Synchronous Oscillatory Neural Ensembles for Rules in the Prefrontal Cortex

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Synchronous Oscillatory Neural Ensembles for Rules in the Prefrontal Cortex

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SUMMARY
Intelligent behavior requires acquiring and following rules. Rules define how our behavior should fit different situations. To understand its neural mechanisms, we simultaneously recorded from multiple electrodes in dorsolateral prefrontal cortex (PFC) while monkeys switched between two rules (respond to color vs. orientation). We found evidence that neural ensembles for the rules were formed by oscillatory synchronization of local field potentials (LFPs): there were rule-specific increases in synchrony at ‘beta’ (19-40 Hz) frequencies between electrodes. In addition, individual PFC neurons synchronized to the LFP ensemble corresponding to the current rule (color vs. orientation). Furthermore, the ensemble encoding the behaviorally dominant orientation rule showed increased ‘alpha’ (6-16 Hz) synchrony when preparing to apply the alternative (weaker) color rule. This suggests beta-frequency synchrony selects the relevant rule ensemble while alpha-frequency synchrony de-selects a stronger, but currently irrelevant, ensemble. Synchrony may act to dynamically shape task-relevant neural ensembles out of larger, overlapping, circuits.
INTRODUCTION

A critical cognitive ability is the flexibility to change one’s behavior based on context. Day-to-day life is full of such situations. For example, one often answers their phone when it rings but mutes it in a lecture. These context-dependent stimulus-response mappings are called “rules”. By allowing us to quickly adapt to specific situations, rules endow the cognitive flexibility crucial for intelligent behavior.

The prefrontal cortex (PFC) is key to rule-based behaviors (Miller and Cohen, 2001). Rule-based tasks, especially those involving rule-switching, activate the human PFC (Dove et al., 2000; MacDonald, 2000; Sakai and Passingham, 2003) and are impaired following PFC damage (Milner, 1963; Stuss and Benson, 1984). Many PFC neurons encode task rules (White and Wise, 1999; Wallis et al., 2001), and can “multiplex”: encoding different task information (rule, stimulus, etc.) in different contexts (Rainer et al., 1999; Cromer et al., 2010). Recent theoretical work suggests that this diversity of PFC neuron properties underlies the capacity to encode a large number of diverse rules (Rigotti et al., 2010).

But this diversity raises the question of how PFC circuits satisfy two competing demands: Form the neural ensembles that represent a current rule while allowing for their flexible reconfiguration when the rule changes. One proposed solution is synchronized network oscillations. Oscillations can establish ensembles of neurons in a task-dependent, flexible, manner (Akam and Kullmann, 2010), allowing ensembles to be dynamically ‘carved’ from a greater, heterogeneous, population of neurons. In addition, coincident activity has a supralinear effect on downstream neurons (Aertsen et al., 1989), increasing the impact of neural ensemble activity on function (Fries, 2005).

To investigate the neural mechanisms underlying cognitive flexibility, we trained two monkeys to switch between two rules: respond to either the
color or orientation of a stimulus (Figure 1A). After acquiring a central fixation target, a rule-cue indicated whether the color or orientation rule was now relevant. Two different cues were used for each rule in order to disassociate neural selectivity for the cue from the rule (see Materials and Methods). After a brief, randomized, interval, a test stimulus appeared. The test stimulus consisted of small shapes that were either red or blue and were either vertically or horizontally aligned (Figure 1A). Depending on the current stimulus and rule, monkeys made a leftward or rightward saccade (color rule: red=left, blue=right; orientation rule: horizontal=left, vertical=right; Figure 1A). On most trials (70%), the color and orientation of the test stimulus signaled incongruent responses to ensure that the animals consistently followed the rule (e.g. a red/vertical cued different saccade directions under different rules). The same rule was repeated for at least 20 trials before a probabilistic switch.

RESULTS

Behavioral and Single Unit Evidence for the Dominance of the Orientation Rule

Monkeys performed well (~90% of trials were correct) but, like humans, were slower to respond on the first trial after switch, compared to repeated rule trials (Allport et al., 1994; Rogers and Monsell, 1995; Caselli and Chelazzi, 2011). This reaction time “switch cost” is thought to reflect the cognitive effort needed to change rules. However, it was only observed after a switch from orientation to color rule and not vice-versa (Figure 1B; p=1.61*10^{-4}, GLM, Table S1). This suggests the orientation rule was behaviorally dominant, as the animals had more difficulty switching away from it.

We quantified neural information about the cued rule using a bias-corrected percent explained variance statistic ($\omega$PEV, see Supplemental Information for details). The majority of PFC neurons carried rule
information (Figure 2A, PFC: 225/313, randomization test, cluster corrected for multiple comparisons, see Figure S1A for an example neuron). Similar numbers of neurons had higher firing rates during orientation and color rule trials (108 and 117 respectively, p=0.25, binomial test). Across the population of PFC neurons, rule-selectivity increased following the rule cue, although some baseline rule information was observed due to the task-design: the rule repeated for multiple trials before a switch (Figure 2A). PFC neurons were also selective for the color or orientation of the test stimulus (104/313, 33%; 126/313, 40%, respectively). Orientation was behavioral dominant (see above) and neural selectivity for it was more common than color (p= 3.9*10^{-3}, binomial test), stronger across the population (Figure 2B and Figure S1C), and appeared slightly earlier (41.1 vs. 47.6 ms after stimulus onset; p=0.0026, permutation test).

Rule-Selective LFP Synchronization between Pairs of Electrodes
We found rule-selective oscillatory synchronization of local field potentials between individual PFC electrode pairs. There were significant differences in synchrony between the rules in two frequency bands during two separate trial epochs: ‘alpha’ (6-16 Hz) after the rule cue and ‘beta’ (19-40 Hz) after test stimulus appeared (179/465 and 207/465 recorded pairs at p<0.05 in alpha and beta, respectively; Figure 3A and Figure S2A, alpha/beta shown as solid/dashed outlines). This was not due to differences in evoked potential (Figure S2E) or oscillatory power (see Supplemental Experimental Methods). It was also not due to simple volume conduction of an evoked potential: many rule-selective electrode pairs were spatially interspersed with electrodes with either the opposite or no synchronous rule preference (22/79 or 28%, see Supplemental Experimental Methods for details) and rule-selective synchrony did not monotonically decrease with distance (Figure S2C).

Beta oscillations increase with cognitive effort (Buschman and Miller, 2007; Pesaran et al., 2008; Kopell et al., 2010). Thus, we sorted electrode pairs by
which rule elicited significantly stronger beta synchrony. This identified two networks: one synchronized during the orientation rule (N=117 out of 465 pairs, p<10^{-15}, binomial test against the number expected by chance) and one during the color rule (N=90, p<10^{-15}, binomial test). There were significantly more electrode pairs with significantly stronger beta synchrony for the orientation rule than the color rule (Figure 3B, p=8.8*10^{-4}), again consistent with orientation being dominant. The magnitude of rule-selective increases in synchrony were comparable to those previously observed during attention (Figures 4 and S3; Buschman and Miller, 2007; Gregoriou et al., 2009).

Rule-selective synchrony between electrodes was not between isolated electrode pairs. Rather, synchrony occurred within interconnected networks: electrode sites were synchronized to an average of 2.6 and 1.8 other sites (out of a maximum of 5.0) for the orientation and color rule networks, respectively (p<10^{-3} for both, permutation test against random networks, see Supplemental Information). These rule-dependent networks were highly overlapping spatially (see Figure S2D for anatomical localization of networks). The majority of recording sites that selectively increased synchrony with one set of electrodes during one rule also increased synchrony with a different set of electrodes during the other rule (58% of electrodes participating in an orientation-rule-preferring pair, 52% of color-rule-preferring, see Supplemental Information).

**Task-Relevant Neurons were Synchronized to the Current Rule-Network**

LFP synchrony may reflect functional networks of spiking neurons (Fries, 2005). Indeed, we found that both stimulus- and rule-selective neurons showed rule-dependent spike-LFP synchrony. When the orientation or color rule was relevant, neurons with selectivity for the relevant test stimulus modality (Figure 5A) and/or the current rule (Figure 5B) were more synchronized to the currently activated beta-band color or orientation ensemble (see Supplemental Information for details). Spike-
field synchrony was largely observed at beta-band frequencies, particularly for orientation rule trials (Figure 5, left column). During color rule trials synchrony was shifted slightly towards higher frequencies (Figure 5, right column). This may reflect differences in the underlying architecture of the rule-selective network either locally or between PFC and sensory/motor regions (Siegel et al., 2012).

**Beta Orientation Network Shows Stronger Alpha Color Selectivity**

Alpha synchrony increases were primarily limited to color rule trials. Figure 3B shows that most of the electrode pairs that showed significant increases in synchrony in the alpha band did so when the color rule was cued. To examine this more closely, we plotted the beta-synchrony defined orientation and color ensembles separately (Figure 6). When separated, it is clear that while increases in alpha synchrony were on color trials they were primarily limited to the orientation rule ensemble (Figure 6, left column). Indeed, electrode pairs with increased alpha synchrony during the color rule were more likely to show increased beta synchrony for the orientation rule than color rule (55/117 and 24/90 pairs, respectively; p<10⁻⁵, permutation test).

Synchronized alpha activity may reflect inhibition of task-irrelevant processing (Ray and Cole, 1985; Klimesch et al., 1999; Pfurtscheller, 2001; Palva and Palva, 2007; Haegens et al., 2011b). Thus, alpha synchrony during color trials may reflect “de-selection” of the dominant (but currently irrelevant) orientation network, allowing the weaker (but currently relevant) color network to be boosted. Indeed, alpha increases in the orientation rule ensemble were associated with enhancement of individual color-rule neurons. Alpha power during the preparatory interval of color trials was positively correlated with the activity level of color-rule-preferring, but not orientation-rule-preferring, neurons during rule application to the test stimulus (Figure S4, correlation coefficient of 0.014, p=0.0019 vs. 0.003, p=0.47, for color and orientation rule preferring
neurons, respectively, for 100 ms following stimulus onset; color>orientation, p=0.047, see Supplemental Information for details). There was no direct evidence for suppression of the orientation network (e.g. a negative correlation between alpha power and the activity of orientation-preferring neurons on color trials). However, these neurons are already suppressed during the color rule, so further suppression may be hard to detect.

**Rule-Dependent Synchrony Correlates with Behavioral Reaction Time**
Synchrony at both alpha and beta was correlated with behavioral reaction time, further suggesting their functional role. There was significantly stronger rule-selective synchrony in both bands on trials with shorter reaction times (Figure 7; alpha: p=3.43*10^{-10}, beta: p=2.71*10^{-3}, Wilcoxon signed-rank test), even after controlling for the effects of preparatory time and rule on reaction time (see Table S1). This stronger synchrony with faster reaction times occurred prior to test stimulus for both alpha and beta (Figure 7; stronger selectivity in Beta: -20 to 0 ms, Alpha: -240 to 0 ms prior to stimulus onset, Wilcoxon signed-rank test, p <.05, Bonferroni correction), suggesting preparatory facilitation of test stimulus processing.

**DISCUSSION**

**Linking Task-Relevant Neurons with Rule-Dependent Synchrony**
Our results suggest distinct, synchronous, PFC networks support different rules. Rule-selective beta-band synchrony may help to dynamically link neurons in order to support task performance. Indeed, task-relevant (rule- and stimulus-selective) neurons were more synchronized to the corresponding network for the current rule. Similar organization of neural activity by synchronous population oscillations have been seen during sensory processing (Lakatos et al., 2008), and attention (Buschman and Miller, 2009). This synchrony-based linking of neurons into networks could
be an ideal mechanism for cognitive flexibility, allowing ensembles of task-relevant neurons to be dynamically formed and reformed (Sejnowski and Paulsen, 2006; Womelsdorf et al., 2007).

Our results are consistent with recent evidence from humans and monkeys suggesting that beta oscillations play a major role in top-down organization of neural processing (Engel and Fries, 2010; Oswal et al., 2012). There is enhancement of beta oscillations in human sensorimotor cortices when maintaining posture (Gilbertson et al., 2005; Androulidakis et al., 2007), and when competing movements need to be inhibited (Pfurtscheller, 1981; Swann et al., 2009). Beta synchronization between frontal and parietal cortices increases during top-down attention (Gross et al., 2006; Buschman and Miller, 2007, 2009) and with increased working memory load (Babiloni et al., 2004; Axmacher et al., 2008). Further, beta synchronization increases in anticipation of an upcoming stimulus and is stronger when a stimulus is more predictable (Liang et al., 2002; Gross et al., 2006; Zhang et al., 2008). Similarly, we observed rule-selective beta synchronization in anticipation of the test stimulus was correlated with the animal’s reaction time.

Coordination of Neural Ensembles
Orientation seemed to be the dominant modality. This may be due to its relative saliency, much like word-naming in the Stroop test (MacLeod, 1991). We found the orientation network, which was synchronized at beta-band frequencies during the orientation rule, had increased alpha-band synchrony when color was relevant. Recent studies in humans have suggested a role for alpha oscillations in working memory (Jensen et al., 2002; Freunberger et al., 2008; Palva et al., 2011) and visual attention (Von Stein et al., 2000; Sauseng et al., 2005; Sadaghiani et al., 2010). In particular, alpha oscillations during attention are suppressed in the task-relevant sensorimotor cortices, enhanced in the task-irrelevant cortices, and can influence discriminability of stimuli (Worden et al., 2000; Gould et
al., 2011; Haegens et al., 2011a). Because of this, it has been suggested that enhanced alpha synchronization creates an inhibition of irrelevant processes (Klimesch et al., 2007; Mathewson et al., 2011). Our study is consistent with this model: alpha synchronization may allow the weaker color network to be activated over the stronger (orientation) network when color is relevant. In support, we observed an increase in the activity of color-selective neurons following an increase in alpha in the orientation network. These results suggest a dual model of competition between networks of neurons: beta synchrony selects the relevant network while alpha may de-select the irrelevant, but dominant, network so that a weaker, relevant, one can be established. Similar dual mechanisms may bias competition between stimuli during focal attention, leading to high-frequency synchronization of neural activity representing attended stimuli (Fries et al., 2001) and slower-frequency synchronization of neural activity representing unattended stimuli (Cohen and Maunsell, 2009; Mitchell et al., 2009).

In sum, our results suggest that synchronous oscillations allow dynamic selection of currently relevant neural ensembles. This may be particularly important in prefrontal cortex, where neurons have highly diverse properties and thus a particular ensemble must be formed from neurons that are also members of other ensembles (Rigotti et al., 2010). The dynamic nature of synchronized oscillations may provide a substrate for the ensembles that allows that their rapid selection and de-selection and, hence, cognitive flexibility.

EXPERIMENTAL PROCEDURES

Recording Locations and Techniques
Two macaque monkeys, one male (CC, *Macaca fascicularis*) and one female (ISA, *Macaca mulatta*), were trained on a cued task switching paradigm (Figure 1A). Neural activity was simultaneously recorded during task performance from two frontal regions: the dorsolateral prefrontal cortex (PFC, area 9/46) and the anterior cingulate cortex (ACC, areas 24c and 32).
Only data from the dorsolateral prefrontal cortex is reported here. The recording well targeting PFC was placed in the left hemisphere and was centered approximately 28 mm anterior to the interaural plane and 21 mm lateral from the midline. Stereotaxic positioning of the well was guided by structural magnetic resonance imaging.

Neural activity was recorded during 34 sessions (11 for monkey CC, 23 for monkey ISA). Arrays of up to sixteen epoxy-coated tungsten electrodes (FHC Inc, Bowdoin ME) were lowered into the PFC during each recording session (median # of electrodes with well-isolated single neuron activity was 5.5 per session). Electrodes were lowered in pairs by a custom built microdrive assembly and spaced at least 1 mm apart. Electrodes were lowered acutely each day through an intact dura and allowed to settle before recording. This ensured stable isolation of the single neuron activity. After each recording session, the electrodes were retracted and the microdrive assembly was removed from the well.

A Plexon Multichannel Acquisition Processor (MAP; Plexon Inc, Dallas, TX) was used to perform electrophysiological recordings. The signal from each electrode was filtered by the pre-amplifier between 154 Hz and 8.8 kHz to isolate spiking activity and between 3.3 and 88 Hz to isolate the local field potential. Both spiking activity and local field potentials were referenced to earth ground (although the same results were observed when re-referencing locally, within PFC). The raw spiking waveforms were digitized at 40 kHz and subsequently sorted into single units offline, based on waveform shape characteristics and principal components analysis (Offline Sorter, Plexon Inc, Dallas, TX). During recording, electrodes were lowered to maximize the signal-to-noise ratio of spiking activity and were not guided by the task-relevance of neural responses. This ensured a representative sample of neural activity without selection bias. A total of 313 neurons were recorded in the PFC (99 in monkey CC and 214 in monkey ISA). The average firing rate of neurons recorded in PFC was 7.4 Hz (inter-quartile range of firing rate was 1.7 to 10.1 Hz). Only local field potentials from electrodes with at least one isolated unit were used for all of our analyses, ensuring the electrode was in the appropriate cell layer.

Animal eye position was monitored using an infrared eye-tracking system (Eyelink, SR Research Ltd., Ontario, Canada) which sampled the eye position at 240 Hz. Behavioral control was handled by Cortex (http://www.cortex.salk.edu). Animal procedures followed all guidelines set by the Massachusetts Institute of Technology Committee on Animal Care and the National Institute of Health. Code used in the analysis was custom-written in Matlab (Mathworks, Natick, MA) or R (R Foundation for Statistical Computing, Vienna, Austria).

**Behavioral Task**

The task began with the presentation of a fixation spot at the center of the screen. The monkeys were required to acquire and maintain fixation within three degrees of this spot until making a behavioral response. Immediately after fixation was acquired, both the rule cue and response targets appeared and remained on screen for the duration of the trial. The rule cue was a
colored border around the display indicating the feature of the stimulus the monkey needed to discriminate on the current trial. The animals were trained to perform two different rules: color and orientation. Each rule was associated with two different cues in order to distinguish rule-related activity from cue-related activity (see Figure S1A for example neurons encoding the rule and not the individual cues). After the presentation of the rule cue, the animals were required to maintain fixation for a ‘preparatory’ time-period before the onset of the stimulus. The duration of the preparatory period was randomized for each monkey (227 – 496 ms for monkey CC, 86 – 367 ms for monkey ISA; different ranges were the result of iteratively lowering the preparatory period during training while equalizing performance between animals).

At the end of the preparatory period, a test stimulus, oriented either vertically or horizontally and colored either red or blue, appeared at the center of the screen. The test stimulus consisted of small shapes (colored and aligned appropriately). The identity of these small items changed from session to session, ensuring the animals generalized the rules. After the onset of the stimulus, the monkeys were free to make their response: a single saccade to either the left or right target. The correct saccade direction depended on both the stimulus identity and the current rule in effect (Figure 1A). For the color rule, a red stimulus required a saccade to the right, a blue stimulus a saccade to the left. For the orientation rule, a horizontal stimulus required a saccade to the right, a vertical stimulus a saccade to the left. As each stimulus consisted of both an orientation and color dimension, the correct saccade for the two rules could either be the same (congruent trials) or different (incongruent trials). For example, a red vertical stimulus is incongruent, requiring a rightward saccade under the color rule and a leftward saccade under the orientation rule. In contrast, a red horizontal stimulus requires a rightward saccade for both rules. The majority (70%) of trials were incongruent, ensuring the animal always followed the rule. After the animal made the correct saccade, a juice reward was delivered via a juice tube. There was an inter-trial interval of approximately 100 ms before the next trial began.

Although the rule was cued on each trial, the rule in effect was blocked into groups of trials. Each block consisted of a minimum of 20 trials of the same rule. After 20 trials, the rule switched randomly – with a 5% or 10% chance of switching rules on each trial for monkey ISA and CC, respectively. The average block consisted of 39 trials of the same-rule for ISA and 30 for CC.

**Behavioral and Neural Analysis Methods**

A generalized linear model (GLM) was used to quantify the effect of multiple task-related covariates on the animals’ behavioral reaction time. A Gamma distribution was used in the model as it is ideal for fitting strictly positive data with a constant coefficient of variation, such as reaction times (McCullagh and Nelder, 1989). The link function, which defines a non-linear transformation between the linear predictors and the mean of the observations, was chosen to be the log function to enforce the requirement that reaction times be strictly positive.
A complete model was developed, fitting the reaction time with the all task-related covariates: the rule (color/orientation), preparatory period, congruency of stimulus-response association across rules, monkeys, time in session, and whether it was a switch trial (see Supplemental Information for details).

A bias-corrected percent explained variance statistic (ωPEV) was used to evaluate neural selectivity. ωPEV determines the portion of variance of a neuron’s firing rate explained by a particular task variable (e.g. the current rule) but is analytically corrected for upward bias in percent explained variance with limited observations. Significance was determined by a permutation procedure (see Supplemental Information for details).

**Synchrony Analysis Methods**

The local field potential (LFP) was transformed into the time-frequency domain using Morlet wavelets. Synchrony was estimated by computing the spectral coherence between pairs of electrodes. Significant differences in coherence between the two rules were determined with a permutation test. The null-hypothesis is that no significant difference exists between rules, therefore a null-distribution was generated by permuting color and orientation trials and recalculating the coherence (this process was repeated at least 100 times for each pair of electrodes). The mean and variance of this null-distribution was used to estimate the likelihood of the observed synchrony (captured by a z-score statistic). Z-scores greater than 1.96 or -1.96 indicated significant changes in coherence for the color and orientation rule, respectively (see Supplemental Information for details). Time-frequency regions of interest (e.g. the ‘alpha’ and ‘beta’ bands) were defined such that they encapsulated the peaks in rule-selective changes in synchrony (Figures 2 and S3). Although the bands were not pre-defined they closely follow the alpha and beta-bands defined in other studies, supporting conclusions about common mechanisms (see Discussion).

Phase locking value (PLV) was used to estimate spike-field synchrony. The phase-locking of task-relevant neurons (as identified by ωPEV, see above) to the LFP of electrodes participating in either the color or orientation network was estimated in a 200 ms window around the time of stimulus onset (-50 ms to 150 ms). In order to correct for the strong sample size bias in estimating spike-field synchrony, a stratification procedure was used (requiring 200 spikes in the window). Significant differences were determined by a permutation test, as above (see Supplemental Information for details).

The relationship between rule-dependent LFP synchrony and reaction time was determined by first regressing-out the effect of preparation time on reaction time (see Supplemental Information for details). The resulting reaction time residuals were sorted into ‘fast’ and ‘slow’ trials (defined as the 65th-95th and 5th-35th percentile of the residual distribution for each session, respectively). As above, a permutation test was used to estimate a z-score of the observed rule-selective differences in synchrony (see Supplemental Information for details).
Significant differences in rule-selectivity between fast and slow trials were determined by comparing the average absolute z-score in the beta (or alpha) frequency bands using a Wilcoxon signed rank test. To preclude dependence between electrodes recorded in the same session, we bootstrap resampled the electrode pairs 1000 times. After establishing rule-selectivity was stronger on average in the alpha and beta bands respectively, we examined rule-selectivity for differences over time by testing for differences in rule-selectivity at each time point, again using a Wilcoxon signed rank test (see Supplemental Information for further details).

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EKM conceived of and designed the experiment; CD designed the experiment, trained monkeys and collected neural data; TJB and ELD conceived of, implemented, and executed data analysis; TJB, ELD, DB and EKM wrote the manuscript.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes 4 Supplemental Figures and Supplemental Methods

**REFERENCES**


Author Information

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**FIGURES**

**Figure 1.** Task design and behavioral performance. (A) Task timeline. Eye position indicated by blue circle. Animals initiated trial by fixating the center dot. Following presentation of a border-cue indicating the rule, the stimulus was presented. The animal integrated the rule and stimulus in order to make a decision about the required saccade: under the color rule, red stimuli meant saccade left and blue meant saccade right; under the orientation rule, vertical meant saccade right and horizontal meant saccade left. The rule in effect was blocked and switched randomly after a minimum of 20 trials. (B) An asymmetric cost was observed when switching between rules, reflected in the speed at which the animals performed the task. Switching from orientation to color was significantly slower, but no cost was observed when switching from color to orientation. This suggests orientation was behaviorally dominant. All error bars are SEM. ***p ≤ 10^{-3}, ** p ≤ 0.01, *p ≤ 0.05
Figure 2. PFC neurons encode task-relevant information, including the current rule and stimulus. (A) Information about the current rule (black line) is captured using a bias-corrected percent-explained variance statistic (y-axis) and is determined in a sliding-window across the trial (x-axis). Shaded region indicates 95% confidence interval. As the rule often repeated on consecutive trials (see Figure 1A) there was some expectancy of the rule encoded by PFC neurons before rule-cue onset (although not significant across the population of recorded PFC neurons). (B) PFC neurons encode stimulus identity, both its orientation (green line) and color (blue line). Shaded regions indicate 95% confidence interval. Information about the orientation of the stimulus was more strongly represented across the population, possibly leading to the behavioral dominance of the orientation rule (see Figure 1B).
Figure 3. Rule-selective Synchrony in PFC. (A) Synchrony between electrodes within prefrontal cortex differs for rules. Synchrony is quantified by the coherence in simultaneously recorded local field potentials during each rule. The difference in synchrony (rectified to capture synchrony differences that prefer either rule) was compared to a trial-shuffled null distribution, resulting in a z-score of observed rule difference (color axis). Absolute synchrony differences are shown across time relative to stimulus onset (x-axis) and frequency (y-axis). Two time-frequency regions of interest (ROI) are seen – an ‘alpha’, 6-16 Hz, pre-stimulus ROI (solid outline) and a ‘beta’, 19-40 Hz, peri-stimulus ROI (dashed outline). (B) Percentage of recorded pairs of electrodes with a significant rule-preference during the ‘alpha’ and ‘beta’ time-frequency regions of interest (solid/dashed outlines in A). Significantly more electrode pairs prefer color within the alpha ROI and orientation within the beta ROI.
Figure 4. (A) Individual electrode pairs in the beta ROI are highly synchronous and show significant rule-dependent change. Coherence between rule-dependent pairs of electrodes (pink and purple crosses, main panel; group averages, solid circles) in the beta ROI was high overall (cumulative probability distribution, bottom panel) and generally reflected a 10% or greater change in coherence over the non-preferred rule (histogram, right panel) compared to non-rule preferring electrode pairs (grey x’s, main panel). (B) Average difference in coherence between preferred and non-preferred rules for all beta ROI electrode pairs.
Neurons encoding task-relevant information were more synchronized with the rule-selective ensemble preferring the current rule. Phase-locking of (A) stimulus-selective neurons and (B) rule-selective neurons to electrodes that either participated in the color-preferring ensemble (pink) or orientation-preferring ensemble (purple). Only electrodes that were exclusive to either ensemble were used (i.e. those electrodes participating in both ensembles were excluded). Phase-locking is shown for both orientation trials (left) and color trials (right). Shaded regions indicate 95% confidence intervals. Significant differences in phase-locking between the two ensemble is indicated at each frequency tested (*, p<0.05; **, p<0.01).
Figure 6. Independent, Rule-specific PFC Ensembles. Ensembles within PFC can be identified by rule selective synchrony in the peri-stimulus ‘beta’ ROI (dashed outline). One ensemble is more synchronous during orientation trials (A, left). This difference is significantly greater than expected by chance (B, left). A separate ensemble of electrodes is more synchronous during color trials (A, right). Again, this difference is significant (B, right). Alpha-band synchrony is observed in the orientation ensemble during the competing color rule (left panels, orange/pink), but not in the color ensemble (right) or during the orientation rule (Figure 2B). Axes are the same as Fig. 3A, but now color axes are no longer rectified: orange/pink reflects greater synchrony during color rule trials, blue/purple during orientation rule trials. Please note the color axis of (B) is intentionally non-linear, showing only significant rule selectivity, beginning at a z-score of +/-1.67 (p=0.05) and fully saturated at +/-1.97 (p=0.01).
Figure 7. Strength of prefrontal synchrony selectivity correlates with reaction time. Trials in which the monkeys responded faster (left) showed stronger rule-selective synchrony in the ‘alpha’ and ‘beta’ regions of interest compared to trials with slower reaction times (right). Green lines indicate reaction time quartiles and white lines indicate the corresponding preparatory period quartiles. Black lines on faster-reaction time trials (left) indicate when synchrony in the alpha and beta-frequency bands (gray and black diamonds, respectively) was significantly higher than synchrony during slower-reaction time trials.
Supplemental Information for

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This PDF file includes: Supplemental Figures 1 to 4, Supplemental Table 1, Supplemental Experimental Procedures, and Supplemental References 1-7
**Supplemental Figures**

**Figure S1, related to Figure 2.** (A) Smoothed (20 ms Gaussian) firing rate histogram an example PFC neuron that responds differentially to the color rule (pink lines) and orientation rule (purple lines). Solid/dashed lines indicate response to two cues for each rule. Black line indicates onset of rule cue, red line indicates median time of stimulus onset and green line indicates median reaction time. (B) Distribution of neuron selectivity in population of recorded PFC neurons. Selectivity was quantified for the rule (red), stimulus (green), and switch (blue). Neurons with selectivity in multiple categories combined the appropriate colors. (C) PFC neurons encode more information about the orientation of the stimulus than the color of the stimulus. Information is captured by a percent explained variance (PEV) statistic (y-axis) over time (x-axis). The black line shows the average difference for all recorded PFC neurons (95% CI shown by shaded region). Vertical red line indicates time of stimulus onset, green line indicates median reaction time (with IQR shown by dashed green lines).
**Figure S2, related to Figure 3.** (A) Synchrony within PFC differed depending on which rule was in effect. Color axis indicates the average z-score of the observed difference between synchrony during the color and orientation rule. Synchrony is shown relative to rule-cue onset (gray vertical line) across frequency (y-axis). As seen relative to stimulus onset (Fig. 2), two time-frequency regions of interest were found to carry rule information: a 6-16 Hz ‘alpha’ band (solid outline) and a 19-40 Hz ‘beta’ band (dashed outline). Median time and interquartile range of stimulus onset and saccade are shown in red and green, respectively. (B) Example local field potential traces of a prefrontal electrode pair (3 mm apart) participating in the orientation sub-network during example orientation (left) and color (right) trials. Local field potentials show peri-stimulus beta synchrony (purple) during orientation trials and rule-locked alpha synchrony (pink) during color trials. Red and green vertical lines indicate stimulus onset and time of saccade. (C) Rule-selective synchrony was observed on a high proportion of electrodes (y-axis) over all recorded distances between electrodes (x-axis). Error bars indicate STE over recording sessions. This distribution is not monotonically decreasing, arguing against the possibility the observed effects are due to volume conduction of local field potentials. (D) Spatial distribution and connectivity of synchronous electrodes. PFC electrode pairs within the rule-selective networks are spatially overlapping and often span the principal sulcus. Each circle represents an electrode location and each line represents significant rule selective coherence between two electrode locations. Electrodes from monkey ISA alone (precise anatomical locations for Monkey CC relative to sulci are unknown, only relative position were recorded). PS = principal sulcus, AS-inf = arcuate sulcus inferior, AS-sup = arcuate sulcus superior. (E) Average power distribution of the evoked field in both time and frequency, relative to stimulus onset (top) and rule cue onset (bottom), for all PFC electrodes. White traces show average evoked potential across all PFC electrodes (gray scale bar is 5 μV). The time-frequency response does not show the same structure as observed in the coherence between electrodes (Fig. 3), suggesting the observed rule-selective synchrony is not a direct modulation of the evoked potential.
Figure S3, related to Figure 4. Raw coherence plots showing rule-selective changes in synchrony between pairs of prefrontal cortex electrodes. Color axes indicate the average coherence observed for all rule-selective electrode pairs. Coherence is shown relative to stimulus onset (white vertical line) across frequency (y-axis). Black boxes indicate the two time-frequency regions of interest (ROI) found to carry rule information (see Figure 3). Median time and interquartile range of saccade is shown by vertical green lines. The rule preference of an electrode pair was defined by their beta-band ROI, as for Figure 6. (A) Coherence for all pairs of PFC electrodes that were rule-selective (regardless of rule preference) during color rule trials (left) and orientation rule trials (right). Coherence is dominated by 1/f component due to referencing to earth ground. As both color- and orientation-prefering electrodes (defined by the beta ROI as in Figure 6), changes in coherence are largely canceled by each ensemble. (B) Coherence for all rule-selective electrode pairs during their preferred (left) and non-preferred (right) rules.
Figure S4, related to Figure 6. (A) Trial by trial LFP power at different frequencies (y-axis) was determined for the 200 ms window preceding stimulus onset (indicated by bracket under the x-axis). The power observed on each trial was correlated with the firing rate of rule-selective neurons in 100 ms windows slid over time (x-axis marks the center of this window). The z-score of the resulting correlation is shown for both color-rule-prefering neurons (left) and orientation-rule-prefering neurons (right). (B) Difference in correlation observed for color-prefering neurons and orientation-prefering neurons. Greater pre-stimulus alpha synchrony was significantly more correlated with an increase in firing rate of color-selective neurons later in the trial (after the stimulus appeared and the animal was executing the rule).
### Supplemental Table

| Parameter          | Estimate  | Std. Error | Pr(>|t|) |
|--------------------|-----------|------------|---------|
| Intercept          | 195.00 ms | 0.18 ms    | < 2*10^{-16} |
| Rule               | Orientation | ----     |         |
|                    | Color      | -3.89 ms  | 0.18 ms | < 2*10^{-16} |
| Congruency         | Incongruent | ----     |         |
|                    | Congruent  | -1.88 ms  | 0.19 ms | < 2*10^{-16} |
| Switch Trial       | Repetition | ----     |         |
|                    | Switch (Orientation) | 0.04 ms  | 0.77 ms | 0.96 |
|                    | Switch (Color)       | 4.14 ms  | 1.11 ms | 1.61*10^{-4} |
| Monkey             | ISA         | ----     |         |
|                    | CC          | -26.14 ms | 0.20 ms |         |
| Preparation Time   | Per 1 ms increase in Preparation Time | -0.18 ms | 0.001 ms | < 2*10^{-16} |
| Session Time       | Early-in-Session | ----     |         |
|                    | Mid-in-Session   | -0.13 ms | 0.11 ms |         |
|                    | Late-in-Session  | -0.26 ms | 0.11 ms |         |

Table S1, related to Figure 1. Estimated coefficients (the first level is included in the baseline), standard errors, and p-values from the reaction time GLM. Since the log link function is used, effects are multiplicative rather than additive as in normal linear regression.
Supplemental Experimental Procedures

1. Behavioral Analysis

Both animals were able to perform the task with high performance, well above chance (~90% of trials were correct, p<2.20*10^{-16} for both animals and both rules, binomial test). Performance was maintained even after a switch in rule (Fig. 1B). However, consistent with human behavioral results (Monsell, 2003), there was a cost to switching between rules – both animals were significantly slower to respond when the rule in effect changed (Fig. 1B). This suggests the animals slowed their response to maintain accuracy in the task. To fully quantify the effect of task switching on the reaction time, a generalized linear model (GLM) was fit to the data. A GLM was selected to model the reaction time because it allows for non-constant variance, can account for the effect of multiple time-dependent covariates, and can treat strictly positive data. In particular, we chose the Gamma distribution for our model fit because it is well-suited to model strictly positive continuous data with a constant coefficient of variation (see main text reference McCullagh and Nelder, 1989). For GLMs, the link function defines a non-linear transformation between the linear predictors and the mean of the observations. We chose the log link function to enforce the requirement that reaction times be strictly positive. A complete model was developed, fitting the reaction time with the following covariates:

\[
\log(\text{Reaction Time}) = \text{Intercept} + \text{Rule (levels: Color, Orientation)} + \text{Normalized Preparatory Period} + \text{Congruency (levels: Incongruent, Congruent)} + \text{Monkey (levels: ISA, CC)} + \text{Session Time (levels: Early, Middle, Late in Session)} + \text{Switch Trial (levels: Repetition, Switch)} + \text{Switch Trial: Rule}
\]

Preparatory period was normalized by subtracting the mean preparatory period for each monkey.

As with all of our analysis, trials in which the monkeys broke fixation and trials in which the monkey did not make a consistent attempt – defined as successful fixating in at least 80% of the five trials before the current trial – were excluded. Outlier reaction times (<100ms and >313ms), determined by examination of the raw reaction times, were also excluded. Reaction time analysis included only correct trials.

Table S1 shows the estimated coefficients and standard errors. Similar to Fig. 1B, the detailed GLM revealed a significant effect of switching rules on reaction time dependent on which rule was in effect: the GLM fit found that the switch cost occurred when the monkey switched from orientation to color but not vice-versa (Table S1, Switch Trial). This, along with the stronger neural selectivity (see Fig. 2), suggests orientation might have been the ‘default’ behavior and may explain the differences observed in the synchronous sub-networks (Fig. 6).

Similar to human behavioral results, the preparatory period duration also had a strong effect on reaction time (Monsell, 2003). Longer preparatory periods result in faster reaction times and shorter preparatory periods result in slow reaction times (p<2*10^{-16}, GLM). Finally, the model shows congruent stimuli led to slightly faster responding (Table S1).

Although not included in the GLM, there was a slight decrease in time to respond during the first few trials following a switch into the orientation rule (this effect can be seen in Figure 1B). This likely reflects the animal’s increased certainty for the first few trials following a rule-switch (as they are guaranteed the rule repeats for a limited number of trials). Although this provides further behavioral support for a dominant orientation rule, the effect was not consistent across trials (only reaching significance at p<0.05 for a few trials).
2. Rule-, Stimulus-, and Switch-Selectivity in Prefrontal Cortex Neurons

Single neuron activity was simultaneously recorded from up to 16 electrodes placed across PFC (see above for recording locations and techniques). Waveforms were digitized at 40 kHz for isolation and then spike times for each isolated neuron was decimated to a 1 kHz sampling rate. We were interested in determining if neurons carried information about task-relevant features and if so, the timing of this information. Three features were of interest: the current rule in effect, the stimulus identity, and whether the current trial was a switch trial (versus a repetition). The rule was cued on every trial, although the identity of the rule was blocked into trials of at least 20 of the same rule (see Behavioral Task above). However, exactly when a switch occurred was random, and therefore unknown to the animal. Similarly, the color and orientation of the stimulus was not known to the monkey before stimulus onset. We assessed selectivity for all three task-relevant features for each neuron using a percent explained variance (PEV) statistic (see Fig. S1A for example rule coding neurons).

The PEV reflects how much of the variance in a neuron’s firing rate can be explained by the value of a particular task variable (e.g. whether the current rule is color or orientation). Typically, PEV is measured by eta-squared:

$$\eta^2 = \frac{SS_{Between Groups}}{SS_{Total}}$$

such as in an analysis of variance. Where $SS_{Total} = \sum_i^N (x_i - \bar{x})^2$ and $SS_{Between Groups} = \sum_{group}^G \eta_{group} (\bar{x}_{group} - \bar{x})^2$. However, the eta-squared statistic has a strong positive bias, particularly for lower sample sizes. Therefore, we used the omega-squared statistic ($\omega_{PEV}$) for determining neural selectivity instead:

$$\omega^2 = \frac{SS_{Between Groups} - d.f.*MSE}{SS_{Total} + MSE}$$

where d.f. is the degrees of freedom (i.e. the number of groups, G, minus 1) and MSE is the mean squared error, $MSE = \sum_i^N (x_i - \bar{x}_{group})^2$. Omega-squared is an unbiased measure (Keren and Lewis, 1979), resulting in a zero-mean statistic when there is no information (e.g. baseline of Fig. S2 is zero). This is crucial for averaging the selectivity across a population of neurons.

The time course of $\omega_{PEV}$ was calculated in a sliding window (a Gaussian with 20 ms standard deviation for rule and switch information and 10 ms for stimulus information, allowing for greater temporal resolution). As used here, the $\omega_{PEV}$ statistic makes the assumption that neurons encode information by modulating their average firing rate within the analyzed window of time. Importantly, the statistic does not make any assumption about the consistency of neural response over time or the nature of the change relative to other time periods. To determine whether and when the observed level of $\omega_{PEV}$ was significantly different from chance, we used a randomization test. The association between neural activity and the identity of the task-relevant variable was randomly permuted and the $\omega_{PEV}$ was re-calculated. By repeating this process 1000 times a null distribution was constructed. A cluster-correction technique was used to correct for multiple comparisons across time. First, a time-varying threshold was set as the 95th percentile of the null distribution over time. Continuous periods of time when the observed $\omega_{PEV}$ exceeded the 95th percentile threshold were identified as clusters. The size of the cluster was then determined by integrating the area between the observed $\omega_{PEV}$ and the threshold. The same process was repeated for each randomly permuted $\omega_{PEV}$ time course. Only the maximum cluster size was taken for each $\omega_{PEV}$ permutation. This corrects for multiple comparisons across time (Nichols and Holmes, 2002) and creates a null distribution of cluster size. The observed clusters are then compared to the null distribution in order to determine the likelihood of observing a cluster of that size. Neurons were classified as carrying...
significant information if they contained at least one observed cluster with a low probability of occurring by chance \((p \leq 0.05)\).

Selectivity was determined for each neuron for all three task-relevant variables: rule, stimulus identity (either color or orientation), and switch/repetition. A significant number of PFC neurons carried information about each of the three variables (Fig. S1B). Individual PFC neurons often carried information about multiple dimensions of the task, with some neurons encoding all three (the white area of Fig. S1B).

### 3. Time course of Neural Selectivity

After the population of selective neurons were identified in each region, the time course of selectivity was determined for stimulus identity (Fig. 2B and S1C). We were interested in determining the time at which the average information across PFC’s population of selective neurons exceeded chance, and whether these times were significantly different for color and orientation information. First, each neuron’s selectivity (as measured by \(\omega_{PEV}\)) was normalized by the randomly permuted, null distribution to create a z-score of \(\omega_{PEV}\) over time. This allowed for selectivity to be weighted appropriately across the population. The time point when the population carried significant information was taken to be when this average z-\(\omega_{PEV}\) was significantly above zero (corrected for multiple comparisons across time) for at least 15 ms. It is important to note that the absolute time to significance is affected by non-physiological parameters (such as the threshold chosen or the smoothing kernel used). Therefore, all statements about timing are relative between different neural populations where these parameters were held constant.

In order to estimate the uncertainty about the time to significance, we used a bootstrapping procedure. A pseudo-population of neurons was created for each area by drawing randomly, with replacement, from the population of observed neurons. The time at which this pseudo-population exceeded chance was then determined. This process was repeated 1000 times in order to generate a distribution around the observed time to significance for each region. Following this process, we determined that orientation information occurred at 41.1 ms after stimulus onset, significantly earlier than color information (47.6 ms, \(p=0.0026\)).

### 4. Time-Frequency Decomposition of Local Field Potentials

The estimation of coherence during the two rules (Figs. 2, S2, 3, 4, and 6) and the estimation of spike-field synchrony (Fig. 5) rely on decomposing the local field potential (LFP) into its time-frequency components. The time-varying spectrum of the LFP was estimated by convolving the filtered signal with a series of Morlet wavelets:

\[
\psi(f, t) = Ae^{t^2/2\sigma^2}e^{2\pi ift}
\]

where \(t\) is time, \(f\) is the center frequency, \(A\) is a normalizing constant to ensure unitary power, and \(\sigma^2\) is the smoothness of the kernel in time. In time-frequency analyses, there is a necessary tradeoff between temporal and spectral resolution. Therefore, the smoothness in time (\(\sigma^2\)) is directly related to the smoothness in frequency, \(\sigma_f = \frac{1}{2\pi \sigma}\). The tradeoff between temporal and spectral resolution is captured by the constant \(q\), such that \(\sigma_f = f/q\). We set \(q = 3\) to balance good frequency resolution (FWHM \(\sim ¾\) of an octave) with good temporal specificity (FWHM \(\sim 9/8\) of that frequency’s wavelength) across a wide range of frequencies. For example, our ‘beta’ band (19-40 Hz) is smoothed in time with a Gaussian with a full-width half-max of 38 ms. No further smoothing in time or frequency was done for any of the spectral analyses. The choice of \(q\) directly impacts the
spread of rule-selective changes in synchrony both in time and frequency (e.g. Figure 3). In particular, in order to achieve good temporal resolution at low frequencies we necessarily lose some degree of frequency resolution, leading to a slightly wider ‘alpha’ band (6-16 Hz) then what is typically reported (8-12 Hz, see Discussion). Note that this does not impact the center frequency (10 Hz in both cases).

The convolution of the Morlet wavelet with the local field potential estimated both the amplitude and phase of the ongoing LFP signal for each frequency and for each time-point during the trial. Synchrony between two simultaneously recorded LFP signals was estimated using the coherence statistic:

$$C_{t,f}^{XY} = \frac{S_{t,f}^{XY}}{\sqrt{S_{t,f}^{XX} S_{t,f}^{YY}}}$$

Where $S_{t,f}^{XY} = X_{t,f} Y_{t,f}^*$ is the cross-spectrum for two time-frequency transformed signals $X_{t,f}$ and $Y_{t,f}$ and $S_{t,f}^{XX}$ and $S_{t,f}^{YY}$ are their respective power spectra. $X^*$ indicates the complex conjugate of $X$.

5. Identification of Synchronous Sub-networks

For each trial, the coherence between each pair of simultaneously recorded electrodes was determined for the color rule and orientation rules separately (see Fig. 3 for population distribution). Note that because we examined coherence on a trial-by-trial basis, extremely low frequency oscillations (< 3 Hz) are essentially “filtered out” of our analysis since one full cycle of the oscillation is slower than the length of the trials in the task (average trial length was 434 ms between monkeys). While oscillations at these frequencies are likely not functionally relevant on a trial-by-trial basis in this task, it is possible that these slower oscillations play a role across trials. However, our particular task and analysis leave us unable to comment on the their relevance as the oscillations may reflect the rhythm of the task itself.

A permutation test was used to determine for each pair whether synchrony in color and orientation was significantly different. The null hypothesis is that there is no difference between rules, and therefore the observed coherence is not the result of a special grouping of trials into color and orientation. Therefore, to generate a null distribution, trials were randomly assigned to either the color or orientation groups (with the relative number of trials in each group held constant) and the coherence statistic and its difference between groups was re-computed. This process was repeated 100 times to estimate the mean and variance of the difference statistic under the null hypothesis. These were then used to estimate the relative likelihood of our observed difference in coherence under the null hypothesis (quantified in the z-score of the coherence statistic). The average absolute-value of the z-coherence across the population of pairs of simultaneously recorded PFC electrodes can either be aligned on rule-cue onset (Fig. S2A) or stimulus-onset (Fig. 3A). Both alignments show two time-frequency periods of interest where the synchrony between electrodes significantly differed between the two tasks: a 6-16 Hz, ‘alpha’ band that is time-locked to the onset of the rule cue and ends around the time of stimulus presentation (solid outline in Fig. 3A and Fig. S2A) and a 19-40 Hz, ‘beta’ band around the presentation of the stimulus (dashed outline in Fig. 3A and Fig. S2A). The differences in time course of the observed rule-selective synchrony when aligning trials on stimulus (Fig. 3A) or rule-cue onset (Fig. S2A) suggests the beta band occurs around the stimulus onset while the alpha band follows rule-cue onset. The average coherence for each pair of electrodes within these regions of interest was compared to the null distribution (see
above), resulting in a z-score of the coherence (zCoh) observed for both the alpha and beta regions of interest. Based upon the zCoh for each pair, the pairs were classified as either being more synchronized during the color or orientation rule (117 pairs significantly preferred the orientation rule, 90 preferred the color rule; see Fig. 3B for population and Fig. S2B for an example electrode pair).

One possible source of the observed rule-selective sub-networks is that the onset of the stimulus evokes a potential that differs between the rules for each group of recording sites. We controlled for this by subtracting each electrode’s average evoked potential (for a given rule) from the local field potential recorded on every trial (of that rule). This was done before calculating the coherence statistic. Therefore, any remaining changes in coherence are due to trial-to-trial variability in the local field potential that co-varies within a subset of recording sites for each rule. In addition, the existence of two separate, but simultaneously observed, sub-networks, each with greater coherence during one of the learned rules, excludes a common, general source of this trial-to-trial variability (such as arousal).

6. Synchronous Sub-networks do not Reflect Differences in Evoked Potential

Two different mechanisms could underlie the observed rule-selective synchrony in the color and orientation networks. One possible mechanism is that the observed rule-selective coherence could reflect a preparatory process, similar to attending to the current rule in effect. Alternatively, the observed synchronous sub-networks could alter the processing of the stimulus (modulating the evoked field) in order to facilitate the execution of a given rule. Our current results provide evidence for a preparatory mechanism: although the beta-band synchrony occurred around stimulus presentation, Fig. 3A shows an early peak in coherence before the stimulus onset, excluding the possibility of a purely evoked response. We isolated this peak by defining the sub-networks using only the pre-stimulus beta-band coherence (i.e. a window of -50 to 0 ms instead of the -50 to 100 ms window previously used). Indeed, the majority of electrodes pairs were still significantly rule-selective in both sub-networks (81/117 for orientation, 55/90 for color). Finally, the time-frequency power distribution of the average evoked field itself does not show the same structure as our observed coherence (Fig. S2E), suggesting the frequency response of the synchronous sub-networks are not just modulations of the stimulus response.

5. Quantification of Synchronous Sub-network Structure

As noted in the main text, our results indicate that abstract rules are not only encoded by the activity of single neurons in frontal cortex, but also in the pattern of synchronous activity within a sub-network. In addition to being rule-selective, these sub-networks showed non-random structure. For the orientation network each ‘node’ (i.e. recording site) in the network was synchronized with an average of 2.57 other sites (in other words, the average ‘degree’ of the orientation sub-network was 2.57). In contrast, each recording site in the color network was synchronized with 1.76 other sites. The average number of possible pairs that an electrode could participate in was 5.05. The degree of both networks was greater than expected when compared to a randomly connected network with the same edge likelihood (p<10^-3, randomization test where the observed coherence values are randomly assigned to pairs of electrodes). In addition, the observed network degree was significantly greater than random networks generated by shuffling coherence values within a given recording day (a more stringent test, p<10^-3 for orientation, p=0.032 for color).

Although the orientation network had more pairs of synchronous recording sites, each site was synchronized with a greater number of other sites, resulting in less individual recording sites participating in the network (N =
91 for orientation, N = 102 for color). As noted in the main text, the two networks were not exclusive at the level of individual recording sites: the majority of recording sites that selectively increased synchrony during one rule with one set of electrodes also increased synchrony during the second rule with a different set of electrodes (N=53). However, to fully test this possibility, we also limited our analysis to recording sites that showed no rule-selective changes in local LFP power. Although this quartered our population of electrode pairs (N=108), we still found a highly significant number of pairs of these sites were synchronized in a rule-selective manner (p=0.0015 for orientation, p = 8.1*10^{-5} for color, binomial test). Furthermore, across the entire population of electrode pairs, there was no obvious correlation between the rule-preference (if any) of the local LFP power at each recording site in a pair and their rule-selective coherence. For example, 28% of pairs of recording sites where both electrodes, individually, showed an increase in beta LFP power during the orientation rule, were more synchronized with each other during the color rule, again arguing against rule-selective differences in local power as the sole explanation for the observed rule-selective synchrony and highlighting the dynamic nature of the observed sub-networks. As noted in the main text, such dynamic re-organization of neural activity is ideal for supporting cognitive flexibility.

Further support for our hypothesis that these rule-selective networks play a functional role comes from analysis of the electrode locations and their relative distances. Estimates of the area of integration for cortical field potentials vary from 250 μm to 3 mm (Berens, 2008; Katzner et al., 2009) meaning high spatial clustering of sub-network electrode pairs within this range would indicate our observed coherences and networks are spurious (although see Kajikawa and Schroeder, 2011 for a challenge to the locality of the field potential). However, half of the sub-network electrode pairs are greater than 3 mm apart (color sub-network interquartile range = [2 mm, 3 mm, 4.24 mm], orientation sub-network interquartile range= [3 mm, 3.16 mm, 5.62 mm]) and we observe rule-selective synchrony as far as 10 mm apart in both sub-networks (see Figure S2 for full distribution). Moreover, many of the sub-network electrode pairs (with known anatomical location) were located on opposite sides of the principal sulcus and there was little spatial difference between the networks (Fig. S2D). Therefore, even under more liberal estimates for field potential integration area (3 mm), our analysis of electrode locations indicates that the observed networks are not entirely the result of common field signals at nearby electrodes.

One final possibility is that there are remote processes in other brain areas generating fields that differentially affect the recorded electrodes, causing the observed differences in coherence. However, several observations about the nature of our networks discount this possibility. First, the interdigitated nature of the rule-selective sub-networks (Fig. S2D) argues against a remote process, because presumably, a remote process should affect electrodes in the same way spatially. This is not what we observed. We quantified this more carefully by examining the selectively of electrode pairs spatially located in-between rule-selective electrodes. Because “spatially in-between” can be difficult to define, we restricted our search to electrode pairs on the same columns, rows and diagonal of the recording array as the current electrode pairs (Recall that recording sites are spaced in a 1 mm grid located over the principal sulcus, see Fig. S2D). As reported in the main text, many electrode pairs had at least one pair of electrodes spatially interposed with either no differences in synchrony between the rules or the opposite preference. Second, we observed no rule-selective synchrony in the nearby anterior cingulate cortex, which should be affected by remote processes as well. Third, our results were qualitatively similar when using a common average reference instead of earth ground. Finally, as discussed in the main text, task selective neurons synchronize more with the electrodes that showed task selective coherence (Fig. 5) which would be unlikely if the coherences were not intrinsic to prefrontal cortex.
6. Alpha-Band Synchrony May Reflect aSuppressive Mechanism

Previous work suggests oscillations in the alpha-band (6-16 Hz) represent a de-selection process during sensation (see main text references, particularly Palva and Palva 2007). Our results extend this model to cognitive processing: we observe increase alpha-band synchrony in the sub-network of the ‘dominant’ orientation rule during the competing color rule. We test two predictions of this model. First, we show that greater alpha coherence is correlated with a faster reaction time (Fig. 6, see below for details on methods). Second, we show synchrony in the alpha-band during color trials is positively correlated with the strength of color rule representation later in the trial (Fig. S4). As coherence is a measure of correlation, it is difficult to estimate on a trial-by-trial basis. Therefore, for this analysis we used LFP power at a given frequency as our measure of synchrony. The LFP power on each trial was estimated for each frequency during a 200 ms window before the onset of the stimulus (i.e. during the preparatory period, see Fig. S4A). The trial-by-trial variability in this power was then correlated with the firing rate of rule-selective neurons on the same electrode. A shuffle-correction was used to remove the effect of correlations over time due to co-varying baselines. This process also determined the z-score of the observed correlation (z-correlation). The average z-correlation was determined for rule-selective neurons that either preferred the color-rule (greater firing rate during color trials over orientation trials, Fig. S4A, left) or rule-selective neurons that preferred the orientation-rule (Fig. S4A, right). As can be seen in Fig. S4B, left, alpha-power before stimulus onset was more strongly correlated the activity of color-preferring neurons later in the trial, after stimulus onset. This difference was significant (Fig. S4B, right, p-value determined by unpaired t-test between z-correlation values). Summary correlation statistics presented in the main text were taken from the first 100 ms after stimulus onset. The observed correlation between power and firing rate is consistent with our model: preparatory alpha power during color trials increases the strength of color-rule representations later in the trial, during rule execution.

7. Rule-Selective and Stimulus-Selective Neurons Synchronize with Currently Relevant Rule Sub-network

Synchrony between the spiking activity from individual neurons and the ongoing local field potential were estimated for simultaneously recorded, neighboring electrodes (N = 465 pairs). Spikes were taken from a 200 ms wide peri-stimulus time period starting 50 ms before the onset of the stimulus. This time-period was selected for when the greatest differentiation of rule-selective sub-networks is observed (Fig. 3). Within this time window spike-field synchronization was estimated using the phase-locking value (PLV) statistic:

\[ PLV(f) = \left| \frac{1}{N_s} \sum_{s \in S} e^{i \varphi(f, s_t)} \right| \]

Where \( \varphi(f, s_t) \) is the phase of the local field potential for frequency \( f \) at the time of the spike \( (s_t) \), as estimated from the wavelet-based time-frequency decomposition, \( N_s \) is the number of spikes, and \( S \) is the set of all observed spike times.

The phase-locking value is known to be strongly biased by the number of observations (e.g. a single spike would mistakenly be taken as perfect phase-locking). Therefore we required a minimum of 200 spikes to be observed for inclusion in the dataset. Furthermore, the total number of spike-phase estimates was balanced for all comparisons using a stratification procedure. When comparing across different neuron populations all estimates of PLV were made with the required minimum number of 200 observations. A null-distribution of phase-locking
values was estimated by shuffling the trial associations between the neural activity and LFP, disrupting any trial-by-trial co-variation, and recalculating the PLV. This process was repeated 100 times and the mean and standard deviation of the resulting distribution was used to normalize observed phase-locking values.

One hypothesis is that the observed rule-selective sub-networks act to dynamically structure neural activity in order to support the current behavior. To test this hypothesis we determined whether neurons involved in the task were significantly more synchronized to the local-field potentials of electrodes involved in the currently cued rule sub-network (Fig. 5). Both stimulus-selective (Fig. 5A) and rule-selective (Fig. 5B) neurons were significantly synchronized with the color- and orientation-preferring sub-networks in the beta-band. Furthermore, which network these neurons were synchronized to shifted with the current task: during execution of the orientation rule (Fig. 5A/B, left column) both populations of neurons were more synchronized to the orientation-preferring sub-network. This preference was reversed during color trials (Fig. 5A/B, right column).

8. Sub-network Synchrony Changes with Behavior

Our results suggest the observed rule-selective synchronous sub-networks encode the current rule and organize the activity of single neurons carrying task-relevant information (Fig. 5). If true, then the animal’s ability to perform the task should be correlated with the strength of synchrony in the observed sub-networks. In order to determine whether this was the case, we compared the rule-selective coherence in each sub-network for trials when the animal responded quickly or slowly (Fig. 6). This procedure is detailed here.

The largest impact on reaction time was the preparation time between rule-cue onset and stimulus onset. Longer preparation times resulted in faster reaction times and shorter preparation times resulted in slower reaction times. In addition, there were slight differences in the animal’s reaction time for the two rules (Table S1, Fig. 1B). However, we were interested in the relationship between the strength of synchrony in the rule-selective sub-networks and the animal’s behavioral performance, not the preparatory time or current rule. Therefore, we accounted for the effect of the rule and preparatory time on reaction time by regressing out their effect. Specifically, we fit the linear model $\log RT_i = A \ast (Preparatory\ Period_i) + B \ast (Rule_i) + C$ where log of the reaction time was used to stabilize the variance of the skewed reaction time distribution. After fitting this model, the residual difference between the observed reaction time and the fit reaction time captures the intrinsic variability in the animal’s performance. These residuals were sorted into ‘fast’ and ‘slow’ trials (defined as the 65th–95th and 5th–35th percentile of the residual distribution for each session, respectively) and the rule-selective coherence was determined, as before (see above).

As noted in the main text, synchrony in both the preparatory ‘alpha’ band and the rule-execution ‘beta’ bands was significantly greater when the animal performed the task quicker (Fig. 6). Strength of rule selectivity was determined by taking the absolute value of the average zCoh within the alpha and beta regions of interest. A Wilcoxon signed rank test compared the zCoh values for fast and slow reaction times at each time point during the trial. We required at least 2 consecutive time points for the rule selectivity to be considered significant. As the black (significant beta differences) and grey lines (significant alpha differences) on Figure 6 indicate, faster reaction times were accompanied by stronger selectivity in both the alpha and beta bands (p<0.05, Bonferonni corrected for multiple comparisons) before the onset of the test stimulus. These results support the hypothesis that the observed synchronous sub-networks are involved in representing and implementing the current rule.
Supplemental References


