Functional Connectivity Between Cerebellum and Primary Motor Cortex in the Awake Monkey

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Holdefer, R. N., L. E. Miller, L. L. Chen, and J. C. Houk. Functional connectivity between cerebellum and primary motor cortex in the awake monkey. J Neurophysiol 84: 585–590, 2000. Simultaneous single neuron and field potential (LFP) recordings were made in arm-related areas of the cerebellar nuclei (CN) and primary motor cortex (M1) of two monkeys during a reaching and button pressing task. Microstimulation of focal sites in CN caused short latency (median = 3.0 ms) increases in discharge in 25% of 210 M1 neurons. Short-latency suppressive effects were less common (13%) and observed at longer latencies (median = 9.9 ms). Stimulation in CN also caused reciprocal facilitation and suppression in averages of antagonist muscle electromyograms (EMGs). The latency of these effects was 8–11 ms. In contrast to the selectivity of unit and EMG effects, stimulation-evoked changes in LFP occurred over a broad range of sites. There were no significant short-latency effects detected in cross-correlation histograms between single neurons in CN and M1. However, CN spikes triggered averages of M1 LFPs were observed in a few cases (10% of 126 cases). In one-half of these, there were effects both before and after the CN spikes, which may reflect causal effects from M1 to CN, as well as from CN to M1. Overall, these results demonstrate a spatially specific, short latency, primarily excitatory pathway from CN to M1. The relatively rare effects at the single neuron level may have resulted from the difficulty in achieving optimal alignment between cerebellar and cerebral sites because of the specificity of these connections.

INTRODUCTION

The existence of a projection to the primary motor cortex (M1) from the cerebellar nuclei (CN) is well established (Allen and Tsukahara 1974; Hoover and Strick 1999). The most direct synaptic linkage is known to be a disynaptic excitatory pathway through ventral thalamus, and the characteristics of the pathway have been studied extensively in the anesthetized cat and monkey (Jortell and Ekerot 1999; Na et al. 1997; Sasaki et al. 1976; Shinoda et al. 1993; Steriade 1995). However, the strength and spatial organization of the projections to motor cortex have not been similarly investigated in the awake animal. This is an important issue since the capacity of the cerebellum for regulating the spatiotemporal patterns of voluntary movement commands is likely to be dependent on the microscopic features of this pathway (Houk et al. 1993). We therefore sought to study the specific functional connections from individual neurons or small clusters of nuclear cells to individual neurons or local regions of cortex, using paired placements of microelectrodes in CN and M1. We specifically sought paired sites that were vigorously active in a limb-reaching and button-pushing task to enhance the likelihood of functional connectivity. We report that neuronal activity can be appreciably increased (and often subsequently decreased) at latencies of a few milliseconds. There also seems to be considerable spatial specificity in these connections. Some of our results were briefly presented in abstract form (Holdefer et al. 1999).

METHODS

Paired recordings and stimulus-triggered averages were made from sites within the arm areas of the M1 and CN of two rhesus monkeys. Each monkey faced a panel containing nine buttons arranged in a circle surrounding a central button. They were trained to press buttons in sequence as they were lighted to receive a liquid reward. A head holder and two stainless steel recording chambers were implanted on each monkey, one over the arm area of M1 and the second over the interpositus and dentate nuclei. All animal care, surgical procedures, and research procedures were approved by the Institutional Animal Care and Use Committee of Northwestern University.

To reject stimulus artifacts, M1 recordings were made differentially between a pair of tungsten electrodes glued together with one tip ~200 microns deeper than the other. Action potentials of single neurons were discriminated, and the local field potential (LFP) from M1 was band-pass filtered (10–300 Hz) and sampled at 2,000 Hz. Biphasic stimulus pulses, each phase 200 μs in duration and ranging from 10 to 100 μA, were delivered continuously at 10 Hz throughout the entire time each data file was collected (typically 2 min). Current was passed through the shallow tip of the CN electrode with respect to ground in order not to damage the recording properties of the deeper tip. A limited number of electromyograms (EMG) were recorded from one animal using surface electrodes. These signals were band-pass filtered (75–1,000 Hz) and sampled at 2,000 Hz.

Electrode penetrations in CN were reconstructed from 50-μm-thick histological sections after identifying the Prussian blue reaction products of small electrolytic lesions. M1 recording sites were identified from the location of pins left in the chamber at the time of perfusion.

RESULTS

Recordings were made throughout both chambers at the outset of these experiments to locate regions that were well modulated by limb movement. Subsequent recording and stimulation experiments were focused on paired sites with strong

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movement-related modulation. In monkey G, most of the M1 recordings were concentrated in the crown and anterior bank of the central sulcus. In monkey I, recordings were made more uniformly within a region from the central sulcus to 6 mm anterior of the sulcus.

Stimulus-triggered unit discharge effects

The strength and timing of the CN stimulus effects were evaluated by calculating peristimulus time histograms of M1 discharge. Figure 1, A–C, demonstrates the effects of several different stimulus intensities on one neuron. The left axis of each panel indicates the number of spikes within each 0.5-ms bin and the right axis indicates the likelihood of spike discharge expressed as a percentage of the mean firing rate throughout the 2-min data file. The peak in Fig. 1A with 100 μA current reached 350% of the mean but was confined to only 2–3 bins. Slightly less effect was noted at 50 μA, while no effect was evident for 25-μA pulses. For a small number of other neurons, effects were seen at this low current. Increased discharge was the predominant effect across all tested sites, but the effects were not limited to excitation. Figure 1, D–F, depicts stimulus effects from three other pairs of sites. These included effects at slightly longer latencies (D and E), effects that spanned greater time (F), and facilitatory effects that were followed by suppression (D and F). Although we did not compile duration data quantitatively, the remarkably brief facilitatory effects in Fig. 1, A and B, were not uncommon among the strongest examples.

Statistically significant effects were detected by a two-step process. To compensate for a slowly varying background, a 4th order polynomial was fitted through the histogram bins and used as a baseline to detect the single largest peak and trough. Z scores for both the peak and the
within the fastigial nucleus. These cells had significantly smaller modified z scores ($z = 4.7$) compared with those recorded during stimulation of sites in dentate or interpositus ($z = 13.3, P < 0.005$).

Significant suppression effects occurred rarely by themselves. The typical pattern was that of Fig. 1, D and F. It was of interest to know whether the magnitudes of facilitation and suppression were independent of one another. Figure 2B shows that this was not the case. Cells with large facilitation tended also to have large suppression ($r = 0.53$ and 0.60 for monkeys G and I, respectively). Note that the y-axis is plotted from 100 (no change) to 0 (complete suppression). Several outlier points for both monkeys were not included in the panel, having facilitation effects between 1,000 and 2,000 and suppression near 0.

The relative timing of facilitation and suppression was also highly constrained. Among the 22 neurons having both effects, facilitation always preceded suppression with one exception. The facilitation in this case was the weakest of all significant effects from monkey G. Across the group of cells, the onset latencies for the two types of effects had relatively little overlap (Fig. 2C). Most of the excitatory effects fell within the 1.5- to 5.0-ms bins (median latency 3.0 ms). The single, shortest latency was 1.7 ms and three others occurred at < 2 ms. Suppression effects spanned a broader range and most were clustered between 5 and 12 ms (median latency 9.9 ms). These results reflect a system that is tightly focused both temporally and perhaps also spatially to produce strong excitation within a limited number of output sites.

**Stimulus-triggered LFP and EMG effects**

Unlike the rather specific neuronal discharge effects, statistically significant fluctuations in M1 LFP were common for many paired sites of CN stimulation and M1 recording. Although we did not systematically explore the extent of M1, we were able to evoke LFPs at > 90% of the sites that we investigated. During three experimental sessions, recordings were made from a single, monopolar electrode referenced to a fixed electrode at the dural surface. These recordings were made in combination with our bipolar recordings to provide a direct comparison. The resultant depth profiles aided in the interpretation of the bipolar recordings and confirmed that points of sign reversal in the monopolar recordings corresponded to peaks in the bipolar recordings.

An example of a stimulus-triggered average of bipolar LFP is shown in the upper portion of Fig. 3A. Following the small stimulus artifact at time 0, there was a sharp negative-going component beginning at 2.0 ms. The first significant inflection point after the artifact was considered to be the onset of the effect. The sign of this early component was nearly always negative. Following this component, the response was often quite varied, both in timing and sign, for recordings made with different stimulus or recording sites. Figure 3B shows a second example illustrating this point. Both examples, however, were associated with significant increases in unit discharge (Fig. 3, bottom of each panel). Although we typically used 50- or 100-μA currents, significant LFP effects were occasionally evoked with current as low as 15 μA.

The stimulus-evoked discharge and LFP effects were clearly related to one another in timing and magnitude. As a group, the
onset latencies of the LFPs were similar to those of the excitatory effects on unit discharge, although the earliest LFP effect always preceded the onset of discharge effects, sometimes by less than a millisecond. We calculated the absolute area between onset and 15 ms (Fig. 3, A and B, hatched area) which served as an overall measure of the strength of any effects from cerebellar nucleus to cerebral cortex. There was a significant correlation between the percent increase in unit discharge and the area of the evoked LFP across sites ($r = 0.69, P < 0.001$; Fig. 3F).

Figure 3, C and D shows similar stimulus-triggered averages made not from LFP but from rectified EMG signals. These substantial effects underscore the strength of the connection from cerebellar nucleus to cerebral cortex. There was a significant correlation between the percent increase in unit discharge and the area of the evoked LFP across sites ($r = 0.69, P < 0.001$; Fig. 3F).

Spike-triggered effects

Stimulation within CN, even at the lowest currents, presumably activated a cluster of neurons synchronously, with correspondingly large output effects. We also calculated averages with respect to single, discriminated CN action potentials. Cross-correlation histograms with 0.5 ms binwidth were calculated between simultaneously recorded neurons in CN and M1. Among 233 tested pairs of single neurons that had at least 2,000 trigger spikes (138 pairs had >4,000 triggers), we detected no significant correlations.

Although there were no detectable effects between pairs of neurons, we did detect significant CN spike-triggered effects on M1 LFP. Among 126 pairs of sites there were 12 cases. Although there was some variation in the shape of these averages, the overall pattern was quite consistent and two examples are shown in Fig. 3E. Most frequent was a waveform with significant positivity prior to the time of the CN spike and significant negativity after the spike (6 of 12 cases). Four cases had only the negative-going component after time 0, and two cases had only the positive component before zero.
DISCUSSION

The discharge patterns of neurons in CN and M1 during reaching show many similarities. The two regions generate movement-related bursts that are largely overlapping in time, and both are broadly tuned across movement direction (Smith et al. 1993; Thach 1975). The extensive interconnections between these areas may underlie the similarities in discharge and could play a fundamental role in burst generation. By studying these effects in the awake animal during behavior, we can be confident that the multisynaptic pathways are near or above threshold and in a physiologically relevant state. Our results demonstrate a strong, yet spatially specific, short latency, primarily excitatory pathway from CN to M1.

Strength and timing of effects from CN to M1

Recurrent feedback circulating in reciprocal loops between CN and M1 may provide an important driving force for burst generation and the production of movement commands in the corticospinal tract (Houk et al. 1993). The strong, short latency effects demonstrated here are consistent with this hypothesis. The timing of the fastest excitatory effects is consistent with a disynaptic pathway (Na et al. 1997), but the longer latency effects might well traverse a greater number of synapses. Suppression, which often closely followed the excitation, may have served to decrease its duration (Fig. 1D).

The correlation between the magnitude of facilitation and suppression suggests a common mechanism rather than largely separate pathways for the two types of effects. If this is the case, the suppression may be mediated by inhibitory interneurons within M1 (Na et al. 1997). Alternatively, inhibitory circuits at the level of the motor thalamus may play a role (Ando et al. 1995). We did not attempt to test whether the strength or timing of these effects was modulated across distinct phases of the monkey’s behavior. Such variation in strength is possible and would be of considerable interest. However, because of the brief duration of the facilitation when averaged across the entire behavior, there could have been very little variation in timing.

The CN stimulus-triggered averages of EMG activity further underscore the strength of this pathway in generating movement commands. While it is likely that these effects resulted from the CN to M1 pathway, we cannot rule out the possibility that a CN to magnocellular red nucleus (RNm) pathway also contributed. The timing of these effects was 1–2 ms longer than those typical of EMG averages from M1 (McKiernan et al. 1998). This difference is very close to the shortest onset latencies which we observed for CN-evoked discharge effects in M1. Furthermore, the averages resembled spike- and stimulus-triggered averages obtained from M1 more than RNm because they demonstrated facilitation and reciprocal inhibition of antagonistic muscle pairs (Cheney et al. 1985; Fetz et al. 1989). However, it is interesting to note that the CN-evoked averages were often biphasic, closely resembling the time-course of the stimulus evoked M1 discharge effects. Such a profile is never described for EMG averages triggered from M1 spikes (Cheney et al. 1991). This observation further supports the hypothesis that the CN stimulus-triggered EMG effects were mediated by the CN to M1 pathway and affected by its biphasic dynamics.

Topology of the effects

The presence of oligosynaptic spike-triggered averages of M1 LFPs is indicative of potent CN to M1 connections, whereas their infrequency is consistent with topological (i.e., functional) specificity. The effects after time 0 are likely to correspond to cortical phenomena caused by CN spikes. In addition, one-half of the cases included effects prior to the spike as well. Such effects could reasonably be interpreted as a cortical phenomenon that gives rise to the subsequent CN spike. This provides at least indirect evidence for effects in both directions. The rarity of these effects probably results from the difficulty in achieving optimal alignment between the CN and M1 sites, suggesting a considerable degree of topological specificity in the projections. The relatively frequent occurrence of bi-directional effects among these spike-triggered LFPs supports the hypothesis that these pathways may not only be highly specific in a topological sense but perhaps also organized in a reciprocal fashion. That these interconnections are potentially reciprocal has been known for some time (Allen and Tsukahara 1974). Recent results using transsynaptically transported viral tracers demonstrated that the arm- and face-related pathways between the cerebellar cortex and M1 are reciprocal at least at a macroscopic level (Kelly and Strick 1999). Although that study did not address the analogous maps within the deep cerebellar nuclei, it is likely they are organized in a similar fashion.

These results may seem at odds with the ubiquitous stimulus-driven LFP effects that we and others have described (Sasaki et al. 1976; Steriade 1995). However, although the general effects are widespread, finer-grained maps have also been described in which particular CN sites induced cerebral effects across a broad area of cortex, yet had one or two spatially restricted foci (Jorntell and Ekerot 1999). The correlation we noted between the magnitude of LFP and single unit effects suggests that the unit effects occurred at these focal points within M1 where the largest number of activated thalamocortical terminal fields converged.

If the cerebrocerebellar interconnections are both highly specific and reciprocal, there must be some mechanism driving developmental plasticity to achieve this architecture. Recent modeling work has demonstrated that a simple, biologically plausible network will develop organized, reciprocal loops analogous to those hypothesized between the cerebellum and cerebral cortex using only Hebbian learning and weight normalization, coupled with random spontaneous activity (Hua and Houk 1997; Li et al. 1999). While these experiments deal predominately with the functional properties of the pathway from CN to M1, we also seek to understand the M1 to CN pathway and the extent to which reciprocity can be observed more directly.

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