Title:
Synchronization patterns suggest different functional organization in parietal reach region and the dorsal premotor cortex

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SC and AG designed the study; SC collected the data; SC and PMV analyzed the data; SC, PMV and AG wrote the paper.

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Running Head:
Synchronization in PRR and PMd

Keywords:
Cross-correlation analysis, posterior parietal cortex, visually guided reaching

Word Count:
Abstract 230
Introduction 859
Materials and Methods 3278
Results 2338
Discussion 2340

Page Count:
Number of pages 38
Number of tables 0
Number of figures 9

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Abstract

The parietal reach region (PRR) and dorsal premotor cortex (PMd) form part of the fronto-parietal reach network. While neural selectivity profiles of single cell activity in both areas can be remarkably similar, other data suggest that both areas serve different computational functions in visually guided reaching. Here we test the hypothesis that different neural functional organizations characterized by different neural synchronization patterns might be underlying the putatively different functional roles. We use cross correlation analysis on single-unit activity (SUA) and multi-unit activity (MUA) to determine the prevalence of synchronized neural ensembles within each area. First, we reliably find synchronization in PRR but not in PMd. Second, we demonstrate that synchronization in PRR is present in different cognitive states, including ‘idle’ states prior to task-relevant instructions and without neural tuning. Third, we show that local field potentials (LFPs) in PRR but not PMd are characterized by an increased power and spike-field coherence in the beta-frequency range (12-30Hz), further indicating stronger synchrony in PRR compared to PMd. Finally, we show that neurons with similar tuning properties tend to be correlated in their random spike rate fluctuations in PRR but not in PMd. Our data supports the idea that PRR and PMd, despite striking similarity in single cell tuning properties, are characterized by unequal local functional organization, which likely reflects different network architectures to support different functional roles within the fronto-parietal reach network.
Introduction

The primate fronto-parietal reach network includes the parietal reach region (PRR) and the dorsal premotor cortex (PMd), two areas that are densely and reciprocally interconnected (Johnson et al. 1996; Johnson et al. 1993; Tanne-Gariepy et al. 2002; Wise et al. 1997). For visually guided reaching, the network integrates visual information with task rules to determine viable reach goals (Caminiti et al. 1998; Gail and Andersen 2006; Kalaska et al. 1997; Wise et al. 1997). Although single neurons in both cortical areas can have strikingly similar spatiotemporal response profiles during rule-based visually guided reaching (Andersen et al. 2004; Gail et al. 2009), anatomical and neuropsychological data suggest that PRR and PMd serve different functions in sensorimotor integration. Here we ask if distinct local organization beyond the immediate response properties of individual neurons within both areas potentially supports these distinct functions, despite the largely overlapping single-cell tuning properties.

Anatomically, the two areas receive inputs from different cortical sources. Parietal cortex has access to visual information from retinotopically organized areas in the visual cortex (Blatt et al. 1990; Colby et al. 1988; Galletti et al. 1999a; Galletti et al. 1999b; Pandya and Kuypers 1969; Shipp et al. 1998), while PMd receives visuospatial information mainly via parietal areas (Pandya and Kuypers 1969; Wise et al. 1997). PMd, instead, receives direct projections from the prefrontal (Barbas 1988; Barbas and Mesulam 1985; Cavada et al. 2000; Luppino et al. 2003; Petrides and Pandya 1999; Selemon and Goldman-Rakic 1988) cortex, involved in abstract rule representation (Wallis and Miller 2003a; b) as well as the anterior cingulate cortex (Arikuni et al. 1994; Morecraft and Van Hoesen 1993; Pandya et al. 1981). Previous studies suggest that both PRR as well as PMd are involved in the spatial representation of motor goals (Hartje and Ettlinger 1973; Mountcastle et al. 1975; Murata et al. 1996; Seal and Commenges 1985; Weinrich and Wise 1982; Wise et al. 1986), including dual representations of potential motor goals during ambiguous reach planning (Cisek & Kalaska 2005; Klaes et al. 2011). Apart from such similarity, PMd was selectively activated when abstract rules are used to construct reach targets (Moisa et al. 2012; Rowe and Passingham 2001; Toni et al. 2001;
Wallis and Miller 2003a). At the single neuron level, motor goals are encoded mainly in gaze-centered co-ordinates in PRR (Batista et al. 1999; Buneo et al. 2002; Cohen and Andersen 2002; 2000; Cohen et al. 2002) but in a combination of eye, hand and goal centered co-ordinates in PMd (Batista et al. 2007; Pesaran et al. 2006). They appear earlier in PMd than PRR, in the specific instances when they have to be determined either using abstract rules or using the monkey’s free choice (Pesaran et al. 2008; Westendorff et al. 2010). Further, on the population level, it has been shown that in PRR, there is on average a 20% stronger spatial selectivity during direct visual cueing of reach goals (pro reaches) when compared to indirect rule based cueing (anti reaches). By comparison, in PMd, the amplitude of neural responses during anti reaches tend to be 15% higher than pro reaches indicating the preference of PMd neural populations for indirect rule based motor goal construction (Gail et al. 2009). Finally, PRR lesions affect the ability to reach to specific spatial locations but not the rule-based selection between motor goals (Hwang et al. 2012; Padberg et al. 2010; Rushworth et al. 1997a; b; Yttri et al. 2014), while PMd lesions impair the acquisition of new arbitrary stimulus-response associations (Passingham 1986; Petrides 1982).

The abovementioned evidence from a combination of lesion, inactivation, anatomical and physiological studies point to an important role of PRR in spatial motor goal construction and encoding whereas PMd seems to be primarily involved in the construction of motor goals using abstract cognitive rule based cues. At the same time, both areas show highly similar sustained motor goal tuning at the neuronal level in a variety of behavioral tasks. Of course, different functional relevance despite similar local encoding of two areas could be explained by the fact that both areas engage in functional interactions with other brain areas due to their different anatomical interconnections. But additionally, there might also exist different local neural network properties in the two brain regions that do not manifest in single neuron tuning but in the local functional interactions and which are capable of supporting different functions. A synchronized, oscillatory and recurrent network in posterior parietal cortex has been hypothesized to be advantageous in the maintenance of working memory (Jensen et al. 2007; Pesaran et al. 2002; Van Der Werf et al. 2008) and could
indeed facilitate the sustained encoding of spatial goals (spatial memory). To test if PMd and PRR differ in terms of local functional organization, we used cross correlation analysis to determine the degree of synchronization within each of these two brain regions, as well as additional signal analyses. We demonstrate substantial differences in neuronal synchronization patterns in both areas. Differently connected neural networks in each of these two areas with characteristically different signatures of neural synchrony could therefore contribute to the different function, despite similar spatial motor goal tuning at the level of individual neurons.

Materials and Methods

Behavioral Task

Three adult male rhesus monkeys (*Macaca Mullata*) henceforth referred to as Monkeys A, S and F, were trained in a memory-guided center-out reach task. We chose this task since both PRR and PMd are well known to show sustained direction-selective responses during the movement planning period of such task, and we wanted to test if neural synchronization differs in both areas during such a prototypical sensorimotor task. The animals were seated in primate chairs approximately 35-40 cm in front of a monitor which was used to display the stimuli (19 inch ViewSonic LCD VX922). As illustrated in Figure 1A, a trial was initiated by the animals, by fixating a small red square in the center of the screen (eye fixation tolerance, 2.0-4.0° VA diameter; 224 Hz CCD camera; ET-49B; Thomas Recording, Giessen, Germany) while touching an adjacent white square of the same dimensions (hand fixation tolerance: 2.0-6.0° VA, touchscreen mounted directly in front of the display monitor; IntelliTouch; ELO Sytems, CA). After a period of 500-2000ms (fixation period; FIX), a peripheral spatial cue (white filled circle) was flashed on the screen for 200ms (cue period; CUE) at one of four possible positions (0, 90, 180, 270°) with an eccentricity of 9 cm (14.5° VA) for monkeys A and S or 8 cm (11.3° VA) for monkey F. The animals were simultaneously presented with a green frame around the hand and eye fixation points to indicate that they should later reach...
towards the peripheral spatial cue. For monkeys A and S, these direct reach or ‘pro’ trials were randomly interleaved with ‘anti’ trials, in which an identical blue frame instructed the animal to later reach to the diametrically opposite direction (Gail et al. 2009; Westendorff et al. 2010). However, only data from the direct reach, ‘pro’ trials will be analyzed in this study, since these were also available for monkey F. Following the presentation of the two cues the monkeys had to maintain ocular and hand fixation for a further 800-2000ms (memory period; MEM). Next, a disappearance of the hand fixation spot served as the go-signal (GO) and the animals had to reach towards the previously instructed goal within a maximum of 800-1000ms (movement period; MOV; 3.0-7.4° VA reach tolerance) and hold the goal for a further 100-300ms (feedback period; FDB). Visual feedback was then provided in the form of a filled circle of the same dimensions as the spatial cue and the same color as the frame. Eye fixation had to be maintained throughout the trial, failure resulting in trial abortion. Liquid reward and auditory feedback indicated correct (high pitch tone, reward) or incorrect (low pitch tone, no reward) trials.

Animal preparation

In preparation for neural recordings, all 3 monkeys were implanted with a titanium head holder and two magnetic resonance imaging (MRI) compatible, recording chambers custom-fit to the monkeys’ skulls (3di, Jena, Germany). Chamber placement above PRR and PMd was guided by pre-surgical structural MRI (Figure 1B) and confirmed by post-surgical MRI. Sustained direction selective neural responses during center-out reach planning under eye fixation were used as a physiological confirmation of the regions of interest. Both chambers were implanted contralateral to the handedness of the monkeys (monkey A, left hemisphere; monkey S and F, right hemispheres). Horsley-Clarke coordinates of the chamber centers on the scull were as follows (all numbers in millimeters). PRR_A: 8.5L x 9.5P, PRR_S: 6.0R x 10.0P, PRR_F: 7.0R x 13.0P, PMd_A: 13.5L x 19.8A, PMd_S: 13.0R x 17A, PMd_F: 20R x 20A. All surgical and imaging procedures were conducted under general anesthesia and in accordance with German laws governing animal use. Further details of these procedures have been previously described (Gail et al. 2009).
Neural recordings

For extracellular recordings, between 3 and 5 glass coated tungsten-iridium microelectrodes with impedances between 1 and 3 MΩ and horizontal inter-electrode separations of 300-1500 μm, were lowered in each cortical area through stainless steel guide tubes, using the 5-channel Eckhorn mini-matrix system (Thomas Recording, Giessen, Germany). The inter-electrode separation and the configuration of the matrix heads were identical across areas. Neural data was recorded using a Plexon MAP recording system (Plexon Inc, Dallas, TX). Signals were initially pre-amplified (20×) before they were band-pass filtered [0.7-300Hz for local field potentials (LFPs); 100Hz-8kHz for spiking data] and amplified (50×). LFPs were digitized at 1kHz whereas the spiking data was stored as two separate data types. Single unit spike waveforms as isolated during online spike sorting, but including part of the background activity (“noise” cluster) were further amplified at gain 12-16× and digitized at 40 kHz for the duration of 800 μs and time-stamped whenever crossing a manual-set voltage threshold (single-unit activity; SUA). These spike waveforms were then later sorted into individual clusters using principal component analysis (PCA) using a time-resolved k-means cluster identification algorithm with manual supervision using Plexon Offline Sorter. The inter-spike interval histograms were monitored for each identified single unit cluster to ensure less than 0.01% of discharges occurred within a 2 ms refractory period. In parallel, the broadband filtered time-continuous data was digitized and stored at 20 kHz (multi-unit activity; MUA). In this paper we will refer to all putative spikes extracted from the MUA signal via simple thresholding as 'events' (see following paragraph for more information). The terms 'spikes' or 'neurons' will only be used in reference to single units where offline spike sorting was performed to identify single unit spiking waveforms.

Extraction of multi-unit activity (MUA)

Threshold-crossing events were extracted from MUA data using a set of multiple variable amplitude thresholds per trial. Each set of thresholds was variable in time to account for drifts in the signal-to-noise ratio over the course of the recording. For each trial, the nearest neighboring trials
that represented the three other reach directions were identified and a mean ($\mu$) and standard
deviation ($\sigma$) of the voltage amplitude across all four trials were computed. Trials with reaches to all
different directions were combined to avoid systematically lowered amplitude thresholds during
trials with fewer events such as those in which the animal executed a reach to the non-preferred
direction. A set of 7 thresholds ($\xi_k$) was then defined for each trial using

$$\xi_k = \mu - k \cdot \sigma, \quad 2 \leq k \leq 8 \quad \text{Equation}(1)$$

Thus, $\xi_2$ would imply a low amplitude threshold (“small” events) whereas $\xi_8$ would be a high
amplitude threshold (“large” events). To detect the negative-going extracellular events, the minimum
value of each data segment that crossed this threshold was detected, time-stamped and defined as a
MUA event, as indicated in Figure 2A. For each channel of MUA data, 7 sets of putative spike events
were extracted per trial, each corresponding to one of the above thresholds $\xi_k$. Note that the events
in these sets are not complementary, but rather each set included all events of the sets with the
higher index number, plus some additional lower amplitude events. The idea of these sets was to
achieve variable selectivity of the MUA signal in terms of how many neurons contribute to it. All of
the following analyses on pairwise correlations were performed on MUA events from different
channels and never between signals obtained from the same channel.

**Different measures of correlation**

In this study, the term ‘correlations’ was used to describe the relative timing between spikes
or events on two different spike trains (SUA or MUA channels). To ease reading, in this paragraph we
will only use the term “spikes” and “neuron”, but the statements are also true for the “events” and
MUA channels. We used different types of correlations which will briefly be motivated here and
defined explicitly in the next section. Two independent spike trains with no common inputs should
fire independent of each other and the occurrence of spikes on one train should not influence the
probability of spikes on the other. However, if both neurons respond to the same stimulus in a
transient fashion, we might expect correlated spikes on both trains time-locked to the stimulus. Such
correlations are likely to be provoked by common input and consistent spike latencies in response to the stimulus across trial repetitions in both areas. These correlations were termed stimulus-locked correlations and since they do not imply mutual functional connectivity, care was taken to disregard this type of correlation for assessing within-area functional network connectivity (shuffle predictor subtraction; see below).

Next, correlations between neurons can occur on several time-scales (Smith and Kohn 2008) and were defined in keeping with accepted nomenclature. Correlated firing between two spike trains occurring at the time scale of several milliseconds and quantified by the peak of corrected cross-correlograms (see below) was termed synchrony ($r_{ccg}$). Alternatively, slow trial-by-trial co-varying fluctuations in firing rate between two spike trains across trials with identical task conditions were described as slow co-variations or noise correlations ($r_{sc}$). Finally, the correlation between the tuning curves of two neurons, a measure of tuning similarity, was termed signal correlation ($r_{signal}$) and computed after averaging spike counts over trials with identical task conditions. Investigations on neural network organization typically focus on synchrony and noise correlations as indicators of functional connectivity. Signal correlations, meanwhile, are a measure of tuning curve similarity, i.e., are commonly not directly associated with functional connectivity. For a detailed review please see Cohen and Kohn (Cohen and Kohn 2011).

**Joint peri-stimulus histogram (JPSTH) and cross correlogram (CCG) construction**

To measure synchrony and its temporal modulation, the joint peri-stimulus time histogram (JPSTH) as first described by Aertsen and colleagues (Aertsen et al. 1989; Eggermont 1994) was implemented. Briefly, pairs were constructed using simultaneously recorded spike trains from two separate electrodes, and for each such train, the spike/event counts in each trial were binned in bins of 10ms each. The correlation $r_{jpsth}$ for a given pair of time bins $t_1$ and $t_2$ was then calculated using,

$$r_{jpsth_{(t1,t2)}} = \frac{E[N_{1,t1}N_{2,t2}] - E[N_{1,t1}]E[N_{2,t2}]}{\sqrt{\lambda_{1}\lambda_{2}}} \quad \text{Equation (2)}$$
where, $N_{1,t1}$ and $N_{2,t2}$ are the number of events on spike trains 1 and 2 in bins $t1$ and $t2$, respectively.

$E$ is the expected value (mean) computed across all eligible trials and $\lambda_1$ and $\lambda_2$ are the mean firing rates of each neuron/event over the entire analysis window and across trials (Kohn and Smith 2005; Smith and Kohn 2008). The second term in the numerator, the cross-product of the PSTHs, is identical to an all-way trial shuffle predictor. It represents the expected correlations arising from non-simultaneous trial pairings and is used to estimate stimulus-locked correlations. Such $r_{jpsth}$ functions calculated for all time bins within an analysis window yielded a matrix showing the temporal progression of correlations at different time lags (raw JPSTH, Figure 2B). The main diagonal in such a JPSTH represents those cases in which $t1=t2$, thus reflecting synchrony in both spike trains.

The cross correlogram (CCG) was obtained by computing the average JPSTH along and parallel to its main diagonal, (Aertsen et al. 1989; Eggermont 1994) according to,

$$r_{ccg\tau} = \frac{1}{T - 2\tau} \sum_{t=\tau}^{T-\tau} r_{jpsth}(t + \tau, t), \quad \tau \geq 0 \quad \text{Equation(3)}$$

where, $r_{ccg\tau}$ is the correlation coefficient at lag $\tau$, $r_{jpsth}$ is the correlation per time-bin measured with the JSPTH (Equation 2), $T$ is the total time and $t$ is the time within the trial. The $r_{ccg\tau}$ of an autocorrelogram at $\tau = 0$ would therefore equal 1 indicating perfect synchrony, by definition. The CCG resulting from the raw JPSTH example shown in Figure 2B is represented in Figure 2E. The resulting raw CCG is characterized by a sharp and narrow peak at $\tau = 0$, which sits atop a much broader elevation evident by $r_{ccg\tau}$’s greater than 0 at $\tau = 0.15s$ in this typical example.

Many reports have shown that CCGs, independent of shuffle predictor subtraction, will represent a mixture of correlations at different time scales. The central peak is thought to reflect synchrony whereas the broad increase is thought to result from slow co-variations (Ben-Shaul et al. 2001; Brody 1999a; b; Grun et al. 2003; Ventura et al. 2005). We used a recently described method of jittering spike trains to produce surrogates which eliminate the effect of such slow co-variations from the raw CCG (Louis et al. 2010; Smith and Kohn 2008). Briefly, each spike/event on each spike train...
was jittered by a random value drawn from a homogenous distribution, within a window of 50ms to produce surrogate spike trains which were identical to the original spike trains in terms of spike/event count and slow co-variations, but for which synchrony occurring at a time scales smaller than 50ms was artificially destroyed. Following such jittering, JPSTHs were calculated as described above using the surrogates. For each spike train, n=1000 surrogates were generated resulting in an equal number of JPSTHs. Averaging across all such JPSTHs, the mean surrogate JPSTH (Figure 2C) and CCG (Figure 2F) were obtained. In the example, the mean surrogate CCG clearly captured the broad increase noted in the raw CCG but lacked a zero lag narrow peak. The jitter-corrected JPSTH (Figure 2D) and CCG (Figure 2G) were obtained by subtracting the mean surrogate JPSTH or CCG from their raw counterparts described earlier. The standard deviations of the different surrogate JPSTHs and CCGs were multiplied by 1.96 to yield 95% confidence intervals superimposed on the jitter corrected CCG shown in Figure 2G (assuming the JPSTHs and CCGs surrogates are drawn from a normal distribution). A pair of spike trains was significantly synchronized if the \( r_{ccg} \) values at \(-10 \leq \tau \leq 10\) milliseconds exceeded these confidence intervals. Whereas the correlations at zero lag were a measure of synchrony those at the lags represented at the edges of the CCGs (> 100ms) which were very rare in our dataset, represented correlations that have been described to have different origins and hence will not be classified here as synchrony (Eggermont et al. 1993; Lampl et al. 1999; Singer and Gray 1995). It is important to note that when a pair of single units were defined by offline spike sorting the MUA channel (colored waveform insets, Figure 2A), the resulting CCGs (Figure 2E-G, red) were remarkably similar in shape to those obtained from the MUA data. This indicates that our method of MUA thresholding approximated the sum total of SUA activity quite accurately. JPSTHs and CCG’s were constructed from all MUA channel pairs and all single unit pairs where each single unit had a minimum of 50 spikes in the last 500 ms of the memory period across trials.

Earlier authors have reported that slow spike count variations across trials can lead to an over estimation of synchrony (Brody 1999a; b). Even though the choice of our method for computing cross-correlations should prevent such effects, we still tested whether such confounding effects
could be seen in our data. Our results show that this is not the case. Figure 3 shows the trial by trial
spike counts for all single and multi units over the course of their individual recording times for both
PRR and PMd in the last 500 ms of the memory period, converted into z scores. Overall, non-
stationary spike rates were rare and no obvious differences were found between PRR and PMd. We
therefore rule out the possibility that differences in synchrony could arise from a systematic
difference in spike stationarity between areas.

To measure the temporal progression of synchrony, trials were aligned in time to either the
presentation of the visual cue or the go signal, respectively, before computing JPSTHs. The main
diagonal of the JPSTHs was then averaged across all significantly synchronized pairs for each brain
area. For each pair of neurons/channels and time bin, we then tested if this zero lag synchrony
deviated significantly from the mean synchrony of the same pair in a reference window (last 300ms
of the fixation period; one sided paired t-test).

Calculation of noise correlation \((r_{sc})\) and signal correlation \((r_{signal})\)

To determine slow co-variations \((r_{sc})\) we used a measure termed noise correlations which
has been previously used to quantify trial-by-trial co-fluctuations in the firing rate of two neurons
(Bair et al. 2001; Shadlen and Newsome 1998; Smith and Kohn 2008). The total spike count for each
neuron was calculated per trial for the analysis time window, in our case, the last 500 ms of the
memory period. Trials with spike counts greater than 3 standard deviations from the mean were
excluded (Zohary et al. 1994). This was done to reject trials with extremely high firing rates which
could be due to neuronal burst firing or other non-stationary signals and affected less than 1% of
trials in our data. The trial-by-trial spike counts for each reach direction were standardized (z-score)
as follows.

\[
N_k^i = \frac{x_k^i - E[x_k]}{\sigma[x_k]}, k = [0^\circ, 90^\circ, 180^\circ, 270^\circ] \quad Equation(4)
\]
where \( x \) is the spike count for trial \( i \) having reach direction \( k \), and \( E \) and \( \sigma \) are the expected value (mean) and standard deviation computed across all trials with reach direction \( k \). The noise correlation, \( rsc \), for each reach direction \( k \), was then calculated using

\[
rsc_k = E[N1_kN2_k] \quad \text{Equation (5)}
\]

where \( N1 \) and \( N2 \) are the standardized spike counts for neurons 1 and 2 as described above, and \( E \) is the expected value across all trials with that reach direction. To approximate a normal distribution, the bounded \( rsc \) values for each reach direction were Fisher transformed:

\[
rsc \text{ (Fisher)} = \frac{1}{2} \ln \left( \frac{1 + rsc}{1 - rsc} \right) \quad \text{Equation (6)}
\]

The mean of the Fisher-transformed correlation values across reach directions was then used as the representative correlation value \( rsc \) for a given pair of neurons for further computations.

To determine the similarity of tuning between neurons we used the signal correlation \( (rsignal) \) measure. For this, the mean spike counts for each neuron were obtained per reach direction as described above and then the Pearson correlation coefficient was calculated for the two sets of mean spike counts. The \( rsignal \) value simply indicates a correlation between two neurons’ tuning curves with an \( rsignal \) of 1 indicating identical tuning curves and an \( rsignal \) of -1 indicating tuning curves shifted by 180°. \( rsignal \) values were also converted into z scores according to Equation 6.

**Analysis of local field potentials (LFPs)**

To measure the spectrum of the LFPs, trials were aligned to the presentation of the go signal and the amplitude density spectra of the LFP signals then calculated using the Fast Fourier Transform (FFT) with a Hamming window for the 500ms prior to the go cue after subtracting the mean amplitude. Spike field coherence within each area was computed using the Neuran Chronux toolbox in Matlab (Jarvis and Mitra 2001). Briefly, trials were aligned either to the visual cue presentation or the go signal and a multi-taper window technique was used with sliding windows of 400ms length.
advanced every 10ms. Spike field coherence was always tested for all possible combinations of
spikes on one electrode and the LFP on another electrode. The spike field coherence values for PRR
and PMd were compared using a non-parametric Wilcoxon rank sum test and the p values corrected
for multiple comparisons, resulting from the different sliding time windows, using the Bonferroni
method.

Calculation of peristimulus time histograms (PSTH’s)

Peri-stimulus time histograms or PSTH’s were calculated as follows. For each reach direction
all trials for SUA or MUA activity were used to calculate separate PSTHs by convolving each spike with
a causal kernel resembling an EPSP which was defined as follows

\[ R(t) = \frac{\tau_g + \tau_d}{\tau_d^2} \left( 1 - e^{-\frac{t}{\tau_g}} \right) e^{-\frac{t}{\tau_d}} \quad \text{Equation (7)} \]

where \( R(t) \) is the spike density at time point \( t \), \( \tau_g \) is the rise time constant, set to 2 ms and \( \tau_d \) is the
decay time constant set to 20 ms (Monosov et al. 2008; Thompson et al. 1996; Westendorff et al.
2010). For each single unit or MUA channel, two separate PSTH’s were calculated using the visual cue
onset and the go cue as alignment points respectively. For each single unit and each MUA channel,
the reach directions were then sorted according to the mean amplitudes in the last 300 milliseconds
of the memory period. PSTH’s for the preferred and anti-preferred reach direction sorted as
described above were then averaged over all single units or MUA channels for both areas.

Results

Data were collected from 3 adult male rhesus monkeys. MUA data, as described above was
obtained from monkeys S and F, whereas SUA data was available for all three monkeys. In total, 240
single units were recorded from PRR (Monkey A: 123, Monkey F: 51, Monkey S: 66) and 165 from
PMd (Monkey A: 40, Monkey F: 40, Monkey S: 85) yielding a total of 217 PRR neuronal pairs (Monkey
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A: 87, Monkey F: 58, Monkey S: 72) and a total of 195 PMd neuronal pairs (Monkey A: 26, Monkey F: 58, Monkey S: 111). In addition, we recorded 150 channels of MUA data from PRR (Monkey F: 67, Monkey S: 83) yielding 179 channel pairs (Monkey F: 77, Monkey S: 102) and 169 channels of MUA data from PMd (Monkey F: 67, Monkey S: 102) yielding 254 channel pairs (Monkey F: 106, Monkey S: 148).

Average firing rates over time across animals and across the two brain areas were similar both for MUA and for SUA. Figure 4 shows mean population PSTH's for all single units and channels of MUA data (threshold at $\xi_2=\mu-2\sigma$) for both areas in all three monkeys. The PSTH's were further sorted by mean firing rate in the last 300 ms of the memory period to show responses to the preferred and the diametrically opposite (anti-preferred) reach directions. Following the fixation period and the visual cue presentation, firing rates in both areas were tuned to the reach direction in sustained fashion throughout the memory period, followed by a rapid increase during movement before collapsing to pre-stimulus fixation period values. Importantly, the similarity of the population PSTH's in both areas rules out biases in firing rate that could potentially confound a comparative analysis of synchronization between both areas. In Monkey F, the SUA data shows lower firing rate in PMd compared to PRR already during the baseline fixation period. However, despite this difference, as will be shown later, our results on synchronization are consistent across all three monkeys.

Stronger synchrony in PRR than PMd

Using JPSTH’s and CCGs, we first quantified the incidence of synchrony and its strength within both cortical regions in the MUA data of monkeys S and F for the last 500ms of the memory period. We chose this time window for our first analysis, since directional selective reach goal encoding typically is most prominent during this trial period in both brain areas. Synchronization was stationary throughout this time window as well as stronger in PRR than in PMd, as the comparison of the mean JPSTHs averaged across all statistically significantly synchronized channels (with a threshold at $\xi_2=\mu-2\sigma$) in each cortical area showed (Figure 5, A,B). The higher values in the central diagonal of
the JPSTHs indicated robust zero lag correlations which can be better estimated and quantified with CCGs (Figure 5, C,D). The stronger synchrony in PRR as compared to PMd is shown by the statistically significant central zero-lag peak in the CCGs obtained from PRR irrespective of whether the CCGs were compiled from all available pairs (black) or only those showing statistically significant synchrony (red). In one of the two monkeys, the CCGs in PRR, but not in PMd were characterized by an oscillatory pattern (15-35 Hz) with troughs and secondary peaks flanking the zero lag peak. Such oscillatory rhythms in the CCG have previously been described in the visual system (Eckhorn et al. 1988; Eckhorn and Obermueller 1993; Engel et al. 1991; Gray et al. 1992; Gray et al. 1989) as well as in motor cortex (Engelhard et al. 2013).

The higher incidence of synchrony in PRR was observed over the range of MUA thresholds, but was most obvious for low-amplitude MUA thresholds (Figure 5 E,F). The incidence of synchrony in PRR was significantly higher than that in PMd ($\chi^2$ test, $p < 0.05$) in more than one threshold in both monkeys.

Pairs of PRR MUA were not only synchronized more often, but also more strongly. A comparison of the zero-lag peak of all neuron pairs (Figure 5, G,H), showed a similar trend to that of the incidence of synchrony. Again, the strength of synchrony in PRR was significantly higher (unpaired t-test, $p < 0.05$) than that of PMd at almost all different MUA thresholds. The strength varied as a function of threshold and gradually diminished at very high amplitude thresholds for monkey F.

We repeated the same analyses with the SUA data from all three monkeys. As shown in Figure 6, CCG’s of PRR single units of all three monkeys showed statistically significant synchronization, i.e. exceeding 95% confidence intervals at lag zero (Figure 6 A-F). Only one neuron pair in Monkeys F and A PMd reached statistical significance and therefore the population data for statistically significant PMd SUA pairs is not shown in Figure 6E,F. Again, PRR was characterized by a significantly higher incidence of synchrony ($\chi^2$ test, $p < 0.05$) which was significantly stronger...
(unpaired t-test, p < 0.05) than that seen in PMd (Figure 6 G-L). The SUA dataset of one of Monkey A showed an oscillatory pattern reminiscent of Monkey F’s MUA data (Figure 5).

The auto-correlogram functions calculated for all the above datasets showed similar features such as decay rates between both brain areas (data not shown). This strengthens the notion that the differences in the observed CCGs were not confounded by differing spike firing characteristics in each area but rather a true reflection of synchronized firing of neural ensembles in PRR, not PMd.

In summary, the comparative quantification of synchrony in PRR and PMd revealed several differences. First, the incidence of synchrony was significantly higher in PRR than in PMd. Second, the strength of synchrony was also significantly larger in PRR than in PMd. Third, these findings were consistent across monkeys and signal types analyzed, although the effects were stronger and therefore, easier to quantify for MUA data with low-amplitude thresholds. Taken together, these results indicate that PRR neurons are often synchronized, whereas PMd neurons are typically not. It should further be noted that although the firing rate in the SUA dataset for Monkey F was different between PRR and PMd, this did not affect the robust synchrony results in this particular dataset.

The thresholds of $\tilde{\xi}_2 = \mu - 2\sigma$ and $\tilde{\xi}_3 = \mu - 3\sigma$ showed the largest incidences of synchronization in both monkeys, whereas the number of synchronized pairs in the SUA data lacked statistical power, especially in PMd. Further, it has been reported earlier that the MUA-MUA cross correlation function being in essence a sum of all SUA-SUA cross correlation functions in the vicinity provides a better approximation of the true synchrony within an area (Roelfsema et al. 2004; Super and Roelfsema 2005). For these reasons, the remainder of the analyses in this paper will focus on the MUA data at the 2- and 3-sigma thresholds.

Modulation of synchrony by cognitive state

Given the difference in synchronization between PRR and PMd during motor planning, we wanted to test how strongly the observed synchrony is related to the cognitive requirements of the
behavioral task. For this, we tested whether the synchronization within PRR varied as a function of the different task epochs. This was achieved by analyzing the modulation of zero-lag correlation over time (Figure 7).

First, and quite surprisingly, the difference in synchrony between PRR and PMd was not restricted to a particular epoch of the trial. For example, during the memory period during which PMd and PRR typically show substantially higher firing rates compared to baseline with sustained motor-goal tuning (Fig. 4), synchrony was at a comparable level as in the fixation period when no task-specific instructions had yet been presented. In both the fixation and the memory periods synchrony in PRR was higher than in PMd. These results indicate that synchrony in PRR is higher than in PMd even before the fronto-parietal reach network engages in task-specific reach planning. We therefore suspect that the differences in synchrony are due to differences in the inherent neural network properties of the two areas.

Second, during the course of the trial, the synchronization within PRR was modulated by the occurrence of transient events, but not by different trial epochs with instructed waiting periods for the monkey (Figure 7, A, C). This de-synchronization was apparent immediately following the visual cue stimulus and around the time the monkey initiated the reach. The level of synchronization after both transient events was significantly lower than the mean synchronization during fixation (one-sided paired t-test, p<0.01; Figure 7, dotted lines). In monkey S, the de-synchronization was a transient phenomenon after both events, whereas in monkey F it was more emphasized after both events and sustained after the hand release. By comparison, PMd synchronization remained at a low baseline level showing almost no modulation except for a small significant upward trend following cue presentation (Figure 7, B,D). From this, it is clear that synchronization is modulated transiently by changes in cognitive states such as during cue presentation and movement execution, hence our measure is sensitive to changes. Yet, all “hold” states in which the monkeys kept a certain behavioral state and awaited the next instruction yielded basically the same pattern of synchronization strengths across both areas, independent of the type of hold state.
Differences in LFP power between PRR and PMd and its co-variation with spike synchrony

Previous studies have shown that LFP often reflects spike synchronization with synchrony occurring more often during periods characterized by oscillations in the LFPs (Denker et al. 2011; Eckhorn et al. 1988; Engelhard et al. 2013; Murthy and Fetz 1996). Given the differences between PRR and PMd in spike synchronization, we next tested the hypothesis that PRR and PMd also exhibited different signature patterns in LFPs. Figure 8A and D show power spectra of the LFPs from the two different monkeys in which we recorded MUA data. The power spectra of the LFPs showed a predominance of power in the $\beta$-frequency range (12-30Hz) in PRR but not in PMd of both monkeys.

Given the synchronization in PRR and the elevated power in LFP-$\beta$, we tested whether the spiking in this area had a systematic dependency on the LFP oscillations in this frequency range. In agreement with our hypothesis, we found spike-field coherence between PRR spikes and LFP from different electrodes within the same cortical area (Figure 8B,E). In both monkeys, this spike-field coherence was significantly stronger in PRR than PMd, as measured by a Wilcoxon rank sum test after correcting for multiple comparisons using the Bonferroni method (Figure 8B, E). The temporal modulations of this spike-field coherence, especially the decrease in coherence following the visual cue and also the go-signal, closely parallel temporal modulations in spike correlation shown above (Figure 7). Notably, in both monkeys, the de-synchronization in PRR during movement is preceded by a transient increase in spike-field coherence in PMd in the low frequency range (<15Hz; Figure 8C,F).

Second, the difference in spike-field coherence between the two areas was present even in the fixation period similar to that observed with differences in spike synchrony. Third, the monkey with the stronger synchrony (monkey F) also showed a stronger power in the LFPs as well as stronger spike-field coherence.

In summary, the LFP data support the view that local ensembles of neurons synchronize in PRR but not PMd, especially during working memory phases of instructed delay.

MUA channels with similar motor-goal tuning show stronger noise correlations in PRR
Different groups of neurons might be preferentially connected to each other according to a variety of established network models. One hypothesis, which is widely prevalent in different cortical regions across species, is that neurons with similar tuning properties are interconnected either anatomically or functionally by virtue of belonging to the same neural ensemble (Cohen and Maunsell 2009; de la Rocha et al. 2007; de Oliveira et al. 1997; Ecker et al. 2010; Fries et al. 2001; Kohn and Smith 2005; Lampl et al. 1999; Nelson et al. 1992; Smith and Kohn 2008; Zhang and Alloway 2004; Zohary et al. 1994). We tested this “like-links-to-like” hypothesis in our data by measuring the strength of different correlation measures as a function of similarity in tuning.

Two separate types of correlation measurements with different temporal ranges can be used to evaluate the probability of neurons with similar stimulus preferences sharing functional connections. Synchrony refers to correlations of spike timing at the millisecond timescale. Noise correlations are a measure of spike count correlations on a longer timescale (see Methods). We first tested the dependency of synchrony on tuning similarity calculated using the pair-wise signal correlation. Signal correlation is simply a correlation of the tuning curves of both neurons. No reliable dependency of synchrony on tuning similarity could be found (data not shown). As discussed below, this is not surprising given that many of our electrode pairs had separations of greater than 1mm, and beyond distances of 1mm synchrony is hardly detectable even in monkey primary visual cortex V1 (Grinvald et al. 1994; Smith and Kohn 2008). We next tested the noise correlation rsc (see Methods), which is less dependent on distance (Smith and Kohn 2008). Figure 9 shows the noise correlations between MUA activity recorded from different channels as a function of their signal correlation. In PRR, in both monkeys, there was a significant positive correlation between these two variables indicating that MUA signals with similar tuning properties on average showed a stronger spike-rate co-variation with each other than signals with dissimilar tuning (Figure 9. A,D). In contrast, there was no such relationship consistently present in PMd, indicating that noise correlation in PMd did not depend on tuning similarity. Consequently, the Spearmann’s correlation coefficient between noise and signal correlation was higher in PRR than in PMd (Figure 9.B, E) at most thresholds. The
significant positive noise correlation in PRR and its absence in PMd could be seen across several threshold levels (Figure 9. B-F). This means that more reliably in PRR, but less so in PMd, neurons with similar tuning properties co-vary in firing rate, which indicates common or mutual connectivity, equivalent to previous observations made by other investigators in V1.

Discussion

In this study, cross correlation analysis on spike trains recorded simultaneously from the parietal area, PRR and the frontal area PMd, show markedly different patterns of neural synchronization within each of these two areas indicating different local functional organization. First, neural synchronization was significantly stronger and more prevalent in PRR than in PMd. Second, synchronization strength was significantly stronger in PRR during 'hold' states, including the fixation period when the animal had to maintain the current status. Third, synchronization was selectively disrupted during transition phases from one motor plan to another. Fourth, local field potentials in PRR exhibited significantly stronger power in the β-range (12-30Hz) in PRR than in PMd and were highly coherent with surrounding spiking activity. Finally, MUA channels with similar preferred directions showed stronger noise correlations than channels with dissimilar tuning in PRR but not in PMd. Taken together these results indicate that PRR, but not PMd, has a functional organization reminiscent of organized sensory cortical areas where similar neurons form neural ensembles that synchronize preferentially with each other. Additionally, due to its temporal evolution over the course of a trial, we speculate that the synchronized and oscillatory activity in PRR might serve the sustaining of spatial working memory in the context of motor planning, while this is not the case in PMd.
Different putative roles of PRR and PMd

The two cortical areas PRR and PMd are reciprocally connected components of the primate fronto-parietal reach network (Buneo and Andersen 2006; Buneo et al. 2002; Johnson et al. 1996). As mentioned earlier in the introduction, several earlier studies have pointed to very different putative roles for these two brain regions within the fronto-parietal reach network.

Probably the most compelling of this evidence, and hence worth reiterating, comes from the lesion literature collected from monkeys and human patients. PRR lesions (Padberg et al. 2010; Rushworth et al. 1997a; b) and inactivation (Hwang et al. 2012; Yttri et al. 2014) have repeatedly shown that reach trajectories and endpoints are severely affected by a compromised parietal cortex although the ability to select between two different types of movement ('push' versus 'pull') that were instructed by different cognitive rules (colors) remained unaffected (Rushworth et al. 1997a). Human patients with parietal lesions have long been known to suffer from optic ataxia and the inability to reach precisely to specific targets, part of the Balint's syndrome [for review see (Andersen et al. 2014)]. Further, although affecting spatial selection, parietal lesions did not compromise the ability of these patients to learn associations between abstract cues and specific movements (Halsband and Freund 1990). By contrast PMd lesions had the opposite effect. Early peri-arcuate lesions in monkeys by Petrides, specifically affected the animals ability to perform movements cued by abstract symbols but not simple visually guided reaches (Petrides 1985; 1982). Passingham repeated these experiments with PMd lesions and found the same effects when two movements were instructed by means of abstract cues only (Halsband and Passingham 1985; Passingham 1986). Human patients with PMd lesions showed similar results with inabilities to perform movements cued by abstract associations (Halsband and Freund 1990).

Despite this body of evidence pointing to a critical role of PRR in spatial and one of PMd in abstract rule based target selection, single unit activity in both areas have consistently showed remarkably similar spatial tuning profiles (Gail and Andersen 2006; Gail et al. 2009; Klaes et al. 2011;
Westendorff et al. 2010) in rule-guided memory reach tasks. However, when examined at the population level, important differences between the neuronal activity in both areas begin to become apparent. First, in PRR the spatial selectivity of neurons or their tuning strength was found to be stronger when reaches were directly cued (pro) rather than when they had to be inferred using an arbitrary associative rule (anti) (Gail et al. 2009). Second, indirectly inferred motor goals were represented in PMd with a stronger gain than in PRR (Gail et al. 2009). Third, simple center-out reach goals were encoded with the same latency in PRR and PMd, while motor goals requiring integration of learned task rules with non-standard stimulus-response associations appeared first in PMd (Westendorff et al. 2010). Last, fMRI studies have shown that PMd is selectively activated during motor tasks that involve arbitrary visuomotor associations (Moisa et al. 2012) whereas PRR is activated during tasks requiring spatial memory (Rowe and Passingham 2001).

The representation of spatial motor goals in PMd at the single neuron level and their remarkable similarity to the single unit spatial tuning profiles in PRR seems to be in disagreement with the body of evidence pointing to distinct functional roles for these two brain regions within the reach network. Obviously, two brain areas with identical local encoding strategies could support different functions just by the fact that they both project to different target areas in the brain. Here we show that beyond this possibility, the local functional organization in PRR and PMd is different, as indicated by a significantly different level of correlation in the spiking activity of pairs of neurons. This means that aside from the fact that both areas project to a different subset of target areas, their local network properties appear to optimized for different neural computations.

**Functional role of synchronization in PRR**

An area like PRR which is important for spatial selectivity and for spatial memory related to movement goals (Hartje and Ettlinger 1973; Mountcastle et al. 1975; Murata et al. 1996; Rowe and Passingham 2001; Van Der Werf et al. 2008; 2010; Wise et al. 1997) could benefit from an interconnected attractor recurrent network with reverberatory activity which has been shown to be
beneficial for maintenance of memories (Amit 1996; Amit et al. 1994; Mongillo et al. 2003). In fact, several investigators have reported oscillatory activity during memory delays in the posterior parietal cortex, the oscillations increasing in amplitude with increasing memory load within the task (Jensen et al. 2002; Palva et al. 2010; Pesaran et al. 2002; Van Der Werf et al. 2008).

In support of this hypothesis, we observed a clear temporal structure of the synchronization in PRR, with on average stronger neural coupling during ‘hold’ states in which the monkeys needed to maintain the current memory and motor status, and weak neural coupling during transition phases in which monkeys needed to update their spatial memory and motor plan. This raises the possibility that the synchronization observed in PRR might be related to the maintenance of a motor plan which in the task design used here, includes a sustained prospective spatial encoding of the intended reach endpoint. This plan could be without spatial translation of the hand, like keeping the hand still during initial fixation, or including a spatially specified reach plan like during the instructed delay after cue presentation. Synchronization stays high until the plan needs to be updated according to an instruction stimulus (de-correlation after cue presentation) or due to movement initiation (de-correlation during movement period). The phasic change in local synchrony in PMd and PRR could reflect neural mechanisms underlying the transition from one synchronized ‘hold’ state to another synchronized ‘hold’ state with a corresponding update of the spatial working memory content.

In addition to synchrony during ‘hold’ states, some datasets also show an additional oscillatory pattern in the cross correlograms recorded from PRR in the β range (15 to 35 Hz, Monkey F MUA and SUA data; Figure 5D and 6E, respectively, Monkey A SUA data; Figure 6F). It is therefore not surprising that this difference in oscillations between PRR and PMd manifests itself as a difference in the LFP power in the β range (Figure 8A,B). Although we have a substantially weaker power in the β range in PMd when compared to PRR it is not absent, especially in Monkey F (Figure 8B). The weaker power in PMd LFP- β is – in retrospect - not surprising given the reports of such activity in the literature, First, LFP- β in premotor cortex is not as robust as in M1. For example,
O’Leary and Hatsopoulos have shown very large inter animal differences in LFP- $\beta$ measured in PMd (O’Leary and Hatsopoulos 2006). Second, LFP- $\beta$ has often been found to be not as reliable as the low frequency bands for decoding performance during reach and grasp tasks (Bansal et al. 2011; Ince et al. 2010) because of its instability. Third, sometimes the LFP- $\beta$ in premotor cortex might be completely absent, unlike in M1 (Spinks et al. 2008). Fourth, PMd LFP- $\beta$ may change substantially depending on exact recording position (O’Leary and Hatsopoulos 2006).

We therefore conclude that our findings indicate that functional connectivities within PMd and PRR are fundamentally different with the latter characterized by strong synchronized networks. We speculate that PRR and PMd although possessing almost identical neuronal tuning properties serve different functional roles by virtue of possessing different functional architectures. We hypothesize that a synchronized network in PRR is crucial for the maintenance of spatially defined movement goals during a sustained memory phase in the absence of visual feedback.

### Computing synchrony with SUA and MUA signals

In this study, we use both SUA as well as MUA signals to extract information about neural synchrony as evidenced by the zero lag correlations observed in jitter-corrected correlograms. Importantly, our main finding, the striking difference in synchrony between the two areas, PRR and PMd, is independent of the signal type studied. Nevertheless, we choose to focus on the MUA signals for further analysis of correlated activity such as the temporal evolution of synchrony.

There are various reasons that deem it advantageous to use MUA signals for purposes of studying synchrony over SUA signals. First, correlograms constructed on SUA signals tend to suffer from a lack of statistical power, simply due to the low spike counts of SUA signals as compared to the MUA signals. Zero lag correlations between two single units might be difficult to detect merely due to a low number of such events. MUA signals on the other hand, since they encompass several neurons in the vicinity of the electrode tend to reflect the co-incident firing of neural events more accurately. Roelfsema and colleagues have demonstrated that under this assumption a MUA-MUA
cross correlation function between two separate electrodes essentially is a sum total of all possible
SUA-SUA cross correlation functions, thereby affording a true estimate of the structure and temporal
evolution of such coincidences (Roelfsema et al. 2004; Super and Roelfsema 2005). Third, systematic
analyses of cross correlation functions of MUA signals and their comparison of identical functions
derived for their SUA components have shown that peak width distributions of MUA and SUA
correlograms overlap entirely (Nowak et al. 1999; Nowak et al. 1995). Peak width is a good estimate
of the integrity of a cross correlation function as weak or spurious correlograms tend to have a flat
peak on a broad elevated baseline (so called 'hill') thereby yielding a large peak width. Our
calculations of the peak half widths of correlograms constructed from SUA and MUA signals showed
similar results, with no significant differences between the two and overlapping distributions (data
not shown but see Fig 2G for example). Due to these reasons, the MUA signal is a preferable signal
for the analysis of synchrony and has been favored by investigators using such analyses for the last
three decades (Brosch et al. 1997; Friedman-Hill et al. 2000; Frien and Eckhorn 2000; Frien et al.
Nowak et al. 1995; Roelfsema et al. 2004; Super and Roelfsema 2005).

The relationship between tuning similarity and synchronzation

We measured the dependence of the synchronization between two neurons on the similarity
between their tuning curves, as measured by their signal correlation. We did not find a relationship
between synchrony, as measured by the zero-lag peak of the cross correlogram, and signal
correlation. Instead, a consistent weak relationship between signal and noise correlations existed in
PRR, but not in PMd. The lack of a positive correlation between synchrony and tuning similarity is not
surprising as even in primate V1, a known topographical area with populations of similarly tuned
neurons spanning greater-than-millimeter distances, synchrony is hardly detectable beyond a
distance of 1 mm (Grinvald et al. 1994; Smith and Kohn 2008). Using a generalized linear model,
(GLM) Stevenson et al. have shown that the firing pattern of a neuron in response to external stimuli
could be explained by its interactions with other neurons without knowledge of its tuning curve
Further, in their model, the observed noise correlations showed a dependency on tuning similarity, but this was not directly related to the true coupling strength. Thus, two neurons in their GLM having similar tuning curves would not necessarily exhibit strong neural coupling (Stevenson et al. 2012). Moreover, Schneidman and colleagues have shown that even relatively weak correlations could imply strongly correlated networks at the population level (Schneidman et al. 2006). Therefore, our weak dependencies of spike count correlations on tuning similarity in PRR should not be interpreted as negating the 'like-links-to-like' hypothesis.

Technical considerations and possible caveats

As with any measurement of correlation, our analyses are open to certain technical caveats which we discuss here. First, the possibility remains that a fundamental difference in the composition of the MUA signal in the two different areas could account for the differences observed in synchronization. We however doubt this given the differences in correlation which were observed in both SUA as well as MUA data. Second, it should be reiterated that the same type of electrodes and spatial electrode configurations (distances) were used to record from each area and the micromanipulators were repeatedly interchanged between areas and across monkeys. Differences in correlation cannot be explained by such trivial biases in the data. Third, we cannot preclude the possibility that some unique aspect of PMd architecture (for example spacing of hypothetical cortical columns) might lead to a difference in observed synchrony. But this would manifest a difference in local functional organization in accordance with our conclusions, rather than marking a confound.
Acknowledgements

The authors acknowledge Stephanie Westendorff, Christian Klaes, and Shenbing Kuang for access to their single unit datasets which augmented the dataset collected for this paper, Sina Plümer for excellent technical assistance and Leonore Burchardt for animal handling during the course of this project. The authors would like to thank Dr. Pieter Roelfsema of the Nederlands Institute for Neuroscience, Amsterdam for helpful correspondence regarding the nature of MUA and SUA correlations. The present address of S. Chakrabarti is Werner Reichardt Centre for Integrative Neuroscience, Hertie Institute for Clinical Brain Research, O. Müller Str. 25, 72076, Tübingen, Germany.

Grants

This work was supported by the Federal Ministry for Education and Research (BMBF, Germany) grants 01GQ1005C, 01GQ0433 and 01GQ0814 awarded to AG, German Research Foundation (DFG) grant SFB-889 awarded to AG, and an Alexander von Humboldt Postdoctoral Fellowship awarded to SC.

Disclosures

The authors declare no conflict of interest
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Figure captions

Figure 1. A. Timeline of the task. A variable fixation period (FIX) was followed by the presentation of a cue (CUE) in one of four directions. Following a variable memory period (MEM), a Go-cue (GO) instructed the monkey to make a center-out reach in the previously instructed direction (MOV). Correct trials were rewarded and feedback provided (FDB). B. Custom fit chambers were implanted on each monkey over dorsal premotor cortex (PMd, blue) and parietal reach region (PRR, red) contralateral to the handedness of the monkey. MRI scans show co-ordinates for chamber placement in one animal (Monkey S).

Figure 2. Extraction of spikes from multi-unit activity (MUA), and computation of synchrony. A. Top panel shows an example of MUA data collected from an electrode in PRR. A simple voltage threshold (horizontal black line, see methods) was applied and the minimum voltage for each data segment crossing this threshold within a refractory period of 2ms was time-stamped and defined as an event (black circles). Gray shaded region has been shown at higher resolution in bottom panel. Inset: Single event (arrow) shown at greater resolution in black. Offline spike sorting on this channel yielded one single unit shown in green (Solid line: mean, shaded region: standard deviation. Scale: 100µV, 0.1ms). B. Raw joint peri-stimulus histograms (JPSTHs) with a simple all-way shuffle correction were constructed to determine correlated discharges between the spike trains in the 500 ms fixation period (n = 99 trials). C. Spike times were jittered within a 50ms window and a mean jittered JPSTH was calculated by averaging across jitters (n = 1000) to compute a jitter predictor. D. A jitter corrected JPSTH was generated by subtracting C from B. E. Each JPSTH when averaged along and parallel to the main diagonal yielded a cross-correlogram (CCG). The corresponding correlogram obtained from the two single units each isolated from one of the two channels shown in red F. The broad elevation in the raw CCG, but not its zero lag peak, was accurately captured in the mean jittered CCG for both signal types. G. The jitter corrected CCG showing an offset of zero at lags
greater than 100 ms. Dashed lines indicate confidence intervals calculated using the standard deviation across the jitters. Note the very similar peak widths.

Figure 3. Trial by trial spike counts show similar patterns in both brain areas. Trial by trial spike counts in the last 500 ms of the memory period for each neuron or multi unit channel that contributed to the neural pairs analyzed for synchrony showed no obvious differences between PRR (left panels) and PMd (right panels) for Monkeys A (A), S(B) and F(C) respectively. Neurons have been sorted according to the number of trials for which they could be maintained in an isolated state and MUA channels are sorted by recording time.

Figure 4. Population PSTH’s of neural activity from both PRR (left column) and PMd (right column) from all single units from Monkey A (A) as well as all multi unit channels at a threshold of $\mu - 2\sigma$ as well as all single units from Monkeys S (B, top row: MUA, bottom row: SUA) and F (C) for comparison of response profiles. Colors indicate the preferred (green) and non-preferred (black) reach directions sorted according to maximum mean values within the last 300 ms of the memory period (thick bar bottom of each panel). Right and left panels of each figure show data aligned to cue stimulus onset and go cue respectively. Lines indicate mean across all channels or units and shaded regions show s.e.m.

Figure 5. Differences in synchrony between PRR and PMd computed from MUA signals. A,B. JPSTH’s computed during the last 500ms of the memory period (Figure 1: MEM) for PRR (left panels) and PMd (right panels) for Monkeys S and F. Only pairs that were significantly synchronized contribute to these figures. C, D. CCGs from the JPSTHs shown in the previous panels. CCGs shown in red were computed from only significantly synchronized neuron pairs and correspond to the JPSTHs in the top panels. CCGs in black were constructed from all neuron pairs, irrespective of statistical significance. Dotted lines indicate 95% confidence intervals and numbers indicate total pairs in each group. E,F.
The fraction of total neuronal pairs that were synchronized at each voltage threshold. Asterisks mark statistical significance (p<0.05) between PRR (black) and PMd (gray) using a $\chi^2$ test. G,H. The mean value of the zero lag peak of all neuron pairs shown for PRR and PMd for each monkey. Asterisks include thresholds where the synchrony between PRR and PMd were statistically different (p<0.05; unpaired t-test). Brackets indicate s.e.m.

**Figure 6.** Identical plots as in Figure 5 with same conventions but now computed using SUA data for all three monkeys. The absence of any red population CCG in PMd of Monkeys F and A (E,F, right panels) is due to the presence of only one such pair.

**Figure 7.** Temporal modulation of synchrony in the different trial epochs. A. Mean value of the main diagonal of the JPSTH averaged across all significantly synchronized MUA channel pairs in PRR for Monkey S. Shaded curve indicates s.e.m. Left and right panels show data from the same trials but correspond to two different alignment points (visual cue onset – left; go cue onset – right, dotted vertical lines). Green vertical dotted line and shaded region signify mean and standard error of the release time when the animal let go of the fixation point in order to execute the instructed reach. Dotted red line shows the p values obtained using a one-sided paired t-test comparing decreases in synchrony against mean values from a reference window (black line). Horizontal dashed line indicate statistical significance at 99% level of confidence. Lower values indicate smaller p values and therefore a smaller probability of the differences occurring by chance. B. Corresponding data obtained from PMd of the same animal. C, D. Identical plots for Monkey F.

**Figure 8.** The power in the beta-range (12-30Hz) of the local field potentials is higher in PRR with accompanying stronger spike field coherence. A. Log plot of power spectrum for the LFPs from PRR (red) and PMd (blue) calculated for the last 500ms of the memory period for monkey S. D. Identical plot for monkey F. Note different scales on y-axis. B, E. Spike field coherence between MUA and LFP...
signals from pairwise different channels in PRR as a function of time and frequency band. Visual cue and go cue onsets are shown with vertical black dotted lines whereas vertical green dotted line and shaded bar represent mean and standard error of release time respectively. Overlaid white dots identify pixels that were significantly stronger in PRR than in PMd (one tailed unpaired t-test, p<0.05). C, F. Identical plots for PMd. Note different color scales for the two animals.

Figure 9. Similarly tuned neurons tend to have stronger correlations in PRR but not in PMd. A. Scatter plots showing the noise correlation (rsc) for all MUA pairs plotted as a function of signal correlation (rsignal) for the last 500 ms of the memory period. Red represents PRR and blue PMd. Thresholds of $\mu-2\sigma$ and $\mu-3\sigma$ are shown for each area for monkey S. B. The Spearman’s correlation coefficient between rsc and rsignal is plotted as a function of MUA thresholds employed for both PRR (red) and PMd (blue). Number of pairs for each threshold shown. C. Log plot of p-values to test the statistical significance of the correlation between rsc and rsignal (Spearman’s correlation) for each of the thresholds. D, E, F. Similar plots with identical conventions for monkey F.
Figure 1
Figure 2
Figure 4

A.

B.

C.
Figure 6

A. monkey S (SUA)  
B. monkey F (SUA)  
C. monkey A (SUA)  

D.  
E.  
F.  

G.  
H.  
I.  

J.  
K.  
L.  

Fraction synchronized

rccg (peak)

Time aligned to go cue (s)

Lag (s)

All pairs significant pairs

n = 72/12 n = 111/4

n = 58/14 n = 26/1

n = 87/8 n = 35/1

*
Figure 7

A. B.

C. D.

Figure 7
Figure 8
Figure 9

- **A.** PRR

- **B.** Monkey S

- **C.** r = 0.38

- **D.** Monkey F

- **E.** r = 0.2

- **F.** PMd

- **G.** r = 0.45

- **H.** r = 0.2

- **I.** r = 0.2

- **J.** r = 0.2

- **K.** p < 0.001

- **L.** p < 0.05

- **M.** p = 0.06