SV40LUC). Also, a pBKRSV, pCMV4, Rous sarcoma virus (RSV)–PP1, CMV-PP2A, CMV-PPIV, or CMV-PPV expression plasmid was cotransfected along with the reporter plasmid. Cells were treated with 1 nM Shh and assayed for reporter gene activity.
25. An internal control containing a 763-bp insert into the Sph I site of the Islet1 cDNA was transcribed in vitro to prepare sense-oriented RNA. One nanogram of this RNA along with 100 ng of total RNA from appropriately treated P19 cells was reverse transcribed using Superscript II, and 1/10th of each reaction mixture was amplified by polymerase chain reaction (PCR). Specific primers for Islet1 (forward primer, 5′ TCA AAC CTA CTT TGG GGT CTT A 3′; reverse primer, 5′ ATC GCC GGG GAT GAG CTG GCG GCT 3′) and for COUP-TFII (forward primer, 5′ GAT ATG GCA ATG GTA GTC AGC ACG TGG 3′; reverse primer, 5′ AGC TTC TCC ACT TGC TCT TGG 3′) were used for PCR amplification (94°C for 1 min, 54°C for 1 min, and 72°C for 1 min). Twenty-two cycles were used for amplifying Islet1 and the resulting products were separated on a 1% agarose gel in tris-acetate EDTA (TAE) buffer. A 427-bp Isl1 fragment and an 838-bp COUP-TFII fragment were used to estimate changes in mRNA quantities.
26. Mouse embryos at embryonic day 9 to 9.5 were dissected in such a way as to remove surface ectoderm and somitic mesoderm, and neural tubes were isolated from the level of rhombomere 8 in the rostral region to the first segmenting somite in the caudal region. Neural tubes were cultured for 24 hours dorsal side down on Millicell-CM membranes and overlaid with Matrigel containing 10 ng of NT-3 per milliliter, with L15 air medium (*4*) in the presence or absence of 5 nM OA. The explants were subjected to whole-mount immunostaining with a monoclonal antibody raised against mouse Islet1 (39.4D5) or NFL (2H3) as described (*29*).
27. H. Sasaki, C.-C. Hui, M. Nakafuku, H. Kondoh, *Development* **124**, 1313 (1997).
28. C-H. Chen, N. Fuse, P. A. Beachy, personal communication.
29. M. C. Mumby and G. Walter, *Physiol. Rev.* **73**, 673 (1993).
30. C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982).
31. Purified Shh-N was provided by J. A Porter and K. Young, purified protein phosphatases PP1 and PP2A were provided by S. Shenolikar (Duke University), PPIV protein and expression plasmid were provided by T-H. Tan (Baylor College of Medicine). CMV-PPV plasmid was provided by M. Chinkers (Oregon University, Health Science Center). PP1 and PP2A expression plasmids were kindly provided by T. Deng (University of Florida). Human Gli and GliRE-CAT reporters were provided by C-C. Hui (University of Toronto, Canada). The authors also wish to thank N. Fuse for communication of unpublished results. This work is supported by an NIH grant.

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Spike Synchronization and Rate Modulation Differentially Involved in Motor Cortical Function

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It is now commonly accepted that planning and execution of movements are based on distributed processing by neuronal populations in motor cortical areas. It is less clear, though, how these populations organize dynamically to cope with the momentary computational demands. Simultaneously recorded activities of neurons in the primary motor cortex of monkeys during performance of a delayed-pointing task exhibited context-dependent, rapid changes in the patterns of coincident action potentials. Accurate spike synchronization occurred in relation to external events (stimuli, movements) and was commonly accompanied by discharge rate modulations but without precise time locking of the spikes to these external events. Spike synchronization also occurred in relation to purely internal events (stimulus expectancy), where firing rate modulations were distinctly absent. These findings indicate that internally generated synchronization of individual spike discharges may subserve the cortical organization of cognitive motor processes.

The classical view of neural coding has emphasized the importance of information carried by changes in the neurons' spike discharge rates (*1*). Thus, relating rate changes of individual neurons to cognitive and motor functions has yielded remarkable insights into neuronal mechanisms of sensory (*2*) and motor (*3*) processing. In parallel, however, the concept emerged that computational processes in the brain could also rely on the relative timing of spike discharges among neurons within functional groups (*4*)—commonly called cell assemblies (*5*). Indeed, it has been argued that the synaptic influence of multiple neurons converging onto others is much stronger if they fire in coincidence (*6*), making synchrony of firing ideally suited to raising the saliency of responses and to expressing relations among neurons with high temporal precision (*4*). For this temporal scheme to work, correlations in spike timing must reflect cooperative interactions among the neurons constituting an assembly. Moreover, to permit neurons to participate in different cell assemblies at different times, depending on the present stimulus context and behavioral demands, these correlations should be dynamic. Indeed, such predicted stimulus- and behavior-dependent modulations of spike correlations with differing degrees of temporal precision were observed in various sensory cortical areas, particularly visual (*7*), auditory (*8*), somatosensory (*9*), and frontal (*10*) areas. However, very little is known about the possible functional role of action potential timing in the motor cortex (*11*), in spite of the fact that this part of the brain directly governs overt behavior. We present evidence for the hypothesis that precise synchronization of individual action potentials among selected groups of motor cortex neurons is involved in dynamically organizing the cortical network dur-

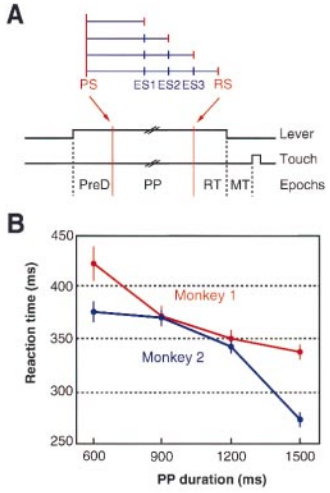

Fig. 1. (**A**) Two macaque monkeys were trained to touch a target presented on a video display equipped with a capacitive touch screen. To start a trial, the animal had to hold down a switch. After a constant delay of 1 s (PreD), a PS forming an open circle (diameter, 3 cm) appeared on the screen, indicating the upcoming target position. After a second delay of variable duration (PP), the RS, indicated by filling the circle, instructed the animal to move its hand to the target and touch it. The PP could last 600, 900, 1200, or 1500 ms; these intervals were presented in random order and with equal probability. Thus, from one possible PP duration (and, hence, ES) to the next, the conditional probability for the RS to occur increased, reaching values of 0.25, 0.33, 0.5, and 1. RT and movement time (MT) were recorded in each trial. Criteria for obtaining the reward were (1) to keep the switch pressed during the PP, (2) to move to and touch the target after RS, and (3) to perform the movement so that neither RT nor MT exceeded 1 s. (**B**) Behavioral results. For each monkey, mean RTs and standard error in relation to PP duration are shown as recorded during the last training sessions (monkey 1, $N = 35$; monkey 2, $N = 59$) when they performed the task with more than 90% correct trials. By using a two-factor (PP duration × training session) analysis of variance, changes in RT were found to be highly significant for both monkeys [monkey 1, $F(3,102) = 38.19$, $P < 0.001$; monkey 2, $F(3,174) = 157.81$, $P < 0.001$].

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ing the planning and execution of voluntary movements. Such synchronization can occur even without modulations of the firing rates, particularly during planning and in relation to internal ("expectation") events. Thus, our findings call for an extension of prevailing models of motor cortical function that rely exclusively on discharge rates in populations of neurons commonly assessed from averaged responses and recorded from one neuron after another (*12*). Preliminary results have been presented in abstract form (*13*).

We designed a delayed-response hand-movement task that allowed us to study neuronal cooperativity in the motor cortex under conditions in which the processing of internal, purely cognitive events could be separated from that of external events, such as presentation of visual stimuli and execution of movements (Fig. 1A). During a trial, two successive visual stimuli, separated by a variable time interval [preparatory period (PP)], were presented to the animal. The first stimulus [preparatory signal (PS)] indicated the target position of the requested hand movement, and the second stimulus [response signal (RS)] instructed the monkey to make the required movement and touch the target. In accordance with earlier findings in human studies (*14*), the reaction time (RT) to onset of movement decreased systematically with increasing duration of the PP (Fig. 1B). We interpret this finding as evidence that the monkey updates its estimate of the probability for the RS to occur. Because this probability attains progressively higher values as the four potential RS-presentation instants (expected signal ES1, ES2, ES3, and RS) pass by, the monkey can adjust its preparatory activities accordingly. During 295 sessions, the activities of two to seven neurons were recorded simultaneously (*15*) in the primary motor cortex of two monkeys (*16*). Instances of near-coincident (5 ms) firing of two or more neurons were detected on a trial-by-trial basis by using the unitary event analysis method (Fig. 2) (*17*). Those coincident spike constellations that occurred significantly more often than expected by chance are referred to as unitary events (UEs) (Fig. 2F, pairs of red dots) (*18*, *19*). We interpret the neuronal composition of a UE as defining the subset of recorded neurons that were momentarily engaged in a cell assembly. The occurrence of a considerable number of UEs is revealed in Fig. 2F. Interestingly, these were not distributed randomly in time. Instead, they appeared in clusters, which were loosely (50 to 100 ms) time-locked to behaviorally relevant events, in this case to expected occurrence times of the RS (Fig. 2F, ES2 and ES3). Comparing the auto- and cross-correlograms of the spike data in epochs with significant UEs with those of the intervening epochs did not reveal any conspicuous signs of periodicities, which suggests that these precise spike synchronizations were not linked to the coherent oscillations reported in (*11*). A total of 359 pairs of neurons were analyzed in this way. In one-third (120 of 359) of them, clusters of precisely coincident (5 ms) UEs were detected. The precise UEs, in turn, were loosely (50 to 100 ms) time-locked to behaviorally relevant events, such as movement events and actually occurring, but also merely expected, stimuli. In a number of cases we even found coincidence precision down to the 1- to 2-ms level, with temporal clustering similar to that at a coincidence precision of 5 ms. This consistent temporal relation between UEs and behavioral events, reflected in the timing and the temporal width of the UE clusters, suggests a functional significance of precisely synchronous action potentials.

If synchronization of neuronal activity is relevant in task-specific computations, not only the occurrence of synchronization but also its strength and composition should be correlated with behavioral aspects such as stimulus probability and RT, motivation and performance, and expectancy. Two examples demonstrate that such relationships were indeed observed (Fig. 3). In the first example (Fig. 3A), we consider UEs occurring in relation to signal expectancy. Two neurons are shown that synchronized their activities each time about 150 to 50 ms before the RS was expected (ES1 to ES3, RS) (top panel). It is clear from the increasing numbers of UEs in successive clusters that the rate of coincident firing grew as the duration of

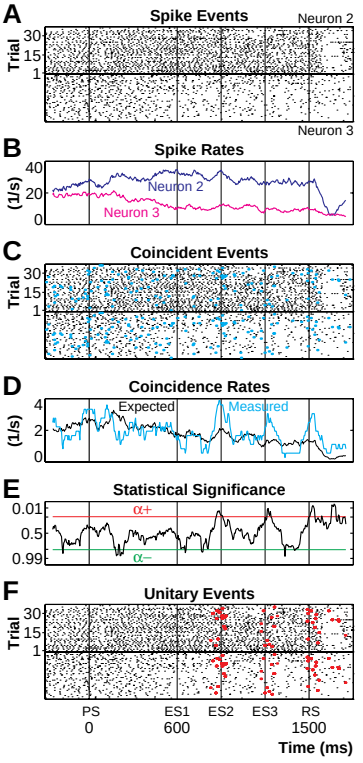

Fig. 2. UE analysis of the spiking activity of two simultaneously recorded single MI neurons. The 36 trials with longest PP duration (1500 ms) were selected in this example. Thus, the monkey could expect the RS to occur at three successive moments (ES1, ES2, and ES3) before it actually occurred at RS. Results of UE analysis over a time interval of 2100 ms, starting 300 ms before PS and lasting until 300 ms after RS, are shown. (**A**) Conventional raster displays of spike discharges of the two neurons (top, neuron 2; bottom, neuron 3). Each dot represents an action potential, and each horizontal line depicts the spiking activity in a single trial. (**B**) Spike discharge rate for each neuron (normalized to spikes per second) was computed by sliding a boxcar window of 100 ms in steps of 5 ms over the spike events [dots in (A)]. (**C**) In a copy of the raster displays from (A), spike coincidences of the two neurons within a precision window of 5 ms are indicated by blue dots. (**D**) Comparison of measured and expected coincidence rates. The measured coincidence rate (blue curve, normalized to coincidences per second) was derived in the same way as the spike rates, by sliding a boxcar window of 100 ms in steps of 5 ms over the coincident events [blue dots in (C)]. The expected coincidence rate (black curve), based on the null hypothesis of independent firing, was calculated as the product of the individual firing rates [curves in (B)]. (**E**) For each time window, the statistical significance for a positive difference between measured and expected coincidence rates was calculated from a Poisson distribution (with the mean set to the expected coincidence rate) as the cumulative probability P of observing the actual number of coincidences or an even larger one by chance. The larger the number of excessive coincidences, the closer P is to 0. Similarly, the larger the number of lacking coincidences, the closer its complement, $1 - P$, is to 0, while P approaches 1. To enhance visual resolution at the relevant low-probability values of P or $1 - P$, we plotted a logarithmic function of the two: $\log_{10}[(1 - P)/P]$. For excessive coincidences, this function is dominated by P; for lacking coincidences, it is dominated by $1 - P$. This procedure, derived from the "surprise" measure (*20*), is comparable to measuring significance on a decibel scale. It yields positive numbers for excessive coincidences (for example, 1 for $P = 0.1$ and 2 for $P = 0.01$) and negative numbers for lacking coincidences, and it changes sign at the chance level ($P = 0.5$). Whenever the significance value of an excess number of observed coincidences exceeded a fixed threshold, α^+ (here $P = 0.05$), this defined an epoch with significantly more coincidences than expected by chance. We marked these significant precise coincidences as UEs, and they are indicated by red dots in the raster displays in (**F**). Occasionally, the significance value dropped below the negative threshold level α^- (here, shortly after PS). Such cases, defining epochs with significantly fewer coincidences than expected by chance, were more rare because of the generally low expected coincidence rates in these data (*21*) and are not considered further here. The widths of the coincidence window and of the sliding time window as well as the significance threshold α^+ could be set to a selected (fixed) value in the analysis; values of 5 ms and 100 ms, respectively, and $P = 0.05$ were used throughout the results reported here.

the PP increased and, hence, the probability for the RS to occur increased. This phenomenon, which was observed in both monkeys, suggests a positive correlation between the growing stimulus expectancy and the occurrence of UEs. A similar correlation was observed when trials for a given PP duration were subdivided into two groups, one characterized by short (top) and the other by long (bottom) RTs. In line with the prediction from the behavioral RT results (Fig. 1B), many more coincidences appeared in the short than in the long RT group. The second example (Fig. 3B) shows a relation between synchronized spike activity and the motivation of the animal. During the trials summarized in the top panel, the monkey performed the task correctly and efficiently; during the trials shown in the bottom panel (collected at the end of the experimental session), the animal had stopped responding to the RS but still initiated the trials, so that stimuli were presented as usual. Observe that epochs of excessive synchronized spike events occurred systematically in relation to the PS during the correctly performed trials (top), whereas such synchronization is completely lacking in the nonresponding trials (bottom). Note that in the latter condition the firing rates had also dropped, but this reduction was taken into account by the UE-analysis technique (*17*). Finally, it is also shown that the composition of the set of the jointly firing neurons may systematically alternate between distinct constellations during successive stages of the trial (Fig. 3B). Before PS, neurons 4 and 6 synchronized their spike events; immediately after PS (from 0 to 250 ms), neurons 2 and 4 synchronized; then (from 250 to 350 ms), neurons 4 and 6 synchronized again; and, finally, all three neurons (2, 4, and 6) coordinated their spiking activity. Such alternating neuron composition suggests a sequential engagement of three different cell assemblies (or cycling through a single, composite cell assembly). In 20 of 43 sessions (46%), the analysis of three or more neurons in parallel revealed such rapid changes in the constellation of synchronously firing cells in systematic relation to behaviorally relevant events.

It is conceivable that synchronization of spiking activity and modulation of discharge rates represent, at least partly, independent computational strategies used by the brain. We tested this hypothesis for all 120 pairs of neurons in which UEs occurred in temporal relation to behaviorally relevant events. We found that spike synchronization could be observed both with and without simultaneous phasic changes in neuronal firing rates (Fig. 4A). Because both PS and RS were predictable, synchronization of spiking activity and changes in discharge rate were both analyzed in relation to the expectancy as well as to the actual occurrence of the PS and RS. The results are summarized (Fig. 4, B and C). Several observations can be made. First, precise spike synchronization was found in relation to both expected and actually occurring stimuli, albeit more often with the latter (70 versus 110). However, the relations between spike synchronization and rate changes were very different in the two cases. If the stimulus was

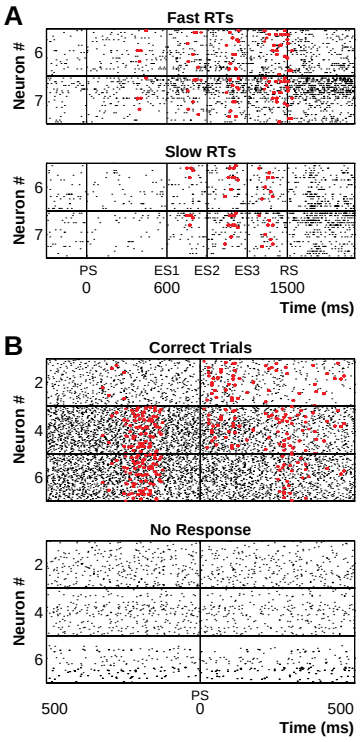

Fig. 3. Synchronized neuronal activity in relation to behavioral aspects of the task. (**A**) UEs (indicated by red dots in the raster displays) in relation to both expectancy and RT during selected trials in which the longest PP was presented. Trials were divided in three approximately equally sized groups: trials with shortest RTs (top; mean = 270 ms, N = 25), intermediate RTs (not shown), and longest RTs (bottom; mean = 340 ms, N = 19). (**B**) UEs in relation to performance of the animal. Trials were divided into two groups: trials during which the animal performed the task correctly (top; N = 107), and trials that were correctly initiated by the animal and, hence, the visual stimuli (RS and PS) occurred in the usual way, but the animal did not respond to the RS (bottom; N = 94). Further details are given in Fig. 2.

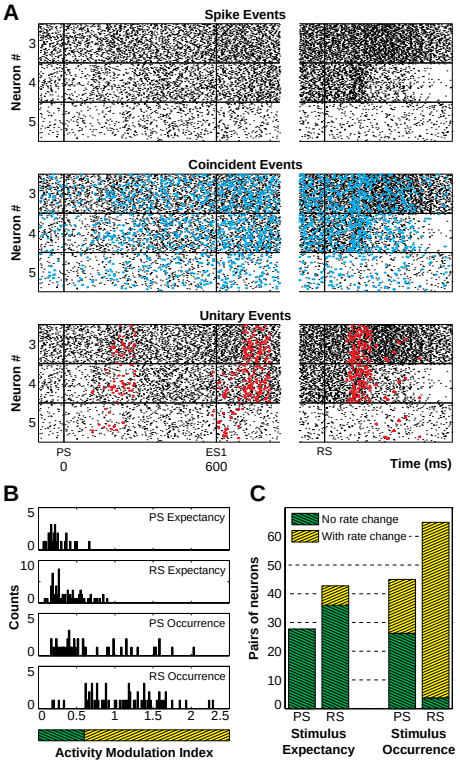

Fig. 4. Relation between synchronization of spike events and modulation of discharge rate. (**A**) Discharge patterns of three neurons recorded simultaneously during 96 trials. UEs are indicated by red dots in the raster diagrams. Data from three types of trials (PP durations, 900, 1200, and 1500 ms) were pooled. The visualization was interrupted 800 ms after PS and continued for the last 600 ms of the trial (ES1, first expected RS; MO, movement onset; ME, movement end) (further details are presented in Fig. 2). (**B**) Distribution of activity modulation indices during epochs with UEs computed for that neuron in the pair that exhibited the strongest rate modulation. Phasic changes in neuronal discharge rate were detected by quantitative analysis of the peristimulus-time histograms in fine-temporal resolution (bin width, 5 ms). The activity modulation index, a quantitative measure adopted from the visual contrast measure, was calculated for each neuron as follows. During epochs with UEs, the minimum (Min) and maximum (Max) values of the discharge rate in each 5-ms bin and the mean (Mean) discharge rate over the entire UE epoch were determined. The activity modulation index was then calculated as (Max–Min)/Mean. Values exceeding 0.6 (for Poisson spike rates of 10 to 50 spikes per second, this corresponds to rate fluctuations beyond 1 to 2 standard deviations from the mean) were considered to reflect significant modulations of discharge rate (yellow bar); for values below 0.6 (green bar) such changes were considered absent. Four distinct epochs during the trial were considered: when either PS or RS was expected and when either PS or RS actually occurred. (**C**) Numbers of pairs of neurons with significant UEs in relation to the visual stimuli (PS and RS) either when these were expected (left) or when they actually occurred (right). Numbers of neuron pairs in which at least one of the neurons changed its discharge rate during UE epochs, as judged from the activity modulation index, are shown in yellow; numbers of neuron pairs in which none of the neurons exhibited such a simultaneous firing rate modulation are shown in green.

expected but did not occur, spike synchronization was never (PS, 0 of 28) or rarely (RS, 6 of 42) accompanied by a change in discharge frequency of any of the participating neurons. By contrast, when the stimulus did occur, excess synchronized spiking was often (PS, 19 of 45) or almost always (RS, 61 of 65) accompanied by a modulation of discharge rate. We wish to emphasize, however, that our procedure to identify UEs takes changes of firing rate into consideration (Fig. 2) (*17*). Hence, the observed UEs are not simply a consequence of rate changes. Instead, these findings indicate that the association between spike synchronization and rate modulation is tightly connected to the actual occurrence of the stimulus. Thus, both internal and external cognitive events are associated with precisely coordinated spike activity, but only external events are also accompanied by discharge rate variation.

Taken together, our findings demonstrate the existence of precise (5 ms) synchronization of individual spike discharges among selected groups of neurons in the motor cortex. This synchronization is associated with distinct phases in the planning and execution of voluntary movements, indicating that it plays a functional role. Moreover, our findings suggest that, under behavioral conditions as studied here, the brain may use different strategies in different contextual situations. To process a purely cognitive (that is, an internal and behaviorally relevant) event, neurons preferentially synchronize their spike occurrences without changing their firing rates at the same time. By contrast, when processing an external, behaviorally relevant event, neurons tend to synchronize their spike occurrences and modulate their firing rates at the same time. Thus, precise synchronization of spike events and modulation of discharge rate may serve different and complementary functions; they act in conjunction at some times but not at others, depending on the behavioral context.

REFERENCES AND NOTES

1. H. B. Barlow, *Perception* **1**, 371 (1972); in *Information Processing in the Cortex: Experiments and Theory*, A. Aertsen and V. B. Braitenberg, Eds. (Springer, Berlin, 1992), pp. 169–174.
2. D. H. Hubel and T. N. Wiesel, *Proc. R. Soc. London* **198**, 1 (1977); W. T. Newsome, K. H. Britten, J. A. Movshon, *Nature* **341**, 52 (1989); M. N. Shadlen and W. T. Newsome, *Curr. Opin. Neurobiol.* **4**, 569 (1994).
3. A. P. Georgopoulos, J. F. Kalaska, R. Caminiti, J. T. Massey, *J. Neurosci.* **2**, 1527 (1982); A. P. Georgopoulos, M. Taira, A. Lukashin, *Science* **260**, 47 (1993).
4. C. von der Malsburg, *Internal Report* 81-2 (Max-Planck-Institute, Göttingen, Germany, 1981); M. Abeles, *Local Cortical Circuits* (Springer, Berlin, 1982); *Corticonics* (Cambridge Univ. Press, Cambridge, 1991); G. L. Gerstein, P. Bedenbaugh, A. M. H. J. Aertsen, *IEEE Trans. Biomed. Eng.* **36**, 4 (1989); G. Palm, *Concepts Neurosci.* **1**, 133 (1990); W. Singer, *Annu. Rev. Physiol.* **55**, 349 (1993).
5. D. O. Hebb, *Organization of Behavior* (Wiley, New York, 1949).
6. M. Abeles, *Isr. J. Med. Sci.* **18**, 83 (1982); W. R. Softky and C. Koch, *J. Neurosci.* **13**, 334 (1993).
7. R. Eckhorn *et al.*, *Biol. Cybern.* **60**, 121 (1988); C. M. Gray, P. König, A. K. Engel, W. Singer, *Nature* **338**, 334 (1989); reviewed in W. Singer and C. M. Gray, *Annu. Rev. Neurosci.* **18**, 555 (1995); P. R. Roelfsema, A. K. Engel, P. König, W. Singer, *J. Cogn. Neurosci.* **8**, 603 (1996).
8. M. Ahissar, E. Ahissar, H. Bergman, E. Vaadia, *J. Neurophysiol.* **67**, 203 (1992); J. J. Eggermont, *ibid.* **71**, 246 (1994); R. C. DeCharms and M. M. Merzenich, *Nature* **381**, 610 (1995); Y. Sakurai, *Neurosci. Res.* **26**, 1 (1996).
9. M. A. L. Nicolelis, L. A. Baccala, R. C. S. Lin, J. K. Chapin, *Science* **268**, 1353 (1995).
10. A. Aertsen *et al.*, *J. Himforsch.* **32**, 735 (1991); M. Abeles, H. Bergman, E. Margalit, E. Vaadia, *J. Neurophysiol.* **70**, 1629 (1993); E. Vaadia *et al.*, *Nature* **373**, 515 (1995).
11. V. N. Murthy and E. E. Fetz, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5670 (1992); J. N. Sanes and J. P. Donoghue, *ibid.* **90**, 4470 (1993).
12. D. R. Humphrey, E. M. Schmidt, W. D. Thompson, *Science* **170**, 758 (1970); A. P. Georgopoulos, A. P. Schwartz, R. E. Kettner, *ibid.* **233**, 1416 (1986); F. A. Mussa-Ivaldi, *Neurosci. Lett.* **91**, 106 (1988); A. V. Lukashin, *Biol. Cybern.* **63**, 377 (1990); A. V. Lukashin and A. P. Georgopoulos, *ibid.* **69**, 517 (1993); J. Wickens, B. Hyland, G. Anson, *J. Motor Behav.* **26**, 66 (1994).
13. A. Riehle, S. Grün, A. Aertsen, J. Requin, *Soc. Neurosci. Abstr.* **21**, 2077 (1995).
14. J. Requin, J. Brener, C. Ring, in *Handbook of Cognitive Psychophysiology: Central and Autonomous Nervous System Approaches*, R. R. Jennings and M. G. H. Coles, Eds. (Wiley, New York, 1991) pp. 357–448.
15. Experiments were performed on two macaque monkeys cared for as described [*The NIH Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD) and the French government regulations]. After training, the animals were prepared for multiple single-neuron recordings. A cylindrical stainless steel recording chamber (inner diameter, 15 mm) was implanted over the contralateral primary motor cortex (MI) under aseptic conditions and general anesthesia. A stainless steel T bar was cemented to the skull to fixate the animal's head during recording sessions. To record multiple single-neuron activity, a multielectrode microdrive [V. B. Mountcastle, R. J. Reitböck, G. F. Poggio, M. A. Steinmetz, *J. Neurosci. Methods* **36**, 77 (1991)] was used to transdurally insert seven independently driven microelectrodes (quartz insulated platinum-tungsten electrodes; outer diameter, 80 μm; impedance, 2 to 5 MΩ at 1000 Hz). The electrodes (spaced 330 μm apart) were arranged in most cases in a circle—that is, one electrode in the middle and six electrodes around—and sometimes in a row (distance between first and seventh electrodes, 1980 μm). From each electrode, single-neuron spikes were isolated by using a window discriminator. Classical histological techniques were used to reconstruct the recording sites and to define the cortical areas [A. Riehle and J. Requin, *J. Neurophysiol.* **61**, 534 (1989)].
16. The activity of 379 neurons recorded in 145 sessions was selected for analysis by the following criteria: a discharge frequency of more than five impulses per second, which was reproducible across multiple trials. Of those, 21 neurons (5.5%) did not change their activity during the task. Of the remaining 358 task-related neurons, 194 (51.2%) changed their activity during the PP, and 351 (92.6%) changed their activity after occurrence of the RS—that is, in relation to execution of movement. The change in PP activity consisted of a phasic modulation of the firing rate after occurrence of the PS (87 neurons, with increased activity in 78 neurons and decreased activity in 9 neurons), a sustained modulation of the firing rate (127 neurons, with increased activity in 82 neurons and decreased activity in 45 neurons), or a phasic change in activity at 600 ms—that is, the moment when the animal expected the first RS (32 neurons). Note that one neuron could exhibit both phasic and tonic changes in activity during the PP.
17. Dynamic changes in synchronicity between neurons were analyzed off-line by determining epochs of statistically significant, excess synchronized firing. The analysis proceeded in several stages (Fig. 2, A to F), details of which are described and calibrated in (*18*) (S. Grün and A. Aertsen, in preparation). Briefly, the occurrences of action potentials elicited by *N* simultaneously recorded neurons were transformed by appropriate binning to *N*-dimensional joint-activity vectors consisting of ones (action potential) and zeros (no action potential). The composition of these vectors represents the various constellations of coincident spiking activity across the *N* neurons. Under the null hypothesis that *N* neurons fire independently, the expected number of occurrences of any joint-spike constellation and the associated probability distribution can be calculated analytically from (and, hence, normalized for) the single neuron firing rates. Using this distribution, we tested the statistical significance of the difference between the observed and the expected numbers of coincident events; those occurrences that exceeded a significance level of 5% are referred to as UEs. Throughout the analyses reported here, the coincidence bin width was fixed to 5 ms; this value was motivated by experimental observations on the temporal precision of spiking in cortical pyramidal neurons (*10*) [Z. F. Mainen and T. J. Sejnowski, *Science* **268**, 1503 (1995)]. In a number of cases (for example, for the data used in Fig. 2), we varied this parameter systematically and found even significant UEs with similar temporal clustering for coincidence precision down to 1 to 2 ms. To normalize for within-trial nonstationarities in the discharge rates of the neurons, the modulations in spike rates and coincidence rate were determined on the basis of short data segments by sliding a fixed time window of 100 ms along the data in steps of the coincidence bin width. This timing segmentation was applied to each trial, and the data of corresponding segments in all trials were then analyzed as one quasi-stationary data set. Throughout the analyses reported here, the sliding window width was fixed to 100 ms; this choice was motivated by the typically observed rates of change of the activity in the recorded neurons and the need to acquire reliable statistics. In a number of cases, this parameter was varied systematically between 50 and 150 ms, with no substantial effects on the results.
18. S. Grün, *Unitary Joint-Events in Multiple-Neuron Spiking Activity* (Harry Deutsch, Thun, Germany, 1996).
19. A. Riehle *et al.*, in *Supercomputing in Brain Research: From Tomography to Neural Networks*, H. J. Herrmann, D. E. Wolf, E. Pöppel, Eds. (World Scientific, Singapore, 1995), pp. 281–288; A. Riehle, S. Grün, A. Aertsen, J. Requin, in *Artificial Neural Networks—ICANN '96*, C. von der Malsburg, W. von Seelen, J. C. Vorbrüggen, B. Sendhoff, Eds. (Springer, Berlin, 1996), pp. 673–678.
20. G. Palm, A. Aertsen, G. L. Gerstein, *Biol. Cybern.* **59**, 1 (1988); A. Aertsen, G. L. Gerstein, M. K. Habib, G. Palm, *J. Neurophysiol.* **61**, 900 (1989).
21. A. M. H. J. Aertsen and G. L. Gerstein, *Brain Res.* **340**, 341 (1985).

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