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RESEARCH NOTE

Franck Grammont · Alexa Riehle

Precise spike synchronization in monkey motor cortex involved in preparation for movement

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Abstract It is commonly accepted that perceptually and behaviorally relevant events are reflected in changes of activity in largely distributed neuronal populations. However, it is much less clear how these populations organize dynamically to cope with momentary computational demands. In order to decipher the dynamic organization of cortical ensembles, the activities of up to seven neurons of the primary motor cortex were recorded simultaneously. A monkey was trained to perform a pointing task in six directions. During each trial, two signals were presented consecutively. The first signal provided prior information about the movement direction, whereas the second called for the execution of that movement. Dynamic interactions between the activity of simultaneously recorded neurons were studied by analyzing individual epochs of synchronized firing ("unitary events"). Unitary events were defined as synchronizations which occur significantly more often than expected by chance on the basis of the neurons' firing rates. The aim of the study was to describe the relationships between synchronization dynamics and changes in activity of the same neurons during the preparation and execution of voluntary movements. The data show that even neurons which were classified, on the basis of the change in their firing rate, to be functionally involved in different processes (e.g., preparation or execution related, different directional tuning) synchronized their spiking activity significantly. These findings indicate that the synchronization of individual action potentials and the modulation of the firing rate may serve different and complementary functions underlying the cortical organization of cognitive motor processes.

Key words Movement preparation · Multiple single-neuron activity · Synchronization · Motor cortex · Monkey

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F. Grammont · A. Riehle
Center for Research in Cognitive Neuroscience, CRNC-CNRS,
31 Chemin Joseph Aiguier, F-13402 Marseille Cedex 20, France

Introduction

It is a generally accepted assumption in the field of cognitive neuroscience that the behaviorally significant changes in the activity of neural structures are indicated by changes in the firing rates of neurons (cf. Barlow 1972, 1992; Shadlen and Newsome 1994). However, in parallel the concept has emerged that cognitive processes and behavioral events could be reflected within neural structures in terms of changes not only in the rate of neuronal firing but also in the cooperative interplay among neurons within large networks (Milner 1974; von der Malsburg 1981; Abeles 1982a, 1991; Gerstein et al. 1989; Singer 1993; Phillips and Singer 1997). Although both concepts are not necessarily exclusive, they should first be approached independently from each other. It is quite conceivable that the cooperative interplay among neurons, induced by sensory and behavioral events, is due to rapid modulations of discharge synchronization. It has been argued that synchronized inputs onto a neuron are much more effective at inducing firing of that neuron than firing inputs which arrive in an uncorrelated way (Abeles 1982b; Softky and Koch 1993; Singer et al. 1997). Because of that non-linear effectiveness of synchrony, small correlation strengths might still cause strong effects. One of the consequences of this hypothesis would be that neurons organize, depending on the momentary computational demand, into functional groups, commonly called "cell assemblies" (Hebb 1949). This would imply that the interactions among neurons should be variable, relatively short-lasting and, in particular, they should be modified in relation to the behavioral state. Each neuron could therefore participate in different cell assemblies at different times.

The concept of cell assemblies has, thus, introduced an additional dimension of neuronal coding mechanisms. In other words, a complementary process – complementary to the change in neuronal firing rate – might also be used in parallel by the central nervous system. The combination of the two different strategies during information processing might provide much more information emerging from one and the same pattern of neuronal activity. In such a way, it might help to increase the flexibility and representational power of a distributed system such as the cerebral cortex.

The question of the functional coupling into cell assemblies was mostly raised by studying the input of sensory structures of the brain, such as visual (Eckhorn et al. 1988; Gray et al. 1989; review by Singer and Gray 1995), auditory (Ahissar et al. 1992; Eggermont 1994; deCharms and Merzenich 1996) or somatosensory (Nicolelis 1995) areas. However, only little is known about the dynamic interactions of neurons in the motor cortex, which is the main output structure governing overt behavior (Murthy and Fetz 1992; Sanes and Donoghue 1993; Baker et al. 1997; Riehle et al. 1997).

A well-known example of a neuronal ensemble activity in the motor cortex during the execution of a motor task is the "population vector" approach (Georgopoulos et al. 1982, 1993). In this approach, the directional tuning properties of a population of motor cortical neurons are combined into joint tuning by appropriate summation over the population response. Here, we want to propose another approach which is inspired by the concept of cell assemblies by evaluating the relative timing of action potentials in such an ensemble of neurons recorded simultaneously. The aim of the study was, thus, to describe the relationships between synchronization dynamics and changes in activity of the same neurons during the preparation and execution of voluntary movements. We recorded simultaneously the activity of up to seven motor cortical neurons of a monkey during preparation and execution of pointing movements in six directions.

Materials and methods

A monkey (*Macaca mulatta*) was trained to perform pointing movements in six directions. It was cared for in the manner described in the *Guiding Principles in the Care and Use of Animals of the American Physiological Society* and the French government regulations. The animal sat in a primate chair in front of a vertical panel on which seven touch-sensitive, light-emitting diodes (LEDs) were mounted, one in the center and six equidistantly in a circle around it. The center target was lit and the animal had to touch it to initiate the trial. Then, after a fixed delay of 500 ms, during which the animal had to continue to press the target, a preparatory signal (PS) was presented consisting of the illumination of one of the peripheral targets in green. After a preparatory period (PP) of 1 s, the green target turned red, serving as the response signal (RS). It instructed the animal to release the center target and to point at the specified target as quickly as possible. During a session of about 150 trials, all 6 movement directions were presented at random with equal probability.

After training, the animal was 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 halothane anesthesia (<1.5% in air). A stainless steel T-bar was cemented to the skull to fixate the animal's head during recording sessions. In order to record extracellularly multiple single-neuron activity, a multielectrode microdrive (Reitböck system, Thomas Recording, Marburg, Germany; cf. Mountcastle et al. 1991) was used to transdurally insert seven microelectrodes (quartz-insulated platinum-tungsten electrodes, outer diameter 80 µm, impedance 2–4 MΩ at 1000 Hz). The electrodes (spaced 330 µm apart) were arranged in a circle, one electrode in the middle and six around it. From each electrode, action potentials of a single neuron were isolated by using a window discriminator.

Dynamic changes in synchronicity between sets of simultaneously recorded neurons were analyzed off-line by calculating the statistically significant epochs of synchronized firing (Grün 1996; Riehle et al. 1997). The simultaneous observation of the occurrences of action potentials elicited by N neurons can be transformed, using an appropriate binning, to N-dimensional joint-activity vectors consisting of ones (action potential) and zeros (no action potential). They reflect the various constellations of coincident spiking activity across neurons ("raw coincident events", cir-

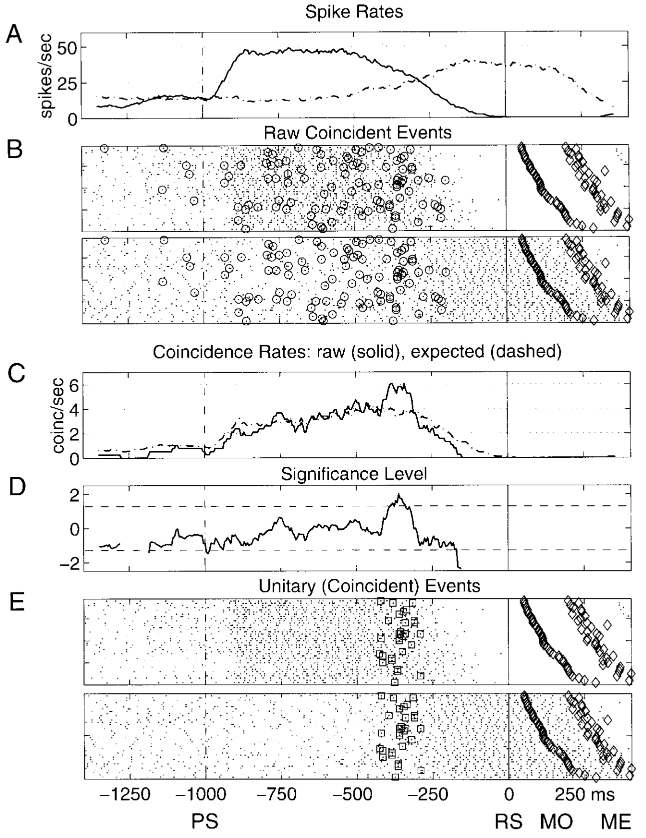


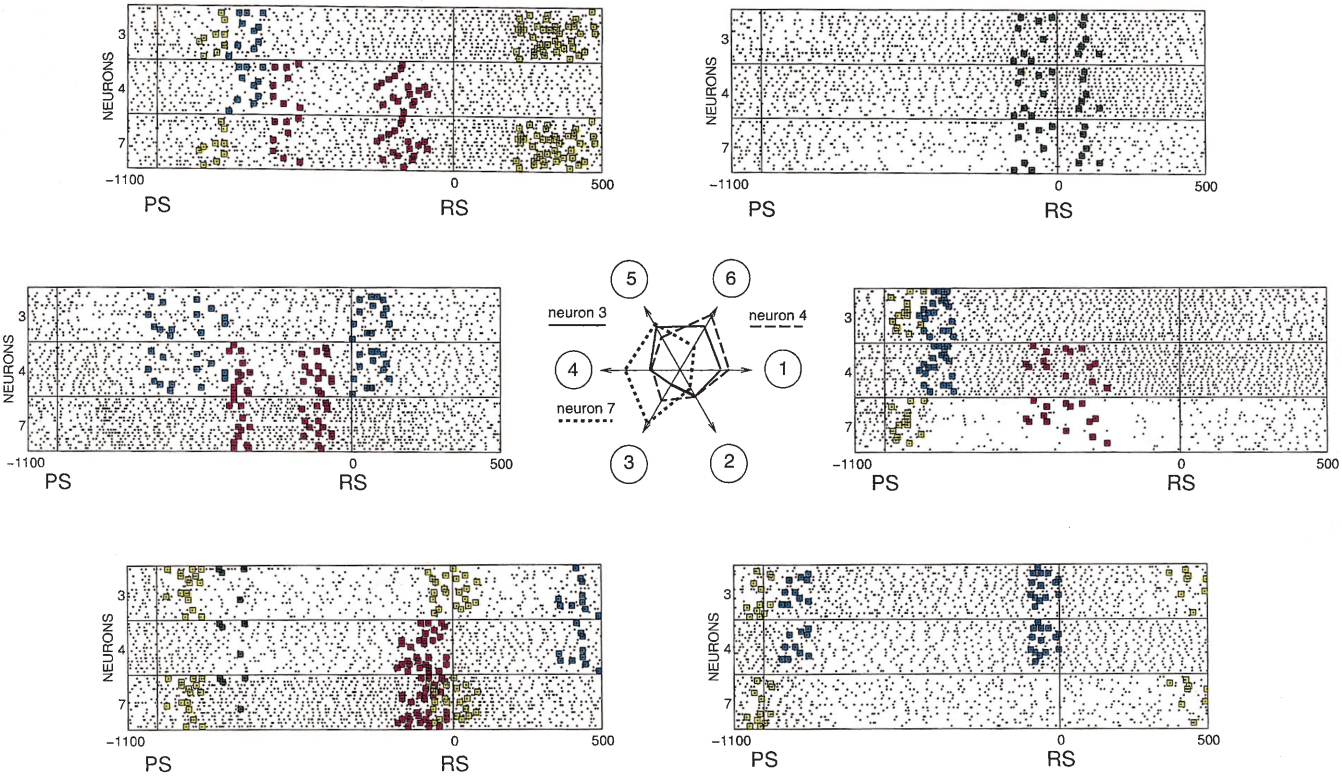
Fig. 1A–E "Unitary event" analysis of the spiking activity of two simultaneously recorded neurons of the primary motor cortex. **A** Firing frequency of each neuron in spikes/s calculated in 5-ms bins. The first vertical (*dashed*) line corresponds to the occurrence of the preparatory signal (*PS*), the second (*solid*) line to the occurrence of the response signal (*RS*). **B** Conventional raster displays of spike discharges of the two neurons. *Each dot* represents an action potential, *each line* a trial, trials being rank-ordered in relation to increasing reaction time. Coincident spikes detected within a 5-ms bin are *marked by circles* in each raster display. In each trial, both movement onset (*MO*) and end (*ME*) are *indicated by diamonds*. **C** Coincidence rates are shown in coincidences/s. **D** For each time window, the statistical significance was calculated for the difference between measured and expected coincidence rates. Whenever the significance value exceeded the threshold (*upper dashed line*, *P*=0.05), this defined an epoch in which significantly more coincidences occurred than expected by chance. Occasionally, this value dropped below the lower dashed line, thus indicating epochs in which significantly less coincidences occurred than expected by chance. **E** The selection of coincident spikes which pass the significance criterion for being "unitary events" are *marked by squares* cles in Fig. 1B). The coincidence bin width was fixed to 5 ms, motivated by experimental observations on the temporal precision of spiking cortical pyramidal neurons (Mainen and Sejnowski 1995). Under the null hypothesis that the N neurons fire independently from each other, the expected number of occurrences ("expected coincidence rate," dashed line in Fig. 1C) of any constellation and its probability distribution can be calculated on the basis of the single neuron firing rates (curves in Fig. 1A). The significance of a positive difference between raw and expected coincidences is assessed from a Poisson distribution (with the mean set to the expected coincidence value) as the cumulative probability P of observing the actual number of coincidences or even a 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. For a better visualization, we plotted a logarithmic function of the two: $\log_{10}[(1–P)/P]$ ("surprise" measure, Palm et al. 1988). It yields positive numbers for excessive coincidences, and negative ones for lacking coincidences (see Fig. 1D). Those occurrences of coincident spiking events which exceed the significance level of 5% (upper dashed line in Fig. 1D) were called "unitary events" (UEs, squares in Fig. 1E). The observation of UEs was used to define neurons that are momentarily engaged in the activation of a cell assembly. To deal with non-stationarities in the firing rate of neurons, synchronicity was estimated on the basis of small time segments, by using a sliding boxcar window (100 ms) that was shifted in 5-ms steps along the data. This timing segmentation was applied to each trial, and the data of corresponding segments in all trials were then analyzed as one stationary data set. Only spike trains were selected for analysis, which reached the following criterion: a discharge frequency of more than 5 impulses/s which was reproducible across multiple trials.

Results

The activity of 229 neurons was recorded in motor cortex. During one session, the activities of two to seven neurons were recorded simultaneously. After the severe application of the selection criteria, 60 pairs of neurons remained for further analysis. Of those, almost all pairs of neurons (56/60, 93%) synchronized significantly their activity during at least one of the periods during the task.

Figure 1 shows an example of two neurons recorded simultaneously during the execution of a pointing movement in one out of the six possible directions. Neuron 1 exhibited a purely preparation-related activity pattern by increasing its activity shortly after the occurrence of the preparatory signal (PS), which lasted up to 250 ms before the response signal (RS) (see Fig. 1A, solid line, Fig. 1B, top). Neuron 2 (Fig. 1A, dashed line, Fig. 1B, bottom) increased its spike discharge only at the end of the preparatory period (PP), that is shortly before RS, where its discharge reached its maximum rate. Neuronal

Fig. 2 Raster displays of three simultaneously recorded neurons are shown during preparation and execution of all six movement directions. Only "unitary events," i.e., precise spike coincidences which occur significantly more often than expected by chance, are *indicated by squares*. Each correlation combination is *indicated by a different color: blue* for neurons 3 and 4, *yellow* for neurons 3 and 7, *red* for neurons 4 and 7, and *green* for all three neurons forming a triplet (*PS* preparatory signal, *RS* response signal). *In the center*, the directional tuning is indicated for each neuron, calculated as mean firing discharge during the execution of each movement direction (RS to movement end). *The length of the arrows* corresponds to the mean firing frequency



activity which was clearly time locked to the execution of the movement stopped with movement end (second line of diamonds in Fig. 1B,E), thus being classified as movement related. However, whereas these two neurons seem to be implicated in two different processes at two distinct moments, their spiking activity was significantly synchronized during PP. Unitary events occurred exactly at the moment when the first neuron was going to stop its activity and the second one began to increase its firing rate (cf. Fig. 1E). Note that during the 150-ms epoch of unitary events both neurons exhibited stationary spiking activity across trials (mean activity in impulses/second ± standard deviation: 34.4±3.8 and 22.2±1.9, respectively; number of trials 38). Furthermore, in order to control the precision of spike coincidences, we compared high-resolution (1- to 2-ms) cross-correlations of the same spike trains at moments when unitary events could be detected with moments with no unitary events. For instance, for the example shown in Fig. 1, the width of the peak of the cross-correlation, centered on zero, was 4 ms, exceeding the shift predictor by 30% and being flanked on both sides by a trough. The classification of these two neurons as preparation (neuron 1) and execution related (neuron 2) based on their discharge rate would by no means allow one to describe the functional link between them which only can be detected by means of the synchronization pattern.

In Fig. 2, the functional cooperativity between three neurons during preparation and execution of movements in six directions is shown. Only synchronizations are indicated which occur significantly more often than expected by chance (unitary events). Indeed, one can observe that the functional coupling between neurons varies on very short time scales. For instance, during the preparatory period in which movement direction 4 was precued, first neuron 4 synchronized its activity with neuron 3 and then with neuron 7. After occurrence of the RS, neurons 4 and 3 were again synchronized. The pattern of synchronization differed for each movement direction in a very complicated manner.

At the center of Fig. 2, tuning curves of all three neurons, calculated as the mean activity during movement execution (from RS to movement end), are shown in a polarplot. Each neuron presented a different preferred direction. However, the change in activity of the neurons during all movement directions does not allow one to establish any systematic relationship between these activity modulations and the functional coupling of the neurons as described by the synchronization patterns. For example, the discharge rate of neurons 3 and 4 was highest for directions 5 and 6; yet, these two neurons did not synchronize significantly their activity during these two movement directions. On the other hand, neurons 4 and 7 were repeatedly coupled by synchrony in directions 3, 4 or 5, despite their different directional preferences.

Discussion

There are many examples in the literature that motor cortical neurons change selectively their activity in relation to preparation and execution of goal-directed movements (Tanji and Evarts 1976; Georgopoulos et al. 1982, 1993; Riehle and Requin 1989; Requin et al. 1992). On the other hand, it has been shown that populations of neurons in monkey motor cortex synchronize their activity (Murphy et al. 1985; Murthy and Fetz 1992; Sanes and Donoghue 1993; Baker et al. 1997). On the level of multiple single-neuron activity, synchronization of single action potentials has been shown to appear on a millisecond timescale (Riehle et al. 1997). There is also evidence that synchronization occurs between sensory and motor areas in monkey (Murthy and Fetz 1992) and cat (Roelfsema et al. 1997). Our results presented here, however, tend to describe how the same motor cortical neurons modify rapidly and selectively not only their discharge frequency, but also their discharge correlation during preparation and execution of pointing movements. Since the probability of observing synchronized activity increases with increasing the firing rate of neurons, this variation in the number of synchronizations might, thus, be only an epiphenomenon of spike discharge (Shadlen and Newsome 1994). However, the statistical analysis technique used in this study (Grün 1996; Riehle et al. 1997) allowed us to detect periods of time during which significantly more coincident spikes could be observed than expected by chance.

Recently, we have shown that such excessive spike synchronization can occur during periods in which neurons do not change their activity, and this in relation to behaviorally relevant events (Riehle et al. 1997). Here, we show that neurons, which are classified in terms of their changes in activity as being members of different functional categories, synchronize their activity significantly. Since we used the observation of excessive spike synchronization to define neurons which are momentarily engaged in the activation of a cell assembly, these data provide evidence that neurons, which do not exhibit necessarily the same patterns in neuronal firing, might be dynamically coupled.

As shown in Fig. 1, the two neurons – the one defined as being preparation related and the other as movement related – were “connected” by synchrony during the transition phase from preparation to movement execution. However, in another example shown in Fig. 2, no systematic relationship can be established between the characteristics of the three neurons in terms of changes in activity and patterns of synchronization. It has been shown by using a population code approach (Georgopoulos et al 1982, 1993; Bastian et al 1998) that each neuron within a population contributes by means of its firing rate to the generation of a goal-directed movement despite its different directional tuning properties. Thus, one could speculate that the effectiveness of the population activity might be increased by increasing the cooperativity among neurons in that population in relation to the behavioral context. This means that during the performance of such a pointing task assemblies of task-related neurons should be activated in order to cooperate in a coordinated way.

If indeed cortical processing relies also on precise temporal relations among the spiking activities of groups of neurons, simultaneous recordings from as many neurons as possible have to be obtained in order to increase the probability of discovering assembly activity. Furthermore, in order to provide evidence that synchronization of spiking activities in ensembles of coactive neurons helps to strengthen the effectiveness within these groups, sets of data recorded during the same behavioral condition need to be quantified and compared with other behavioral conditions. Finally, the transient nature of cell assemblies makes it necessary to study the evolution in time of such dynamic network activities on a single trial level. For these purposes, new analysis techniques need to be developed.

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