Fig. 5 compares the SBR between task lateralities within each area. Figure 5D shows the SBR for the cerebellar cells. The SBR for bilateral trials was greater than that for ipsilateral trials (Wilcoxon signed-rank test, P < 0.01) although the difference in the mean SBR was small (bDCN: 1.74 ± 1.9, iDCN: 1.66 ± 1.9). Both bilateral and ipsilateral SBR were greater than that for contralateral trials (1.0 ± 0.6, Wilcoxon signed-rank test, P < 0.01).

In the case of CC cells (Fig. 5E), there were no significant differences between the distributions (P > 0.19, Wilcoxon signed-rank test), although this could be due to the small number of cells available for analysis. For SMA UIDs (Fig. 5F), ipsilateral SBR (3.5 ± 4.4) was smaller than that for contralateral (5.2 ± 5.1) and bilateral trials (5.3 ± 4.9, P < 0.01, Wilcoxon signed-rank test), but there was no difference between contralateral and bilateral SBRs (P > 0.4, Wilcoxon signed-rank test).

The difference in SBR shown in Fig. 5, A–C, implies that callosal cells will be better suited than cerebellar neurons to signal the time of behavioral events. To provide a quantitative measure of how well a cell’s discharge represented real-time parameters of task performance, we performed a multiple regression analysis (see METHODS). Figure 6 illustrates two example cells. Figure 6, A and B, shows rectified EMG signals from four ipsilateral and four contralateral muscles that modulated their activity clearly during task performance. Underneath is the spike train for each cell (Fig. 6, C and D) recorded simultaneously with the EMG traces above. Figure 6C is from an SMA callosal cell, and D is from a cerebellar neuron. Figure 6, E and G, shows the instantaneous firing rates of the callosal neuron and F and H of the cerebellar neuron. In Fig. 6 E and F, overlaid on the firing rate (black line) is the prediction from the linear regression analysis (red line). The prediction matches the rate much better for the cerebellar neuron, yielding a larger r^2 value.

Figure 6F shows the cumulative distribution of adjusted r^2 values across the different cell populations using the linear regression model. CC cells had smaller r^2 (0.046 ± 0.04) than both UID (0.078 ± 0.044) and DCN cells (0.11 ± 0.07; t-test, P < 0.01; Kolmogorov-Smirnov test for difference of distributions, P < 0.05).

Although a clear difference was seen between CC cells and the other populations, it remained possible that this might be an artifact of assuming a linear relationship between the variables. Accordingly, we tested the effect of fitting nonlinear models that included higher powers of EMG (see METHODS). Figure 6J presents the dependence of the mean r^2 value across the DCN and CC cell populations on model order. Unsurprisingly, more complex models were able to explain an increasing fraction of the variance for both cell classes. However, the difference between these populations also grew with more complex models (Fig. 6J, red line).

Figure 6G and H, shows the prediction of firing rate for the two example cells made using the most complex model tested (up to 5th power of EMG). Although the fit with the actual rate

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**FIG. 5.** Cumulative probability distribution of the signal-to-baseline ratio (SBR) for different cell types. A: SBR ratio of ipsilateral trials for CC and UID but for contralateral for DCN cells. B: SBR ratio for contralateral trials for CC and UIDS but for ipsilateral trials. SBR for bilateral trials for all cell types. Only cells with significant modulations were used for A–C. D: SBR ratio for all DCN cells for trials with different lateralities. E: same as D but for CC cells. F: same as E but for UIDS.
is better in both cases, the improvement is more marked for the cerebellar unit.

Figure 6K is a cumulative plot across the cell populations of the $r^2$ values obtained from the regression model using up to 5th powers of EMG. The $r^2$ values were greater than for the linear model, but still CC cells had the smallest values (0.096 ± 0.043), followed by SMA cells (0.181 ± 0.098) and then DCN cells (0.221 ± 0.11). All three populations were significantly different from each other ($P < 0.05$, Kolmogorov-Smirnov test for difference of distributions). This suggests that, even for nonlinear decoding models, the representation of the moment-by-moment modulation of the behavioral responses by CC cells will be much poorer compared both with neighboring cells in SMA and cerebellar output neurons.

**DISCUSSION**

This paper reports, for the first time, how identified callosal cells in primate SMA fire during a bimanual motor task. By comparing their firing with nearby unidentified cells, and with neurons from the cerebellum, we suggest that these cells have a distinctive role to play in bimanual coordination.

**Neural firing with trials of different lateralities**

Much previous work has concentrated on the modulation of cell activity during performance of tasks with different hands. Unidentified neurons in both SMA and primary motor cortex modulate their discharge during contralateral, ipsilateral and bilateral task performance (Donchin et al. 1998, 2002; Tanji et al. 1988). Cerebellar cells can also exhibit task-dependent firing changes for movements of either hand (Greger et al. 2004; Soteropoulos and Baker 2003). The present data agree with this previous work: the majority of cells examined modulated their firing rate during all three types of task performance. However, there was a clear preference: for SMA cells, the rate modulation was larger during contralateral than ipsilateral trials; the reverse relationship was seen for the cerebellum.

Previous work has also shown that some neurons in SMA fire in specific patterns for particular task lateralities, whereas in some cases, firing during bilateral movements cannot be explained as the simple sum of the activity during left and right unilateral movements (Donchin et al. 2002; Tanji et al. 1988). Our analysis was not intended to examine this in detail, but we have observed some examples of neuronal activity corroborating these studies. Donchin et al. (2002) additionally reported greater average activity during bimanual performance than during contralateral trials. In our data, this was not seen (Fig. 4, C and D). However, the task used by Donchin et al. (2002) was a reaching task involving mainly proximal arm muscles, in contrast to the precision grip employed in our experiments that required mainly distal hand activation. It is likely that these differences in the nature of the task underlie any minor differences between our findings and previous work.

Recordings of identified callosal neurons have a great advantage over those from unidentified cells. For unidentified neurons, the functional role that the cell plays must be inferred from its firing rate modulation during different types of behav-
ior. A cell that fires only during bimanual performance is thus classed a "bimanual neuron." Such an approach is limited as it makes assumptions on what firing patterns are required for movement coordination. By contrast, antidromically identified CC cells are known to project to the opposite hemisphere and must therefore have a role in bilateral coordination. Examination of their rate modulation then provides independent information on what activity is exchanged during uni- and bimanual task performance.

Viewed in this framework, it is interesting that although callosal neurons did often show significant rate modulation during ipsilateral movements, this modulation was on average smaller than for contralateral movements (Fig. 4C). It seems that the SMA callosal system primarily signals when the contralateral hand moves. In the context of bimanual movements, this could drive the close temporal coupling of movements across the midline, which seems to be a hallmark of motor control (Diedrichsen et al. 2003; Kazennikov et al. 1994; Kelso 1984; Kennerley et al. 2002; Perriq et al. 1999). For unimanual movements, several studies suggest that the lack of movement is not simply a lack of excitatory drive but rather a process of active inhibition whereby cortical circuits are prevented from triggering movement (Allison et al. 2000; Newton et al. 2005; Toma et al. 1999; Weiss et al. 2003; Yazawa et al. 1998). Sometimes, this suppression is incomplete: low level muscle activation in the supposedly stationary hand (mirror activation) is a normal part of motor performance (Mayston et al. 1999). Callosal firing during contralateral trials could initiate this active inhibitory process.

If this interpretation is correct, it implies that callosal activity either triggers or suppresses movement initiation by the contralateral hemisphere according to motor set. It is known that SMA has neurons that fire selectively before movement according to the instructed task (Kurata and Wise 1988; Mushiake et al. 1990; Tanji 1985; Tanji and Kurata 1985; Tanji and Mushiake 1996). The function of these cells may be to prime the receiving network to ensure that incoming callosal activity has the correct effect.

Coding constraints imposed by firing rate

Callosal cells in monkey SMA fire at low rates compared with neighboring unidentified cells and to cells recorded from DCN (Fig. 4A). This agrees with previous reports (Beloozerova et al. 2003a,c; Swadlow 1994), but is surprising because we recorded from the cortical area most associated with bimanual coordination (Kermadi et al. 1998; Tanji et al. 1988) during performance of a bimanual task. Two cells even failed to fire spontaneous spikes completely; again, such silent callosal cells have been previously reported (Swadlow and Hicks 1997) but not in primate SMA during a bimanual task. The low firing rates markedly reduced the ability of the callosal neurons to encode continuous parameters with any fidelity (Fig. 6). However, the low baseline rate could enable callosal cells to signal the occurrence of a behavioral event with better signal-to-baseline ratio (Fig. 5). In agreement with this idea, most callosal cells modulated their activity best with the reach or release event (Fig. 4F). These ballistic movements have a rapid, well-defined onset in our task, and a system that signals the time of a discrete event would be expected to modulate most with these.

Although the firing rates of single callosal cells were low, the summed activity of a large population could accurately encode continuous behavioral parameters. Although we cannot rule out such a possibility, the low firing rate must provide an important clue to the function of this system. There is no a priori reason why cells with callosal projections should fire at lower rates than neighboring unidentified neurons. Other pyramidal cells such as pyramidal tract neurons (PTNs) in primary motor cortex fire at least as fast as neighboring UIDs (Baker et al. 2001). PTNs undoubtedly transmit finely-graded muscle activation commands to the spinal cord, and most of our UIDs are likely to be large pyramidal cells due to the well-known recording bias of extracellular recording (Towe and Harding 1970). Information theory shows that the higher the firing rate of a cell, the greater the maximum information that it can transmit (Rieke et al. 1997). If the function of the callosal system was to transmit continually varying parameters of movement performance, it should have evolved to maximize firing rate.

The CC consists of fibers with a wide range of conduction velocities; the majority are slowly conducting (<5 m/s) (Swadlow 1985, 1994; Swadlow and Waxman 1976). Our recordings were biased toward the faster fibers (Fig. 2, H and J) due both to the larger size of these cells and to the lower threshold for activation of their axons by electrical stimulation (Swadlow 1998). Given the observed range of estimated conduction velocities for the cells in this study, all are likely to be myelinated (Waxman and Bennett 1972). However, Swadlow (1998) has previously shown that basal firing rates for slowly conducting cells are even lower than for fast-conducting cells. It seems unlikely that the slowly conducting neurons, undoubtably undersampled in our experiments, would show qualitatively different behavior from the cells which we recorded. Interestingly the conduction velocities of the two "silent" CC cells were at the low end of our sample at 6.1 and 7.1 m/s.

The preceding discussion assumes that neurons use firing rate to encode behavioral information. However, work in the visual system has shown that callosal connections mediate inter-hemispheric oscillatory synchronization in a stimulus-dependent manner (Engel et al. 1991; Munk et al. 1995). It is unlikely that a similar form of communication operates in the motor system during movements, as it is known that network oscillations are abolished by movement and only occur during periods of steady contraction (Baker et al. 1997; Salenius et al. 1997; Salmelin et al. 1995; Stancak and Pfurtscheller 1996). Additionally, we have previously shown that the ability of single neurons to represent oscillations in their discharge is markedly impaired at low firing rates (Baker et al. 2003); the low callosal rates will therefore have similar adverse consequences for the transmission of fine-grained information by oscillatory synchronization as by rate codes. However, during movement, nonoscillatory synchrony does occur within the motor cortex (Baker et al. 2001), and it is possible that information about the timing of a behavioral event could be signaled to the opposite hemisphere by coordinated synchronous firing across many cells. In that case, low rates would serve to reduce the probability of spurious chance synchrony, providing a low synchrony baseline against which to detect event occurrence. The arguments are thus similar whether a
rate code or synchrony code is assumed: low baseline firing increases the detectability of the informative spiking associated with a task event.

To assess how well neurons encoded parameters of the behavioral performance, we used regression analysis between unit discharge and EMG. Because a wide range of EMGs were sampled, they probably provided a fairly good representation of the behavior. Previous work in motor cortex and cerebellum has shown considerable success in predicting EMG from neuron firing using linear decoding (Morrow and Miller 2003; Townsend et al. 2006). In a linear framework, the DCN cells were much better at encoding EMGs than callosal cells. However, linear regression analysis does not allow for the possibility of more complex decoding schemes. When we extended the regression model to include nonlinear effects, we found that although this resulted in better prediction for all cell populations (Fig. 6I), the difference between DCN and CC cells increased. This strongly supports the conclusion that the CC population is worse at coding for continuous behavioral parameters, regardless of the coding scheme.

We cannot entirely exclude the possibility that a more sophisticated decoding scheme would extract even more information from the CC discharge. Additionally, this analysis does not extract “set-related” activity, which precedes behavioral performance. However, it is likely that the low firing rates of CC cells impose a fundamental upper limit on the rate of information that they can transmit (Rieke et al. 1997), which is lower than that carried by SMA UID and DCN cells irrespective of the decoding scheme used.

Conclusions

Our data therefore suggest that the SMA callosal system codes continuous parameters of a behavioral task poorly, whereas the higher firing rates of cerebellar neurons allow them to achieve much better parameter encoding. This may make the cerebellum a better candidate structure than the callosum for the continuous coordination of bimanual movement. By contrast, the low baseline rate of callosal cells could allow them to signal the discrete time of a motor event to the opposite hemisphere with high fidelity. If we were to assign a single function to a single structure based on our results, our conclusions would thus be the opposite to those of Spencer et al. (2003) based on human lesion studies.

Interpreting the deficits produced by lesions is notoriously difficult, given the various behavioral and developmental compensatory mechanisms possible. For example, after lesion of the callosum, other pathways may be able to compensate effectively for its normal function; this could include the cerebellum, which is known to be capable of coordinating the motor plant bilaterally (Brown et al. 1993; Greger et al. 2004; Immisch et al. 2003; Soteropoulos and Baker 2003; Ullen et al. 2003). In addition, it may be erroneous to subdivide motor behavior too rigidly into “discrete” and “continuous” tasks. Even a task such as circle drawing, where kinematic variables appear to vary smoothly and continuously, may have a defined pattern of agonist/antagonist muscle alternation with discrete times of activity onset and offset (Moran and Schwartz 1999).

Patients with callosal lesions are remarkably unimpaired on everyday bimanual tasks (Devinsky and Laff 2003). Such tasks usually require continual coordination: each hand must make on-line correction and allowance for the forces and torques imposed by the movements of the other hand. In agreement with the common experience of these patients, our results suggest that the CC may play little role in such coordination, which could instead involve cerebellar pathways. We suggest that our results may have general applicability, and that low basal firing rates may be a signature of a system preferentially signaling the occurrence time of a discrete event.

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