A multielectrode study of the inferotemporal cortex in the monkey: effects of grouping on spike rates and synchrony

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Sponsorship: Britt Anderson was supported by the Burroughs-Wellcome Fund, Matthew Harrison was supported by an NSF Integrative Graduate Education and Research Traineeship (IGERT) and David L. Sheinberg was supported by the James S. McDonnell Foundation, NSF IIS-0423031 and NIH EY014681.

Received 2 December 2005; revised 22 December 2005; accepted 25 December 2005

We measured spiking activity from 175 pairs of visually selective neurons while a monkey performed a visual classification task in which the same image pairs appeared in informative and uninformative contexts. Spike counts and synchrony counts were higher during fixations of informative configurations. Spike field coherence showed low frequency (~5–15 Hz) desynchronization when viewing informative stimulus configurations. Jitter statistics, which quantify synchrony and control for spike rates, showed less probable, more surprising patterns of synchronies when the monkey made the correct response than when he erred. Our results suggest that changes in spike counts parallel changes in raw synchrony counts and that both track aspects of stimulus context. Synchrony unexplained by rates changes, however, also occurs and correlates with performance. *NeuroReport* 17:407–411 © 2006 Lippincott Williams & Wilkins.

Keywords: coherence, neurons, synchrony, temporal lobe, vision

Introduction

Temporal–spatial patterns of visual stimuli drive individual neurons to fire and produce correlations at early stages of visual processing [1]. The hypothesis of binding by synchrony becomes most controversial [2,3] when it extends these ideas to higher-order visual processing and posits synchrony codes independent of changes in spike rates.

Direct evidence of binding by synchrony for higher-order visual areas has been difficult to obtain for a number of reasons. First, for cells in areas such as the inferotemporal cortex, characterization of the cell's response profiles is quite difficult. This complicates stimulus design. Second, the need to correlate synchrony to a perceptual state requires paradigms in which different configurations of the same basic stimuli require different reports. These tasks are hard to learn [4]. This complicates experimental design. Third, when spike rates vary, synchronies secondary to chance will covary and it is known that spike rates of inferotemporal neurons can vary as a function of salience or attention [5]. This complicates data analysis.

Few reports look at simultaneously recorded single units in the inferotemporal cortex. Aggelopoulos and colleagues [6] recorded spikes using multiple electrodes and reported information theoretic analyses of spike counts and stimulus-dependent correlations, estimated by cross correlation. In their data, spike counts alone provided essentially all the information about the stimulus. Their tasks, however, did not involve an objective behavioral measure of grouping.

In this report, we test the binding by synchrony hypothesis using a new behavioral task and new statistical methods. In our visual classification task, no single image on the computer screen provides information about the correct response. The monkey must respond on the basis of a collection of images made informative by an explicit grouping cue. We introduce the application of 'jitter' statistical methods to quantify synchrony while controlling for differences in background firing rates.

Methods

General

One adult male rhesus macaque (weight: 9.2 kg) was housed and trained in accordance with the established policies and procedures set forth in the US Public Health Service Policy on Humane Care and Use of Laboratory Animals. All experiments were approved by the Institutional Animal Care and Use Committee of Brown University.

Eye movements were recorded using a scleral search coil. Offline eye movement records were smoothed by convolution and saccades were automatically extracted using a velocity-based algorithm.

We used the Thomas Recording five-channel mini-matrix (Thomas Recording GmbH, Giessen, Germany) that incorporates independent quartz-coated tungsten/platinum fiber microelectrodes for electrophysiological recordings. Neural signals were amplified and filtered for the simultaneous

recording of both action potentials (250 Hz to 8 kHz) and local field potentials (1–100 Hz) [7,8]. Analog signals for spike analyses were sampled at 25 kHz and raw traces were displayed online. Spike sorting was done using time amplitude criteria and k-means clustering on waveform samples. The local field potential signals were streamed to disk at 2500 Hz.

Stimulus images were selected from photo and clipart databases. The hardware for stimulus presentation was a dedicated graphics workstation running optimized OpenGL. The display had a resolution of 1024×768 pixels and a refresh rate of 100 Hz.

Behavioral task

A preliminary viewing-only task was used to select effective and ineffective images on a particular day. These images were then used for the remainder of the behavioral testing for that session. The classification task required the monkey to learn a new image pairing in every session. This daily training included three stages.

All trials began with the acquisition of a central fixation spot. In the first training stage, a single pair of images (denoted symbolically as AB or CD, in which each letter represents one image) appeared at a random position on the screen. Each image subtended 1°–2° of visual angle. Both images appeared on top of a filled purple rectangle. Different orientations (horizontal or vertical) were alternated on different days. The monkey learned to associate either a left or a right button press with each image pair for a juice reward.

In the second training stage, two sets of image pairs were simultaneously displayed on the screen in random positions. One of the pairs of images was always either AB or CD and appeared on the purple rectangle. The second pair on the screen was a faux pair. It was made up of either a false pairing (e.g. AC) on a purple rectangle or a true pair (e.g. AB) that was broken by shifting the rectangle such that only one of the pairs was located on it, or dividing it into two small squares with a central gap (see Fig. 1). The correct response for these trials was determined by whether there was an AB or CD pair on an unbroken purple rectangle. This second stage was designed to teach the monkey which *pairs* of images were informative for a given session.

The third stage – the only one used for data analysis – was similar to stage two, except that one of the pairs always appeared in the center of the screen, where the fixation point had been. On one third of the trials, the central pair appeared on the unbroken rectangle. This arrangement provided a time period when the monkey would look at one of the two pairs without a preceding eye movement (see also Fig. 1). The AB and CD pairs for a day appeared equally often. The faux trials were drawn randomly from the other potential image combinations and so each faux pair appeared less often than the reinforced pairs.

Data analysis

We analyzed data on the basis of fixations. For each fixation, we computed the Euclidean distance from the center of gaze to the center of each pair of objects. Fixations positioned within 2° of a stimulus pair were labeled as 'unbroken' or 'broken', depending on the nearer stimulus pair. We

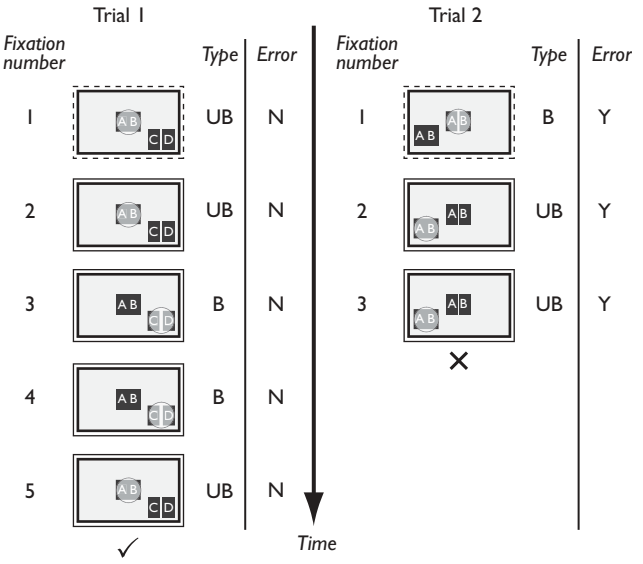

Fig. 1 Final task phase and data analysis setup. Two hypothetical trials are depicted on the left and right. Each gray box denotes a single fixation. Different images are symbolized by the letters, and the light circle designates the fixated pair. Time runs from top to bottom. On the left, there were five fixations. One stimulus group contained an AB pair in an unbroken configuration and the other cluster was made up of a CD pair in a broken configuration. For mock trial 2, both groups were composed of an AB stimulus pair. In one cluster it was unbroken and in the other broken. The dashed box around the first fixation in each trial highlights that these fixations were analyzed separately. To the right of each trial are the fixation labels. Each fixation was coded correct or incorrect. Note that the first fixations of these two mock trials would have been used in comparing the data for broken versus unbroken first fixations of the AB pair, whereas the second fixations would have been used when comparing unbroken AB fixations as a function of error status.

compared synchrony counts between conditions with equivalent total fixation duration or, for spike field coherence (SFC), spike number ($\pm 5\%$).

We limited the neurons used for analyses to those that were visually responsive and selective as determined by comparing empirical mutual information between spike counts and image identity to the mutual information estimates from surrogate, random pairings of spike counts and image labels.

Quantifying synchrony

To quantify synchrony, we counted the number of times a spike from one neuron fell within $\pm 1/2$ bin size (5 or 10 ms) of a spike in a second, simultaneously recorded neuron. In addition, we used the jitter method [9–12]. For each pair of neurons and each fixation, we computed the probability of having seen the empirically observed, or a greater number of synchronies by chance, under the following assumptions: first, that the spike rates (or, more formally, the probability of a spike occurring) were flat on a timescale of 41, 21, 11 or 5 ms. Second, that our neurons had an absolute refractory period of 1 ms and a relative refractory period of 5 ms that declined linearly. Third, that the 'value' of a synchrony was related to its offset in time and that it declined linearly over 5 ms. These probabilities were ranked for all fixations and then these ranks were used in a permutation test.

Spike field coherence
We used established methods [13]. For each spike, we captured the local field potential from another, separate electrode that had recorded a visually responsive spike. We took a 200-ms sample of local field potential centered on the spike time. We applied a Hamming taper to the data to reduce discontinuities at the endpoints. Our frequency resolution was approximately 5 Hz.

Statistics
Our principal statistical method was a permutation test [14]. After calculating the empirical value for a specific measure, the condition labels were shuffled and the analysis repeated. We looked for values that were in the top or bottom 2.5% of the permutation distribution (two-tailed, $P \leq 0.05$) and report the proportion of the population of neuron pairs exceeding this threshold.

Results

Behavioral

The monkey reliably solved the task and typically evaluated both stimulus pairs before responding. For the 34 sessions (12 355 trials) with neural data, the mean proportion of trials correct in a session was 85% (range: 76–98%).

The monkey actively explored the stimulus array. The mean number of visual fixations across all behavioral sessions combined was 3.47 per trial and this was relatively consistent from one behavioral session to the next (minimum: 2.61; maximum: 3.97). The duration of the time from stimulus onset to the first saccade was short (range of median time of first fixation: type unbroken 126–154; type broken 126–150 ms). On 71% of all trials, the monkey visited both stimulus groups before making his response (range: 32–98% across behavioral sessions).

Synchrony

Changes in raw synchrony counts tracked changes in spike rates. We had a total of 175 cell pairs, and for each cell pair we examined separately the fixations of each of the stimulus pairs; this yielded 350 potential stimulus/cell pair configurations for analysis. For the analysis of some conditions, some cell pairs were eliminated because they had no synchronies at all.

First fixation
Statistically significant increases in spike counts and raw synchrony counts were much more common when viewing the unbroken, informative, stimulus pair. For a 5-ms bin size, there were 318 pairs with non-zero synchrony counts. Seventy-seven of these (24% of the population) exceeded the 97.5% cutoff of the permutation distribution. Only three fell below the 2.5% cutoff. The situation was similar when using a 10-ms bin for computing synchronies (86 of 326 eligible comparisons $>97.5\%$, six $<2.5\%$).

Changes in firing rates, however, were more pronounced than differences in synchrony counts. For 62% of the sample, one or both of the neurons had a significantly greater number of spikes when fixating unbroken pairs than when fixating broken pairs ($>97.5\%$ of the permutation distribution); this was roughly double that of synchrony count comparisons. We determined the change in spike counts for each of the two neurons and correlated the larger of these two values with the change in synchrony number. The correlation was 0.60 ($P<0.01$, 10-ms bin size). We achieved similar results if we used the sum of the change in spike counts ($r=0.67$; $P<0.01$).

The jitter analysis [9] computes a probability for synchrony conditioned on the number of spikes observed, so it provides some control for changes in overall firing rate. Repeating the above analyses using a jitter statistic, only 4% of the neuron/image pairs exceed the 97.5% cutoff of the permutation distributions and 14–17% (depending on the jitter window) showed more surprising synchrony in favor of the *broken* pair of fixations.

The jitter results suggest that after controlling for the rate changes there might actually be more unusual configurations of spikes when viewing the broken stimulus pairs. We evaluated the SFC for this same time period in 219 spike–local field potential pairs and for each pair of stimuli. We found (see Fig. 2) an increase in SFC at the lower frequency bands when the monkey was fixating broken stimulus pairs. This suggests that the unbroken stimulus pairs may invoke some mechanism that may actively desynchronize the neuron pairs.

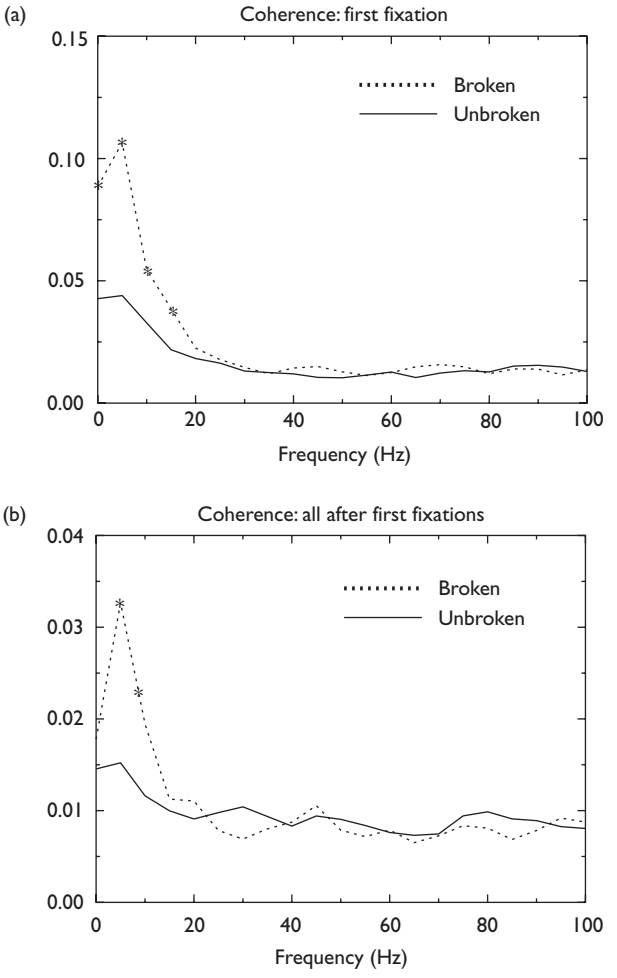

Fig. 2 Spike field coherence (SFC) is shown for the first fixation only (top) and all the fixations after the first (bottom). Note that the scale of coherence is greater at the low frequencies for the first fixation analysis. Asterisks mark frequency bands with a significant difference by a sign test corrected for multiple comparisons.

Table 1 Errors and synchrony

Condition	No.	Raw synchrony		Spike counts		Jitter method	
		C>E	E>C	C>E	E>C	C>E	E>C
Unbroken	281	15%	1%	11%	5%	11%	3%
Broken	307	7%	6%	4%	41%	11%	3%

Condition refers to which fixation condition, unbroken or broken, was being compared as a function of error status. The 'No.' column designates the number of neuron–stimulus pairs. C > E (or E > C) refers to the proportion of the population achieving statistical significance (> 97.5% or < 2.5% of the permutation distribution) in the direction favoring correct over error (or vice versa). All jitter windows (41, 21, 11 and 5 ms) showed similar results.

Subsequent fixations

Results from analyzing the subsequent fixations are similar to the first fixation analysis, but are quantitatively and statistically less extreme. For both the 5- and 10-ms bin sizes, about 10% (34 and 36) of the neuron–image pairs exceeded the 97.5% cutoff of their permutation distribution ($P < 0.001$, binomial test) and seven of each fell below the 2.5% cutoff ($P > 0.05$ binomial test).

Emphasizing the role that spike rate plays, 92 of the neuron–image pairs showed that one or both of the neurons had a significant increase in spike counts when fixating unbroken stimuli compared with broken stimuli. In addition, there was the same pattern of increases in synchronous counts paralleling the differences in spike count (r=0.42 for the 5.0-ms bin size and r=0.56 for the 10-ms bin size).

While the overall SFC values were smaller for later fixations, the pattern was the same with a low-frequency desynchronization seen for the fixations of unbroken stimuli pairs compared with broken stimuli pairs (bottom Fig. 2).

One small difference for this fixation class was that the jitter analysis showed an even split with ~6% greater than 97.5% of the permutation distribution and ~8% falling below the 2.5% cutoff. These proportions are significantly different from 2.5% ($P < 0.05$, binomial distribution). No greater tendency to find pairs with extreme synchrony when viewing broken or unbroken pairs was observed.

Error analyses

Unexpectedly, we found that when controlling for spike rate, more surprising patterns of neuronal synchrony were a correlate of correct task performance. We looked for behavioral–neural correlations by coding each fixation of a stimulus pair as correct or an error depending on the monkey's response on that trial. We compared subsets of fixations matched for total fixation time. From the binding by synchrony hypothesis, we expect the number of synchronies to be greater in correctly grouped views of unbroken pairs than incorrectly interpreted views of unbroken pairs, whereas the opposite is true for fixations of broken pairs. This is what we found (see Table 1). Views of the unbroken stimulus pairs on correct trials are more likely to show increases in synchrony counts, whereas the opposite is true for broken stimulus pair fixations. Controlling for spike counts, however, reveals a very different pattern. Regardless of jitter window size or whether the viewed stimulus pair was of the broken or unbroken type, 10–12% of the neuron–image pairs showed significantly greater synchrony (permutation test).

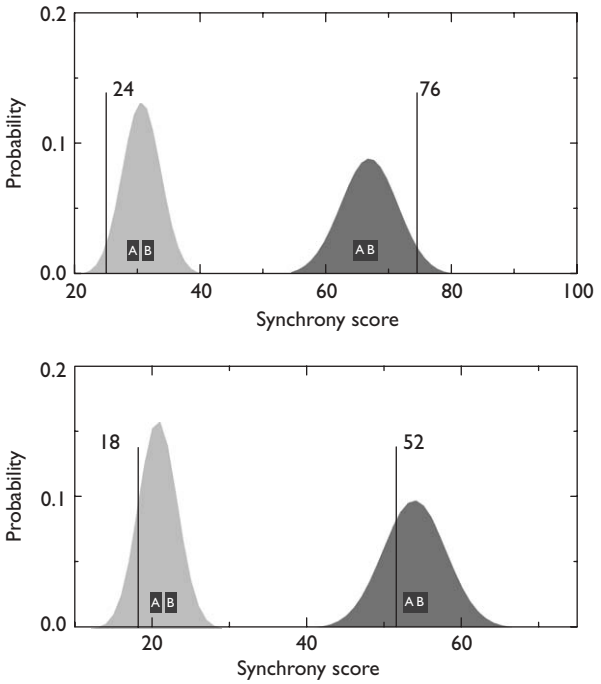

Fig. 3 Synchrony in the context of spike rates. The numbers in the two panels indicate raw synchrony counts for two different cell pairs (10-ms bin size) and two different image pairs. The upper panel includes comparisons from a first fixation-only analysis. The lower panel contains all but the first fixation comparison. The filled curves trace the probability mass functions computed from the jitter statistic (11-ms jitter window) for observing a given number of synchronies in the unbroken (dark gray) or broken (light gray) viewing conditions. As there are more spikes, for the same amount of fixation time, in the unbroken viewing condition it is expected, by chance alone, that there should be a greater number of synchronies. Simply comparing raw synchrony counts across the two conditions can be misleading (lower panel). In the upper panel, the observed number of synchronies while viewing unbroken image pairs is relatively extreme compared with its jitter distribution and therefore the difference is more extreme than expected by chance.

Discussion

Individual neurons respond more actively when the animal fixates configurations of stimuli that are informative or expected [5] than when the animal fixates the same stimuli in other configurations. These increased firing rates are associated with increased raw synchrony counts.

Firing rates correlate with performance. Therefore, so do the raw synchrony counts. When the animal responds to a broken configuration as though it were unbroken, we observe more spikes and more synchronies. These results are consistent with a rate code with the rate computed over the duration of a fixation (~150–250 ms).

SFC is one measure of synchrony that controls for spike rate. The SFC results indicated *less* synchrony when viewing

unbroken image pairs. These data are consistent with models that propose decorrelating correlated inputs [15].

We found an increased proportion of the neuron–pair samples responding with surprising degrees of synchrony when comparing conditions defined by performance. This occurred in favor of the correct viewings, and irrespective of whether the animal fixated a broken or unbroken pair. We suggest that correlated activity, adjusted for rate, contributes to the monkey's ability to discriminate stimuli and correctly decide on a response [16].

We note several implications from these experiments. First, as our results rest on collections of fixations with just a few spikes in each, detection of synchrony is improved by isolating single units. Second, monkeys can make decisions about groups of stimuli in a way that can be practically adapted to studies of binding relevant for the inferotemporal cortex. Third, measures of correlation or synchrony must control for changes in baseline firing rate and not assume trial-to-trial repeatability. We demonstrate the reasons for this caveat graphically in Fig. 3. Cross-correlogram measures do not meet these requirements and are susceptible to both false-positive and false-negative conclusions. False positives occur because correlated changes in rates across trials may give rise to artifactual peaks in cross-correlograms [17]. False-negative conclusions arise because using a measure of neuronal synchrony that is primarily a function of joint rates cannot increase information beyond that present in the rates themselves. Jitter methods and the SFC control for spike rates. Other methods may also be useful [18].

In summary, we find evidence for statistically significant synchrony in a population of inferotemporal neurons responsive to the stimuli used in a grouping task. After using methods that control for background firing rates, these relationships remain, but are more subtle than a simple binding by synchrony concept. The development of new analytical methods and refinement of behavioral paradigms should permit further progress in understanding the relationship between higher-order visual processing and coordination of neuronal activity across visually responsive cell populations.

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