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

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Submitted 31 August 2013; accepted in final form 15 September 2014

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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. 1993, 1996; 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 whether distinct local organization beyond the immediate response properties of individual neurons within these 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, 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 cortex (Barbas 1988; Barbas and Mesulam 1985; Cavada et al. 2000; Luppino et al. 2003; Petrides and Pandya 1999; Seleson and Goldman-Rakic 1988), involved in abstract rule representation (Wallis and Miller 2003a, 2003b), 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 and 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 and Kalaska 2005; Klaes et al. 2011). Apart from such similarity, PMd was selectively activated when abstract rules were 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 coordinates in PRR (Batista et al. 1999; Buneo et al. 2002; Cohen et al. 2002; Cohen and Andersen 2000, 2002) but in a combination of eye-, hand-, and goal-centered coordinates 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 with abstract rules or with the monkey’s free choice (Pesaran et al. 2008; Westendorff et al. 2010). Furthermore, on the population level, it has been shown that in PRR there is on average a 20% stronger spatial selectivity during direct visual cuing of reach goals (pro reaches) compared with indirect rule-based cuing (anti reaches). By comparison, in PMd the amplitude of neural responses during anti reaches tends to be 15% higher than during 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. 2007; Pesaran et al. 2008; Westendorff et al. 2010). Furthermore, on the population level, it has been shown that in PRR there is on average a 20% stronger spatial selectivity during direct visual cuing of reach goals (pro reaches) compared with indirect rule-based cuing (anti reaches). By comparison, in PMd the amplitude of neural responses during anti reaches tends to be 15% higher than during 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. 2007; Pesaran et al. 2008; Westendorff et al. 2010).
MATERIALS AND METHODS

Behavioral task. Three adult male rhesus monkeys (Macaca mulatta; 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 tasks and we wanted to test whether neural synchronization differs in these areas during such a prototypical sensorimotor task. The animals were seated in primate chairs ~35–40 cm in front of a monitor that was used to display the stimuli (19-in. ViewSonic LCD VX922). As illustrated in Fig. 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 Systems). After a period of 500–2,000 ms (fixation period), a peripheral spatial cue (white filled circle) was flashed on the screen for 200 ms (cue period) 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 toward 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 reach later to the diametrically opposite direction (Gail et al. 2009; Westendorff et al. 2010). However, only data from the direct-reach, pro trials are analyzed in this report, since these were also available for monkey F. After the presentation of the two cues the monkeys had to maintain ocular and hand fixation for a further 800–2,000 ms (memory period). Next, a disappearance of the hand fixation spot served as the go signal, and the animals had to reach toward the previously instructed goal within a maximum of 800–1,000 ms (movement period; 3.0–7.4° VA reach tolerance) and hold the goal for a further 100–300 ms (feedback period). 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, with 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 three 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 place-
ament above PRR and PMd was guided by presurgical structural MRI (Fig. 1B) and confirmed by postsurgical 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; monkeys S and F, right hemisphere). Horsley-Clarke coordinates of the chamber centers on the skull were as follows (all numbers in millimeters): PRR_A: 8.5L × 9.5P, PRR_S: 6.0R × 10.0P, PRR_F: 7.0R × 13.0P, PMd_A: 13.5L × 19.8A, PMd_S: 13.0R × 17A, PMd_F: 20R × 20A.

All surgical and imaging procedures were conducted under general anesthesia and in accordance with German laws governing animal use. This research was conducted under license 33.11.42502-04/064/07 reviewed and approved by Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Oldenburg, Germany. Further details of these procedures have been described previously (Gail et al. 2009).

Neural recordings. For extracellular recordings, between three and five glass-coated tungsten-iridium microelectrodes with impedances between 1 and 3 MΩ and horizontal interelectrode separations of 300–1,500 μm were lowered in each cortical area through stainless steel guide tubes with the five-channel Eckhorn mini-matrix system (Thomas Recording). The interelectrode separation and the configuration of the matrix heads were identical across areas. Neural data were recorded with a Plexon MAP recording system (Plexon, Dallas, TX). Signals were initially preamplified (20×) before they were band-pass filtered [0.7–300 Hz for local field potentials (LFPs); 100 Hz—8 kHz for spiking data] and amplified (50×). LFPs were digitized at 1 kHz, whereas the spiking data were stored as two separate data types. Single-unit spike waveforms as isolated during online spike sorting, but including part of the background activity (“noisy” cluster), were further amplified at gain 12–16×, digitized at 40 kHz for a 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 with principal component analysis (PCA) using a time-resolved k-means cluster identification algorithm with manual supervision using Plexon Offline Sorter. The interspike interval histograms were monitored for ter identification algorithm with manual supervision using Plexon manual-set voltage threshold [single-unit activity (SUA)].

These spike waveforms were extracted per trial, each corresponding to one of the above thresholds \( k \). 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 are briefly explained 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 timescales (Smith and Kohn 2008) and were defined in keeping with accepted nomenclature. Correlated firing between two spike trains occurring at the timescale of several milliseconds and quantified by the peak of corrected cross-correlograms (see below) was termed synchrony (rccg). Alternatively, slow trial-by-trial covarying fluctuations in firing rate between two spike trains across trials with identical task conditions were described as slow covariations or noise correlations (rsc). Finally, the correlation between the tuning curves of two neurons, a measure of tuning similarity, was termed signal correlation (rsignal) and computed after averaging spike counts over trials with identical task conditions. Investigations of 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 (2011).

Joint peristimulus histogram and cross-correlogram construction. To measure synchrony and its temporal modulation, the joint peristimulus time histogram (JPSTH) as first described by Aertsen and colleagues (Aertsen et al. 1989; Eggermont 1994) was implemented. Briefly, pairs were constructed with 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 10 ms each. The correlation \( r_{jpsth} \) for a given pair of time bins \( t1 \) and \( t2 \) was then calculated with

\[
r_{jpsth}(t1,t2) = \frac{E[N_{1,t1}N_{2,t2}] - E[N_{1,t1}]E[N_{2,t2}]}{\sqrt{\lambda_1 \cdot \lambda_2}}
\]

where \( N_{1,t1} \) and \( N_{2,t2} \) are the number of events on spike trains \( l \) 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 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 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).
temporal progression of correlations at different time lags (raw JPSTH; Fig. 2B). The main diagonal in such a JPSTH represents those cases in which \( t_1 = t_2 \), 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

\[
\text{rccg}_\tau = \frac{1}{T - 2\tau} \sum_{i=\tau}^{T-\tau} rjpsth(t + \tau, i), \tau \geq 0
\]  

where \( \text{rccg}_\tau \) is the correlation coefficient at lag \( \tau \), \( rjpsth \) is the correlation per time bin measured with the JPSTH (Eq. 2), \( T \) is the total time, and \( i \) is the time within the trial. The \( \text{rccg}_\tau \) 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 Fig. 2B is represented in Fig. 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 \( \text{rccg}_\tau \) greater than 0 at \( \tau = 0.15 \) s in this typical example.

Many reports have shown that CCGs, independent of shuffle predictor subtraction, will represent a mixture of correlations at different timescales. The central peak is thought to reflect synchrony, whereas the broad increase is thought to result from slow covariations (Ben-Shaul et al. 2001; Brody 1999a, 1999b; Grun et al. 2003; Ventura et al. 2005). We used a recently described method of jittering spike trains to produce surrogates that eliminate the effect of such slow covariations 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 50 ms, to produce surrogate spike trains that were identical to the original spike trains in terms of spike/event count and slow covariations but for which synchrony occurring at a timescales smaller than 50 ms was artificially destroyed. After such jittering, JPSTHs were calculated as described above using the surrogates. For
each spike train, $n = 1,000$ surrogates were generated, resulting in an equal number of JPSTHs. Averaging across all such JPSTHs, the mean surrogate JPSTH (Fig. 2C) and CCG (Fig. 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 (Fig. 2D) and CCG (Fig. 2G) were obtained by subtracting the mean surrogate JPSTH and CCG from their raw counterparts described above. 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 Fig. 2G (assuming the JPSTHs and CCGs surrogates are drawn from a normal distribution). A pair of spike trains was significantly synchronized if the $rccg_{k}$ values at $-10 < \tau \leq 10$ ms 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 (>100 ms), which were very rare in our data set, represented correlations that have been described to have different origins and hence will not be classified here as synchrony (Eggermont et al. 1993; Lampi et al. 1999; Singer and Gray 1995). It is important to note that when a pair of single units were defined by off-line spike sorting the MUA channel (Fig. 2A), the resulting CCGs (Fig. 2E–G) 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 CCGs 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 overestimation of synchrony (Brody 1999a, 1999b). 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 units and multunit pairs 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, nonstationary 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 whether this zero-lag synchrony deviated significantly from the mean synchrony of the same pair in a reference window (last 300 ms of the fixation period; 1-sided paired $t$-test).

**Calculation of noise correlation and signal correlation.** To determine slow covariations ($rsc$) we used a measure termed “noise correlations” that has been previously used to quantify trial-by-trial 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 nonstationary signals and affected <1% of trials in our data. The trial-by-trial spike counts for each reach direction were standardized ($z$ score) as follows:

$$N_{k}^{t} = \frac{x_{k} - E[x_{k}]}{\sigma[x_{k}]}, \quad k = [0°, 90°, 180°, 270°]$$  

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 with

$$rsc = E[N_{1}N_{2}^{c}]$$  

where $N_{1}$ and $N_{2}$ 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:

$$rsc(Fisher) = \frac{1}{2} \ln \left( \frac{1 + rsc}{1 - rsc} \right)$$  

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 Eq. 6.

**Analysis of local field potentials.** 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 with the fast Fourier transform (FFT) with a Hamming window for the 500 ms prior to the go cue after subtracting the mean amplitude. Spike field coherence within each area was computed with the NeurAn Chronux toolbox in MATLAB (Jarvis and Mitra 2001). Briefly, trials were aligned to either the visual cue presentation or the go signal, and a multitaper window technique was used with sliding windows of 400-ms length advanced every 10 ms. 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 with a nonparametric Wilcoxon rank sum test and the $P$ values corrected for multiple comparisons, resulting from the different sliding time windows, with the Bonferroni method.

**Calculation of peristimulus time histograms.** Peristimulus time histograms (PSTHs) 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 excitatory postsynaptic potential (EPSP) that was defined as follows:

$$R(t) = \frac{\tau_{c} + \tau_{d}}{\tau_{c} \cdot e^{-t/\tau_{c}}} \times \left( 1 - e^{-t/\tau_{c}} \right) \times e^{-t/\tau_{d}}$$  

where $R(t)$ is the spike density at time point $t$, $\tau_{s}$ 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 PSTHs 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 ms of the memory period. PSTHs for the preferred and antipreferred reach directions sorted as described above were then averaged over all single units or MUA channels for both areas.

**RESULTS**

Data were collected from three adult male rhesus monkeys. MUA data, as described above, were obtained from *monkeys S* and *F*, whereas SUA data were 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 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 PSTHs for all single units and channels of MUA data (threshold at $\xi_2 = \mu - 2\sigma$) for both areas in all three monkeys. The PSTHs 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 (antipreferred) reach directions. After the fixation period and the visual cue presentation, firing rates in both areas were tuned to the reach direction in a sustained fashion throughout the memory period, followed by a rapid increase during movement.
before collapsing to prestimulus fixation period values. Importantly, the similarity of the population PSTHs in both areas rules out biases in firing rate that could potentially confound a comparative analysis of synchronization between the areas. In monkey $F$, the SUA data show a lower firing rate in PMd compared with PRR already during the baseline fixation period. However, despite this difference, as shown below, our results on synchronization are consistent across all three monkeys.

**Stronger synchrony in PRR than PMd.** Using JPSTHs 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 500 ms of the memory period. We chose this time window for our first analysis since direction-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 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 (Fig. 5, A and B). The higher values in the central diagonal of the JPSTHs indicated robust zero-lag correlations that can be better estimated and quantified with CCGs (Fig. 5, C and D). The stronger synchrony in PRR compared with 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 or only those showing statistically significant synchrony. 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 Obermüller 1993; Engel et al. 1991; Gray et al. 1989, 1992) 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 (Fig. 5, E and 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 synchronized not only more often but also more strongly. A comparison of the zero-lag peak of all neuron pairs (Fig. 5, G and H) showed a trend similar 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 in 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 Fig. 6, CCGs of PRR single units of all three monkeys showed statistically significant synchronization, i.e., exceeding 95% confidence intervals at lag zero (Fig. 6, A–F). Only one neuron pair in monkey F and monkey A PMd reached statistical significance, and therefore the population data for statistically significant PMd SUA pairs is not shown in Fig. 6, E and F. Again, PRR was characterized by a significantly higher incidence of synchrony (\( \chi^2 \)-test, \( P < 0.05 \)).
that was significantly stronger (unpaired t-test, \( P < 0.05 \)) than that seen in PMd (Fig. 6, G–L). The SUA data set of monkey A showed an oscillatory pattern reminiscent of monkey F’s MUA data (Fig. 5).

The auto-correlogram functions calculated for all the above data sets showed similar features such as decay rates between the two 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 data set for monkey F was different between PRR and PMd, this did not affect the robust synchrony results in this particular data set.

The thresholds of \( \xi_2 = \mu - 2\sigma \) and \( \xi_3 = \mu - 3\sigma \) 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. Furthermore, it was 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 report focus on the MUA data at the 2\( \sigma \) 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 (Fig. 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 with baseline with sustained motor-goal tuning (Fig. 4), synchrony was at a level comparable to the fixation period, when no task-specific instructions had yet been presented. In both the fixation and 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 (Fig. 7, A and C). This desynchronization 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 (1-sided paired \( t \)-test, \( P < 0.01 \); Fig. 7). In monkey S the desynchronization 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 (Fig. 7, B and 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. However, 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 covariation with spike synchrony.** Previous studies have shown that LFP power 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 8, A 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 (12–30 Hz) 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 dependence 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 (Fig. 8, B and E). In both monkeys, this spike field coherence was significantly stronger in PRR than in PMd, as measured by a Wilcoxon rank sum test after correction for multiple comparisons with the Bonferroni method (Fig. 8, B and 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 (Fig. 7). Notably, in both monkeys, the desynchronization in PRR during movement is preceded by a transient increase in spike field coherence in PMd in the low frequency range (<15 Hz; Fig. 8, C and 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 MATERIALS AND METHODS). We first tested the dependence of synchrony on tuning similarity calculated with the pairwise signal correlation. Signal correlation is simply a correlation of the tuning similarity calculated with the pairwise signal correlation (Smith and Kohn 2008). 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 covariation with each other than signals with dissimilar tuning (Fig. 9, A and 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 Spearman’s correlation coefficient between noise and signal correlation was higher in PRR than in PMd (Fig. 9, B and E) at most thresholds. The significant positive noise correlation in PRR and its absence in PMd could be seen across several threshold levels (Fig. 9, B–F). This means that more reliably in PRR, but less so in PMd, neurons with similar tuning properties covary 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 showed 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, LFPs in PRR exhibited significantly stronger power in the β-range (12–30 Hz) than those 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,

Fig. 8. Power in the β range (12–30 Hz) of the local field potentials (LFPs) is higher in PRR with accompanying stronger spike field coherence. A: log plot of power spectrum for LFPs from PRR (red) and PMd (blue) calculated for the last 500 ms of the memory period for monkey S. D: identical plot for monkey F. Note different y-axis scales. B and 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 dashed lines, whereas vertical green dashed line and shaded bar represent mean and SE of release time, respectively. Overlaid white dots identify pixels that were significantly stronger in PRR than in PMd (1-tailed unpaired t-test, P < 0.05). C and F: identical plots for PMd. Note different color scales for the 2 animals.

J Neurophysiol • doi:10.1152/jn.00621.2013 • www.jn.org
Fig. 9. Similarly tuned neurons tend to have stronger correlations in PRR but not in PMd. A: scatterplots showing the noise correlation ($r_{\text{sc}}$) for all MUA pairs plotted as a function of signal correlation ($r_{\text{signal}}$) 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 Spearmann’s correlation coefficient between $r_{\text{sc}}$ and $r_{\text{signal}}$ is plotted as a function of MUA thresholds employed for both PRR (red) and PMd (blue). Numbers of pairs for each threshold are shown. C: log plot of $P$ values to test the statistical significance of the correlation between $r_{\text{sc}}$ and $r_{\text{signal}}$ (Spearmann’s correlation) for each of the thresholds. D–F: similar plots with identical conventions for monkey F.

A

B

C

D

E

F

SYNCHRONIZATION IN PRR AND PMD

has a functional organization reminiscent of organized sensory cortical areas where similar neurons form neural ensembles that synchronize preferentially with each other. Additionally, because of its temporal evolution over the course of a trial, we speculate that the synchronized and oscillatory activity in PRR might serve to sustain 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 et al. 2002; Buneo and Andersen 2006; Johnson et al. 1996). As mentioned 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, 1997b) and inactivation (Hwang et al. 2012; Yttri et al. 2014) have repeatedly shown that reach trajectories and end points are severely affected by a compromised parietal cortex, although the ability to select between two different types of movement ("push" vs. "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 Balint’s syndrome (for review see Andersen et al. 2014). Furthermore, 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 periarcaute lesions in monkeys by Petrides specifically affected the animals’ ability to perform movements cued by abstract symbols but not simple visually guided reaches (Petrides 1982, 1985). 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 critical roles of PRR in spatial and of PMd in abstract rule-based target selection, SUAs in both areas have consistently showed remarkably similar spatial tuning profiles (Gail et al. 2009; Gail and Andersen 2006; 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 the two 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) than when they had to be inferred with 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 nonstandard stimulus-response associations appeared first in PMd (Westendorff et al. 2010). Finally, 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 seem 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 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 the areas project to different subsets of target areas, their local network properties appear to optimized for different neural computations.

**Functional role of synchronization in PRR.** An area like PRR that 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 end point. 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 (decorrelation after cue presentation) or because of movement initiation (decorrelation 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 data sets also show an additional oscillatory pattern in the CCGs recorded from PRR in the β range (15–35 Hz, monkey F MUA and SUA data, Fig. 5D and Fig. 6E, respectively; monkey A SUA data, Fig. 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 (Fig. 8, A and B). Although we have a substantially weaker power in the β range in PMd compared with PRR, it is not absent, especially in monkey F (Fig. 8D). 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 interanimal differences in LFP-β measured in PMd (O’Leary and Hatsopoulos 2006). Second, LFP-β 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-β in premotor cortex might be completely absent, unlike in M1 (Spinks et al. 2008).

Fourth, PMd LFP-β 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 used both SUA and 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 make 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 because of the low spike counts of SUA signals compared with MUA signals. Zero-lag correlations between two single units might be difficult to detect merely because of 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 coincident 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. 1995, 1999). Peak width is a good estimate of the width of a cross-correlation function, as weak or spurious correlograms tend to have a flat peak on a broad elevated baseline (a 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). For 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 et al. 1994; Frien and Eckhorn 2000; Gail et al. 2000; Gray et al. 1989; Maldonado et al. 2000; Nelson et al. 1992; Nowak et al. 1995, 1999; Roelfsema et al. 2004; Super and Roelfsema 2005).

Relationship between tuning similarity and synchrony. We measured the dependence of the synchrony 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 CCG, 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 primates 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 (Stevenson et al. 2012). Furthermore, in their model, the observed noise correlations showed a dependence 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, Schenck and colleagues have shown that even relatively weak correlations could imply strongly correlated networks at the population level (Schenck 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 subject 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 synchrony. We doubt this, however, given the differences in correlation that were observed in both SUA and 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 micro-manipulators 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 hypothesized cortical columns) might lead to a difference in observed synchrony. But this would manifest itself as a difference in local functional organization in accordance with our conclusions, rather than marking a confound.

ACKNOWLEDGMENTS

The authors acknowledge Stephanie Westendorff Christian Klaes and Shenbing Kuang for access to their single-unit data sets that augmented the data set collected for this paper, Sina Plümer for excellent technical assistance, and Leonore Burchardt for animal handling during the course of this project.


Synchronization in PRR and PMD


