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Dynamical changes and temporal precision of synchronized spiking activity in monkey motor cortex during movement preparation

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Abstract – Movement preparation is considered to be based on central processes which are responsible for improving motor performance. For instance, it has been shown that motor cortical neurones change their activity selectively in relation to prior information about movement parameters. However, it is not clear how groups of neurones dynamically organize their activity to cope with computational demands. The aim of the study was to compare the firing rate of multiple simultaneously recorded neurones with the interaction between them by describing not only the frequency of occurrence of epochs of significant synchronization, but also its modulation in time and its changes in temporal precision during an instructed delay. Multiple single-neurone activity was thus recorded in monkey motor cortex during the performance of two different delayed multi-directional pointing tasks. In order to detect conspicuous spike coincidences in simultaneously recorded spike trains by tolerating temporal jitter ranging from 0 to 20 ms and to calculate their statistical significance, a modified method of the 'Unitary Events' analysis was used. Two main results were obtained. First, simultaneously recorded neurones synchronize their spiking activity in a highly dynamic way. Synchronization becomes significant only during short periods (about 100 to 200 ms). Several such periods occurred during a behavioural trial more or less regularly. Second, in many pairs of neurones, the temporal precision of synchronous activity was highest at the end of the preparatory period. As a matter of fact, at the beginning of this period, after the presentation of the preparatory signal, neurones significantly synchronize their spiking activity, but with low temporal precision. As time advances, significant synchronization becomes more precise. Data indicate that not only the discharge rate is involved in preparatory processes, but also temporal aspects of neuronal activity as expressed in the precise synchronization of individual action potentials. © 2000 Éditions scientifiques et médicales Elsevier SAS

monkey / motor cortex / multiple single-neurone recordings / precise spike coincidences / cell assemblies

1. Introduction

Movement preparation is considered to be based on central processes which are responsible for improving motor performance (for a review see [22]). For that purpose, sensory and contextual information has to be resembled and integrated to shape the motor output. One strong argument in favour of this efficiency hypothesis of preparatory processes is the fact that providing prior information about movement parameters [25, 30] and/or removing time uncertainty about when to move [8, 26] significantly shortens reaction time. On the neuronal level, it has been shown that motor cortical neurones change their activity selectively in relation to prior information about movement parameters [27, 28, 35]. Furthermore, it has been demonstrated that the activity of single motor cortical neurones is highly predictive for performance speed [28, 29]. In this context, it is widely accepted that sensorimotor functions including preparatory processes are based on joint processing in neuronal networks distributed over various brain structures (cf. [23, 24]). However, it is much less clear, how these networks organize dynamically in space and time to cope with momentary computational demands. The concept emerged that computational processes in the brain could also rely on the relative timing of spike discharges among neurones within such functional groups [1, 3, 11, 19, 32, 34], commonly called cell assemblies [17]. An essential ingredient of the notion of co-ordinated ensemble activity is its flexibility and dynamic nature. To critically test if such a temporal scheme is actually implemented in the central nervous system, it is necessary to simultaneously observe the activities of many neurones, and to analyse these activities for signs of temporal co-ordination. One type of temporal co-ordination may be defined by coincident spiking activities. In order to detect and evaluate the occurrence of coincident spikes we used the modified 'Unitary Events' analysis [13–16]. Basically, this technique allows one to determine epochs containing spike coincidences which violate the assumption of independence of

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the participating neurones. The interdependence is then interpreted as a signature of a functional cell assembly [5]. The statistical null-hypothesis is formulated on the basis of the individual firing probabilities and allows to calculate the number of expected coincidences. The statistical significance of the measured number of coincidences is evaluated by comparing it with the expected number. By application of this analysis in a sliding window one can determine epochs of significant synchronized activity. The temporal precision of spike synchronization is obtained by additionally varying the allowed coincidence width in the analysis [16]. If cell assemblies exist and are involved in cortical information processing, they should be activated in systematic relation to the behavioural task. Our analysis technique allows us to describe a detailed relationship between spike synchronization, i.e. the activation of a cell assembly [5], rate modulation and behaviourally relevant events [12, 26]. The aim of the study was thus to provide a phenomenological description of this relationship by comparing, in the same neurones, the frequency of occurrence of significant synchronization, its modulation in time and its modification in temporal precision with the modulation in firing rate in the context of the performance of two different delayed multi-directional pointing tasks.

2. Materials and methods

2.1. Behavioural procedures

Two rhesus monkeys were trained to perform a multi-directional pointing task. They were 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 centre and six placed equidistantly on a circle around it. In each trial, two signals were presented successively. The first, the preparatory signal, provided prior information about the target which has to be pointed after occurrence of the second, the response signal. First, the centre target was lit and the animal had to press it for initiating the trial. Then, after a fixed delay of 500 ms, the preparatory signal was presented by illuminating one of the targets in green. After another delay (preparatory period), during which the animal still had to continue to press the centre target, the colour of the peripheral target turned to red. This served as response signal requesting from the animal to perform the movement as fast as possible, in order to be rewarded by a drop of fruit juice. Two conceptually different experimental procedures were used by manipulating the temporal aspects of the task. In task 1, there was no temporal uncertainty, i.e. the delay between the preparatory and the response signal remained fixed at 1 000 ms. In task 2, the delay between the two signals was manipulated such that the response signal could occur either after 600 ms or after 1 200 ms. The two durations were presented at random, but with equal probability, i.e. the conditional probability for the response signal to occur was 0.5 during the first 600 ms of the preparatory period, but was 1 after passing 600 ms. In task 1, six types of trials corresponding to the 6 movement directions were presented at random and with equal probability. In order to dispose of enough trials for data analysis, a behavioural session comprised about 150 trials. In task 2, however, in which not only six movement directions, but also two different durations of the preparatory period were presented, a behavioural condition had necessarily to comprise twice as much trials.

2.2. Electrophysiology

After training, the animals were prepared for multiple single-neurone 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 anaesthesia ($< 2.5\%$ 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 extra-cellularly single-neurone activity from multiple sites, a multi-electrode microdrive (Reitböck system, Thomas Recording, Giessen, Germany, [18]) was used to transdurally insert seven micro-electrodes (quartz insulated platinum-tungsten electrodes, outer diameter 80 μm, impedance: 2–5 MΩ at 1 000 Hz). The electrodes were arranged in a circle, one electrode in the middle and six around it (equally spaced 330 μm apart). From each electrode, electrical signals were amplified and band-pass filtered (300 Hz–10 kHz). Action potentials of only one single neurone per electrode were then isolated by using a window discriminator. Neuronal data along with behavioural events (e.g. occurrence of preparatory and response signals, including target information,

reaction and movement times, reward, trial start and end, errors) were stored for off-line analysis with a time resolution of 1 kHz.

2.3. Data analysis

Dynamic changes of synchronous activity between sets of simultaneously recorded neurones were analysed off-line by calculating the statistically significant epochs of synchronized firing ('Unitary Event' method, [13]). There is recent experimental evidence that the timing accuracy of spikes, which might be relevant for higher brain functions, can be as precise as 1–5 ms [4, 26]. To detect such precise synchronous spikes of variable coincidence width, we developed an extension of the 'Unitary Event' method, the 'multiple shift' method [16]. It treats the data in their (original) high time resolution while searching for coincidences with larger coincidence width. Technically, coincident spikes are detected in simultaneously recorded pairs of neurones by shifting the spike trains against each other over the range of allowed coincidence width (ranging from zero to 20 ms) and integrating the number of exact coincidences (on the time resolution of the data) over all shifts. In order to evaluate the significance of the coincidences, the outcomes ('raw coincidences') are compared to the expectation. Assuming that the spike trains are generated by stationary Poisson processes and under the null-hypothesis of independent firing, the expected number of occurrences ('expected coincidences') and its probability distribution can be estimated on the basis of the single neurone firing rates. The predictor is given by the total number of time steps (number of shifts times length of the data segment) multiplied by the product of the marginal probabilities. The significance of a positive deviation 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 coincidence 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 use a logarithmic function of the two: $\log_{10}[(1-P)/P]$ ('surprise' measure, [20]). This yields positive numbers for excessive coincidences, and negative ones for lacking coincidences. Those occurrences of coincident spikes which exceed the significance level of 5% were called 'Unitary Events'. We will use the term 'synchronicity' for defining synchronous activity in terms of the statistical significance level, i.e. the joint-surprise which is by definition independent of the firing rate of the participating neurones. To deal with non-stationarities in the firing rate of neurones, synchronicity was estimated by using a sliding window (usually 100 ms) that was shifted in steps of 5 to 20 ms along the data. This timing segmentation was applied to each trial, and the data of corresponding segments in all trials were then analysed as one stationary data set.

Applying the above method, non-stationarities of spike counts over trials can in principle lead to an underestimation of the expected number of coincidences and thereby to incorrect joint-surprise values. This problem can be overcome by generating coincidence count distributions taking full account of the spike count in individual trials. Using simulations, independent spike trains with exactly the same spike counts in each trial and window are generated and analysed for the occurrence of coincident events. From many repetitions of such simulations, distributions of coincidence counts can be constructed for each window. We checked for all data sets that using the elaborate distributions described above does not considerably change the time course of the joint-surprise measure (related potential problems are discussed in [21]).

In the same way, fast rate changes on a time scale below the width of the analysis window can lead to an underestimation of the expected number of coincidences. The standard procedure of trial shuffling leaves the rate profile intact but destroys potential spike time correlation. We checked for all data sets that the temporal structure of the joint-surprise measure vanishes if artificial data generated from trial shuffles are compared to the theoretical distribution.

Only data sets were selected for analysis which reached the following criteria: (i) a discharge frequency of more 7 impulses·s^{-1} [31], (ii) stationarity of rate changes across multiple trials of one behavioural condition, (iii) a minimum of about twenty trials per condition (depending on the firing rate of the participating neurones, the higher the firing rate, the less trials are needed) and (iv) the criteria have to be fulfilled by at least two simultaneously recorded neurones.

In order to quantify synchronous activity, the probability to find significant epochs at each instant in time was determined by averaging over behavioural conditions, e.g. movement directions. First, for each coincidence width a binary vector

representing the original temporal resolution was constructed that indicates by '1' a significant epoch and by '0' a non-significant one. Significance is tested within a window centred at the corresponding time step. Second, from all binary vectors constructed for a given coincidence width in different behavioural conditions we calculated the probability for significant epochs per time step to obtain a 'probability of significant synchronization' as a function of time.

3. Results

In both tasks, about a quarter of the task-related neurones recorded in the primary motor cortex fulfilled the criteria described in Section 2 and were thus selected for further analysis. During one experimental session, the activity of two to seven neurones was recorded simultaneously. In task 1, among 229 task-related neurones, we selected 56 of them (recorded in twenty sessions) for constituting sixty pairs. In task 2, among 120 task-related neurones 35 of them (recorded in fifteen sessions) were selected for constituting 24 pairs. A single neurone could participate in several pairs.

3.1. Modulation of synchronous spiking activity in time

The first question we asked was whether or not the strength of synchronization remains constant over time. In other words, does synchronization, as determined by the number of coincidences per second, depend only on the firing rate of the participating neurones or is it, in addition, context-dependent? In *figure 1*, an example of a pair of neurones is shown, recorded in task 1. During the preparatory period, i.e. the delay between preparatory and response signal, neurone 2 does virtually not change its activity, whereas the firing rate of neurone 6 increases progressively (*figure 1A*). Both stop firing during reaction time, about 150 ms after presentation of the response signal. Since the number of expected coincidences is calculated, window by window, on the basis of the mean firing rate of the participating neurones (see Section 2), the rate of the expected coincidences necessarily reflects the discharge rate of both neurones. Surprisingly, when comparing in *figure 1C* the rate of expected coincidences (dashed curve) with the rate of measured (raw) coincidences (solid curve), it can clearly be seen that the measured coincidence rate modulates strongly as compared to the expected level. The modulation of synchronous activity, corrected for firing rate levels, is reflected in the joint-surprise, shown in *figure 1D*. If this value fluctuates around zero, the spiking activities of the two neurones are not correlated, i.e. neurones fire independently from each other, the number of synchronous spikes corresponds to the number expected by taking into account the instantaneous firing rates. Positive excursions indicate more synchronization than expected, and vice versa, negative excursions indicate less synchronization than expected. Thus, before the presentation of the preparatory signal, less coincidences occur than expected, whereas at 250 and 500 ms after it many more coincidences than expected are detected, exceeding by far the significance level. Finally, from there on approximately as many coincidences as expected are detected. Thus, the modulations in the number of coincidences cannot be explained by the modulations of firing rates generated by our moving window estimator (*figure 1A*).

In *figure 2*, one of the two neurones (neurone 4), as in the example shown in *figure 1*, does hardly change its firing rate during the preparatory period, whereas the other (neurone 2) strongly increases its activity, starting after occurrence of the preparatory signal. In this case, synchronicity modulates more or less rhythmically during the whole delay period between significantly positive and negative values, with increasing amplitude of the significance level, reaching a maximum at the end of the preparatory period (*figure 2C*).

In *figure 3*, another example of modulating synchronicity is shown recorded in task 2. In this task, the duration of the preparatory period was manipulated such that a response signal was expected at 600 ms (ES, expected response signal) with a probability of 0.5. Once this moment passed and the response signal was not presented, its probability to occur at 1 200 ms was 1. There is an increase in the firing rate in both neurones shortly after the moment when the response signal was expected, but did not occur. Interestingly, synchronicity is at chance level during the first 600 ms of the delay (see *figure 3D*). Then, at the moment of signal expectancy, it begins to modulate rhythmically with increasing amplitude towards the end of the preparatory period. After occurrence of the response signal, i.e. during movement execution, the joint-surprise drops down to a highly negative value indicating that neuronal activity is significantly less synchronized than expected by chance, although the firing rate of one of the neurones increases.

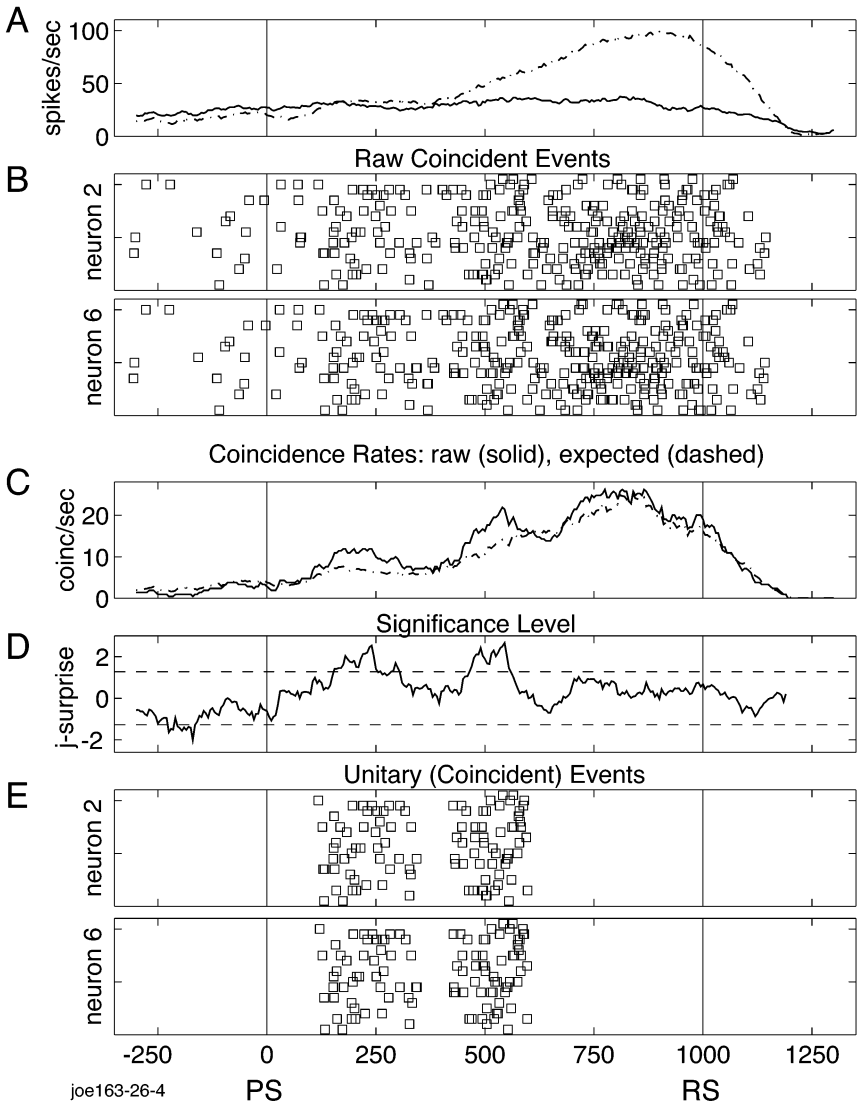

Figure 1. Dynamic changes of synchronous spiking activity of two simultaneously recorded neurones during the execution of trials of one type (movement direction 4) in task 1. All calculations are made by using a sliding boxcar window of 100 ms duration that was shifted in 5 ms steps along the data. Time is running along the x-axis and is indicated in ms. **A**, Firing rate of each neurone in spikes s^{-1}. The first vertical line corresponds to the presentation of the preparatory signal (PS), the second line to that of the response signal (RS). **B**, Conventional raster displays of spike discharge of both neurones. Each dot represents an action potential, each line a trial, trials being rank-ordered in relation to increasing reaction time, indicated by thick dots. Coincident spikes were detected by allowing a jitter of 3 ms and marked by squares in each raster display. **C**, Raw (solid) and expected (dashed) coincidence rates are shown in coincidences s^{-1}. **D**, For each sliding window, the statistical significance (joint-surprise value) was calculated for the difference between raw and expected coincidence rates. The result of each window was placed in its centre. 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 ($P < 0.05$) 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. Synchronicity increases after the occurrence of PS to reach a first maximum (significance level of < 0.01 corresponds to a joint-surprise value of > 2) at about 250 ms and a second one at 500 ms. At the end of the preparatory period, neurones are considered to fire independently from each other, i.e. the significance level is around zero.

3.2. Changes of the temporal precision of synchronous activity

In *figure 1*, synchronization was determined for a coincidence width of 3 ms, i.e. coincident spikes were detected by allowing a jitter ranging from 0 to 3 ms. In *figure 4*, the same spike trains were analysed for varying coincidence widths ranging from 0 to 20 ms. For each allowed coincidence width we calculated the joint-surprise curve. The results are represented in a three-dimensional colour matrix, time is running along the x-axis and coincidence width along the y-axis. The colour code indicates the joint-surprise values, ranging from dark blue (negative values) over green (zero) to dark red (positive values). The threshold of statistical significance of $P = 0.05$ for excess coincidences corresponds to a value of 1.28, for lacking coincidence to -1.28. It can clearly be seen that synchronicity changes not only in strength, but also in temporal precision throughout the trial. With a latency of 200 ms after the occurrence of the preparatory signal, neuronal activity becomes significantly synchronized, however with low temporal precision (at a coincidence width of about 15 ms). Precision then increases to reach a minimal value of 2–4 ms in the middle of the preparatory period at 500 ms. During the remaining 500 ms of the preparatory period, neurones desynchronize their activity and joint-surprise values modulate around zero, indicating that neurones fire more or less independently from each other.

In *figure 5* the evolution of synchronicity both in time and precision is very different. The spiking activity of the analysed pair of neurones was already show in *figure 2*. Synchronization modulates rhythmically, whereas precision increases from step to step during the whole delay period to reach a maximal precision of 2 ms at a maximal strength of synchronicity (joint-surprise value = 2.39) about 120 ms before the presentation of the response signal.

3.3. Quantifying correlated activity: comparison with firing rate

In the next analytical step, we compared for pairs of neurones the time course of the probability for significant epochs with the underlying firing rates. Both measures are averages over different behavioural conditions. This type of analysis is shown in *figure 6* for the neurones shown in *figures 1* and *4*. In *figure 6A*, a grey scale intensity code is used to indicate the probability for being significantly synchronized by quantifying over all six movement directions (see Section 2). At the beginning of the preparatory period, after a latency of about 200 ms, the probability for being signifi-

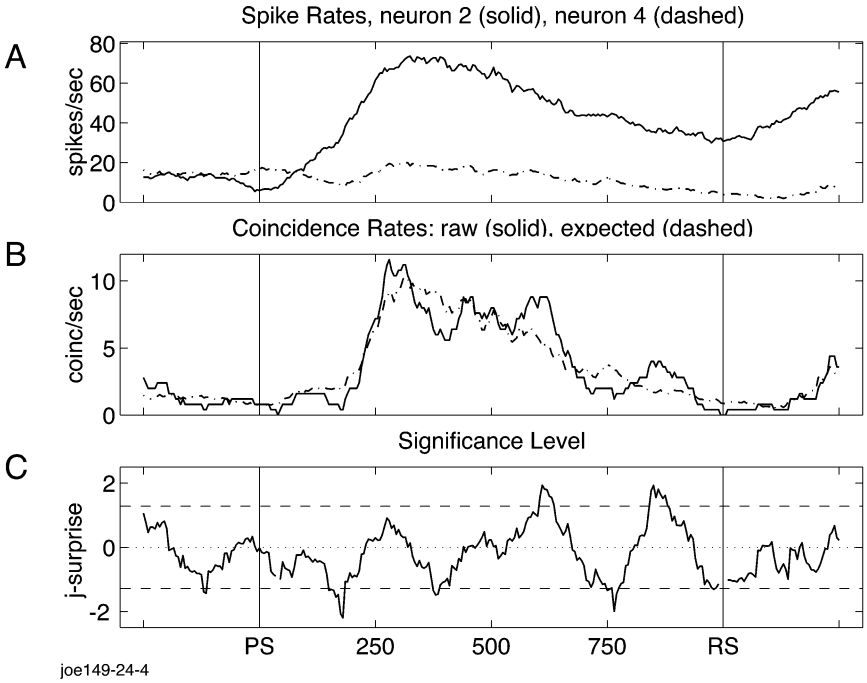

Figure 2. Dynamic changes of synchronous spiking activity of another pair of neurones during the execution of one type of trials (direction 4) in task 1. Only firing rates (**A**), coincidence rate (**B**) and the significance level (**C**) are shown. For calculation, a sliding window of 100 ms was shifted along the spike trains in 5 ms steps. The allowed coincidence width was 3 ms (for details, see *figure 1*). Synchronicity modulates throughout the whole preparatory period between significantly negative and positive values with increasing amplitude.

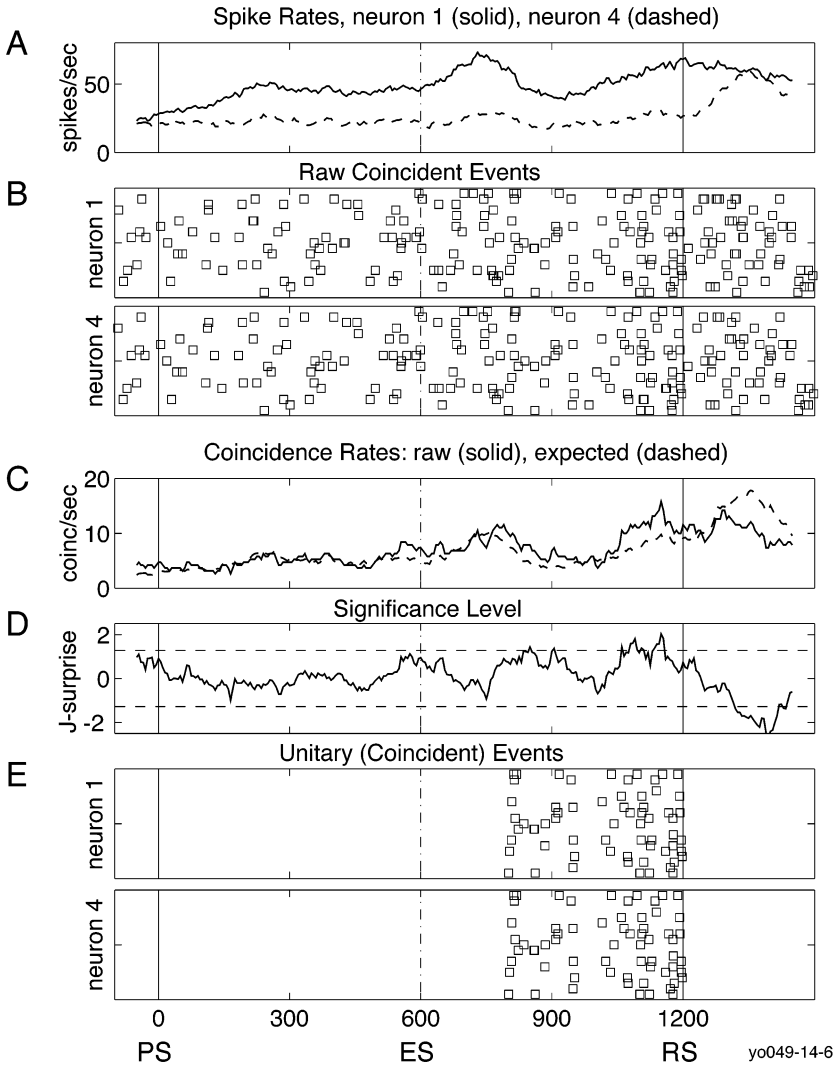

Figure 3. Dynamic changes of synchronous spiking activity of another pair of neurones during the execution of one type of trials in task 2 (long preparatory period, direction 6). For calculation, a sliding window of 100 ms was shifted along the spike trains in 5 ms steps. The allowed coincidence width was 2 ms. PS: preparatory signal; ES: expected (response) signal; RS: response signal; the delay between PS and RS was 1 200 ms. For further details, see *figure 1*. Synchronicity is at chance level during the first 600 ms of the preparatory period and starts then to increase. It modulates for the remaining period with increasing amplitude to reach a maximum just before the presentation of the response signal. During movement execution, neuronal activity is considered to be anti-synchronized, i.e. there are significantly less coincidences than expected.

cantly synchronized is highest. However, this synchronization occurs with very low temporal precision of about 15–16 ms. As time goes on during the delay, precision increases to reach a value of 2 ms at the end of the preparatory period. Synchronicity is then compared, in *figure 6B*, with the averaged firing rate of both neurones in corresponding time windows along the trial. It is interesting to note that when neuronal activity is significantly synchronized with a high temporal precision, firing rate is maximal. In *figure 7*, the same type of quantification is shown for a pair of neurones recorded in task 2 by averaging over all movement directions for trials presenting a long preparatory period. Remember, in these trials a response signal was expected at 600 ms with a probability of 0.5. It can clearly be seen that 200 ms before that moment, neurones become significantly synchronized to reach a first peak exactly at 600 ms. When this moment passed and no signal was presented, neurones immediately stop synchronizing. Another increase of synchronized activity then evolves to reach the highest probability value for being significantly synchronized about 200 ms before the actual presentation of the response signal, which now will occur with absolute certainty. The temporal precision at the maxima of synchronicity increases from about 12–15 ms at 600 ms to 6–8 ms before the actual signal occurrence. Finally, although the probability for being

synchronized is very low at movement initiation, its precision is extremely high (1 ms).

The mean firing rate of the same two neurones (*figure 7B*) exhibits very different dynamics, firing rate generally succeeds synchronicity. At signal expectancy, synchronicity is slightly preceding neuronal activity by about 100 ms. However, whereas synchronicity peaks 200 ms before the actual presentation of the response signal and drops then almost to zero, the mean firing rate reaches its maximum at movement onset.

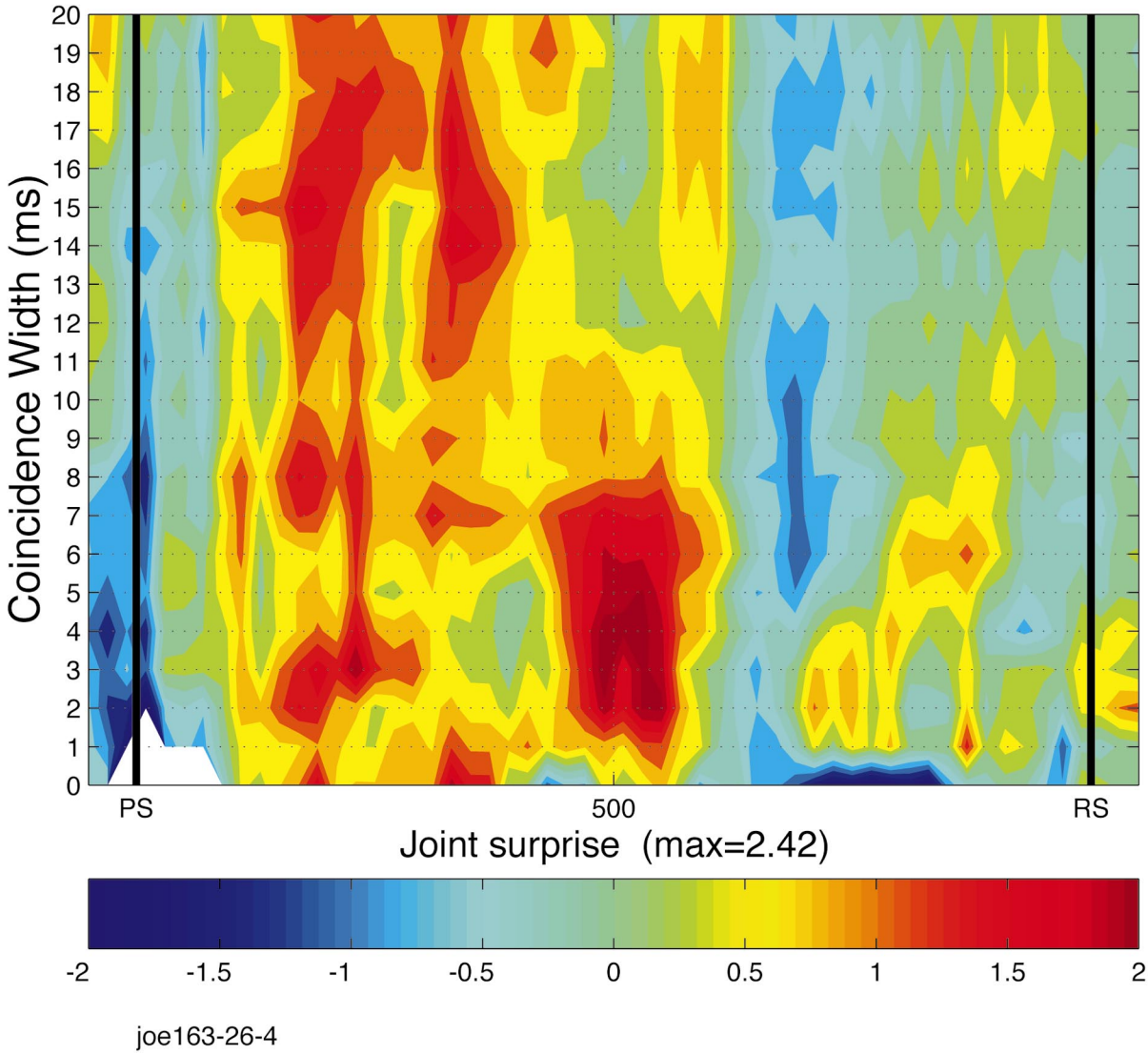

Figure 4. The same spike trains as in *figure 1* (task 1) were analysed for varying coincidence widths ranging from 0 to 20 ms (y-axis). For each coincidence width, the significance level (joint-surprise) was calculated separately. The colour code indicates the joint-surprise values, ranging from -2.19 (dark blue) over 0 (green) to 2.42 (dark red). For symmetry, values were clipped at -2 and 2. The threshold of statistical significance for 0.05 for excess coincidences corresponds to the value 1.28, and for lacking coincidences -1.28. For calculation, a sliding window of 100 ms was shifted along the spike trains in 20 ms steps. PS preparatory signal; RS: response signal; the delay between the two signals was 1 000 ms. As in *figure 1*, significant coincidences only occur during the first half of the preparatory period with increasing precision, reaching the strongest synchronicity value at 500 ms with a precision of 2–4 ms (dark red spot).

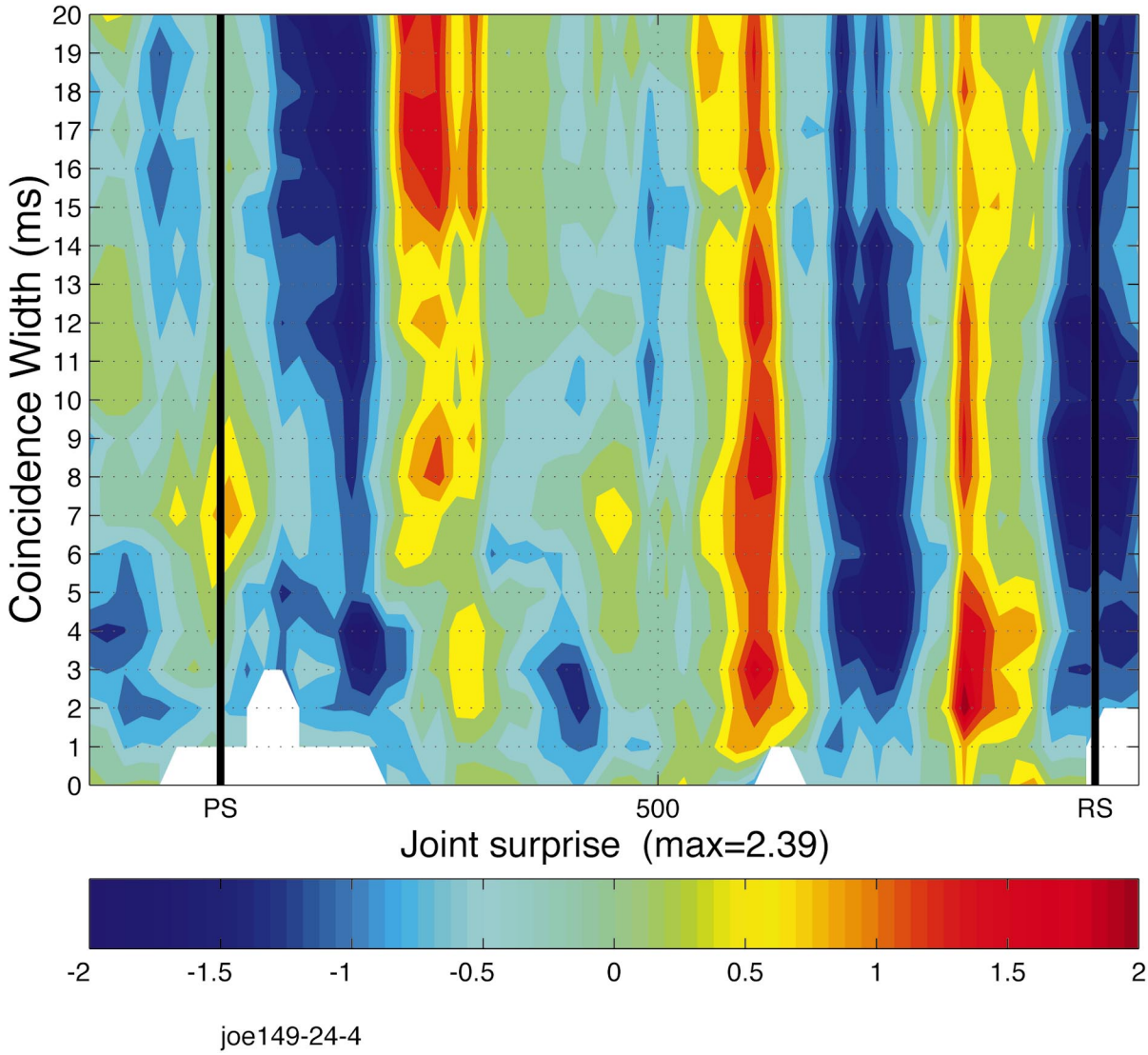

Figure 5. The same type of colour plot representation as in *figure 4* for the pair of neurones presented in *figure 2* (task 1). For calculation, a sliding window of 100 ms was shifted along the spike trains with 20 ms steps. PS: preparatory signal, RS: response signal; delay between PS and RS was 1 000 ms. The colour code indicates the joint-surprise values, ranging from −2.97 (dark blue) over 0 (green) to 2.39 (dark red). For symmetry, values were clipped at −2 and 2. Synchronized activity modulates rhythmically throughout the whole preparatory period with increasing temporal precision.

4. Discussion and conclusion

The aim of the present paper was to critically test the presence of synchronous spiking activity and to characterize its dynamics during movement preparation. Although the results do not yet allow final conclusions about the role of synchronous spiking activity for movement preparation, there are several non-exclusive ways to interpret the presented dynamics of synchronicity. The analysis of the data revealed two main results. First, synchronous spiking activity in motor cortical areas of the monkey during preparation for action is not maintained at a significant level for more than 100 to 200 ms. Periods of synchrony, however, may occur several times during the same behavioural trial, with a more or less regular pattern. Second, for many pairs of neurones, the temporal precision of synchronicity changes over time. If such changes occur, temporal precision typically in-

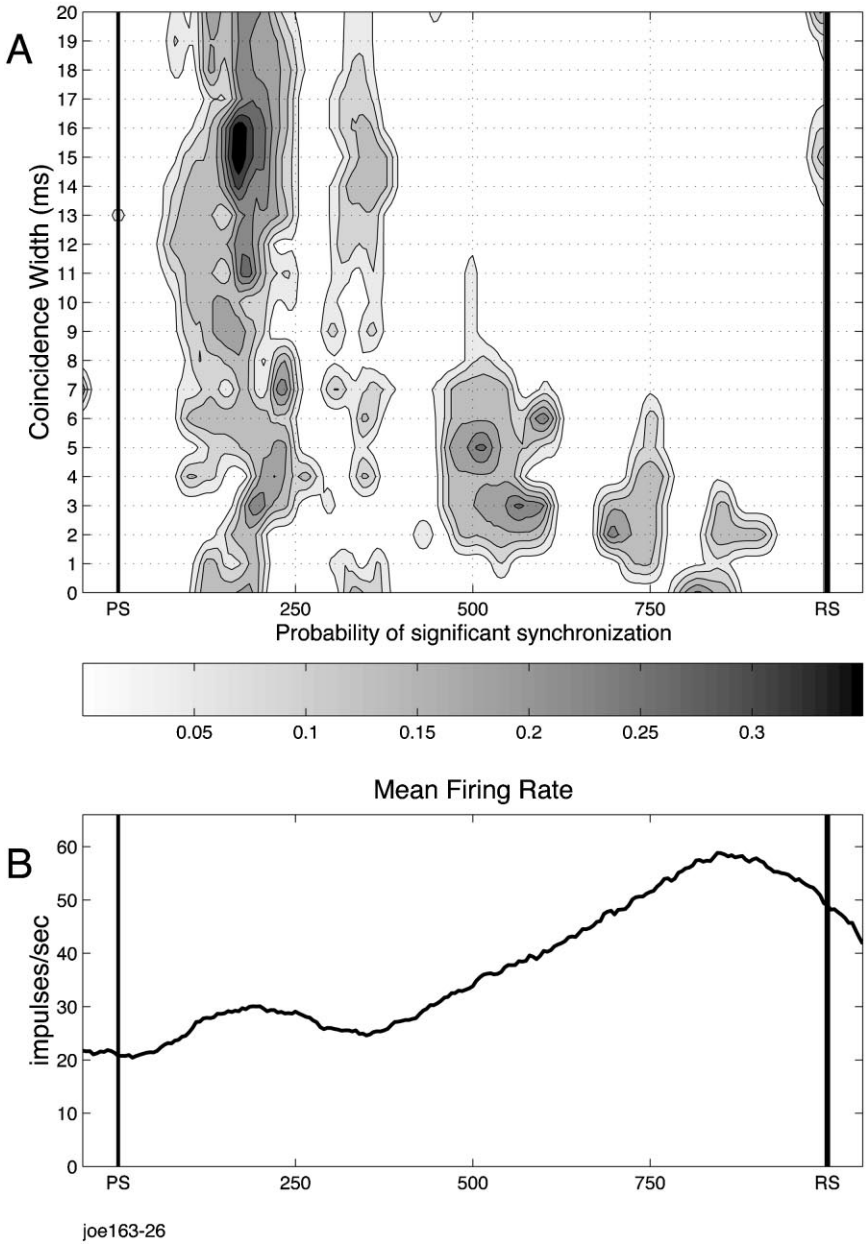

Figure 6. Quantification of correlated activity of the same pair of neurones as presented in *figures 1* and *4* (task 1), now pooled over all six movement directions. **A**, For each movement direction (parameters: sliding window of 100 ms, shift offset 5 ms) a similar matrix as in *figures 4* and *5* was calculated (coincidence width varied along the *y*-axis, time along *x*-axis), but now containing only entries of 0's and 1's. An entry of '1' indicates that the data analysed in the window contained significantly more coincidences than expected (i.e. joint-surprise ≥ 1.28), an entry of '0' that in the window are not significantly more coincidences than expected (i.e. joint-surprise < 1.28). The matrices from the different behavioural conditions (six different movement directions) were then averaged to calculate the probability matrix. It contains per entry position the probability for being significantly synchronized across behavioural conditions (from white to black, probabilities ranging from 0 to 0.4, see grey bar). This probability modulates in intervals of about 200 ms during the preparatory period. High probability is accompanied with low temporal precision early in the preparatory period (black spot at about 200 ms with highest probability at a coincidence width of 15 ms), and sharpens to high temporal precision throughout the trial, reaching the highest temporal precision at about 200 ms before the presentation of RS. **B**, The averaged mean firing rate (calculated within sliding windows of 100 ms width shifted in 5 ms steps) from the two neurones in the same behavioural conditions increases during the preparatory period from about 20 to 55 spikes·s^{-1}.

creases during the preparatory period to be highest towards its end. In other words, shortly after the presentation of the preparatory signal neurones start to synchronize significantly their spiking activity, but with low temporal precision. As time advances, synchronization becomes more precise.

4.1. Modulation of synchronicity

In almost all pairs of neurones, the strength of synchronous activity modulates over time. This suggests that interaction between neurones as indicated by their synchronous spiking activity is not constant throughout a behavioural trial. These

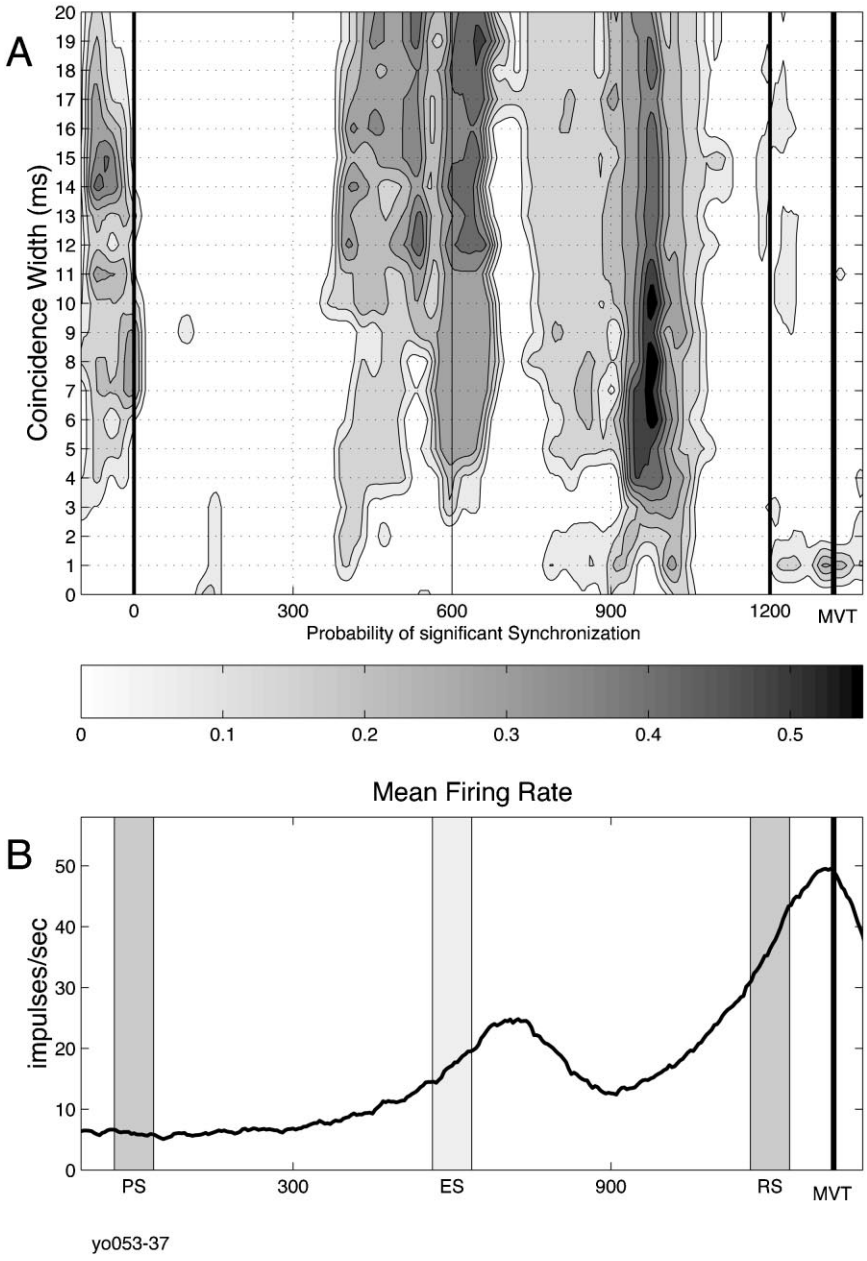

Figure 7. Quantification of correlated activity of a pair of neurones recorded in task 2 during the execution of long trials, pooled over all six movement directions. **A**, The probability for the occurrence of significant epochs (see details for calculation in *figures 6* and *7*) is high before PS, then drops to zero at PS, and increases to high levels starting at about 200 ms before a response signal was expected at 600 ms (ES), which did not occur, reaching a maximum shortly after this moment with low precision (between 12–15 ms). Then it drops nearly to zero probability and increases again from about 150 ms after ES to reach a maximum at about 250 ms before RS, however now with higher temporal precision (between 6–8 ms). Between 150 ms before RS until movement onset (MVT), hardly no significant epoch occurs. The highest probability value is about 0.6. **B**, The mean firing rate of the two neurones during the same conditions shows a complementary behaviour: it increases slightly during the first 600 ms of the preparatory period, reaches a first maximum shortly after the ES, and increases again from about 300 ms before RS to reach a peak value at about movement onset.

modulations may be assigned to either or both of the two informative attributes of the preparatory signal. Recall that in both tasks, the preparatory signal provided prior information about 'where' (i.e. movement direction) and 'when' (i.e. temporal aspects) the target will occur. The problem of time uncertainty relates to the nature of the preparatory processes that are activated when a subject anticipates a behavioural demand. In task 1 there was no uncertainty about the temporal aspects of target occurrence. The preparatory period was kept constant at 1 000 ms. In task 2, however, the target could occur with equal probability either 600 or 1 200 ms subsequent to the preparatory signal. Although neither of the two attributes of the preparatory signal was necessary to correctly perform the task, the behavioural output and the pattern of neuronal activity indicated that this

information was processed. On the behavioural side, reaction time measures in task 2 revealed latencies of approximately 100 ms longer for short preparatory periods than for long preparatory periods; presumably because conditional probability of target occurrence increases from 0.5 for the short preparatory period, to 1 for the long preparatory period (data not shown here, but see [26]). The same phenomenon was also observed in reaction time experiments with humans [8, 22]. Similarly, prior information about movement direction also reduces reaction time [27].

In the context of a visually-guided movement, visual information, provided by the preparatory signal, has to propagate from visual areas along the visuo-motor pathways to the motor cortical areas and be transformed into visuo-motor information (for a review see [37]). Previous research has shown that motor cortical neurones change their activity at least as early as 100 ms subsequent to a visual preparatory signal [25, 36]. Many of these neurones modulate their activity in relation to prior information about movement direction [27, 28, 35]. The sensitivity of these neurones to the 'where' aspect of the preparatory signal is also reflected at the population level, where movement direction is reliably represented throughout the whole delay period [6, 9, 10, 38].

However, how the temporal aspects of a preparatory period in a sensorimotor task are represented by central structures is still unclear. The present results are suggestive of one such possibility. The 'when' aspect of the preparatory signal could be exploited through a continuous update of the time constraints by dynamically constituting functional cell assemblies reflected in the modulation of synchronicity. This idea of a continuous update of temporal information for the prediction of target occurrence is not new. A similar idea has been proposed by early works in experimental psychology [7], where behavioural data suggested that when no immediate action is required, such as during a preparatory period, 'decision units' of a life-time of a few hundreds of milliseconds may serve to update this temporal information. The other hypothesis proposes that the preparatory signal induces a continuous adjustive process. To our knowledge, there are no data available yet which allow to decide between these two alternative hypotheses. This thus remains a challenging subject for future research.

4.2. Dynamics of temporal precision of synchronous activity

Previous data showed that not only timing and amplitude of mean activity, measured over trials, were related to prior information about movement direction, but also trial-by-trial analyses revealed that in about 40% of motor cortical neurones the amount of activity at the end of the preparatory period is significantly correlated with reaction time [28, 29]. The increase of temporal precision of synchronous activity during the same period in time may serve to strengthen this effect. Indeed, it has been argued that the synaptic influence of multiple neurones converging onto others is much stronger if they fire in coincidence [2, 33], thus making synchronous firing ideally suited to facilitate the efficiency of the motor output.

4.3. Comparison of synchronized activity with rate modulation

It is interesting to note that the modulation of synchronicity is by no means predictable by simply inspecting the firing rate of the same neurones (see also [12, 26, 39]). It has been shown that neurones, which were classified on the basis of the change in their firing rate to be functionally involved in different processes (e.g. being preparatory- or movement-related, exhibiting a different directional tuning), synchronize their spiking activity in a systematic way [12]. Here we show that neurones which significantly synchronize their activity during movement preparation may de-synchrononize it at movement onset, although their firing rate reaches a maximum (cf. *figures 3* and *7*). This is in agreement with the hypothesis [26] that neurones preferentially synchronize their spiking activity in relation to internal, cognitive processes, such as movement preparation or expectation of (future) events, whereas external processes including movement production are rather coded by rate modulation. However, two aspects of synchronous activity have to be considered, the strength of synchronicity, expressed in the joint-surprise value, and its temporal precision. We have shown in the present data that both these aspects do not necessarily behave in parallel. Neurones might be most strongly synchronized at the beginning of the preparatory period, but with low precision, whereas less strongly at the end of the delay, but with high precision (see *figure 6*). However, there

are examples in which both the strength and the precision of synchronicity increase during time (see *figure 7*). It is thus still an open question if there is a systematic relationship between these two aspects of synchronous activity, firing rate and behaviour.

In conclusion, data indicate that not only the discharge rate is involved in preparatory processes (cf. [6, 27]), but also temporal aspects of neuronal activity as expressed in the precise synchronization of individual action potentials. The combination of the two strategies of neuronal processing, i.e. rate code, on the one side, and temporal code, on the other, allows the retrieval of much more information from one and the same pattern of neuronal activity and, thus, to increase the dynamics and flexibility of a distributed system such as the cerebral cortex.

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