Temporally irregular mnemonic persistent activity in prefrontal neurons of monkeys during a delayed response task

Albert Compte$^{1,2}$, Christos Constantinidis$^3$, Jesper Tegnér$^{1,4,5}$, Sridhar Raghavachari$^1$, Matthew V. Chafee$^3$, Patricia S. Goldman-Rakic$^3$ and Xiao-Jing Wang$^1$

$^{1}$Volen Center for Complex Systems, Brandeis University, Waltham, MA 02454
$^{2}$Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, 03550 San Juan de Alicante, Spain
$^{3}$Section of Neurobiology, Yale School of Medicine, New Haven, CT
$^{4}$Stockholm Bioinformatic Center, SCFAB, S-10691 Stockholm, Sweden
$^{5}$Computational Biology, Department of Physics, Linköpings Institute of Technology, S-581 83 Linköping, Sweden

Running head: Temporally irregular neuronal PFC activity during working memory

Contact Information: Albert Compte
Instituto de Neurociencias
Universidad Miguel Hernández-CSIC
03550 San Juan de Alicante
Spain
Tel: +34 965919367
Fax: +34 965919547
Email: acompte@umh.es

Keywords: principal sulcus, working memory, bursting, oscillation, power spectrum
ABSTRACT

An important question in neuroscience is whether and how temporal patterns and fluctuations in neuronal spike trains contribute to information processing in the cortex. We have addressed this issue in the memory-related circuits of the prefrontal cortex by analyzing spike trains from a database of 229 neurons recorded in the dorsolateral prefrontal cortex of 4 macaque monkeys during the performance of an oculomotor delayed-response task. For each task epoch, we have estimated their power spectrum together with inter-spike interval histograms and autocorrelograms. We find that (1) the properties of most (~60%) neurons approximated the characteristics of a Poisson process. For about 25% of cells, with characteristics typical of interneurons, the power spectrum showed a trough at low frequencies (< 20 Hz) and the autocorrelogram a dip near zero time lag. About 15% of neurons had a peak at < 20 Hz in the power spectrum, associated with the burstiness of the spike train; (2) a small but significant task dependence of spike-train temporal structure: delay responses to preferred locations were characterized not only by elevated firing, but also by suppressed power at low (< 20 Hz) frequencies; and (3) the variability of inter-spike intervals is typically higher during the mnemonic delay period than during the fixation period, regardless of the remembered cue. The high irregularity of neural persistent activity during the delay period is likely to be a characteristic signature of recurrent prefrontal network dynamics underlying working memory.
INTRODUCTION

The ability to hold and manipulate information in memory for short periods of time has been termed working memory and constitutes a crucial element of higher cognitive functions. Imaging techniques in humans (Jonides et al., 1993; Courtney et al., 1997; McCarthy et al. 1994; Leung et al. 2002) and electrophysiological experiments in monkeys (Fuster and Alexander, 1971; Kubota and Niki, 1971; Funahashi et al., 1989) have identified the prefrontal cortex as an area critically involved in working memory. A significant proportion of prefrontal neurons show firing rates that are tuned to the position or identity of a sensory stimulus and maintain this tuning persistently across the delay period of a working-memory task, when the stimulus is no longer available to the senses. This tuned persistent activity is regarded as the neural correlate of working memory (Goldman-Rakic 1987, 1995; Wang 2001).

Most previous neurophysiological studies in monkeys have investigated the effect of different stimuli and their maintenance in memory in terms of changes in the mean discharge rate evoked by these events. However, temporal characteristics of the spike train, for example oscillations, inter-neuronal synchronization, or bursting could, in principle, convey information about task related events. Oscillatory discharges in particular have received considerable attention over the last decade as the mediator of such functions as attention, figure-ground segregation, memory maintenance and conscious awareness (Crick, 1994; Singer and Gray, 1995; Treisman, 1996). Oscillations have been reported in the primary visual cortex of the cat and monkey (Eckhorn et al., 1988; Gray et al., 1989;
Frien et al., 1994; Livingstone, 1996; Friedman-Hill et al., 2000), while other studies have contested their prevalence and significance (Ghose and Freeman, 1992; Young et al., 1992; Kiper et al., 1996). Fewer studies have examined the existence and possible role of oscillations outside the primary visual cortex, also with conflicting positive (Kreiter and Singer, 1996; Fries et al., 2001), and negative (Tovee and Rolls, 1992; Bair et al., 1994; Cardoso de Oliveira et al., 1997) results. Notice, however, that positive results are usually observed in population measures like multiunit recordings or local field potentials, while single unit activity typically shows little oscillatory behavior. It is conceivable that temporal properties not observed at the neuronal level could emerge from the population collective dynamics (Csicsvari et al. 1999, Brunel 2000, Brunel and Wang 2003).

Recently, there are reports suggesting that oscillatory firing may be especially significant in neuronal discharges associated with active memory maintenance in delayed response tasks, compared with baseline spontaneous activity (Pesaran et al., 2002; Tallon-Baudry et al., 2001). Persistent activity during the memory period is believed to be sustained by reverberatory dynamics in a recurrent network (Amit and Brunel, 1997; Camperi and Wang, 1998; Lisman et al., 1998; Wang, 1999; Compte et al., 2000; Durstewitz et al., 2000; Wang 2001) and it is reasonable to assume that a strongly reverberatory network could promote oscillatory firing under certain conditions, such as when the recurrent excitation is much faster than feedback inhibition (Wang, 1999; Compte et al. 2000; Tegnér et al. 2002). To examine this possibility, we have here applied unbiased analytic methods to investigate the presence of rhythmic activity and its possible correlation with task-related events on a database of neurons recorded in the dorsolateral
prefrontal cortex of monkeys as they performed an oculomotor working memory task.
METHODS

Animals

The data used for this study were recorded from four male rhesus monkeys (*Macaca mulatta*), weighing 6-12.5 kg. Detailed descriptions of the experimental techniques as well as the firing rate modulations of recorded neurons in terms of their firing rate modulations have been published previously (Chafee and Goldman-Rakic, 1998; Constantinidis et al., 2001). Recordings were obtained from the dorsolateral prefrontal cortex that included both the frontal eye fields and area 46 in all four monkeys. Two animals (JK and AZ) had additional recordings from areas 7a and 7ip in the posterior parietal cortex. Monkeys were implanted with a scleral eye coil to monitor eye position and a head bolt to stabilize the head during performance of the task (Judge et al., 1980). Surgery and training protocols were in accord with guidelines set by the National Institutes of Health, reviewed and approved by the Yale University Animal Care and Use Committee.

The animals were trained to perform an oculomotor delayed response task (ODR). They initiated a trial by fixating a central point 0.1-0.2° in size, for 1 s and continued to maintain fixation as a cue stimulus subtending 0.5-1° flashed for 500 ms at an eccentricity of 13-14°. A delay period lasting 3 s followed the presentation of the cue, at the end of which the fixation point was extinguished and the monkeys were trained to make a saccade to the remembered target location. The cue could appear at 1 of 8 possible locations, equidistant around the fixation point, and randomly interleaved from trial to trial. Ten to twelve correct trials were typically recorded for each location.
Neurophysiological Recording

Neuronal activity was monitored using varnish-coated tungsten electrodes of 1-4 MΩ impedance, at 1 kHz, as described in Chafee and Goldman-Rakic (1998) and Constantinidis et al. (2001). One or more electrodes were placed in stainless steel guide tubes and advanced into the cortex with the aid of a microdrive. Glass-coated Elgiloy metal microelectrodes (0.6-1.5 MΩ at 1 kHz) were used for a few experiments. Neuronal activity was amplified and band-pass filtered between 400 Hz and 10 kHz. For two animals (MAR and COD), the conditioned signal was sampled with a temporal resolution of 30 µs and waveforms were sorted into separate units using a template-matching algorithm (CED, Cambridge, UK). The data acquisition system was configured to classify voltage deflections into separate spikes if the peaks of the deflections were separated by approximately 1.5 ms, which is shorter than the absolute refractory period of most cortical neurons. Action potentials occurring at shorter intervals could not be correctly resolved. For the other two animals (JK and AZ) neurophysiological activity was sampled at 2 ms resolution and action potentials were classified into separate units using a waveform discriminator (Signal Processing Systems, 8701 waveform discriminator, Prospect, South Australia). In practice, action potentials could not be stored as successive samples using this system, so the minimum separation of recorded action potentials was 4 ms.

Neurons were classified as Regular Spiking (RS) or Fast Spiking (FS), putative pyramidal neurons and interneurons, respectively, based on their spike width and baseline firing rate, as described in detail elsewhere (Constantinidis and Goldman-Rakic, 2002). Briefly, plotting the baseline firing rate against the width of spike waveform of each unit
revealed two classes of neurons, one characterized by a longer spike duration and low firing rate and one characterized by shorter spike duration and high firing rate. Approximately 70% of the neurons located in the extreme of the two distributions were classified as FS or RS, respectively.

**Neuron selection**

Neurons analyzed in this study were selected from our database of recordings if they satisfied two criteria. They displayed activity that was significantly elevated during the delay period relative to the baseline and also exhibited spatial tuning, i.e., this activity varied significantly for the 8 spatial locations tested with the ODR task (ANOVA test, p<0.05). Some of the analytical techniques for evaluating the spectral properties of the spike train, described below, required a minimum total number of spikes. Given that 10-12 trials were collected per condition in most experiments, this essentially constituted a third requirement of firing rate greater than approximately 4 sp/s. A total of 229 prefrontal cortical neurons from the four monkeys (169 from COD, 22 from MAR, 21 from JK, and 17 from AZ) which met these criteria were analyzed. For these neurons, we computed autocorrelograms, inter-spike interval (ISI) histograms and power spectra for three conditions: (1) the last 500 ms of the fixation period; (2) delay period activities for preferred targets, i.e. cue locations that elicited a firing rate above the average delay rate across all cues (solid circles in tuning curves of Fig. 4); and (3) nonpreferred cue positions, those eliciting firing rates below the average delay rate (hollow circles in tuning curves of
Fig. 4). Additionally, we also applied our analyses to 27 available multiunit recordings (from 2 monkeys) from which 2 or 3 units could be typically isolated.

**Power spectrum analysis**

In all cases power spectra were computed for each spike train considered, normalized by the expected power of a Poisson spike train at the same firing rate, and then averaged to reduce the variance. Averaging was thus always performed in the frequency domain and on normalized spectra. We computed the power spectrum of a neuronal spike train as described by Jarvis and Mitra (2001). These methods assume the stationarity of the point process analyzed. For this reason, we cut all the spike trains in overlapping 0.5-second windows and treated each window as a different trial of an independent stationary point process. Implicit is thus the assumption that non-stationarities in the data appear only at time-scales longer than 0.5 seconds. A continuous process was built from the spike train by replacing each spike by a delta function and subtracting the average rate, and we subsequently processed these data with Fourier transforms to obtain the power spectra.

The raw estimate of the spectrum, i.e. the magnitude-squared Fourier transform of the spike-train data is a biased and inconsistent (i.e. the variance does not decrease with increasing data length) estimate. Windowing the data with a suitable envelope function or taper reduces the bias. This taper function $h(t)$ is non-zero only within the time-window of the data and is properly normalized: $\int_0^\tau h(t)^2 dt = 1$ (Percival and Walden, 1993). In order
to reduce the variance of the estimate (the windowed estimator is distributed as \( \chi^2 \)), one can either average over nearby frequencies (lag-window averaging) or use a set of envelope functions that are a complete set of basis functions for time- and frequency-limited functions. One such set of taper functions are the Slepian functions: a set of orthogonal basis functions that maximize the energy in a given time-frequency interval. Since the windows are an orthonormal set, the estimates of the spectra from each window are independent, and hence their average results in a low-variance estimate of the spectrum. We computed Fourier transforms on our spike trains windowed by the first 4 tapers of the Slepian sequences (Percival and Walden, 1993). The power spectrum of the spike train estimated in this way was then normalized to the expected power of a Poisson spike train of the same mean rate and spectra from multiple behavioral trials were averaged together. The variance of the normalized spectra was calculated using a jackknife method (Thomson and Chave, 1991). The jack-knife variance is a powerful method of assessing the variance of samples without any assumptions about the underlying distributions, similar to the bootstrap. The resulting jack-knife statistic is \( t \)-distributed. For Poisson data, the jack-knife variance is a good approximation of the theoretical variance. Specifically, a series of spectrum ensembles were generated by leaving out one trial at a time and averaging over the remaining spectra. The jackknife variance was estimated as the variance across ensembles scaled by the number of degrees of freedom used to compute each member of the ensemble \( \times (N_{\text{trials}} - 1) \). The jackknife variance is a robust estimate, even in the presence of non-stationarities.
Coefficient of variation

From the series formed by the inter-spike intervals (ISIs) of each spike train we computed two measures of the ISI variability: the coefficient of variation (CV) and its local counterpart, \( CV_2 \). CV is obtained directly from the ISI histogram, by dividing its standard deviation by its mean, \( CV = \left\langle \frac{(\text{ISI} - \langle \text{ISI} \rangle)^2}{\text{ISI}} \right\rangle^{1/2} \). The CV measures how close the spike train is to an ideal Poisson spike train (for which \( CV = 1 \)), assuming that the data are stationary. Since this assumption was violated by many of our datasets, we used a second measure of ISI variance, which we refer to as \( CV_2 \). \( CV_2 \) is computed by comparing each ISI (ISI\(_n\)) to the following ISI (ISI\(_{n+1}\)) to evaluate the degree of variability of ISIs in a local manner (Holt et al. 1996):

\[
\left\langle CV_2 \right\rangle = \frac{1}{N-1} \sum_n CV_2(n), \quad CV_2(n) = \frac{2|\text{ISI}_{n+1} - \text{ISI}_n|}{\text{ISI}_{n+1} + \text{ISI}_n}
\]

A Poisson spike train has \( \left\langle CV_2 \right\rangle = 1 \). For a Poisson spike train with absolute refractory period \( t_r \), however, \( \left\langle CV_2 \right\rangle \) is always smaller than 1. A straightforward calculation departing from the calculations in (Holt et al. 1996) yields for a Poisson spike train of rate \( r \) and absolute refractory period \( t_r \):

\[
\left\langle CV_2 \right\rangle = 1 - rt_r \int_{-\infty}^{\infty} e^{-x} \frac{e^{-x}}{rt_r} dx,
\]

which is a non-monotonic function of \( rt_r \). This formula is used to compute \( t_r \) once \( \left\langle CV_2 \right\rangle \) and \( r \) are known for a
given spike train, these values then define the Poisson spike train with absolute refractory period $t_r$ that best approximates the data. Furthermore, the probability density of the quantity $CV_2(n)$ for adjoining ISIs is given by

$$P(CV_2) = \frac{1}{2} e^{-rt_{r,2(2-CV_2)}} + \frac{r t_{r,2}}{2} e^{-rt_{r,2}} \int_{rt_{r,2}}^{\infty} \frac{e^{-x}}{x^{CV_2/(2-CV_2)}} dx$$

Thus, for a Poisson spike train with absolute refractory period, $CV_2$ depends on the firing rate in a non-trivial fashion.

**Bursting measures**

In order to assess the degree of burstiness of a given collection of spike trains, we binned all inter-spike intervals (ISIs) and defined the following measures: fraction of ISIs smaller than 5 ms relative to the fraction of ISIs smaller than 5 ms in a Poisson spike train of the same mean firing rate ($B_1$), fraction of ISIs smaller than the mean ISI divided by 10 ($B_2$), or fraction of ISIs smaller than 5 ms ($B_3$). Measures $B_1$ and $B_2$ applied to a Poisson spike train do not change as its rate is varied (in this sense, these measures are rate-independent). However, the measure of burstiness, $B_3$, of a Poisson spike train grows with its firing rate. All the results reported here for the autocorrelogram measure $A$ (see below) were paralleled by $B_1$ and $B_2$. We therefore chose to report only the results for $A$ for the sake of clarity.
**Autocorrelograms**

We calculated the autocorrelogram of a spike train as the histogram of the time intervals between any two spikes from which the shuffle predictor was subtracted (the shuffle predictor was obtained as the average crosscorrelogram of spike trains corresponding to different trials) and the result was normalized to the standard deviation of the shuffle predictor at each time lag (see Aertsen et al. 1989). Thus, the autocorrelogram is expressed in units of the shuffle predictor standard deviations. Our measure of burstiness/refractoriness in a spike train ($A$) is computed from the autocorrelogram: it is the average height of the central bins that correspond to a time lag smaller than 5 ms.

**Cell type classification**

We used two criteria to classify spike trains as "Poisson", "refractory" or "bursty": The departure of the power spectrum in the 5-60 Hz band relative to a Poisson spectrum of equal firing rate by more than one standard deviation (bursty: excess power, refractory: suppressed power, Poisson: no structure more than one standard deviation away), and the average height of the central autocorrelogram bins ($A$). We classified spike trains as bursty if their power spectrum exceeded the Poisson spectrum by more than one standard deviation and $A$ was greater than 1 (central peak more than one standard deviation above the shuffle predictor). Spike trains were classified as Poisson if their power spectrum did not deviate from their Poisson power spectrum by more than one standard deviation and the value of $A$ fell between -1 and 1. Finally, we classified spike trains as refractory if their power spectrum was suppressed relative to the Poisson spectrum by more than one standard deviation and $A$ was smaller than -1 (central trough more than one standard deviation away).
deviation depressed with respect to the shuffle predictor). Notice that some power spectra could satisfy both the "burtsy" and the "refractory" criterion and lead to a non-disjoint classification of spike trains. In those cases, we classify spike trains as "bursty" since spectral peaks are often accompanied by power suppression at adjoining frequencies. In order to classify one neuron according to its spiking characteristics as "Poisson", "refractory" or "bursty", we required that the corresponding classification criterion was satisfied for spike trains recorded in at least one of the three task conditions (fixation, delay after preferred targets and delay after non-preferred targets). Notice that this classification is non-exclusive: a given cell can belong to various classes. The use of the power spectrum shape to determine the burstiness of a spike train has been discussed by Bair et al. (1994).

Spectral classification techniques: Isomap

In order to compare spectra in an unbiased way we used the classification scheme Isomap, described by Tenenbaum et al. (2000). As in the case of multi-dimensional scaling (Mardia et al., 1979), the purpose of Isomap is to provide a visual representation of the pattern of proximities (i.e., similarities or distances) among a set of objects (here, power spectra). However, in contrast to multi-dimensional scaling (MDS), Isomap does not implicitly assume that distances are taken on an Euclidean manifold but allows for the more general case of geodesics on smooth non-Euclidean manifolds. Thus, it is a powerful method to detect the underlying structure of the data without making a priori assumptions about its classifying aspects, and it succeeds in many more cases than classical MDS. In brief, pairwise distances between spectra are determined by using the Itakura-Saito distance:
\[ D = \log(\langle S_1(f)/S_2(f) \rangle) - \log(S_1(f)/S_2(f)) \] , where \( \langle \cdot \rangle \) denote an average over frequencies. The Itakura measure is a standard tool for calculating distances between spectra for automatic speech processing. Only segments connecting each spectrum to its 4 closest neighboring spectra are then retained to construct a graph, along which all pairwise distances among spectra are recalculated. The table of distances along this graph is then fed into a classical MDS algorithm (Mardia et al., 1979) to find the best 2-dimensional embedding that preserves the geodesic distance. The output of Isomap consists of the coordinates of each spectrum in this 2-dimensional space. We will focus on the axis that accounts for most of the variance in the population of power spectra (in our graphs, the x-axis) and we will use this coordinate for each spectrum to compare its position in relation to other spectra and thus assess the significance of task-dependencies in the shape of the power spectra.

**Statistical tests**

We used various statistical tests in order to assess the statistical significance of our results. When we used ANOVA tests, we first determined whether the data distributions, or their transformed values, deviated significantly from a Gaussian distribution (Jarque-Berra test, \( p<0.01 \)). We also tested the hypothesis of homogeneity of variances of our distributions through Bartlett's test, and we made sure that it could not be rejected at the significance level \( p<0.01 \). Interaction effects of the factors considered in 2-way ANOVA tests were further analyzed by looking at the corrected means (Harwell, 1998). When statistical tests showed that the data (or any transformation checked) was not compatible with a Gaussian
hypothesis within our significance level, we used the non-parametric Kruskal-Wallis test (non-parametric version of one-way ANOVA) to assess significance, and we represented the data dispersion as median ± semi-interquartile range. Post-hoc analyses were performed by means of a multiple comparison procedure.

All our data analysis was performed in the software package Matlab 6.
RESULTS

Discharge Patterns

A wide range of discharge patterns was observed among the 229 neurons in our data base. Stereotyped patterns of discharge were classified as Poisson, refractory and bursting as assessed through their autocorrelograms and their power spectra (see Table 1). The most frequent pattern in our database (Poisson discharge) had a virtually flat power spectrum, approaching that which would be expected for an ideal Poisson spike train. An example neuron is shown in Fig. 1A, exhibiting a flat autocorrelogram, an exponentially decaying inter-spike interval histogram, and a flat power spectrum. Approximately 89 percent of neurons (204/229) displayed (see Methods for details on neuronal classification) a flat power spectrum ranging within one standard deviation of the expected power of a Poisson spike train with the same firing rate. Sixty eight percent of neurons (155/229) had a central peak in the autocorrelogram that did not differ by more than one standard deviation (see Methods) from their shuffle predictor (-1 < A < 1). In 63 percent of neurons (145/229), this stereotyped discharge pattern was present in both measures - the power spectrum, and the autocorrelogram. This is the subpopulation of neurons classified as "Poisson" for our subsequent analyses.

{Figure 1 about here.}

Another pattern observed in the sample was characterized by autocorrelograms displaying a central dip and power spectra with significant troughs at low frequencies (Fig. 1B). A significant power spectrum trough in the band 5-60 Hz was identified in 38 percent
of our neurons (87/229). Central dips in the autocorrelogram (A<1) were clear in 39 percent of neurons (90/229). Neurons that could be characterized as "refractory" by both measures constituted 26 percent of all cells in our sample (60/229) and they constitute the class of "refractory" neurons in this paper.

Bursting behavior was also present in our database. A cell displaying a representative bursting power spectrum is shown in Figure 1C. The bursting behavior can be inferred by the sharp central spike of the autocorrelogram, the long tail of the ISI histogram and by the power spectrum peak in the 20 Hz range. Bursting in this example did not depend on the epoch of the task, despite the marked differences in firing rates during the fixation period and the delay period, or on whether activity was recorded for the preferred or non-preferred target. 17 percent of neurons (39/229) displayed power spectra with the power enhanced significantly over the expected value for a Poisson spike train in the band 5 Hz to 60 Hz. Autocorrelograms showed a marked central peak (A > 1) in 42 percent of the neurons (96/229). Overall, 14 percent of the cells in our sample (32/229, henceforth constituting the class of "bursty" neurons) displayed a "bursting" discharge pattern according to both the power spectrum and the autocorrelogram.

**Task dependent modulation of inter-spike intervals**

We next tested whether the temporal patterns of prefrontal cortical neuron discharge varied systematically across different task conditions. Analysis of the coefficient of variation (either its global CV or local version $\langle CV^2 \rangle$, see Methods) was higher during the delay than the fixation period but it was not very different between the delay conditions
following preferred and non-preferred cues (Fig. 2). This finding indicated that the coefficient of variation of the inter-spike interval was not a function of firing rate alone, as one would expect to observe a similar CV for fixation period and delay period after presentation of a non-preferred target, which did not differ substantially in rate (13 Hz in fixation and 11 Hz in delay/non-preferred, average across all cells), and a significantly different CV for delays between preferred and nonpreferred targets, the rates of which were markedly different (18 Hz for delay/preferred and 11 Hz for delay/non-preferred, average across all cells). This is confirmed in the lower right panel of Fig. 2A, where CV and $\langle CV_2 \rangle$ are shown not to depend on the mean inter-spike interval (correlation coefficient -0.03 for CV, and -0.2 for $\langle CV_2 \rangle$). From the distribution of $CV_2$ for all adjoining ISIs across neurons belonging to each of the classes (Poisson, refractory and bursty in Fig. 2B, lower right panels), it shows that firing statistics of prefrontal neurons classified as "Poisson" are quite well described by a Poisson spike train with absolute refractory period, neurons in the "refractory" class have some systematic departures from this model but follow it qualitatively, and bursty neurons have a completely different $CV_2$ probability density, not captured by the model (as it should be expected). A second task-dependent effect was associated with the degree of bursting in the spike trains (Fig. 3). Based on the central peak in the autocorrelogram (A), spike trains in the delay period following a non-preferred target were typically more bursty than either the fixation or the delay period after a preferred target. This held also for neurons classified as Poisson or bursty. However, for cells in the "refractory" class the magnitude of the zero-lag dip increased significantly in
the delay period after a preferred cue as compared to the other two conditions (similar results were obtained from burstiness measures derived from the ISI histogram, not shown). These results indicate that some temporal patterns of spike trains, e.g. burstiness, are independent of firing-rate modulation.

{Figure 2 about here.}

{Figure 3 about here.}

Task dependent modulations of power spectra

To explore more systematically the modulations in temporal structure of spike trains, we focused on their power spectra. Power spectra can be normalized and error bars can be computed for each point, thus making them a convenient analytical tool for comparisons across task epochs and for determining significant differences. From visual inspection, marked changes in power spectra were not evident in the various epochs of the task. However, weak task modulations were apparent in many cases. Figure 4 shows three examples of task dependent modulations in the power spectrum shape observed in individual neurons of our database. The most frequent task-dependency (36/229 neurons, 16%) was a marked deepening of a low-frequency trough in the delay after a preferred cue (Fig. 4A). This was the most salient task-dependent effect observed when we averaged power spectra across the population of neurons (Fig. 5). The effect was consistent across all monkeys and was observed both in the dorsolateral prefrontal cortex, as shown here, as well as in the posterior parietal cortex (monkeys JK and AZ, data not shown).
In contrast, only a few cells showed power spectra with clear peaks. The neuron in Fig. 4B had a clear peak in the power spectrum that was slightly decreased during the delay period following a preferred target (dependency shared by 6/229 neurons, 3%). Another task-dependency observed (6/229 neurons, 3%) was an enhancement of a peak in the spectrum during the delay, following the preferred target (Fig. 4C).

An averaged spectrogram of 229 neurons was computed for trials in which the cue elicited a delay firing rate above average (preferred cue condition, Fig. 6A) and also for non-preferred cues (Fig. 6B). We observed very little difference between the spectral properties of neuronal firing before and after cue presentation except for a suppression of power at low frequencies (< 20 Hz) after the presentation of preferred cues (Fig. 6A). The spectrogram shows the time course of this suppression of power: it began during cue presentation and persisted throughout the delay period. For trials with non-preferred cues only a slight power increase at low frequencies was observed (< 20 Hz, see Fig. 6B).

Classifying power spectra shapes

In order to make a more unbiased comparison of the spectra across task epochs, we used the classification scheme Isomap (Tenenbaum et al., 2000). The classification obtained this way is unbiased in the sense that the major differences between the spectra
are decided by the algorithm rather than a pre-determined spectral feature that we are interested in (for details see Methods). The resulting two-dimensional map is shown in Fig. 7. Note that the principal axis, which accounts for most of the variance (x-axis), separates spectra that show a peak (large positive values of x) from those that show a pronounced trough (large negative values of x). This is shown more clearly in Fig. 8A, where the position of a spectrum on the x-axis of the Isomap was found to correlate remarkably well with the average power in the 5-20 Hz band, whereas it correlated only weakly with the average power in the band 20-60 Hz. Even this weak correlation with the 20-60 Hz power range can be accounted for by the correlation of the latter with the power in the 5-20 Hz band (Figure 8A, right panel). This result confirms our previous observation that the most significant structures in the power spectra occur at low frequencies (below 20 Hz) and it is in that frequency range that the spectra distinguish themselves from one another.

We proceeded to test whether there was any correlation between the principal coordinate of each spectrum on the Isomap and the degree of bursting of the corresponding spike trains. Bair and colleagues (1994) have reported that bursty cells typically show a peak in the power spectrum. If peaks in the spectra of our database are primarily caused by bursty firing rather than by non-bursty periodic firing we would expect to see a pronounced correlation between the principal coordinate of the spectra and the bursting of the spike trains. This was indeed so, as shown in Fig. 8B, right panel

{Figure 7 about here.}

{Figure 8 about here.}
We also investigated how the electrophysiologically identified classes of regular and fast spiking neurons related to the classifications of the spike trains that we have used here: Poisson, refractory and bursty neurons. This classification showed a significant effect, in that fast-spiking neurons are over-represented among refractory neurons (Fig. 9). This result is similar to the observation from behaving rats that fast-spiking putative interneurons tend to be characterized by autocorrelations with a large dip at zero time lag (Csicsvari et al. 1999). Also, an overrepresentation of RS neurons was observed among neurons with very high bursting (for A>5 there are 18 neurons classified as RS and 1 as FS, the same effect is seen in the power spectra, see histogram tails in Fig. 9), in agreement with previous results (Constantinidis and Goldman-Rakic, 2002).

We then examined whether the shape of power spectra, as classified by the Isomap algorithm, varied depending on the task conditions (Fig. 10). In order to assess that, we considered for each cell the Isomap location of its spectra corresponding to fixation, delay after preferred targets and delay after non-preferred targets (two examples in left upper panels of Fig. 10). For each set of three power spectra associated with a given neuron, we calculated their center of mass along the principal axis (crosses in Fig. 10) and recorded the signed distance \( r \) between the location of each of the spectra and their center of mass, along the Isomap principal axis. A positive value of \( r \) indicates that a spectrum corresponding to a particular task condition was shifted towards the "peak"-side of the Isomap with respect to the other task conditions. The two examples shown in Fig. 10
indicate the $r$ value associated with spectra shown in Fig. 4. The two spectra displayed opposite sign $r$-values, as the Isomap classification distinguishes peaks and troughs. When the $r$ values for spectra in each of the behavioral conditions were computed, the resulting empirical distributions were tested against the hypothesis of the equality of means (Fig. 10). There was a clear tendency, consistent across monkeys, of power increase at low frequencies (positive $r$) in the delay period following non-preferred targets, whereas trough deepening (negative $r$) was typical in the delay period after preferred targets. Spectra for spike trains recorded during fixation were typically close to the center of mass of the three conditions.

{Figure 10 about here.}

Finally, we analyzed a smaller sample of multiunit recordings along the main lines of our single unit analysis. The results are illustrated in Fig. 11. Because of the small sample, some effects observed in single units did not reach significance (task dependency of burstiness, Fig. 11B; and task dependency of spectral shape, Fig. 11C right panel). Other results, however, were indeed replicated: variability of the inter-spike intervals ($CV$ and $\langle CV^2 \rangle$) also increased significantly in the delay (Fig. 11A), and the main feature of the power spectra was their structure at frequencies below 20 Hz (Fig. 11C, left panel. As for single units in Fig. 8A, the high-frequency index did not correlate more with the principal coordinate than with the low-frequency index, not shown), which correlated very well with the degree of burstiness of the spike trains (Fig. 11C, middle panel). No overall increase of
spectral peaks or troughs relative to single-unit recordings was revealed in the multi-unit records.

{Figure 11 about here.}
DISCUSSION

We performed an analysis of the temporal structure of prefrontal neuron spike trains during the fixation and delay periods of a working memory task. Our main findings are threefold. First, the variability of inter-spike intervals for a neuron is typically higher during the mnemonic delay period than during the fixation period, regardless of whether the cue is preferred or non-preferred. Second, the spectral properties of neuronal discharges in most neurons approximated the characteristics of a Poisson process. In a minority of cells that deviated significantly from the expected Poisson pattern, some showed a trough at low frequencies of the power spectrum and a pronounced dip near zero time lag of the autocorrelation function; others displayed a peak (at < 20 Hz) in the power spectrum associated with the burstiness of spike discharges. Third, we observed a small but significant task dependency in the spike-train temporal structure. Neuronal firing during the delay period for preferred locations was characterized not only by elevated firing rate, but also by spectral power of low (< 20 Hz) frequencies suppressed below the expected power spectrum of Poisson spike train. This power suppression was most prominent in fast spiking neurons (putative interneurons).

General characteristics of spike train temporal properties

We identified two types of deviations from the expected power spectrum of a spike train with Poisson characteristics: spectral peaks and troughs. Both of these were almost always observed in frequencies below 20 Hz. Significant spectral peaks were strongly correlated with increased burst firing. Previous studies have shown that firing of action
potentials in bursts produces power spectral peaks, even when bursts themselves are not repeated periodically, with a constant inter-burst interval (Bair et al., 1994). Our current results are in agreement with these findings. Burst discharges are characteristic of a subclass of pyramidal neurons in the cortex (Connors and Gutnick 1990, Gray and McCormick 1996). Recent work suggests that bursts may subserve special types of coding and computations in sensory neurons (Kepecs et al 2002, Doiron et al 2003). What may be the significance of burst firing to the working memory circuits in the cortex? One possibility stems from the evidence that excitatory synapses in the prefrontal cortex exhibit short-term facilitation (Hempel et al 2000). Hence, bursts may represent a reliable neural signal through unreliable yet facilitating synapses (Lisman 1997, Wang 1999), thereby contributing to the synaptic reverberation in the prefrontal circuits.

Our data shows that a great proportion of neurons showing a significant dip in the autocorrelation function and power suppression at low frequencies are classified from the shape of the recorded spikes as fast spiking neurons (putative interneurons). This is consistent with a physiological study of behaving rats reporting that fast-spiking interneurons tend to show a pronounced dip at zero time lag of their autocorrelations (Csicsvari et al. 1999). Such a dip in the autocorrelation and power suppression at low frequencies are usually related to an effective refractoriness in the neuronal spike train. Therefore, interneurons apparently engage more importantly in this task-induced enhancement of the refractoriness of their output. The cellular and synaptic mechanisms, and functional implications, of this effect are currently unknown. Inhibition has been identified by computational models (Camperi and Wang 1998; Compte et al. 2000; Brunel
and Wang 2001; Tegnér et al. 2002) as well as neurophysiological studies (Funahashi et al. 1989) as a mechanism responsible for the suppression of firing with respect to baseline for those pyramidal neurons non-selective to the cue during the delay. The contribution of temporal dynamics of interneuronal spike discharges to the generation of such suppressive inhibition is an important issue to be elucidated in the future.

Absence of oscillatory discharges in the autocorrelation and power spectrum

Since the original descriptions of oscillatory firing in the primary visual cortex of the anesthetized cat (Eckhorn et al., 1988; Gray et al., 1989), there have been several studies in the cat and monkey that failed to detect oscillatory firing, or reported that such oscillations were largely unrelated to the properties of visual stimuli (Ghose and Freeman, 1992; Tovee and Rolls, 1992; Young et al., 1992; Bair et al., 1994). Other experimenters have detected oscillatory firing but in a smaller proportion of the neurons tested (Schwarz and Bolz, 1991; Nowak et al., 1995; Bringuier et al., 1997; Cardoso de Oliveira et al., 1997). Our present results add to this debate as truly oscillatory discharges were very rare in the spike trains of prefrontal cortical neurons. Some obvious factors that could be responsible for discrepancy among the various studies, such as the use of anesthesia, stimulus-induced periodicities, analysis criteria and species differences have been addressed by subsequent studies (Eckhorn et al., 1993; Frien et al., 1994; Gray and Viana Di Prisco, 1997; Friedman-Hill et al., 2000; Fries et al. 2001).

The present study was designed to test the central hypothesis that elevated firing rate during the delay period following a target in the neuron's memory field was associated
with oscillatory discharges, as suggested by Lisman and Idiart (1995) and Tallon-Baudry et al. (2001). Our present results suggest that, at least in individual neurons of the prefrontal cortex, this is clearly not the case. Power enhancement over the expected value for a Poisson spike train occurred only at frequencies below 20 Hz, and they were not related systematically to the behavioral task in any of four monkeys. This is in contrast to similar analysis performed on single neurons recordings and local field potentials in posterior parietal cortex (Pesaran et al. 2002). Pesaran and colleagues detected significant task-related power increase in the gamma band in single parietal neurons. The spectral techniques employed there are analogous to the ones that we apply here. The only differences in the technical aspect of the analysis are their inclusion of non-memory-selective (according to the firing rate) neurons, the database size (40 neurons in their study vs. 229 neurons in the present study) and our unbiased classification through the Isomap method. It seems unlikely that these reasons can entirely account for such contrasting results. This raises the possibility that neurons in the prefrontal cortex have less propensity to display oscillations than those in the parietal cortex. A number of computational studies have shown that oscillations are detrimental to working memory function sustained by local cortical reverberation (Wang 1999; Compte et al. 2000; Tegnér et al. 2002). For example, suppression of oscillatory activity results in a greater tolerance to intervening stimuli (Compte et al. 2000). There is evidence that working memory-related activity in the prefrontal cortex is more resistant to distracting stimuli than in other cortices, like the inferotemporal cortex and posterior parietal cortex (Sakai et al. 2002; Miller et al. 1996; Constantinidis and Steinmetz 1996). Thus, the absence of oscillatory activity in prefrontal
single units during a delayed response task could reflect the stability of persistent activity supported by local cortical reverberation; and, at the same time, resistant to distracting stimuli.

Studies with recordings from motor cortex reported maximal rhythmic firing during periods preceding the onset of a cue and reduction of spectral power after the instruction for a particular movement, even though the firing rate modulation of single neurons follows the opposite pattern (Sanes and Donoghue, 1993; Murthy and Fetz, 1996; Baker et al., 2001). These results led to the suggestion that oscillatory firing in the motor cortex may be associated with a higher level of attention or arousal, in anticipation of a sensory event, rather than with the encoding of stimulus information per se. However, we do not find oscillatory firing in prefrontal neurons recorded during the oculomotor delayed response task, even though this task requires substantial attention allocation throughout each trial. Furthermore, the power suppression detected during delay for preferred cues cannot possibly be associated with a release of attention, since that is the period of the task most demanding in terms of attentiveness for the animal.

The possibility remains that oscillations at the population level are not easily detectable at the level of firing patterns of individual neurons (Csicsvari et al. 1999, Brunel 2000, Brunel and Wang 2003). Indeed, the data we analyzed here correspond to single neuron activities. Moreover, we noted that the results held for a smaller sample of multinuit recordings incorporating 2 or 3 units each (Fig. 11). On the other hand, the identification of task-related oscillatory activity has found its strongest evidence in measures of global activity of a large ensemble of neurons, such as local field potential
(Pesaran et al. 2001) or intracranial EEG recordings in the occipital and parietal lobes
Oscillation may be more readily apparent in multi-unit recordings since such recordings
increase the probability that one of the units will display oscillations, and even more so in
local field potentials. Unit isolation therefore may be partly responsible for the large
differences in percentage of neurons displaying oscillatory firing in different studies.
Therefore, it would be interesting to undertake local field potential recordings from the
prefrontal cortex during activation of working memory. The functional significance of
rhythmic firing, detectable only in the activity of large numbers of neurons, as exemplified
by local field potentials, but not in the spiking output of individual neurons, remains
unclear at the present time.

*Power spectral changes related to memory function*

Neurons in our database showed little change in the spectral characteristics of their
spike trains between the fixation and the delay periods, irrespective of whether the cue
was preferred or not. Assuming that the spectral features of a spike train are primarily
affected by the degree of attentiveness required for a task, the fact that there were no
major differences in modulation of power spectra between the fixation and delay periods
in the present data base could be explained on the basis that the fixation requirement in an
ODR task also requires substantial sustained attention.

Nevertheless, small but significant spectral effects of the task could be detected. We
used the Isomap method to quantify the power spectrum's structure. We found that the
first first principal coordinate of the Isomap correlates well with the average power in the 5-20 Hz band, and distinguishes the presence of a trough or peak in that frequency range. Using this measure, and consistently for all four monkeys, we found that the spectral signature of active memory maintenance in single prefrontal neurons is a suppression of power at low frequencies (< 20 Hz) if the cue presented is preferred, and a slight enhancement of power if the cue is non-preferred (Fig. 10). A similar phenomenon has been observed in posterior parietal cortex during this same task (Fig. 5 in Pesaran et al. 2002), although there a power increase in the gamma band was also very prominent. It is thus possible that refractoriness is a shared signature for single neurons during working memory across cortical areas, whereas gamma band oscillations are a result of the local network connectivity and much more robust in parietal cortex than in prefrontal cortex. The comparison of our results with those of Pesaran et al. (2002) leads to the hypothesis that, in spike trains recorded during working memory, task-dependent power suppression below 20 Hz and gamma-band power increases are due to distinct mechanisms. As discussed below, we also provide suggestive evidence that interneurons might be involved in the generation of the power suppression effect below 20 Hz.

*Temporally irregular nature of persistent neural activity*

We found that the inter-spike interval's coefficient of variation (CV) of a prefrontal neuron is consistently high (see also Shinomoto et al. 1999). Previous studies have reported that the ISI CV of visual cortical neurons in response to a fixed sensory stimulus is close to 1, the value for a Poisson process (Sofkty and Koch 1993). When the stimulus
is naturalistic and time-varying (videos), the CV of V1 and inferotemporal neurons is as high as 1.8-1.9 (Baddeley et al. 1997). In the latter case, the large CV may be directly related to the slow temporal changes of the neural firing rates. Here, we report that during the delay period of an oculomotor task, when there is no external stimulus and the monkey’s eye position is maintained, the CV of mnemonic activity of prefrontal neurons is above 1, on average, and larger than during the fixation period, regardless of whether the cue is preferred or not. Single neuron modeling shows that, generally, the CV is larger when the neuron fires at a lower rate (Sofkty and Koch 1993, Troyer and Miller 1997, Liu and Wang 2000) or is more bursty (Wilbur and Rinzel 1983). Therefore, when the cue is non-preferred, a larger CV of the delay activity may be explained by a lower firing rate (compared to the fixation period) and perhaps by a slightly higher propensity of burstiness across the neural population (as observed in Fig. 3). On the other hand, when the cue is preferred, the neuronal firing rate is higher (up to more than 60 Hz), and burstiness is smaller, yet the CV is still larger. Whether this high CV of prefrontal neurons is a result of slow temporal changes of delay period activity (see, for example, Chafee and Goldman-Rakic 1998; Romo et al. 1999), or reflects the balanced dynamics between synaptic excitation and inhibition (Shadlen and Newsome 1993) in the working memory circuit, remains to be explored in the future. Also, the incidence of task-dependent burstiness could be significant, as it has been shown that fluctuations of neural activity and synaptic inputs at low or high frequencies may have differential impacts on the reverberatory network dynamics (Nowak et al. 1997).
The observed irregularity of persistent activity has potentially important implications. Since high variability of neural discharges is likely to originate from stochastic synaptic bombardments (Shadlen and Newsome 1993, van Vreeswijk and Sompolinsky 1996, Shu et al 2003), our result is consistent with a network mechanism for generating mnemonic delay activity in the prefrontal cortex. By contrast, it is unclear how the high ISI variability could be explained if persistent activity is a single neuron phenomenon (Egorov et al 2002). Moreover, the scenario of balanced synaptic excitation and inhibition (Shadlen and Newsome 1993, van Vreeswijk and Sompolinsky 1996), proposed to account for a high CV close to 1, does not seem to be compatible with the premise of multistability between a resting state and active memory states (Renart 2000, Renart et al 2003). Therefore, the fact that delay activity of prefrontal neurons in behaving monkeys indeed shows a high variability, as reported here, highlights a major inadequacy of the existing working memory models. Resolution of this issue will help to elucidate the microcircuit organization and dynamics of working memory cortical networks.
ACKNOWLEDGMENTS

We wish to thank M. N. Franowicz who participated in some of the recordings, YingHui Liu for contribution to the initial phase of data analysis, and B. J. Pesaran for helpful comments on the manuscript. This work was supported by the NSF, the NIH, the Alfred P. Sloan Foundation and the Swartz Foundation (X-J W.); NIMH grant MH38546 (P.S.G.-R); a McDonnell-Pew Program in Cognitive Neuroscience Award (C.C) and support from the Wennergren foundation, the Swedish Medical Research Council, and the Fernstroms Foundation (J.T.)
REFERENCES


Constantinidis C, Steinmetz MA. Neuronal activity in posterior parietal area 7a during the delay periods of a spatial memory task. *J Neurophysiol* 76:1352-1355, 1996.


Softky WR, Koch C. The highly irregular firing of cortical cells is inconsistent with 

Tallon-Baudry C, Bertrand O, Fischer C. Oscillatory synchrony between human 
extrastriate areas during visual short-term memory maintenance. *J Neurosci* 21: 


Tenenbaum JB, de Silva V, Langford JC. A global geometric framework for nonlinear 


Tovee MJ, Rolls ET. Oscillatory activity is not evident in the primate temporal visual 


Troyer T and Miller KD. Physiological gain leads to high ISI variability in a simpl 

van Vreeswijk C, Sompolinsky H. Chaotic balanced state in a model of cortical circuits. 


FIGURE LEGENDS

Figure 1. Representative discharge patterns observed in the database: Poisson (A), refractory (B) and bursting (C). In all panels the first row represents the last 500 ms before cue onset (fixation epoch), the second row the delay period after preferred cues and the third row the delay period after presentation of a non-preferred cue. Columns depict, respectively, sample spike trains, post-stimulus time histograms (y-axis in Hz), autocorrelograms, inter-spike interval histograms (arrowheads on x-axis indicate mean ISI) and power spectra with the associated error bars (dashed lines) positioned at one standard deviation from the mean (thick solid line). The vertical units of the power spectra indicate power relative to the expected power of a Poisson spike train at the same firing rate.

Figure 2. Task-dependency in the variability of the neuronal responses. Both the global (CV, in A) and local (\(\langle CV_2 \rangle\), in B) measures of ISI variability increase significantly during the delay period as compared to the fixation epoch. In each panel the upper row of graphs shows histograms for CV or \(\langle CV_2 \rangle\) (data from all neurons in the database) for each task condition. Lower graphs on the left show mean and standard deviation in each task condition (F=fixation, PR=delay after preferred cue, NP=delay after non-preferred cue). This is also shown separately in the case of CV (not shown for \(\langle CV_2 \rangle\) to avoid redundancy) for the three classes of neurons identified using both the burstiness measure A and the power spectrum.
In the lower right graph in A, $CV$ and $\langle CV^2 \rangle$, in gray versus mean inter-spike interval is plotted for all neurons and task conditions in the database. The lower right graphs in B show the probability density of $CV_2$ as estimated from the empirical data (gray histograms, data from adjoining ISIs in cells and trials combined, for each population of cells identified using both the spectrum and A) or as expected for a Poisson spike train with absolute refractory period of the same mean rate and same $\langle CV^2 \rangle$ (black solid line, computed using formulae in Methods. The resulting refractory times $t_r$ are indicated). Stars indicate that the significance level for rejection of the null-hypothesis was reached and the location of the star with respect to the corresponding error bar indicates for what adjoining task condition the difference of means reached significance. Significance was determined using a 2-way unbalanced ANOVA ($p<0.01$) and a multiple comparison procedure on data transformed according to $\log(CV-0.5)$ in panel A and $\langle CV^2 \rangle^2$ in panel B, and for factors task and monkey in left histograms and task and cell-type in right histograms in A. No significant interaction found for task and monkey nor between cell-type and task.

Figure 3. Task dependency in the occurrence of bursts. Our measure of burstiness A shows that burstiness is enhanced significantly during the delay if the cue was presented at a non-preferred location. Upper panels show an example for a bursty neuron (sample spike trains, ISI histogram with logarithmic x-axis and autocorrelogram). In all other graphs, thick bars show the median across all cells or
the indicated subpopulation of cells (Poisson, refractory or bursty; see classification
details in Methods), and the error bars indicate the magnitude of the semi-
interquartile range, a star indicates that the null assumption of equal medians could
be rejected (see Methods for the meaning of stars' locations) at the significance
level \( p < 0.01 \) using a multiple comparison procedure after a Kruskal-Wallis test. \( F = \)
fixation, \( PR = \) delay after preferred cue, \( NP = \) delay after non-preferred cue.

Figure 4. Sample task-dependent changes in the power spectra of single neurons. The most
frequent task-dependency was a suppression of power at low frequencies during the
delay epoch after presentation of a preferred cue (panel A, found in 36 of 229
neurons). Other task-dependencies observed more rarely involved suppression
(panel B, 6/229) or the enhancement (panel C, 6/229) of power during the delay
period after a preferred cue. First panels on the left show tuning curves for each of
the cells shown here. Second panels from the left show spectra computed from
spike trains recorded during fixation. Third panels are power spectra from the delay
period after preferred cue presentation (i.e. all cues that elicited a higher average
firing rate than the average across all 8 cues and all trials, solid circles on the tuning
curve) and rightmost panels are obtained from delay period data, when the
presented cue was none of the cues preferred by the cell (hollow circles on the
tuning curve). In the power spectra, solid lines follow the mean spectra across trials
and dashed lines are error bars obtained by adding and substracting one standard
deviation to the mean. All spectra are in log units on the y-axis and are normalized
to the power expected for Poisson firing.
Figure 5. The major task dependency observed by averaging together power spectra from different cells is a power suppression at low frequencies in spike trains recorded during the delay epoch of the task after the presentation of a preferred stimulus. Panel A: Spectra averaged across all 229 neurons in the database for each task condition (fixation, delay-preferred, delay-nonpreferred). B: spectra averaged across all cells on a monkey by monkey basis. Average firing rates ($\langle r \rangle$) across the populations are indicated in each panel. Because of the difference in neuron number recorded from each monkey, the grand average is dominated by the power spectra from COD.

Figure 6. Power suppression at low frequencies after the presentation of preferred cues starts at cue onset and is maintained throughout the delay period as shown through the spectrogram averaged over all neurons in the database (panel A). For non-preferred cue presentation (panel B) the spectrogram does not vary systematically for neurons of our database in the course of the task. Power spectra features at very low frequencies ($< 5$ Hz approx.) are not reliable because of the finite length of our spike trains. Spectrograms were computed by averaging normalized power spectra calculated over 500-ms-long windows overlapped 250 ms from the recorded spike trains.
Figure 7. An unbiased, two-dimensional classification of all power spectra in the database (Isomap, see Methods) neatly separates peaks (positive $x$-coordinate) from troughs (negative $x$-coordinate) and clusters the majority of the spectra on a central region corresponding to Poisson-like power spectra. As explained in Methods, the algorithm Isomap represents each power spectrum as a point in a multidimensional functional space, and computes the two-dimensional projection map that best preserves the geodesic distances between spectra (shown by blue dots, one dot per spectrum). Some of the power spectra are explicitly plotted on the map next to their location (highlighted with a red circle) to show how the output of Isomap responds quite accurately to our intuition of separating spectra showing peaks and troughs. The units of the map axes are arbitrary and their values are only used to compare spectra locations to each other.

Figure 8. The locations ($x$-coordinate or principal coordinate) of the spectra on the map of Fig. 7 correlate well with the average power in the band 5-20 Hz (low-freq. index, left panel in A) but not with the average power in the band 20-60 Hz (high freq. index, middle panel A) since all the correlation observed there can be accounted for by the correlation between the high frequency index and the low frequency index (right panel A). The principal coordinates of spectra correlate also poorly with the firing rate of the associated spike trains (left panel B). In contrast, the coefficient of variation ($CV$) and especially the burstiness measure A strongly correlate with the Isomap principal coordinate (middle and right panels B). Correlation coefficients ($R$) are indicated in each panel.
Figure 9. The abundance of electrophysiologically identified fast-spiking neurons relative to regular spiking neurons was significantly larger among those cells classified as "refractory" by any of our criteria (see Methods). The upper panel shows the proportion of fast-spiking (FS) putative interneurons, and regular spiking (RS) putative pyramidal neurons, as classified based on their action potential and firing rate characteristics, in all electrophysiologically identified neurons (first bar), and in each classification of their spike trains according to both our spectral and burstiness measures (Poisson, refractory, bursty). When either classification criterium (burstiness and spectral shape, see Methods) was used alone, similar results were obtained. The lower panel shows the location on the map of Fig. 7 of RS (black dots) and FS (gray crosses) neurons and the histogram of their $x$-coordinate (RS: black hollow histogram, FS: gray solid histogram), showing a slight but significant shift of FS neurons' locations towards the left of the map. Statistical significance in the upper panel was assessed by using $\chi^2$ to analyze the corresponding contingency table. The null hypothesis that the probability of recording a RS or FS neuron is independent of the burstiness and spectral properties (in the 5-60 Hz range) of their spike trains was rejected at a significance level of $p<0.02$. In the lower panel, we used the non-parametric Wilcoxon rank sum test to reject the hypothesis of equality of means for the locations of FS and RS neurons in the map of Fig. 7 ($p<0.01$).

Figure 10. The task dependency in the location of the spectra on the Isomap of Fig. 7 reveals significant effects. For all monkeys, delay period after a preferred cue was
characterized by low frequency (< 20 Hz) power suppression (or shift to the left along the principal coordinate axis) and delay after non-preferred showed typically a shift to the right along the principal coordinate axis (consistent with the bursting effect observed in Fig. 3). Task dependency was assessed by means of the quantity \( r \). As illustrated in the examples of the upper left panels (same neurons as in Fig. 4), \( r \) is the distance along the principal axis of each spectrum to the center of mass (crosses) of the 3 spectra that were obtained for that given neuron in the various task conditions (F, PR, NP). Bar plots show, for all monkeys together and for each one of them (COD, MAR, JK, AZ), the mean value of \( r \) for each task condition (gray bars) and its standard deviation (error bars). The null assumption of equal means across the data was tested by means of a 2-way unbalanced ANOVA and a multiple comparison procedure within a significance level \( p < 0.01 \). No interaction effect was observed between task and monkey and the hypothesis of equal means across monkeys could not be rejected. There was, however, a consistent effect in the task showing that \( r \) is typically larger for delay after non-preferred cues than otherwise. (F = fixation, PR = delay-preferred, NP = delay-nonpreferred)

Figure 11. A smaller sample of multiunit recordings (n=27) reveals similar results to single units when statistical significance is reached. Panel A: both CV and \( \langle CV_2 \rangle \) increase significantly (ANOVA \( p < 0.02 \)) during the delay period, irrespective of whether the target elicited a high (PR) or a low (NP) firing rate. Panel B: Burstiness of multiunit activity showed a tendency to the significant result obtained for single units (enhanced burstiness for delay after non-preferred cue), but did not reach
significance. Panels C: the Isomap classification also showed very good correlation with the power at low frequencies (5-20 Hz, left panel) and with the burstiness of the spike trains (middle panel). However, the weak task dependency of the principal coordinate observed for single units (see Fig. 10 for details) did not reach significance for multiunit activity.
Table 1. Classification of neuronal trains in the database according to two measures (Spectrum, \(A\)) and concurrently by both of them (Spectrum+\(A\)) for the three task conditions: fixation period, delay after preferred cues and delay after non-preferred cues. The power spectrum identified neurons as belonging to one of the three classes depending on whether the power spectrum estimate in the frequency range 5-60 Hz was within one standard deviation (Poisson), below (Trough) or above (Peak) by more than one standard deviation, compared to the power expected for a Poisson spike train of the same firing rate. If a power spectrum showed a significant peak, it was counted in the “Peak” class, regardless of whether it also displayed a significant trough at a different frequency (see Methods). The central bins of the autocorrelogram as indexed by \(A\) (see Methods) classified neurons as Poisson for \(-1<A<1\), as refractory for \(A<-1\) or as bursty for \(A>1\).
### TABLE 1: Classification of spike trains

<table>
<thead>
<tr>
<th>Measure</th>
<th>Classification</th>
<th>Task conditions</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fixation</td>
<td>Preferred</td>
<td>Non-preferred</td>
</tr>
<tr>
<td>Spectrum</td>
<td>Poisson</td>
<td>81% (185)</td>
<td>49% (112)</td>
<td>76% (175)</td>
</tr>
<tr>
<td></td>
<td>Trough</td>
<td>10% (22)</td>
<td>35% (81)</td>
<td>15% (35)</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>10% (22)</td>
<td>16% (36)</td>
<td>8% (19)</td>
</tr>
<tr>
<td>Autocorr (A)</td>
<td>Poisson</td>
<td>52% (119)</td>
<td>38% (87)</td>
<td>41% (94)</td>
</tr>
<tr>
<td></td>
<td>Refractory</td>
<td>20% (45)</td>
<td>32% (73)</td>
<td>18% (42)</td>
</tr>
<tr>
<td></td>
<td>Bursty</td>
<td>26% (59)</td>
<td>30% (69)</td>
<td>40% (92)</td>
</tr>
<tr>
<td>Spectrum + A</td>
<td>Poisson</td>
<td>48% (111)</td>
<td>24% (56)</td>
<td>35% (81)</td>
</tr>
<tr>
<td></td>
<td>&quot;Refractory&quot;</td>
<td>6% (14)</td>
<td>24% (56)</td>
<td>10% (22)</td>
</tr>
<tr>
<td></td>
<td>&quot;Bursty&quot;</td>
<td>8% (19)</td>
<td>12% (28)</td>
<td>8% (18)</td>
</tr>
</tbody>
</table>
FIGURE 1
FIGURE 2
A  
Spike train burstiness
Sample:

[Sample spike train data]

ISI histogram

autocorrelogram

B  
Population data:

[Population data bar charts]

FIGURE 3
FIGURE 4
**Figure 5**
A  PREFERRED CUES

B  NON-PREFERRED CUES

FIGURE 6
FIGURE 7
A low–freq index vs. pral coord. 

1st principal axis 

R=0.86 

high–freq index vs. pral coord. 

1st principal axis 

R=0.43 

high–freq index vs. low–freq. index 

low–freq. index 

B mean firing rate (Hz) vs. pral coord. 

1st principal axis 

R=−0.13 

CV vs. pral coord. 

1st principal axis 

R=0.47 

burst index A vs. pral. coord. 

1st principal axis 

R=0.79 

FIGURE 8
FIGURE 9
FIGURE 10
MULTIUNIT RECORDINGS (n=27)

C. Isomap classification:

FIGURE 11