A Spectrum From Pure Post-Spike Effects to Synchrony Effects in Spike-Triggered Averages of Electromyographic Activity During Skilled Finger Movements

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Schieber, Marc H. and Gil Rivlis. A spectrum from pure post-spike effects to synchrony effects in spike-triggered averages of electromyographic activity during skilled finger movements. J Neurophysiol 94: 3325–3341, 2005. First published July 13, 2005; doi:10.1152/jn.00007.2005. During individuated finger movements, a high proportion of synchrony effects was found in spike-triggered averages (SpikeTAs) of rectified electromyographic activity aligned on the spikes discharged by primary motor cortex (M1) neurons. Because synchrony effects can be produced even if the trigger neuron itself provides no direct synaptic connections to motoneurons, such nonoscillatory synchrony effects often are discounted when considering control of motoneuron pools. We therefore examined the distinctions between pure postspike effects and synchrony effects. The criteria usually applied to distinguish pure and synchrony effects—onset latency and peak width—failed to separate the present SpikeTA effects objectively into distinct subpopulations. Synchrony effects generally were larger than pure effects. Many M1 neurons produced pure effects in some muscles while producing synchrony effects in others. M1 neurons producing no effects, only pure effects, only synchrony effects, or both pure and synchrony effects did not fall into different groups based on discharge characteristics during finger movements. Nor were neurons producing different types of SpikeTA effects segregated spatially in M1. These observations suggest that neurons producing pure and synchrony SpikeTA effects come from similar M1 populations. We discuss potential mechanisms that might have produced a continuous spectrum of variation from pure to synchrony effects in the present monkeys. Although synchrony effects cannot be taken as evidence of mono- or disynaptic connections from the recorded neuron to the motoneuron pool, the functional linkages indicated by synchrony effects represent a substantial fraction of M1 input to motoneuron pools during skilled, individuated finger movements.

INTRODUCTION

Spike-triggered averages (SpikeTA) of rectified electromyographic (EMG) activity can provide evidence of a functional linkage between the discharge of action potentials by the triggering neuron and the discharge of motoneurons in the pool producing the recorded EMG. A significant peak or trough appearing after multiple segments of EMG have been first aligned at the spike (trigger) times and then averaged indicates that the motoneuron pool received facilitatory or suppressive synaptic inputs, respectively, at a consistent latency relative to the discharge of action potentials by the trigger neuron. If the neuron comes from a population known to make monosynaptic excitatory or disynaptic inhibitory connections to motoneurons, if the peak or trough begins at a latency consistent with conduction times from the recorded neuron to the motoneuron pool and then to the muscle, and if the peak or trough is narrow enough to reflect monosynaptic excitatory postsynaptic potentials (EPSPs) or disynaptic inhibitory postsynaptic potentials (IPSPs), then the peak or trough may be taken as evidence that the trigger neuron provided direct (monosynaptic) or relatively direct (disynaptic) connections to the motoneuron pool (Fetz and Cheney 1980). Such a peak or trough is referred to as a postspike effect (PSpE). In macaque monkeys, PSpEs have been demonstrated from neurons in the primary motor cortex (M1), red nucleus, spinal gray matter, and dorsal root ganglia (Fetz and Cheney 1980; Flament et al. 1992; Lemon et al. 1986; Mewes and Cheney 1991; Perlmutter et al. 1998).

Many SpikeTAs from the same neuronal populations show significant peaks or troughs that do not meet these criteria, however. Although the latency of the peak maximum or trough minimum may be appropriate for a PSpE, the onset may be too early to be consistent with the minimal conduction time from cortex to muscle and may even precede the trigger. In addition, the peak or trough may last too long to be consistent with a single synaptic input (Baker and Lemon 1998). Such peaks or troughs generally are referred to as synchrony effects because they indicate that synaptic inputs from other neurons arrived in the motoneuron pool synchronized with the discharges of the trigger neuron. Synchrony effects therefore can appear in SpikeTAs even when no mono- or disynaptic connection exists from the trigger neuron to the motoneuron pool. Synchrony effects in SpikeTAs typically are not oscillatory and probably represent a form of synchrony in the nervous system different from the coherent oscillatory synchrony at 15–40 Hz that can be demonstrated in the motor cortex and EMG (Baker et al. 1997; Conway et al. 1995; Donoghue et al. 1998; Kilner et al. 1999, 2000; Murthy and Fetz 1996b).

Because synchrony effects in SpikeTAs cannot be taken as evidence of direct connections to motoneurons, neurons producing synchrony effects often are discounted when considering control of muscle activity. Synchrony effects nevertheless provide evidence that, on average, excitation or inhibition arrived in the motoneuron pool at a short, consistent latency relative to the discharge of action potentials by the trigger neuron. Given that a great deal of the corticospinal control of...
muscles arrives in the motoneuron pools over nonmonosynaptic connections (Kuypers 1987) and that nonmonosynaptic inputs may be sufficient to control even fine finger movements (Ropper et al. 1979; Sasaki et al. 2004), we examined the distinctions between pure PSPs and synchrony effects in SpikeTAs obtained from M1 neurons as monkeys performed skilled, individuated finger and wrist movements.

METHODS

Most of the methods used in the present study for behavioral training, data collection, and initial analyses have been described in previous reports (Poliakov and Schieber 1999; Schieber 1991, 2002; Schieber and Poliakov 1998) and are summarized here as needed. A separate description of training-related differences in SpikeTA effects observed among the present monkeys has been published previously (Schieber 2002).

Animals and behavioral procedures

All care and use of these purpose-bred monkeys complied with the U.S. Public Health Service Policy on Human Care and Use of Laboratory Animals and was approved by the University Committee on Animal Resources at the University of Rochester. Each monkey was trained to perform visually cued individuated flexion and extension movements of the right hand fingers and/or wrist (Schieber 1991). The monkey sat in a primate chair with the upper arm approximately vertical, and the elbow, bent at ~90°, restrained in a molded cast. The horizontal forearm was placed in mid-position between pronation and supination (radial aspect up), and the hand was placed in a pistol-grip manipulandum that separated each finger into a different slot. At the end of each slot, the fingertip lay between two microswitches. By flexing or extending the digit a few millimeters, the monkey closed the volar or dorsal switch, respectively. The manipulandum, in turn, was mounted on an axis that permitted flexion and extension wrist movements. Each monkey viewed a display on which each digit (and the wrist) was represented by a row of five light-emitting diodes (LEDs). When the monkey flexed or extended a digit, closing a microswitch, the central yellow LED went out and a green LED to the left or right, respectively, came on, cueing the monkey to which switch(es) had been closed. Red LEDs to the far left or right, respectively, were illuminated one at a time, under microprocessor control, instructing the monkey as to which instructed digit (1 = thumb through 5 = little finger, w = wrist), and the first letter of the instructed direction (f = flexion; e = extension). For example, “4f” indicates instructed flexion of the ring finger.

Data collection

Data were collected from monkey A starting after 1 yr of training. Monkey A performed instructed movements 1f, 2f, 3f, 4f, 2e, and 3e with the wrist axis locked. Monkeys C and G each were studied after 5 yr of training; both performed all 12 instructed movements, flexion and extension of each digit and of the wrist.

Aseptic surgery under isoflurane anesthesia was used to perform a craniotomy over the left central sulcus at the level of the hand representation, to implant a rectangular Lucite recording chamber over the craniotomy, and to implant head-holding posts. Once the monkey had recovered from this procedure and had become accustomed to performing the finger movement task with its head held stationary, EMG electrodes made of 32 gauge, Teflon-insulated, multi-stranded stainless steel wire (Cooner AS632, Chatsworth, CA) were implanted percutaneously using aseptic technique in 8–16 forearm and hand muscles under ketamine anesthesia, using techniques adapted from those of Cheney and colleagues (McKiernan et al. 2000). For forearm muscles, each wire was stripped of insulation for 1 mm, passed retrograde for 2 mm into a 23-gauge hypodermic needle, the needle was passed percutaneously into the muscle belly, and then the needle was withdrawn, leaving the wire tip fish-hooked in the muscle belly. To implant intrinsic muscles of the hand, wires were tunneled separately for each muscle from a 5-mm incision on the dorsal aspect of the forearm to a 5-mm incision on the dorsal aspect of the hand, wire tips were stripped and inserted into the muscle belly using hypodermic needles, and then the incisions were closed with subcuticular, absorbable suture. For both forearm and intrinsic muscles, two wires were placed 5–10 mm apart in the long axis of each muscle belly to provide a bipolar recording configuration. Four bipolar pairs were led to a single external connector. Appropriate location of each bipolar pair was confirmed by observing the movements evoked with intramuscular stimulation (1-s trains of 100-Hz biphasic, constant current pulses, 200 μs/phase, 10–1000 μA). Externally, the wires and connectors were held in place with elastic adhesive tape (Elastikon, Johnson & Johnson), covered with a self-adhesive wrap (Vetrap, 3M), and the monkey was placed in a jacket (Alice King Chatham, Hawthorne, CA) with heavy Cordura sleeves to prevent removal of the electrodes. During subsequent recording sessions, the sleeve and self-adhesive wrap were removed, exposing the connectors held in the elastic adhesive tape. Implanted electrodes typically functioned well for 6–8 wk after which they were removed by first loosening the elastic adhesive tape with acetone and then gently pulling each wire out through the skin. After 2 wk, another set of EMG electrodes was implanted. Muscles implanted typically included 8–16 of the following: thenar eminence (Thenar), first dorsal interosseus (FDI), hypothenar eminence (Hypo), flexor digitorum profundus, radial region (FDR), flexor digitorum profundus, ulnar region (FDPu), flexor digitorum profundus, proximal ulnar region (FDPpu), flexor digitorum superficialis (FDS), flexor carpi radialis (FCR), palmaris longus (PL), flexor carpi ulnaris (FCU), abductor pollicis longus (APL), extensor pollicis longus (EPL), extensor digiti secundi et tertii (ED23), extensor digitorum communis (EDC), extensor digiti quarti et quinti (ED45), extensor carpi radialis (ECR), extensor carpi ulnaris (ECU), and supinator (Sup).

Thereafter in daily recording sessions, conventional techniques were used to record single M1 neurons simultaneously with EMG activity from the implanted forearm and hand muscles (EMG amplification: 2,000–100,000 times, band-pass: 0.3–3 kHz, sampling frequency: ~4 kHz/channel) as the monkey performed individuated finger and wrist movements. M1 neurons with large-amplitude action potentials, the firing rate of which is modulated intensely in relation to movement execution, are most likely to produce SpikeTA effects. During recording, our selection of neurons for study therefore intentionally was biased in favor of such neurons. One data-acquisition interface was used to store data to disk on one host PC, which also provided a scrolling display of all neuron and EMG recordings (Power1401 interface, Spike2 software, Cambridge Electronic Design). A second identical data-acquisition interface and host PC running AVE software (courtesy L. Shupe, E. E. Fetz, and P. D. Cheney) were used concurrently to form initial on-line averages of rectified EMG for each channel with data segments extending ±50 ms from the time of all neuron spikes. As more segments are averaged, EMG activity that is not time-locked to the neuronal spike evens out toward a baseline, whereas EMG activity that is time-locked to the spike accumulates progressively, forming a facilitatory peak or suppression trough (Fetz and Cheney 1980). M1 neurons that had produced no effect in the on-line averages after accumulating 4,000 spike triggers were recorded further only long enough to obtain 10 correctly performed trials of each instructed movement. M1 neurons that...
produced an effect evident in the on-line average of at least one muscle were recorded until 10,000 spikes had accumulated or as long as satisfactory isolation could be maintained. Often, additional recordings then were made as single-pulse intracortical microstimulation (single biphasic pulses, 0.2 ms/phase, 5–20 μA) was delivered through the microelectrode at inter-pulse intervals varying continuously between 60 and 80 ms (Park et al. 2001). Stimulus-triggered averages formed on-line for each EMG in these recordings confirmed that the recorded neurons were in M1 (Schieber 2002).

Analysis of SpikeTA effects

SpikeTAs were obtained for 318, 132, and 166 neurons in the M1 hand representation of monkeys C, G, and A, respectively. These same populations formed the basis of a previous report (Schieber 2002), although because of a book-keeping error the number of neurons from monkey G was reported then as 136. Off-line, SpikeTAs were formed for each EMG channel using custom software to average segments of rectified EMG activity from 30 ms before to 50 ms after each spike of the M1 neuron. Significant effects in SpikeTAs were identified with multiple-fragment statistical analysis (Poliaakov and Schieber 1998). This approach divides the spike train into multiple fragments, forms a triggered average using the spikes in each fragment, and subtracts the mean value of the average in a test window from the mean in immediately preceding and following control windows. If this difference on average is significantly different from 0 across all the fragments, the peak (or trough) in the test window is statistically significant. Because previous studies have shown that the peaks and troughs of postspike effects in SpikeTAs typically occur at latencies from 6 to 16 ms after the M1 neuron spike (Cheney and Fetz 1985; Fetz and Cheney 1980; Kasser and Cheney 1985; Lemon et al. 1986; McKiernan et al. 1998), statistically significant peaks (or troughs) were identified in that fixed temporal window. Although on rare occasion we observed SpikeTAs with peaks preceding the spike time, or oscillations extending on both sides of the spike time, potentially significant peaks or troughs occurring at latencies outside the fixed 6- to 16-ms window were not examined in the present analysis.

Initial averages were formed using all spikes recorded from a given M1 neuron. If any muscle showed a peak (or trough) still significant at the P < 0.05 level after Bonferroni correction for testing multiple EMG channels, the neuron was accepted as producing effects. Then each EMG in that recording with a peak (or trough) significant at P < 0.05 without correction was submitted to the following analysis. To eliminate contributions of sweeps containing only noise, a second, filtered average was formed, using spikes as triggers only if the RMS value of the EMG from 30 ms before to 50 ms after the trigger was >5 μV, i.e., greater than the typical noise level (McKiernan et al. 1998). If the peak (or trough) in this filtered average remained significant at P < 0.05 (without Bonferroni correction), the effect was retained for further analysis. Any baseline ramp in the filtered average was subtracted, and the filtered average was smoothed with a flat five-point finite impulse response filter.

A computer algorithm performed the following computations for each EMG sweep-filtered, ramp-subtracted, smoothed SpikeTA with a significant peak or trough occurring 6–16 ms after the trigger (Schieber 2002). As illustrated in Fig. 1 for two different SpikeTAs, the mean ± 2 SD of the average was calculated over a baseline period from 30 to 10 ms before the time of the trigger. The maximum value of the peak (or minimum of the trough) was identified, and the average was followed backward and forward until it fell within 2 SD of the baseline mean. These times were defined as the onset and offset, respectively, of the SpikeTA effect, and their latencies were corrected for the time from spike onset to the trigger pulse used for averaging. The mean percent increase (MPI) of the SpikeTA effect was calculated by averaging the amplitude of the waveform from onset to offset, subtracting the mean during the baseline period, and then dividing the result by the baseline mean and multiplying by 100. The peak percent increase (PPI) of the SpikeTA effect was calculated by finding the maximum (for a peak, or minimum for a trough) of the SpikeTA waveform between the onset and offset, subtracting the baseline mean, and then dividing the result by the baseline mean and multiplying by 100. The normalized area of the SpikeTA effect was calculated by multiplying the MPI by the duration of the effect, from onset to offset. Finally, the peak width at half-maximum (PW=H) of the SpikeTA effect was determined by computing half the height of the peak above (or trough below) the baseline mean and measuring the width of the peak (or trough) at this level. Although these measured features of SpikeTA effects all may vary to some degree depending on both the level of background EMG activity (Bennett and Lemon 1994) and the instantaneous spike frequency of the neuron (Lemon and Mantel 1989), controlling for background EMG and instantaneous spike frequency vastly reduces the amount of data included in the SpikeTA for each neuron-muscle pair. We therefore chose to quantify effects in SpikeTAs compiled using all spikes available after EMG sweep-filtering, which best reflects the neuron-muscle interactions averaged over all the present individuated finger and wrist movements. These analyses above were performed using custom Spike2 scripts (Cambridge Electronic Design). Subsequent analyses described in RESULTS were accomplished in MATLAB (MathWorks, Natick, MA). As most of the parameters compared were not normally distributed, statistical comparisons were performed with the Kolmogorov–Smirnov (KS) test.

We evaluated potential cross-talk between simultaneously recorded EMGs using previously described procedures (Buys et al. 1986; Kasser and Cheney 1985). The largest motor units in a given EMG channel were used as triggers to create a triggered average of unrectified EMG in every EMG channel. The ratio of the peak-to-peak amplitude of the averaged motor unit waveform in a test channel to that in the trigger channel then provides an estimate of cross-talk from the trigger to the test channel. We considered that the SpikeTA effect recorded in a test channel might be attributable to cross-talk from motor units recorded in the trigger channel if the ratio of the SpikeTA MPI in the test channel to that in the trigger channel was less than twice the ratio of the averaged motor unit waveform in the test channel to that in the trigger channel. When significant SpikeTA effects were identified in multiple muscles in the same recording, one
of any pair of effects potentially resulting from cross-talk between EMG recordings was eliminated from the present data set.

Analysis of M1 neuron activity patterns

To summarize the activity pattern of each M1 neuron during the 6 or 12 individuated finger and wrist movements performed by a given monkey, we compiled for each instructed movement a separate histogram (20-ms bin width) of spike firing rate during all correctly performed trials, aligning the data at the end of the movement (time of switch closure) in each trial. These histograms (compiled using custom Spike2 scripts) then were exported to MATLAB and were used to measure the baseline firing rate, the peak firing rate, and the change (peak-baseline) in firing rate for each movement. Three neurons from monkey C and four from monkey G could not be used in these analyses either because the monkey was unable to perform 1 of the 12 movements the day the neurons were recorded or because a cable failure corrupted the behavioral event codes during recording.

Cluster analysis was performed in a fashion similar to previous work (Poliakov and Schieber 1999). Monkey G had not been studied at the time of that report, and recording from monkey C had not concluded. Of the 318 neurons reported here from monkey C, 146 were included in the prior cluster analysis. Of the 166 neurons reported here from monkey A, 159 were included in the prior cluster analysis. The activity pattern of each neuron across the instructed movements was characterized by a 12-dimensional (monkeys C and G) or 6-dimensional (monkey A) vector consisting of the change in firing rate for that neuron during each of the 12 (or 6) movements performed. To search for neurons with similar relative patterns of discharge across the movements rather than similar absolute discharge, we normalized the vector for each neuron to unit length by dividing each change in firing rate by the root of the sum of squares of the 12 (or 6) values. The vectors of all neurons thus were normalized to lie on the surface of an imaginary multi-dimensional sphere. Single-linkage clustering then was performed on these unit vectors using Euclidean distance as the similarity measure. Using the cosine of the angle between vectors as the similarity measure yielded similar results.

Reconstruction of electrode penetrations

After data collection was completed in each monkey, a microelectrode was used to make electrolytic lesions at selected locations. One to 2 wk later, the monkey was killed by barbiturate overdose and perfused transcardially with heparinized saline followed by 10% formalin, 10% sucrose, and 30% sucrose. A 30-gauge needle mounted on the same micropositioner used for microelectrodes then was used to inject India ink into the cortex at selected coordinates around the recording sites. Thereafter the brain was removed from the skull, photographed, sunk in 30% sucrose, blocked, and 50-μm sections were cut on a freezing microtome. Every section through the region of microelectrode penetrations was mounted and stained with cresyl violet for Nissl substance. Microscopic examination confirmed that the majority of electrode penetrations had passed down the cortex in the anterior bank and lip of the central sulcus. Some penetrations also had passed through the crown of the precentral gyrus. A few other penetrations had been made into the postcentral cortex, and neurons recorded in these penetrations were excluded from subsequent analyses.

Because numerous penetrations were made over many months in each monkey, individual identification of each penetration in the histologic material was not possible. We therefore reconstructed the position of each neuron in three dimensions using the micropositioner/microdrive coordinates at the time of recording. Because the brain may be shifted upward toward the craniotomy when intracranial venous blood volume increases during moments of elevated intrathoracic pressure (Valsalva effect). The depth coordinate at which the advancing electrode touched the dura mater, for example, typically varied ±5 mm from day to day. For these reasons, when coordinates of recorded neurons lay either superficial or deep to the typical boundaries of the cortical gray matter, the positions of all neurons recorded in that penetration were adjusted by the same offset to bring their reconstructed position within the cortical gray matter. Depth was corrected in this way by 1–2 mm for 46 of 318 neurons recorded in 17 of 183 penetrations in monkey C, by 1–3 mm for 27 of 132 neurons recorded in 10 of 85 penetrations in monkey G, and 1–2 mm for 4 of 165 neurons in 2 of 93 penetrations in monkey A. Uncertainty in the anteroposterior and mediolateral coordinates was substantially less, and these coordinates were not altered. The location of the central sulcus on the hemispheric surface was estimated in the same coordinate system by comparing penetration locations with the histologic material. These coordinates for the central sulcus, and those for each M1 neuron tested for SpikeTA effects (see RESULTS) were plotted in three-dimensions using MatLab. Views of these reconstructions were rendered using Persistence of Vision Ray Tracer (POV-Ray).

RESULTS

SpikeTAs were obtained for 318, 132, and 166 neurons in the M1 hand representation of monkeys C, G, and A, respectively. In these three monkeys, 3,561, 2,764, and 1,649 neuron-EMG pairs were tested for SpikeTA effects. Significant effects were found for 467 neuron-EMG pairs from 159 M1 neurons in monkey C, 212 pairs from 51 neurons in monkey G, and 47 pairs from 27 neurons in monkey A.

Classifying SpikeTA effects as pure versus synchrony

Although in theory a categorical distinction may be drawn between pure and synchrony SpikeTA effects, in practice, a sharp demarcation may be difficult to identify using objectively quantifiable parameters. As described in the INTRODUCTION, two features of the peaks or troughs in SpikeTAs have been used to distinguish pure versus synchrony effects: onset latency and peak width. Figure 2 shows the distributions of onset latency and PWHM for all significant SpikeTA effects obtained from each of the three monkeys during individuated finger movements. For each of these distributions ( ), separate daughter distributions are shown for facilitatory effects ( ) and suppressive effects ( ).

The onset latency distribution from each monkey (left) differs from the others, but none of the three are bimodal in a way that would permit pure effects to be distinguished categorically from synchrony effects. Defining a critical onset latency to make this distinction is best achieved by stimulating the corticospinal tract directly and observing the minimal latency of EMG responses. Because we did not employ implanted pyramidal stimulating electrodes, we estimated the earliest possible onset latency as follows. In macaque monkeys, the minimal conduction time from M1 to the cervical enlargement is ~1.4 ms (Humphrey and Corrie 1978), the minimal segmental latency from arrival of action potentials in the cervical lateral column to monosynaptic EPSPs in motoneurons is ~0.5 ms (Maier et al. 1998), and the latency from cervical motoneuron discharge in the spinal cord to motor unit action potential in the EMG of forearm muscles is ~2.8 ms (Perlmutter et al. 1998), which totals ~4.7 ms for the minimal
latency from M1 neuron spikes to EMG activity (Fetz and Cheney 1980; Lemon et al. 1986). We therefore have used a value of 5 ms.

PWHM has been used as another criterion for distinguishing pure versus synchrony SpikeTA effects. In theory, synchrony effects should be more dispersed in time than pure postspike effects. Peak width is measured at half-maximum rather than at the base of the peak because the measurement at half-maximum is affected less by the noise in the triggered average than measuring the duration close to baseline. Based on theoretical considerations and computer simulations, a critical value of 7–9 ms has been suggested for distinguishing pure versus synchrony effects.

FIG. 2. Onset latency and peak width at half-maximum (PWHM) distributions. Separate histograms show the distributions of onset latency (left) and PWHM (right) for the population of SpikeTA effects obtained in each monkey (C, top; G, middle; and A, bottom). For each parent histogram (■), 2 daughter histograms show the distributions for facilitatory (MPI >0, □) and suppressive (MPI <0, △) SpikeTA effects. Neither onset latency or PWHM consistently showed a bimodal distribution that would indicate distinct subpopulations of pure and synchrony SpikeTA effects.
synchrony effects (Baker and Lemon 1998). The shorter value of 7 ms would separate purely monosynaptic corticomotoneuronal (CM) effects from those that might include any element of synchrony, whereas the longer value of 9 ms would include most CM effects with the possibility of including those that additionally represent some degree of synchrony. In theory, if multiple M1 neurons had been recorded simultaneously through independent electrodes, a more precise PWHM criterion could have been derived based on examination of the cross-correlation kernels between synchronized M1 neurons. The present data, however, were collected with single electrodes. Figure 2 (right) shows the PWHM distributions for all SpikeTA effects from the present monkeys. Only for monkey G did the distribution suggest two subpopulations separated by a nadir at 7–8 ms. Although the PWHM distributions are not clearly bimodal, we therefore have used the more liberal value of 9 ms suggested by Baker and Lemon (1998) to distinguish pure versus synchrony effects.

Neither of these two objective criteria—onset latency or PWHM—separated the present populations of SpikeTA effects into distinct pure and synchrony subpopulations. Rather, the features of onset latency and PWHM each covered a continuous spectrum from values consistent with pure postspike effects to values characteristic of synchrony effects. Because the combination of the two parameters might have separated pure and synchrony effects more clearly than either parameter alone, we also examined the two-dimensional distribution of these parameters for each monkey. Figure 3 shows this distribution for the population of SpikeTA effects from each monkey as a scatterplot in which facilitatory (+) and suppressive (●) effects are distinguished. To better visualize variation in the density of points, a two-dimensional histogram of the same data combining the facilitatory and suppressive effects has been plotted as well, using a color scale to represent the number of effects with both onset latency and PWHM falling into particular 1-ms bins. Cross-hairs have been drawn in each plot at the critical values of onset latency (5 ms) and PWHM (9 ms).

These two-dimensional distributions fail to show two distinct subpopulations separated by a nadir near the critical values. Nor would shifting one or both critical values a few milliseconds in any direction mark a more distinct nadir. In monkey C, from which the largest number of points was available, two regions of high-density might be considered (yellow-green, one around onset latency 9, PWHM 5; another around onset latency 7, PWHM 9), but these are bridged by the bin with highest density (red, onset latency 7–8, PWHM 6–7). The two-dimensional distributions from monkeys G and A showed even less indication of two subpopulations of SpikeTA effects. We also examined these distributions separately for intrinsic hand muscles and forearm muscles because the extra conduction distance renders the onset latency of effects in intrinsic hand muscles 3–4 ms longer than in forearm muscles (Lemon et al. 1986). But these plots (not illustrated) resembled

**FIG. 3. Two-dimensional histograms of onset latency vs. PWHM.** For each monkey—C, G, and A—a scatterplot shows each SpikeTA effect as a point located at coordinates representing its onset latency (abscissa) vs. its PWHM (ordinate). +, facilitatory effects (MPI > 0); ●, suppressive effects (MPI < 0). A 2-dimensional histogram shown as a colorscale indicates the number of SpikeTA effects with different combinations of onset latency and PWHM falling into 1-ms bins. The same colorscale (inset in the bottom plot for monkey A) was used for the data from all 3 monkeys. Although the population does not appear to be bimodal, crosshairs have been drawn at onset latency ~ 5 ms and PWHM = 9 ms, the values used here to separate pure and synchrony effects. Using these 2 parameters in tandem arbitrarily separates the population into 4 categories—pure, pure + synchrony, synchrony and late widening—described in the text.
those of Fig. 3 in showing no bimodal separation of pure and synchrony effects. Overall, these observations support the notion that in these monkeys SpikeTA effects had a continuous spectrum of features spanning the characteristics of pure and synchrony effects.

We can use crosshairs drawn at onset latency = 5 ms and PWHM = 9 ms, however, to consider various combinations of SpikeTA effect features in somewhat greater detail. Points falling in the lower right quadrant represent SpikeTA effects with both onset latency >5 ms and PWHM <9 ms, which hence meet both criteria for pure postspike effects, an example of which is shown in Fig. 9A. Points falling in the upper left quadrant represent effects with both onset latency <5 ms and PWHM >9 ms, meeting both criteria for synchrony effects, an example of which is shown in Fig. 9F.

But points falling in the remaining two quadrants meet one criterion for pure effects and one criterion for synchrony effects. Points falling in the lower left quadrant represent SpikeTA effects with narrow peaks but early onsets. This occurs when a narrow peak appears superimposed on a wider base with the narrow peak accounting for at least half the amplitude of the SpikeTA effect. An example is shown in Fig. 9C. Using only the PWHM criterion, these effects would be classified as pure effects as they do indicate direct input from the recorded neuron to the motoneuron pool. Using only the onset latency criterion, these effects would be classified as synchrony effects as they do indicate some degree of input to the motoneuron pool produced by other neurons synchronized with the trigger neuron. A number of studies therefore have interpreted such effects as indicating the combination of direct input from the recorded cell, plus additional inputs from other synchronized neurons arriving in the same motoneuron pool (Flament et al. 1992; McKiernan et al. 1998; Perlmutter et al. 1998). Such SpikeTA effects then may have termed pure + synchrony effects.

Other points falling in the upper right quadrant represent SpikeTA effects that began at an onset latency consistent with direct input from the recorded neuron but had a wide peak. Using only the onset latency criterion, these effects would be classified as pure postspike effects although their wide peaks indicate some additional inputs to the same motoneuron pool that arrived later from other synchronized neurons. Previous reports have not emphasized such SpikeTA effects, which we term late widening effects. Examples are shown in Fig. 5 (PL, ED45, and ECU) and Fig. 9C.

Consideration of pure + synchrony and late widening effects is useful when thinking of potential mechanisms producing different features of SpikeTA effects (see DISCUSSION). Like the pure and synchrony categories, however, the pure + synchrony and late widening categories are parts of a continuous spectrum in the present populations of SpikeTA effects rather than distinct classes. To simplify further comparisons below, however, we have classified any SpikeTA effect with both onset latency >5 ms and PWHM <9 ms as a pure effect; all other SpikeTA effects have been grouped together as synchrony effects. Pure + synchrony and late widening effects thus were included with standard synchrony effects. Based on this classification, in monkey C, 225 effects were classified as pure, and 242 as including some form of synchrony; in monkey G, 47 pure and 165 synchrony; and in monkey A, 37 pure and 10 synchrony.

Because corticospinal neurons are uniformly excitatory, even pure suppressive SpikeTA effects are thought to represent at least a disynaptic pathway from the recorded M1 neuron to the motoneuron pool, inhibitory interneurons being positioned between the corticospinal neuron and the motoneuron pool. In the one-dimensional histograms of onset latency or PWHM (Fig. 2), the daughter distributions for suppressive effects were remarkably similar to the distributions for facilitatory effects, many of which presumably were monosynaptic. The scatterplots of Fig. 3 suggest, however, a tendency for suppressive effects to occur somewhat later than facilitatory effects. At any particular PWHM, the effects with the earliest onsets tended to be facilitations (+), whereas the effects with the latest onsets tended to be suppressions (●), considerable overlap being evident in between.

**Strength of pure versus synchrony SpikeTA effects**

The amplitude of a SpikeTA effect gives some indication of the total strength of synaptic inputs arriving in the motoneuron pool at a consistent latency relative to the spikes discharged by the recorded neuron. Because of multiple factors—including temporal facilitation at corticomotoneuronal synapses, persistent inward currents in motoneuron dendrites, the proportion of motoneurons facilitated or suppressed, the recorded waveforms of contributing motor unit action potentials, the number of motor units recorded by the EMG electrodes, cancellation of some action potentials by superimposition with others, and the general background EMG level—the relationship between synaptic input strength and SpikeTA effect amplitude may not be linear (Baker and Lemon 1995; Bennett and Lemon 1994; Botteron and Cheney 1989; Botteron and Cheney 1989; Hultborn et al. 2003; Lemon and Mantel 1989; Muir and Porter 1973). Nevertheless, more synaptic input in general would be expected to lead to discharge of more motoneurons and a larger SpikeTA effect. We therefore compared the amplitudes of pure and synchrony SpikeTA effects.

The amplitude of SpikeTA effects most often has been quantified as the mean percent increase (MPI) of average EMG activity above the baseline, from the onset to the offset of the SpikeTA effect. MPI distributions for pure versus synchrony effects differed significantly in monkeys C and G (P < 0.05) although not in monkey A. To identify the source of this difference, we compared the MPI distributions of pure versus synchrony effects separately for facilitatory (MPI > 0) and suppressive (MPI < 0) effects in each monkey. Pure versus synchrony facilitatory MPI distributions differed significantly in monkeys C and G (P < 0.01), though not in monkey A (P > 0.05); pure versus synchrony suppressive MPI distributions also differed significantly in monkeys C and G (P < 0.005), whereas insufficient data were available in monkey A. Given these differences in the distributions, we calculated the mean MPI for pure and for synchrony facilitatory and suppressive SpikeTA effects in each monkey. As shown in Table 1, the absolute value of the mean MPI was greater for synchrony effects than for pure effects, both for facilitatory and for suppressive effects, in each of the three monkeys. Another commonly used measure of SpikeTA effect amplitude, the peak percent increase (PPI), showed a similar pattern: the mean PPI was consistently greater for synchrony than for pure effects (Table 1), and the PPI distributions were significantly different.
both for facilitatory and for suppressive effects in *monkeys C* and *G* (*P* < 0.001) although not in *monkey A*. These observations suggest that although synchrony effects do not result simply from synapses made directly by the recorded M1 neuron, synchrony effects on average represent total inputs arriving in the motoneuron pool somewhat stronger on average than those originating directly from single corticomotoneuronal (CM) cells.

Using PPI or MPI to quantify the amplitude of SpikeTA effects may underestimate the differences between pure and synchrony effects, however. The PPI measures a single point, and the MPI averages amplitude over the duration of the SpikeTA effect. By definition, synchrony effects begin earlier and/or last longer than pure effects. Indeed, the duration (offset time–onset time) of pure versus synchrony effects was significantly different in each of the three monkeys (*P* < 0.00001 for *monkey C* and *G*, *P* < 0.01 for *monkey A*, means in Table 1). The total amount of facilitation or suppression arriving in the motoneuron pool time-locked to the spikes discharged by an M1 neuron may be represented more accurately by the area under the peak (or trough) of the SpikeTA effect than by the MPI or PPI.

We therefore calculated a normalized area for each effect as MPI multiplied by duration and compared this normalized area of pure versus synchrony effects in each of the three monkeys. The absolute value of normalized area was greater for synchrony than for pure effects in all three monkeys (Table 1), and these differences were significant for both facilitatory and suppressive effects in *monkeys C* and *G* (*P* < 0.00001) and for facilitatory effects in *monkey A* (*P* < 0.01). Furthermore, as shown in Fig. 4, many facilitatory (positive values) and suppressive (negative values) synchrony effects had substantially greater areas than did pure effects.

To exclude the possibility that amplitude differences between pure and synchrony effects resulted simply from EMG amplitude variation across different electrode implants, we compared amplitudes within the largest single set of effects obtained in recordings made through the same pair of implanted wires. In *monkey C*, 16 pure and 10 synchrony effects were obtained through the same pair of wires in FDP. Mean absolute values of MPI, PPI, and normalized area all were larger for synchrony than for pure effects. With this small sample, the difference in MPI and PPI did not attain significance, but the difference in normalized area was significant (*P* < 0.01, 1-way ANOVA). We conclude that the spikes discharged by M1 neurons that produced synchrony effects indicated, on average, the arrival (within a few milliseconds) of substantially more input in the motoneuron pool than was

![Table 1. Mean amplitude measurements of SpikeTA effects](image)

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Facilitations</th>
<th>Suppressions</th>
<th>Facilitations</th>
<th>Suppressions</th>
<th>Facilitations</th>
<th>Suppressions</th>
<th>Mean Duration, ms</th>
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<td>Mean PPI</td>
<td>Mean Normalized Area</td>
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</tr>
<tr>
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<td>-67.40</td>
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</table>

SpikeTA, spike-triggered average; MPI, mean percent increase; PPI, peak percent increase.

![Fig. 4. Normalized area of SpikeTA effects](image)
indicated by the spikes of neurons that produced pure effects.

**Pure and synchrony SpikeTA effects from the same M1 neuron**

Previous studies have shown that single premotoneuronal neurons from several regions of the nervous system can produce pure postspike effects in the SpikeTAs of multiple muscles (Fetz and Cheney 1980; Flament et al. 1992; Lemon et al. 1986; Mewes and Cheney 1991; Perlmutter et al. 1998). Such premotoneuronal neurons are thought to have axons that branch in the spinal cord to innervate multiple motoneuron pools as has been demonstrated anatomically and physiologically for some M1 neurons (Shinoda et al. 1979, 1981). An M1 neuron that produced pure SpikeTA effects through monosynaptic connections to motoneurons of one or more muscles therefore might be assumed to produce only pure effects. Conversely, an M1 neuron that produced synchrony effects in some muscles might be assumed to have had no monosynaptic connections to any motoneuron pool and therefore to be incapable of producing pure effects. Previous studies have documented cases, however, in which the same M1 neuron produced a pure postspike effect in some muscles, while producing a synchrony effect in at least one other muscle (Jackson et al. 2003; McKiernan et al. 1998).

We likewise observed numerous instances in which M1 neurons that produced pure postspike effects in some muscle, simultaneously produced synchrony effects in other muscles. For example, Fig. 5 shows SpikeTA effects produced in four muscles by the same M1 neuron. These four effects began at latencies after the M1 neuron spike (vertical line) ranging from 7.8 to 10.0 ms, all consistent with conduction times from the M1 neuron to muscle via a monosynaptic connection to motoneurons. Whereas the peak in FDPu was quite narrow (PWHM = 4.8 ms), the peaks in PL, ED45 and ECU were substantially wider (ranging from 9.4 to 10.8 ms). This neuron thus produced a pure postspike effect in FDPu, plus late widening effects in three other muscles. The pure SpikeTA effect in FDPu allows us to infer that this neuron, C0107, was a CM cell, with direct connections to FDPu motoneurons. C0107 also discharged spikes synchronously with other neurons that provided inputs to the PL, ED45, and ECU motoneuron pools. Had we not recorded simultaneously from FDPu, we would not have been able to infer that C0107 was a CM cell. Conceivably, C0107 also might have made direct connections to one or more of the other three motoneuron pools, although these inputs would have been synchronized with additional inputs from other neurons that arrived in the motoneuron pool slightly later.

To examine the frequency with which single M1 neurons produced different numbers of pure and synchrony effects, we compiled two-dimensional histograms (Fig. 6). In these histograms, vertical bars along the “pure” axis represent the count of neurons that produced only pure effects in various numbers of muscles. Bars along the “synchrony” axis represent counts of neurons that produced only synchrony effects in various numbers of muscles. All other bars represent counts of neurons that produced pure effects in some muscles while simultaneously producing synchrony effects in some other muscles. In each monkey, a sizeable fraction of these M1 neurons produced pure effects in some muscle(s) while producing synchrony effects in others. The ratio of the number of neurons that produced only pure effects, to the number that produced only synchrony effects, to the number that produced both pure and synchrony effects was 48:41:70 in monkey C; 12:20:19 in monkey G; and 18:3:6 in monkey A. These ratios differed significantly among the three monkeys (P < 0.001, χ² test), consistent with the greater prevalence of synchrony effects in monkeys C and G (Schieber 2002). That so many M1 neurons produced pure effects in some muscles while producing synchrony effects in others suggests that in the present monkeys M1 neurons that produced synchrony effects were not categorically distinct from M1 neurons that produced pure SpikeTA effects.

**Discharge characteristics of M1 neurons producing pure, synchrony, or both types of effects**

Whereas M1 neurons that produced pure SpikeTA effects can be inferred to be corticospinal neurons with direct projections to motoneurons, neurons that produced only synchrony SpikeTA effects might have come from noncorticospinal sub-
populations in M1. A number of recent studies have shown that different subpopulations of motor cortex neurons have distinctive patterns of activity. During locomotion over a level surface, for example, the discharge of rabbit corticospinal neurons modulates with the step cycle (Beloozerova et al. 2003). In contrast, corticocortical neurons (projecting to the ipsilateral somatosensory cortex, or to the contralateral motor cortex) and corticothalamic neurons show relatively little modulation. Similarly in monkeys, M1 neurons the axons of which project through the cerebral peduncle have a higher tonic frequency, and discharge more intensely during limb movement, than do corticostriatal neurons (Turner and DeLong 2000). We therefore classified the M1 neurons recorded in each monkey into four categories—neurons that produced no detected SpikeTA effects (none), neurons that produced only pure effects (pure), neurons that produced only synchrony effects (synchrony), and neurons that produced both pure and synchrony effects (both)—and then compared the discharge characteristics of these four M1 subpopulations.

First, we examined the baseline discharge of each neuron. From histograms formed by aligning all the correctly performed trials of each movement, we took the mean discharge frequency from 500 to 400 ms preceding the end of the movement (switch closure), and averaged these values across all the movements performed by the monkey (12 movements for monkeys C and G, 6 for monkey A) to provide a measure of the baseline firing rate for each neuron. The mean and SD of these baseline firing rates for neurons in each of the four SpikeTA effect categories—none, pure, synchrony, and both—from each monkey are given in the upper portion of Table 2. Although the mean baseline firing rates differed among monkeys, for none of the monkeys did we find significant differences in baseline firing rates depending on the type of SpikeTA effects produced by the M1 neurons.

Next, we examined the maximal movement-related discharge frequency for each neuron. From the histograms formed by aligning correctly performed trials, we calculated the average firing rate during the 100 ms immediately preceding the end of each movement, an interval in which M1 neurons typically attained their highest firing rates. For each neuron we then found the maximal firing rate during any of the movements performed by the monkey. The mean and SD of these maximal movement-related firing rates for neurons in each of the four SpikeTA effect categories—none, pure, synchrony, or both—from each monkey are given in the lower portion of Table 2. Only in monkey C did the movement-related firing rates of neurons that produced both pure and synchrony SpikeTA effects differ significantly from the firing rates of neurons that produced either pure effects or no effects ($P < 0.01$ after Bonferroni correction for 6 pairwise tests).

Finally, we examined whether neurons in each of the four SpikeTA effect categories—none, pure, synchrony, or both—fell into different groups with similar patterns of discharge across the various individuated finger and wrist movements performed by each monkey. We previously have used cluster analysis to look for groups of M1 neurons with similar discharge patterns (Poliakov and Schieber 1999). We therefore performed a single linkage cluster analysis on the discharge patterns of the M1 neuron populations from each of the present monkeys (Fig. 7). The top display in Fig. 7 for each monkey

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**FIG. 6.** Distribution of M1 neurons producing both pure and synchrony effects. Two-dimensional histograms for each monkey show the number of M1 neurons (Count) that produced different numbers of pure and synchrony effects. Counts along the Pure axis (to the right) represent neurons that produced only pure effects in different numbers of muscles. Counts along the Synchrony axis (to the left) represent neurons that produced only synchrony effects. Counts falling between the 2 axes represent neurons that produced both pure effects in some muscles and synchrony effects in others.
shows a matrix in which each neuron is represented by a column of cells, the color of which indicates the relative change in neuronal discharge frequency during each of the 12 (or 6) movements, from 1f (thumb flexion) at the bottom through we (wrist extension) at the top, normalized to a unit vector. In these displays, groups of neurons with similar patterns of discharge across the 12 movements appear as regions several columns wide with horizontal bands of similar colors. In keeping with previous findings, the largest and most consistent group in all three monkeys consisted of neurons that increased discharge during most if not all of the movements (broad field excitation, BFE). The second largest, consistent group consisted of neurons that decreased discharge during most of the movements (broad field inhibition, BFI). Other small groups of neurons appeared that discharged most intensively for a particular movement (e.g., 5f, 2f or 5e in monkey C), but these groups were not found consistently in all monkeys and varied depending on how discharge was measured and which clustering algorithm was used. That more movement-specific groups appeared in monkey A than in monkeys C or G probably reflects the smaller number of movements performed by monkey A. Many M1 neurons in each monkey failed to fall into any definable group. Of note, at the time of our previous report, only 177 M1 neurons had been recorded from monkey C. Clustering using all 318 neurons now available from monkey C produced similar findings. Furthermore, clustering the M1 population from monkey G, which had not been studied at the time of our previous report, also produced similar findings. These observations support the notion that these findings reflect the fundamental physiology of M1 during performance of skilled individuated finger movements.

The type of SpikeTA effects produced by each M1 neuron is shown in the bottom display for each monkey in Fig. 7. The color of the vertical line aligned with the column representing each neuron in the unit vector matrix above indicates whether each neuron produced no SpikeTA effect (white), only pure effects (red), only synchrony effects (green), or both pure and synchrony effects (blue). Neurons in these four categories appeared randomly intermingled. The larger BFE and BFI groups included neurons from all four categories as did the smaller, movement specific groups (e.g., 5f, 2f, 5e). In monkey A, M1 neurons that produced only pure effects (red) appeared to fall largely into movement-specific groups, which may have resulted from the combination of the smaller number of movements performed, the smaller fraction of neurons producing effects, and the larger fraction of pure effects in monkey A. M1 neurons in each SpikeTA effect category also were found among those neurons that fell into no particular group. The activity patterns of M1 neurons that produced only pure effects (red), thus did not fall into clear groups distinct from the activity patterns of those that produced both pure and synchrony effects (blue), those that produced only synchrony effects (green), or those that produced no effects in the sampled muscles (white). Whereas a pure effect in the SpikeTA indicates a relatively direct connection from the CM cell to the motoneuron pool, we infer that the activity of other M1 neurons may promote similar patterns of modulation in the motoneuron pools, albeit through connectivity somewhat (synchrony effect) or much less direct (no SpikeTA effect).

Spatial location of M1 neurons producing pure, synchrony, or both types of effects

ICMS studies of the macaque M1 upper extremity representation have shown a nested horseshoe arrangement with an inner core of distal representation in the anterior bank of the central sulcus surrounded medially, laterally and anteriorly by outer horseshoes of more proximal representation (Kwan et al. 1978; Park et al. 2001). The distal representation core and surrounding proximal representation are inter-linked by horizontal intracortical connections, which include collaterals of corticospinal neurons (Ghosh and Porter 1988; Huntley and Jones 1991). Indeed, cross-correlations of simultaneously recorded spike trains indicate significant interactions between some neurons in the proximal and distal representations (Kwan et al. 1987). Neurons recorded in the outer proximal representation therefore might be included in assemblies synchronized with CM cells in the distal representation core. In the present study, proximal representation neurons therefore might have produced many of the synchrony effects in forelimb and hand muscles. If so, we would expect neurons producing pure SpikeTA effects to be located within a central core in the anterior bank of the central sulcus with neurons producing synchrony effects distributed further medially, laterally, and anteriorly.

We examined this possibility by reconstructing the three-dimensional spatial location of the M1 neurons tested for...
SpikeTA effects in each monkey. In Fig. 8, each neuron is represented as a sphere, colored to show whether that neuron produced no SpikeTA effect (white), only pure effects (red), only synchrony effects (green), or both pure and synchrony effects (blue). For each monkey, three orthogonal views of the reconstruction are shown: surface, viewing from above looking along the line of electrode travel; edge on, looking along the central sulcus; and face on, viewing the anterior bank of the central sulcus as if from the frontal pole, with medial to the left and lateral to the right.

The surface and edge-on views demonstrate that the majority of the present M1 neurons in each monkey were located in the anterior bank and lip of central sulcus. We found few neurons related to performance of the individuated finger movement task on the surface of the precentral gyrus. The face-on view permits comparison of the mediolateral location along the central sulcus of neurons producing different types of SpikeTA effects. Neurons producing pure effects (red) or both pure and synchrony effects (blue) appear intermingled with those producing only synchrony effects (green). Although few neurons in our populations were sampled from the surface, little if any indication can be seen of a central core of pure effects with surrounding medial and/or lateral flanks of synchrony effects. We conclude that the cortical territories containing M1 neurons that produced pure effects only, both pure and synchrony effects, and synchrony effects only, all were coextensive.

**DISCUSSION**

In contrast to the categorical distinction described in other studies, the populations of SpikeTA effects obtained as the
present three monkeys performed individuated finger and wrist movements formed a continuous spectrum from pure postspike effects to synchrony effects. Neither onset latency nor peak width was distributed bimodally, and using these two parameters together also failed to distinguish discrete subpopulations. In many cases, the same M1 neuron produced pure effects in some muscles and synchrony effects in others. Furthermore, the firing rates, activity patterns, and spatial distribution of M1 neurons that produced synchrony effects, or both pure and synchrony effects, were similar to those that produced only pure effects.

We have reported previously that in monkeys C and G, each of which trained for >5 yr and performed 12 different finger and wrist movements, synchrony effects were significantly more prevalent than in monkey A, which trained for <1 yr and performed only 6 movements (Schieber 2002). We have in-
ferred that the long-term training of monkeys C and G to perform a large repertoire of skilled movements was associated with a substantial increase in the synaptic inputs to motoneuron pools synchronized with spikes discharged by M1 neurons. Such an increase in synchrony may have changed what would have been a categorical distinction between pure and synchrony effects into a continuous spectrum in the present populations of SpikeTA effects. We therefore re-examine potential mechanisms that might account for this spectrum of SpikeTA effects.

**Potential mechanisms producing a spectrum of SpikeTA effects**

Pure postspike facilitations indicate that the recorded M1 neuron had a corticospinal axon which made excitatory monosynaptic connections to spinal motoneurons contributing to the recorded EMG. During voluntary activity, motoneurons that are already near threshold may be brought to discharge an action potential by the arrival of an EPSP from such a corticomotoneuronal (CM) cell (Fetz and Cheney 1980). Because...
the EPSPs from a given CM cell arrive in the motoneuron pool at a fixed latency after the somatic spike in the cortex and because the conduction latencies from individual motoneurons to their muscle fibers are constant, an average of rectified EMG triggered from the cortical spike may show a narrow peak occurring after the spike, a pure postspike facilitation (Fig. 9A).

Not all pure postspike effects necessarily reflect monosynaptic connections, however. Some SpikeTAs show a narrow trough a few milliseconds after the cortical spike. Because the corticospinal output is entirely excitatory, such postspike suppression is thought to involve an inhibitory spinal interneuron between the corticospinal neuron and the motoneuron pool. These disynaptic postspike suppressions still may meet the onset latency and PWHM criteria for pure postspike effects. In a strict sense, even for postspike facilitations, only those effects with the shortest latencies (e.g., 5–6 ms) can be regarded as definitive evidence of a monosynaptic connection from a fast-conducting CM cell. Narrow peaks at longer latencies might arise either from monosynaptic (direct) connections made by more slowly conducting CM cells or via an excitatory interneuron (relatively direct connections). Nevertheless, both facilitatory and suppressive postspike effects that have an appropriate onset latency and a narrow peak thus can be taken as an indication that the recorded cortical neuron made relatively direct synaptic connections to the spinal motoneuron pool. We have referred to these as pure SpikeTA effects.

Still other SpikeTA effects in the present populations showed both a narrow peak with onset latency appropriate for a corticomotoneuronal connection, and a following tail, as illustrated by the effect in Fig. 9B. This following tail (curved arrow) suggests the arrival of additional inputs in the motoneuron pool synchronized with, but arriving slightly later than, the CM cell EPSPs. As illustrated by the schematic, these later inputs might arise from other CM cells, from rubrospinal neurons, and/or from spinal interneurons that receive collateral inputs from the recorded CM cell. The possibility that late-arriving inputs might contribute to CM cell SpikeTA effects has been suggested previously based on the small number of synaptic boutons provided by a single CM cell axon onto individual motoneurons and by the relatively small size of minimal corticomotoneuronal EPSPs (Lawrence et al. 1985). For the SpikeTA effect shown in Fig. 9B, the following tail reached less than half the height of the peak, however. Despite the tail, this SpikeTA effect therefore met both onset latency and PWHM criteria for a pure postspike effect.

In other SpikeTA effects with stronger late synchronized input, however, the following tail would be expected to be greater in amplitude relative to the pure SpikeTA peak. With enough late synchronized input to the motoneuron pool, the following tail would rise above half the height of the pure peak. The PWHM then would be too long to meet the criteria for a pure effect, making the peak a late widening effect. In still other SpikeTA effects with still more late synchronized input (Fig. 9C), the following tail might attain an amplitude equivalent to that of the pure peak. Any separation between a pure peak and the following tail then might be obscured. Such a late widening effect could be produced by an M1 neuron that itself made no synaptic connections to a motoneuron pool but was synchronized with other neurons that did make such connections (Baker and Lemon 1998), as indicated by in the schematic of Fig. 9C (- - -) Late widening effects therefore cannot be used as definitive evidence of a direct synaptic connection from the recorded M1 neuron to the motoneuron pool although such a connection in fact may have been present.

Yet other SpikeTA effects show evidence of inputs arriving in the motoneuron pool synchronized with but preceding the EPSPs from the recorded CM cell. The SpikeTA effect in Fig. 9D, for example, was similar to that in A or B but included an increase that preceded the sharp peak. Although the onset latency of this effect therefore was <5 ms, neither the early increase nor the following tail exceeded half the amplitude of the sharp peak. The PWHM therefore was <9 ms, making this a pure + synchrony effect. As illustrated by the schematic, synchronized inputs to the motoneuron pool that produce an early increase in the SpikeTA effect (as well as the following tail) might arise from other CM cells that are synchronized with the recorded neuron, through either their own collateral serial connections or common inputs.

As the amount of synchronized input to the motoneuron pool grows larger, the preceding increase and following tail of the SpikeTA effect may increase to a point at which the synchrony base exceeds half the amplitude of the total effect produced by input from a recorded CM cell. Figure 9E shows an example in which, although a sharp CM cell peak appears to ride on a broad synchrony base, both onset latency and PWHM objectively classified this SpikeTA effect as a synchrony effect. Similar effects have been produced by computer simulations involving spike-synchronization among 20–30 CM cells (see Fig. 4 of Baker and Lemon 1998). Although the sharp peak strongly suggests that the M1 neuron of Fig. 9E was a CM cell, and this suggestion can be supported by observing a similar peak in SpikeTAs compiled from different subsets of the same neuron’s spikes, available statistical methods are unable to demonstrate objectively that the narrow peak is distinct from the broader base.

If the synchronized inputs are greater still (Fig. 9F), the early increase and following tail might further obscure any sharp peak. As illustrated by the schematic of Fig. 9F, such a synchrony effect might appear in the SpikeTA, even without any direct input to the motoneuron pool from the recorded M1 neuron (Baker and Lemon 1998). Nor are any direct serial connections among CM cells necessary. Common input shared by the recorded M1 neuron and a large number of CM cells would be sufficient to produce such a broad synchrony effect.

A broad synchrony effect, however, would not rule out the possibility that the recorded M1 neuron was a CM cell, especially if the SpikeTA effect is large. Even when neurons show spike-to-spike synchronization, only a minority of the spikes discharged by any selected trigger neuron actually occur within a few milliseconds of the spikes discharged by another synchronized neuron (Baker and Lemon 1998; Jackson et al. 2003; Smith and Fetz 1989). Any effect added by spike-synchronization therefore tends to be much smaller, on average, than the pure postspike effect of a single CM cell. The temporal dispersion associated with spike-to-spike synchronization makes the effect smaller still. Indeed, in computer simulations a non-CM cell had to be synchronized with 30 CM cells before its synchrony effect reached the amplitude of the pure effect from a single CM cell (see Fig. 4 of Baker and Lemon 1998). Given that in the present study synchrony effects on average were larger than pure effects, many of the present M1 neurons producing synchrony effects either may have had CM connec-
tions to the motoneuron pools themselves and/or were synchronized with relatively large numbers of numbers of neurons providing inputs to the motoneuron pool.

These considerations of the potential mechanisms of SpikeTA effects suggest that as increasing numbers of neurons with inputs to a given motoneuron pool become synchronized, the pure postspike effect of a given trigger neuron with direct connections to the motoneuron pool might either become combined with (pure + synchrony and late widening effects) or entirely subsumed by (synchrony effects) the effects of the other neurons with which that trigger neuron is synchronized. Other, probably smaller, synchrony effects may have been produced by M1 neurons with no relatively direct connections to motoneuron pools. In the present populations of SpikeTA effects, particularly those from monkeys C and G, which were trained for a prolonged period to perform 12 skilled movements, increased levels of synchronization might have resulted in a continuous spectrum from pure to synchrony effects in which categorical distinctions were not objectively evident.

**Influence of M1 neurons on motoneuron pools**

Only a limited fraction of the M1 neurons tested in the present monkeys produced SpikeTA effects (51, 39, and 16% in monkeys C, G, and A, respectively). Many M1 neurons that we selected for recording produced no SpikeTA effects but nevertheless showed firing rates, activity patterns, and spatial locations in M1 similar to those neurons that did produce effects. Many neurons that produced no effects therefore may have been layer V, corticofugal neurons. Although some of these neurons might have produced effects in muscles from which we did not record, others may have influenced motoneuron pools only indirectly.

Of those M1 neurons that did produce SpikeTA effects, a considerable fraction produced synchrony effects, particularly in monkeys C and G. Given the ubiquity of both oscillatory and nonoscillatory synchrony in M1 (Baker et al. 1997; Conway et al. 1995; Donoghue et al. 1998; Kilner et al. 1999, 2000; Kwan et al. 1987; Murthy and Fetz 1992, 1996a,b; Oram et al. 2001; Smith and Fetz 1989), one might have expected even more of the present M1 neurons to show spike-synchrony with CM cells and therefore to produce synchrony effects in SpikeTAs. The production of synchrony effects therefore suggests that these M1 neurons, while linked to the motoneuron pool possibly less directly than via monosynaptic CM connections, nevertheless were linked more directly than many other M1 neurons with similar discharge characteristics that produced no SpikeTA effects. The rarity of SpikeTA effects—pure or synchrony—produced by neurons in the cat motor cortex (Drew 1993) or red nucleus (T. Drew, personal communication) or in the monkey reticular formation (Davidson 2004; Davidson and Buford 2004), all of which participate in control of limb movement, emphasizes the importance for production of SpikeTA effects of some relatively direct connectivity between the recorded neuron and the motoneuron pool.

Previous studies have demonstrated that two CM cells can show spike synchronization in their spike-to-spike cross-correlation function, while both CM cells produce pure postspike effects in the same EMG (Smith and Fetz 1989). Spike synchronization between CM cells actually is more common when the two CM cells produce pure effects in the same motoneuron pool than when they produce effects in different motoneuron pools (Jackson et al. 2003). These observations demonstrate that spike synchronization between two CM cells is insufficient to produce substantial synchrony features in the SpikeTA of a shared target motoneuron pool. Hence the likelihood that an M1 neuron (CM cell or not) could produce substantial synchrony features in a SpikeTA effect simply by virtue of spike synchronization with a small number of CM cells is relatively low. Rigorous computer simulations have suggested, however, that spike synchronization among 10–30 CM cells can produce the features of pure + synchrony, late widening, and broad synchrony effects (Baker and Lemon 1998). Synchrony features in SpikeTA effects indicate, therefore that relatively large numbers of neurons provided synchronized inputs to the motoneuron pool. Hence the schematics of Fig. 9 may underestimate the number of synchronized neurons contributing to the synchrony features of SpikeTA effects.

Only pure SpikeTA effects can be used to identify neurons with direct connections to motoneuron pools, i.e., CM cells. Synchrony effects may be produced in SpikeTAs even though the triggering neuron has no direct connections to the motoneuron pool. Although every spike of a CM cell that produced a pure postspike effect can be assumed to have been followed at a highly consistent latency by postsynaptic potentials (PSPs) in motoneurons, which spikes of an M1 neuron that produced a synchrony effect were followed by PSPs in the motoneurons and which were not is unknown. Moreover, the latency of the PSPs following the spikes of an M1 neuron that produced a synchrony effect presumably jittered in the range of several milliseconds. For these reasons, synchrony effects in SpikeTAs often have been discounted when considering the influence of M1 neurons on muscle activity.

Synchrony effects nevertheless represent a time-locked linkage between the discharge of spikes by the trigger neuron and the arrival of excitation or inhibition in the motoneuron pool. In many cases, synchrony effects may include monosynaptic EPSPs of the trigger neuron. The synchrony effects observed here were larger on average than the pure postspike effects. The discharge times of individual M1 neurons that produced large synchrony effects therefore may represent a greater fraction of the synaptic input arriving in motoneuron pools than do the discharge times of individual M1 neurons that produced smaller pure effects. Although the synchrony effects of M1 neurons may be produced less directly than pure effects, they may represent much of the influence of M1 neurons on motoneuron pools. Understanding how M1 neurons control spinal motoneuron pools therefore will require consideration of the entire spectrum from pure to synchrony SpikeTA effects.

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