Title: Putative pyramidal neurons and interneurons in the monkey parietal cortex make different contributions to the performance of a visual grouping task

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Running head: Activities of different cell classes in visual grouping

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Abstract

Visual grouping of discrete elements is an important function for object recognition. We recently conducted an experiment to study neural correlates of visual grouping. We recorded neuronal activities while monkeys performed a grouping detection task in which they discriminated visual patterns composed of discrete dots arranged in a cross and detected targets in which dots with the same contrast were aligned horizontally or vertically. We found that some neurons in the lateral bank of the intraparietal sulcus exhibit activity related to visual grouping. In the present study, we analyzed how different types of neurons contribute to visual grouping. We classified the recorded neurons as putative pyramidal neurons or putative interneurons, depending upon the duration of their action potentials. We found that putative pyramidal neurons exhibited selectivity for the orientation of the target, and this selectivity was enhanced by attention to a particular target orientation. By contrast, putative interneurons responded more strongly to the target stimuli than to the non-targets, regardless of the orientation of the target. These results suggest that different classes of parietal neurons contribute differently to the grouping of discrete elements.
Introduction

Although it is known that there are many functional classes of cortical neurons, which include excitatory pyramidal neurons and inhibitory interneurons, it remains largely unknown how those two classes each contribute to visual perception and cognition. Recently, several attempts have been made to classify extracellularly recorded neurons according to known differences in the waveforms of their action potentials (e.g., Mitchell et al., 2007). These studies revealed interesting differences between putative pyramidal neurons and interneurons with respect to their relation to the numerical representation, task selectivity and the effects of attention and working memory (Diester and Nieder 2008; Johnston et al. 2009; Mitchell et al. 2007; Rao et al. 1999). They also suggest that classification of neuron type will provide valuable new information that could be crucial to understanding neural processing within local circuits in the cerebral cortex.

We recently showed that neurons in the lateral bank of the intraparietal sulcus (L-IPS) are involved in visual grouping, which is a process whereby multiple discrete elements are bound into a single object, and is an essential component of object recognition. For example, if multiple dots with the same color are arranged along a straight line, these dots are grouped together and recognized as a single linear object. This grouping is caused by the similarity and continuity of the dots; in other words, the image elements are grouped
together based on the relationship among the multiple elements. Visual grouping is also 
affected by top-down factors, such as prior knowledge, past experience with the objects 
(Wertheimer 1950; Palmer 1999). We found that L-IPS neurons exhibit selectivity for 
grouped stimuli, and that the strength of the selectivity is affected by attention, which 
suggests L-IPS neurons play an important role in visual grouping affected by top-down 
attention (Yokoi and Komatsu 2009). These findings are consistent with neurological 
observations made in human patients and in fMRI studies of healthy human subjects, which 
showed that the parietal cortex is important for visual grouping and the attention related to it 
(Himmelbach et al. 2009; Robertson et al. 1988; Warrington and James 1967; Xu 2008; Xu 
and Chun 2007). It remains unknown, however, how different types of neurons are involved 
in visual grouping.

In the present study, we classified recorded neurons using the same procedure 
previously used by Mitchell et al. (2007), and examined how the resultant two classes of 
neurons contribute to visual grouping. We found that putative pyramidal neurons, which had 
broader action potentials (Br), exhibited selectivity for the orientation of the target stimulus, 
and the selectivity was enhanced by attention. By contrast, putative inhibitory neurons, 
which had narrower action potentials (Nr), did not exhibit such selectivity or enhancement.

Instead, these neurons responded more strongly to the target stimuli than to the non-targets.
These results suggest that different classes of parietal neurons contribute differently to the visual grouping of discrete elements.

Materials and Methods

Two monkeys (Macaca fuscata, male, weighing 6.9-8.8 kg, monkey GG and FZ) served as subjects in this study. All procedures for animal care and experimentation were in accordance with the NIH Guide for the Care & Use of Laboratory Animals (1996) and were approved by our institutional animal experimentation committee. The database used for the present study included 107 neurons that were identical to the sample of neurons reported in our previous study (Yokoi and Komatsu 2009). All of these neurons showed significant visual responses during the behavioral task, and all of the experimental procedures were the same as those reported previously, except for the classification of the neurons. We will therefore only briefly describe the visual stimuli and the task.

Visual stimuli and behavioral task

Monkeys were trained to perform a visual grouping task (Yokoi and Komatsu 2009). Visual stimuli were presented on a CRT monitor (frame rate, 100 Hz; Totoku Electric Co., LTD., Tokyo) situated at a distance of 57 cm from the monkey. Visual stimuli composed of 5
square black or white dots (1.2 deg on the edge) arranged in a cross (Fig. 1A) were presented in a rapid sequence, and the monkeys were required to detect the target stimuli. A total of 20 stimulus types were used, of which four were target stimuli containing three dots with the same contrast (either black or white) aligned either horizontally or vertically (Fig. 1A top). The remaining 16 stimuli were non-targets. Four of those are shown in Fig. 1A bottom. The remaining 12 non-targets had the same patterns but had different orientations separated by 90° each.

Figure 1B shows the time course of a trial. The monkeys made behavioral responses using a lever. A trial started when a monkey pressed the lever, and a white fixation spot appeared at the center of the display. During the trial, the monkey was required to fixate on the spot and to maintain its eye position, which was monitored using the scleral search coil technique (Judge et al. 1980; Robinson 1963). Eight hundred ms after the onset of fixation, a visual stimulus was presented for 300 ms and then disappeared. Visual stimuli were presented 3-4 times interleaved with inter-stimulus intervals (ISIs, 300 - 340 ms). A target was presented within a trial in 75% (or 80%) of the trials. In the remaining trials, a target was not presented. The target appeared only once or did not appear at all within a trial. The probability that the target would appear in each step of the stimulus presentation was the same (0.2 or 0.25/step). The center position of the visual stimuli was 7.1° in eccentricity. The
polar angle was in most cases 22.5°, 45.0°, 157.5°, 202.5°, 225.0°, 315.0° or 337.5°. The
monkeys had to release the lever within 600 ms after the onset of the target to obtain a liquid
reward. In a trial in which no target was presented, the monkeys had to keep pressing the
lever until the fixation spot disappeared to obtain a reward.

Because we included a predictable bias in the target orientations, the monkeys
performed the detection task while their attention was directed towards a particular
orientation. Figure 1C shows a matrix indicating the relationship between the target
orientation and the attended orientation. There were two attention conditions: valid and
invalid. In the valid condition, the attended orientation was the same as the target
orientation; in the invalid condition, the attended orientation differed from the target
orientation. The attended orientation was controlled by the orientation of a visual cue for
monkey GG and by the biased block design for monkey FZ (Yokoi and Komatsu 2009). For
the visual cue, two short horizontal or vertical bars positioned symmetrically on opposite
sides near the fixation point served as the visual cue. The orientation of the cue changed
randomly from trial to trial. In the block design, a block lasted about 100 trials and, within a
block, the target had a particular orientation (horizontal or vertical) in 90% of the trials (valid
condition) and a perpendicular orientation in the remaining 10% of trials (invalid condition).

The attended orientation alternated between horizontal and vertical in serial blocks.
Recording methods

While the monkeys performed the grouping detection task, we recorded single unit activities from the L-IPS using tungsten microelectrodes that were inserted horizontally into the cortex. Under sodium pentobarbital anesthesia, we implanted an eye coil and placed a head holder and a recording chamber on the skull using standard sterile surgical procedures. Neural signals were amplified and band-pass filtered at 500 Hz to 10 kHz (Nihon Kohden, Tokyo). These signals were then digitalized and recorded on a PC’s hard disk at a sampling rate of 25 kHz. After recording, we confirmed the spike activity to be a single neuron using a template-matching method, and then conducted off-line analysis using MATLAB (The MathWorks, Inc.). We have not tested neuron activities in the memory-guided saccade task.

Judging from the reported stereotaxic coordinate of these areas (Borra et al. 2008; Janssen et al. 2008; Sereno and Amadar 2006; Toth and Assad 2002), our recording site (A3-P4) seems mainly situated in the anterior part of LIP and possibly includes a part of AIP (Yokoi and Komatsu 2009).

Data analysis

Recorded neurons were classified based on action potential duration using the method
described previously by Mitchell et al. (2007). We computed the average waveform of spikes recorded at a sampling rate of 25 kHz. The averaged waveforms were interpolated using a cubic spline function to give a resolution of 2.5 μs. Action potential duration was defined as the time between the trough and the peak of the averaged waveform.

To compare response properties across neuron types classified according to their action potential duration, we first tested the neurons’ selectivity for visual features (orientation and contrast) of stimuli composed of multiple dots (Fig. 1A). Firing rates were computed based on the activity recorded within a 200-ms period extending from 50 to 250 ms after stimulus onset. To quantify the selectivity for visual features, we first computed the difference in responses between different target orientations (DBO) and the difference in responses between different target contrasts (DBC) as follows:

\[
DBO = \frac{|(WHt + BHt) - (WVt + BVt)|}{2},
\]

\[
DBC = \frac{|(WHt + WVt) - (BHt + BVt)|}{2}
\]

where WHt, BHt, WVt and BVt respectively represent the response to a target in which white dots are aligned horizontally, black dots are aligned horizontally, white dots are aligned vertically and black dots are aligned vertically. DBO and DBC were separately calculated under each attention condition.

We then quantified the selectivity of each neuron for the target orientation and the
target contrast by normalizing the differences between responses to relevant features to the sum of the responses to all features as follows:

Orientation selectivity index = | (WHt + BHt) - (WVt + BVt) | / (WHt + BHt + WVt + BVt)

Contrast selectivity index = | (WHt + WVt) - (BHt + BVt) | / (WHt + BHt + WVt + BVt)

Each selectivity index takes a value between 0 and 1, and a larger value corresponds to stronger selectivity.

To determine the degree to which selectivity was modulated by attention, we calculated a modulation index as follows:

Attention modulation index = (DBOvalid - DBOinvalid) / (DBOvalid + DBOinvalid)

where DBOvalid and DBOinvalid are values of DBO in the valid and invalid conditions, respectively. Modulation indexes ranged between -1 and 1, with a positive value indicating that the selectivity is stronger in the valid condition than the invalid condition.

To examine how the responses to targets differ from those to non-targets, we used rank analysis to compare the responses to the target with those to the non-target. There were 40 stimulus conditions, reflecting 20 stimulus types presented in 2 attended orientations. These stimulus conditions were ranked in the order of the response magnitudes. A stimulus condition ranked 1st was the stimulus condition in which the strongest response was evoked among the 40 stimulus conditions.
Although comparison between correct and error trials would be of interest, the number of error trials for each stimulus was too small to allow systematic analysis. Therefore, except for computing the duration of action potentials, we analyzed only data obtained in correct trials.

Results

Behavioral performance

For both monkeys, the reaction time was significantly shorter in the valid condition than in the invalid condition (monkey GG: median = 328.6 ms vs. 349.3 ms, valid vs. invalid, p < 0.001, Mann-Whitney test; monkey FZ: 315.3 ms vs. 342.5 ms, p < 0.001), and the average detection rates across the recording sessions were significantly higher in the valid condition than the invalid condition (monkey GG: 96.9% vs. 86.8%, valid vs. invalid, p < 0.001, paired t-test; monkey FZ: 98.9% vs. 91.6%, p < 0.001). It is thus clear that attention was properly guided to the instrumental target orientation during this task.

Classification of neuron types

We recorded from 107 single neurons in the L-IPS (82 from monkey GG, 25 from monkey FZ). Of those, 94 (71 from monkey GG and 23 from monkey FZ) showed a typical
extracellular waveform, with a negative deflection followed by a positive deflection, based
upon which we classified these cells as putative pyramidal neurons and interneurons. Figure
2A shows the normalized waveforms of these neurons, while Figure 2B shows the
distribution of the action potential durations. Consistent with previous reports, the histogram
of action potential durations had two peaks, one at 165 $\mu$s and another at 270 $\mu$s, and the
durations distributed smoothly around these two peaks. We classified neurons with action
potential durations shorter than 205 $\mu$s (red) as putative interneurons and will refer to them
as narrow action potential (Nr) neurons in the following text. Neurons with action potential
durations longer than 225 $\mu$s (blue) were classified as putative pyramidal neurons and will
be referred to as broad action potential (Br) neurons. Neurons with duration between 205
and 225 $\mu$s (gray) were excluded from the following analysis because their classification
was ambiguous. In addition, to confirm the stability of the action potential durations over
recording session, we compared the action potential durations recorded within initial 50
trials and that within last 50 trials. Only two neurons (indicated by white bar) switched the
classification, both of which changed from Br classification to Nr classification. We excluded
these neurons from the subsequent analysis. After this, about a one-fourth of the neurons
were classified as Nr neurons (19 of 65 in monkey GG and 5 of 21 in monkey FZ), and the
remaining neurons were classified as Br neurons (46 of 65 neurons in monkey GG and 16 of
The average action potential duration was $161.2 \pm 27.8 \mu s$ (mean ± SD) for the Nr neurons and $295.8 \pm 38.5 \mu s$ for the Br neurons. In addition, Nr neurons tended to exhibit higher firing rates than Br neurons (Fig. 2C). The average firing rate during the epoch between -150 and 0 ms before stimulus onset was $14.2 \pm 10.9$ spk/s for Nr neurons and $8.2 \pm 7.2$ spk/s for Br neurons ($p < 0.05$, Mann-Whitney test; Fig. 2D left), while the average firing rate during stimulus presentation (50-250 ms after stimulus onset) was $28.3 \pm 16.2$ spk/s for the Nr neurons and $19.6 \pm 15.8$ spk/s for the Br neurons ($p < 0.05$, Mann-Whitney test; Fig. 2D right). These results are consistent with earlier findings using the same classification scheme and show that putative interneurons tend to exhibit higher activity than putative pyramidal neurons (Diester and Nieder 2008; Johnston et al. 2009; Mitchell et al. 2007). There was no significant difference in the response latencies between Nr neurons (mean ± SD: $63.0 \pm 35.4$ msec) and Br neurons ($73.3 \pm 32.7$ msec; $p = 0.18$, Mann-Whitney test).

**Selective responses to the target orientation: example neuron**

To examine the respective contributions made by the two classes of neurons to the visual grouping task, we compared the response properties of Br and Nr neurons. We previously reported that L-IPS neurons exhibit selectivity for the target orientation and that
this orientation selectivity was enhanced by attention. Here, we found that there are clear

differences between Br and Nr neurons with regard to these response properties.

Figure 3A-C shows the responses of a representative Br neuron displayed as spike
density functions. The action potential duration for this neuron was 300 μs. In both the valid
(solid line) and invalid (dashed line) conditions, this neuron strongly responded to targets in
which dots with the same contrast were aligned vertically (vertical targets), whereas it did
not clearly respond to targets in which dots with the same contrast were aligned horizontally
(horizontal targets; Fig. 3A). This preference did not depend on the contrast: similar
responses were observed whether the aligned dots were black or white. Although this
selectivity was also observed in the invalid condition, responses to the horizontal targets
were stronger while those to the vertical targets were weaker, making the orientation
selectivity weaker in the invalid condition than in the valid condition.

To quantitatively evaluate target selectivity, we first computed the response magnitudes
during the epoch extending from 50 to 250 ms after stimulus onset in each condition (Fig.
3B). We then computed the strength of the selectivity for the orientation and contrast of the
target stimuli. In the left panel of Fig. 3C, DBOs (see method) are depicted as vertical bars
that correspond to the differences between the mean responses to the horizontal and
vertical targets. The black and gray bars depict the DBOs obtained in the valid and invalid
conditions, respectively. In the right panel of Fig. 3C, the DBCs depicted correspond to the differences between the mean responses to the white and black targets. The values of DBOs and DBCs were, respectively, 17.1 and 1.7 spk/s in the valid condition and 5.8 and 1.3 spk/s in the invalid condition. DBOs were greater than DBC in both attention conditions, and DBOs were greater in the valid condition than in the invalid condition. This neuron was thus more selective for target orientation than target contrast, and the selectivity for the target orientation was enhanced when the target orientation matched the attended orientation (valid condition). These two characteristics were commonly observed across the population of Br neurons, but not the Nr neurons. Figure 3D-F shows the responses of an example Nr neuron. The responses to the target in which white dots were aligned horizontally was stronger than the responses to other target stimuli (Fig. 3D). However, the selectivity across the target stimuli was broad (Fig. 3E), so that selectivity for target orientation or target contrast was not clear (Fig. 3F).

Selective responses to the target features: population analysis

To quantify the degree of selectivity for each visual feature, we calculated an orientation selectivity index and a contrast selectivity index for each neuron. In Figure 4, we compare these two indices across the population of L-IPS neurons in the valid (A) and invalid (B)
conditions. In Br neurons, the selectivity for the target orientation was greater than for the
target contrast (Fig. 4 left column). In the valid condition (Fig. 4A left), a majority of the data
points (80.6%; 50 of 62 neurons) were located above the diagonal line, indicating that the
orientation selectivity tended to be stronger than the contrast selectivity. As such, the mean
orientation selectivity index (0.32) was significantly larger than the mean contrast selectivity
index (0.14; p < 0.001, Wilcoxon signed-rank test).

The results obtained in the invalid condition are essentially the same as in the valid
condition (Fig. 4B left). More than half of the Br neurons (64.5%; 40 of 62 neurons) exhibited
greater selectivity for the target orientation than the target contrast, and again the mean
orientation selectivity index (0.25) was larger than the mean contrast selectivity index (0.17;
p < 0.01, Wilcoxon signed-rank test), which is consistent with the results we previously
obtained for the entire population of L-IPS neurons (Yokoi and Komatsu 2009).

In contrast to Br neurons, Nr neurons did not exhibit stronger selectivity for the target
orientation. In the valid condition (Fig. 4A right), the averages of the orientation and contrast
selectivity indexes were 0.12 and 0.09, respectively, while in the invalid condition (Fig. 4B
right) they were 0.15 and 0.13, respectively. There was no significant difference between the
orientation and contrast selectivities in either attention condition (p = 0.35 in the valid
condition, p > 0.5 in the invalid condition, Wilcoxon signed-rank test), and the selectivity of
Nr neurons for the target orientation was significantly lower than that of Br neurons (p < 0.05, Mann-Whitney test). These results indicate that although many neurons in both classes exhibited selectivity for target orientation (Br neuron, 49/62=79.0%, Nr neuron, 15/24=62.5%, ANOVA, p < 0.05), the selectivity of Nr neurons was on average much weaker than that of Br neurons. The selectivities for the target contrast were not significantly different between Br and Nr neurons (p > 0.1). We also computed the selectivity indexes after subtracting the baseline firing rates. The results were essentially the same as those described in the text.

Effect of attention: population analysis

The example of Br neuron depicted in Fig. 3 showed greater orientation selectivity in the valid condition than in the invalid condition. The effect of attention towards a particular orientation was quantified by computing the attention modulation index for each neuron. The distribution of attention modulation indexes for Br neurons is shown in Fig. 5, left panel. The mean was significantly larger than zero (0.13; p < 0.05, one sample Wilcoxon test), indicating that the selectivity for target orientation was greater in the valid condition than in the invalid condition. By contrast, the average attention modulation index in Nr neurons was -0.04, which was not significantly different from zero (p > 0.5, one sample Wilcoxon test).
These results indicate that Br neurons, on average, have a slight bias towards stronger selectivity in the valid than invalid conditions, but Nr neurons do not. Nr neurons exhibited higher firing rate than Br neurons (Fig. 2C and D). To confirm that the differences of selectivity and attentional modulation between two classes were not attributable to the difference in the firing rates, we examined the correlation between the firing rates during 50-250 ms after stimulus onset and the orientation selectivities or the absolute value of the attentional modulation indexes. With regard to the orientation selectivity, there tended to be correlation between the firing rate and the orientation selectivity in both Br neurons ($r = -0.37, p < 0.01$) and Nr neurons ($r = -0.36, p = 0.09$). Thus, to examine whether the difference in orientation selectivity between two classes of neurons can be simply explained by the difference in the firing rate, we performed an analysis of covariance (ANCOVA) in which the data from Br neurons and that of Nr neurons were fit with separate lines and it was tested whether the slopes and the intercepts were the same or different. The slope of Br neurons was not significantly different from Nr neurons ($p > 0.05$), whereas the intercept of Br neurons was significantly higher than Nr neurons ($p < 0.05$). This result indicates that the difference in the orientation selectivity cannot be attributable to the difference in firing rate between two classes of neurons. With regard to the attentional modulation, we found that there were no significant correlations between the firing rates and
the absolute values of the attention modulation indexes in both Br neurons \( r = -0.043, p > 0.5 \) and Nr neurons \( r = -0.082, p > 0.5 \).

In our previous paper, we have reported that responses of L-IPS neurons tended to be enhanced when the monkey directed its attention to a particular orientation for each neuron (Yokoi and Komatsu 2009). This can be also seen in the example neurons depicted in Figure 3. The responses of the Br neuron depicted in Fig. 3A-C were enhanced when the monkey directed its attention to the vertical orientation regardless of whether the target orientation was horizontal (invalid condition) or vertical (valid condition). Similarly, the responses of the Nr neuron depicted in Fig. 3D-F were enhanced when the monkey directed its attention to the horizontal orientation regardless of whether the target orientation was horizontal (valid condition) or vertical (invalid condition). We examined whether this pattern of response modulation is commonly observed in both classes of neurons by comparing the response modulation for the horizontal target and the vertical target. The response modulation was calculated separately for the horizontal and vertical targets. The response modulation for the horizontal target was calculated by subtracting the average of the responses to the horizontal targets when the monkey directed its attention toward the vertical orientation from that when the monkey directed its attention toward the horizontal orientation. The response modulation for the vertical target was calculated in the same
manner. Figure 6 compares the response modulations for the horizontal targets (abscissa) and vertical targets (ordinate). If the responses of a given neuron were enhanced when the attention was directed toward a particular orientation regardless of the target orientation, the data point should be plotted within the first- or the third quadrant. We found that this was the case for a large majority of both Br neurons (74.2%; 46 of 62 neurons) and Nr neurons (83.3%; 20 of 24 neurons). These results suggest that the response modulation by attention toward a particular orientation is a common property of both Br neurons and Nr neurons recorded in the present study.

Comparison of the responses to the target and non-target stimuli

A rank analysis carried out in our previous study indicated that the distribution of responses to target stimuli was biased towards the extremes (the smallest and the largest ranks) due to selectivity for the target orientation (Yokoi and Komatsu 2009). In the present study, the results described so far indicate that Br neurons, but not Nr neurons, exhibit such orientation selectivity, which suggests that the distribution of ranks should differ between these two classes of neurons. To test this idea, we conducted separate rank analyses for Br and Nr neurons.
Figure 7 presents the results of rank analyses for the example Br and Nr neurons depicted in Fig. 3. The responses of the Br neuron to the target stimuli showed clear orientation selectivity, and were located at the two extremes of the distribution of responses to non-target stimuli (Fig. 7A). This tendency can be readily seen in Fig. 7B, which shows the ranks of the four target stimuli (white boxes) among the 40 stimuli. The responses to the target stimuli in the valid condition were located near either the top or the bottom of the rank. By contrast, the result of the rank analysis for the example Nr neuron showed a quite different tendency. The responses to the target stimuli tended to be stronger than those to non-target stimuli, and three out of four target stimuli fell above the median for the entire rank (Fig. 7C and D).

These characteristics of the example Br and Nr neurons were commonly observed across the population for each class of neurons. Figure 8 shows the distribution of ranks of the target stimuli for the populations of Br (A) and Nr (B) neurons. The responses of Br neurons were clearly skewed toward both the smallest (strongest response) and largest (weakest response) ranks, and deviated from the uniform distribution (dashed horizontal line). By contrast, the responses of Nr neurons skewed only toward the smallest ranks (strong responses; Fig. 8B), which implies that Nr neurons tend to respond strongly to the
target stimuli, regardless of their orientation. In other words, Nr neurons tend to prefer target
over non-target stimuli, but they have little ability to discriminate target orientation.

To exclude the possibility that the bias toward smaller rank in Nr neurons may come
from a small subset of neurons that responded maximally to all targets, we conducted an
additional analysis. We first selected best target and the worst target for each Nr neuron; the
best target is one of four targets that yielded the strongest response in that neuron, and the
worst target is the one that yielded the weakest response. When we examined rank
distribution of the responses to the best target, we found that in all 24 but one Nr neurons,
the rank was above the middle (>= 21). On the other hand, when we examined the rank
distribution of the responses to the worst target, we found that the rank was above the
middle in only 5 of 24 neurons. This implies that, for most Nr neurons, responses to four
target stimuli widely distributed across ranks, and that Nr neurons in general preferred target
over non-target stimuli.

In addition, we examined whether the latency of target detection differs between two
classes of neurons in the following way. For each neuron, we compared the difference
between the trial-by-trial distribution of the responses to the preferred target and that to
non-target stimuli by using receiver-operating-characteristic (ROC) analysis. The ROC value
ranges from 0 to 1, and the value of ROC would be 0.5 if there was no difference between
two groups. To determine whether a given ROC value differed significantly from 0.5, we performed permutation test in which ROC values were re-computed after the correspondence between the stimulus and the firing rate of the original data were randomly shuffled. This process was repeated 2000 times and the confidence interval (95%) was determined from the distribution of the ROC values computed from the shuffled data. We first tested whether a given neuron exhibited significant difference when the entire stimulus presentation period (50 - 250 ms after stimulus onset) was used for computation. A large majority of both Nr neurons (83.3%, 20/24 neurons) and Br neurons (85.5%, 53/62) showed ROC values significantly larger than 0.5. For those neurons, we computed the time course of the evolution of ROC value with 1 ms resolution commencing 50 ms after stimulus onset, and determined the latency of target detection as the earliest time point where the ROC value remained significantly over 0.5 (p < 0.05) for 10 ms period. The latency of the target detection was on average about 16 ms shorter for Br neurons (119.3 ± 43.4 ms, n=53) than Nr neurons (mean ± SD: 135.6 ± 45.2 ms, n=20), but the difference was not statistically significant (p=0.18, Mann-Whitney test).

Discussion

In this paper, we classified neurons as putative pyramidal neurons (Br neurons) or
interneurons (Nr neurons) based on known differences in the extracellular waveforms of their action potentials, and examined the involvement of these two classes of neurons in a visual grouping task. In the following, we will first consider the procedure for classifying neuron types, and then the possible implications of the present results.

In previous studies involving extracellular recording of cortical neurons, narrower action potentials were deemed to have been produced by interneurons, while broader action potentials were from pyramidal neurons (e.g., Mitchell et al. 2007; Rao et al. 1999). This premise was based on intracellular recordings (Connors and Gutnick 1990; Kawaguchi 1993; McCormick et al. 1985) and was further supported by the observed effects of antidromic stimulation of cortical neurons (Johnston et al. 2009). Consistent with several previous studies including a work in the parietal cortex (Maimon and Assad 2009), we found that the distribution of action potential durations was bimodal, and that Nr neurons exhibited higher levels of activity than Br neurons, which suggests that classification of cell types based on action potential duration can be applied to parietal neurons.

We also found that there are clear differences in the response properties of Nr and Br neurons in a visual grouping task. Br neurons showed selectivity for the target orientation, but Nr neurons did not. The orientation selectivity of the responses of Br neurons suggest that these cells encoded visual features of the stimuli, which were comprised of multiple
discrete dots. In addition, the orientation selectivity of Br neurons was enhanced when the 
target orientation matched the attended orientation. Thus the activities of putative pyramidal 
neurons were clearly associated with detection of the grouping target. One potential source 
of differences in selectivity or attentional effects between the two putative cell classes is the 
differences in the receptive fields and that target stimuli were systematically presented at 
non-ideal position in the receptive field of Nr neurons. However, this is highly unlikely 
because Nr neurons exhibited as strong responses as Br neurons.

We found that the proportion of neurons (about 70%) classified as Br neurons across 
the entire population of cortical neurons was higher than that of Nr neurons, which is 
consistent with earlier extracellular recordings using the same classification procedure 
(Diester and Nieder 2008; Johnston et al. 2009; Mitchell et al. 2007). The response 
properties of Br neurons shown in the present study also matched earlier results analyzed 
after pooling the entire population of L-IPS neurons (Yokoi and Komatsu 2009), likely 
because of the large proportion of pyramidal neurons. Our present study clearly indicates 
that L-IPS neurons signal information about the grouped stimulus to other cortical areas. 
Regions in the L-IPS are anatomically connected to the inferior temporal (IT) cortex (Blatt et 
al. 1990; Webster et al. 1994), and the attentional enhancement of signals encoding 
information about grouped objects may facilitate the object’s representation by IT neurons.
Pyramidal neurons in the L-IPS may also provide feedback signals and affect the contextual modulation of V1 neurons. The activities of V1 neurons are modulated by the presence of visual stimuli in the receptive field surround, and this contextual modulation is affected by attention (Gilbert et al. 2000). The L-IPS may be an important source of signals related to such contextual modulation.

Putative interneurons (Nr neurons) exhibited weaker orientation selectivity than putative pyramidal neurons (Br neurons), and the orientation selectivity was not enhanced by attention. The function of Nr neurons is not yet clear, but our rank analysis provides some hint as to the possible function of these cells. In contrast to Br neurons, the responses of Nr neurons skewed toward only one extreme, indicating that the responses to the targets tended to be higher than those to the non-targets. This suggests three possible functions for these neurons in the current task. First, the activities of Nr neurons may be involved in suppressing the saccade toward the location of the target. It is well established that neurons in the LIP are involved in spatial attention and saccadic eye movements (Balan and Gottlieb 2006; Barash et al. 1991; Bisley and Goldberg 2003, 2006; Colby et al. 1996; Snyder et al. 2000); however, recent evidence shows that LIP neurons are also involved in encoding non-spatial information, such as object shape, color and motion (Freedman and Assad 2006; Janssen et al. 2008; Sereno and Maunsell 1998; Toth and Assad 2002). In the present
study, the target stimulus is behaviorally important and draws attention, but monkeys must maintain their eye positions on the fixation spot. Putative interneurons may strongly suppress the activities of the population of LIP neurons involved in making eye movements when the target stimulus appeared, thereby contributing to the performance of the behavioral task, which is mediated using information encoded by the activities of a different population of pyramidal neurons in the same area.

A second possibility is that the activities of putative interneurons modulate the selective responses of Br neurons. We observed that Nr neurons showed broader selectivity than Br neurons, which is similar to earlier observations made in the monkey prefrontal cortex (Constantinidis and Goldman-Rakic 2002; Diester and Nieder 2008). Moreover, studies using multi-channel recording or blockade of GABAergic inhibition suggested that interneurons shape the selectivity of pyramidal neurons (Diester and Nieder 2008; Sato et al. 1996; Tamura et al. 2004; Wang et al. 2000). Although putative interneurons have broad orientation tuning, they may nonetheless contribute to the shaping of selectivity and modulate the orientation selectivity of putative pyramidal neurons.

The third possibility is that interneurons improve the efficiency of signal transmission by pyramidal neurons. It is known that a majority of the neurons exhibiting narrower action potentials are parvalbumin-positive, fast-spiking interneurons (Cauli et al. 1997; Connors
In addition, recent studies using optogenetics have shown that activation of parvalbumin-positive interneurons induces gamma band oscillation and enhances signal transmission in the cortical microcircuitry (Cardin et al. 2009; Sohal et al. 2009). As will be discussed below, Nr neurons may mediate spatial attention to the target stimulus and improve transmission of target information within the cortical microcircuitry and to other areas.

In an earlier study, Mitchell et al. (2007) showed that putative interneurons are more strongly affected by attention than putative pyramidal neurons in V4. The discrepancy may reflect different involvement of V4 and L-IPS in attention: V4 neurons are affected by attention, whereas neurons in the parietal-frontal network which includes L-IPS are thought to be involved in the allocation of attention. Alternatively, this discrepancy may be explained by the difference in the task design in the two studies. In Mitchell et al.’s study, monkeys were required to allocate attention to the spatial location of the target stimulus. In our study, by contrast, monkeys were required to allocate attention to a particular orientation of a grouped object. As described above, the results of our rank analyses suggested that putative interneurons tend to show stronger responses to the target, which may reflect enhancement of the response to the target when spatial attention was drawn to the target stimulus. If so, it may mean that interneurons in the L-IPS function similarly to those in area
V4, and that they are involved in the engagement of spatial attention. Interestingly, fast-spiking interneurons that likely correspond to Nr neurons are involved in the generation of gamma oscillation, which is thought to be related to spatial attention and its interareal coordination (Fries et al. 2001; Gregoriou et al. 2009). In that context, the activities of Nr neurons in L-IPS and V4 may play similar roles in the allocation of spatial attention to behaviorally important stimuli. By contrast, the principal function of the pyramidal neurons may be to encode visual features, and their activities may be enhanced when attention is directed toward a particular visual feature that is related to the feature selectivity of that pyramidal neuron.
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REFERENCES


Fries P, Reynolds JH, Rorie AE, Desimone R. Modulation of oscillatory neuronal

Gregoriou GG, Gotts SJ, Zhou H, Desimone R. High-frequency, long-range coupling

Gilbert C, Ito M, Kapadia M, Westheimer G. Interactions between attention, context and

Himmelbach M, Erb M, Klockgether T, Moskau S, Karnath HO. fMRI of global visual

Janssen P, Srivastava S, Ombelet S, Orban GA. Coding of shape and position in

Johnston K, DeSouza JF, Everling S. Monkey prefrontal cortical pyramidal and putative
interneurons exhibit differential patterns of activity between prosaccade and antisaccade

Judge SJ, Richmond BJ, Chu FC. Implantation of magnetic search coils for measurement

Kawaguchi Y. Groupings of nonpyramidal and pyramidal cells with specific physiological


Figure legends

Figure 1

Visual stimuli and the sequence of the grouping detection task.

Monkeys were required to detect a particular pattern composed of five discrete dots.

A: A total of 20 types of visual stimuli were used. Four of the stimuli served as targets, in which dots with the same contrast were aligned horizontally or vertically (top panel). Target stimuli were characterized by two features: orientation and contrast. Other arrangements were non-target stimuli, four of which are shown in the bottom panel. Note that the contour line of the dot is for illustration purpose only and was not present in the actual stimulus in the experiment.

B: Time course of a trial. During a trial, the monkey was required to maintain gaze at a fixation point (FX) and quickly release a lever when the target was presented. Visual stimuli were presented serially up to 3 (or 4) times within a trial.

C: Matrix showing the relationships between the target orientation and the attended orientation. In the valid condition, the target orientation matched the attended orientation. In the invalid condition, the target orientation was orthogonal to the attended orientation.
Figure 2

Classification of neurons according to action potential duration.

A: Averaged and normalized action potential for each of 94 neurons analyzed. Time was locked at the trough of each action potential.

B: Distribution of action potential durations. Based on the durations of their action potentials, neurons were classified as having a narrow action potential (Nr) or broad action potential (Br). Vertical lines indicate the criteria for classification. Six neurons around the criteria were excluded from analysis (gray, also shown in panel A). Two neurons switched the classification over recording session (white bars), and these were also excluded from analysis.

C: Averaged responses for each class of neurons under all stimulus conditions. The thick horizontal bar indicates the stimulus presentation period. Broken lines represent ±SEM.

D: Cumulative histogram for the firing rate during the epochs -150-0 ms before stimulus onset (left) and 50-250 ms after stimulus onset (right). Classified Br and Nr neurons are indicated by blue and red, respectively.
Figure 3

Responses of representative Br and Nr neurons to the target stimuli.

Neural activities are shown for the valid condition (black solid line and open symbols) and invalid condition (broken line and gray symbols). Panels A-C show responses of a Br neuron that showed orientation selectivity.

A: Spike density functions for the responses to the target stimulus illustrated in the inset in each panel. Spike density functions were obtained by convolving the spike train (1 ms resolution) with a Gaussian kernel (SD = 20 ms). The thick horizontal bars on the bottom indicate the stimulus presentation period.

B: Mean firing rate (with SEM) during the response to each target stimulus.

C: Selectivity for the target orientation (left) and contrast (right). Each plot illustrates the average responses to the two target stimuli indicated below. The vertical bars on the right of each panel represent differences between the responses that are dependent on the target orientations (DBO, left panel) or the target contrast (DBC, right panel).

Panels D-F show the responses of an example of Nr neuron, using the same conventions as panels A-C.
Figure 4

Comparison of the neuronal selectivities.

Left and right panels correspond to Br and Nr neurons, respectively.

A: Responses in the valid condition. Each point represents a neuron and is plotted at a position corresponding to its contrast selectivity index (horizontal axis) and orientation selectivity index (vertical axis). The cross indicates the average of the selectivity indexes.

B: Responses in the invalid condition. Conventions are the same as in A.

Filled squares in A and B show the result obtained with the neurons depicted in Fig. 3.

Figure 5

Distribution of the attentional modulation index. Left and right panels correspond to Br and Nr neurons, respectively. Open triangles indicate the average modulation indexes for each population. A positive value of the index indicates that the neuron exhibited greater selectivity in the valid condition than the invalid condition. The asterisks denote statistical significance (p < 0.05, one sample Wilcoxon test).

Figure 6

Comparison of the response modulation depending on the attended orientation for the
responses to the horizontal target (horizontal axis) and that to the vertical target (vertical axis). Each data point represents the response modulation of a single neuron, which was calculated by subtracting the average of the responses to the target when the monkey directed its attention toward the vertical orientation from that when the monkey directed its attention toward the horizontal orientation.

**Figure 7**

Comparison of responses to the target stimuli with those to the non-target stimuli.

A: Responses of the representative Br neuron depicted in Fig. 3A-C to targets and non-targets. The short horizontal lines to the right depict the magnitudes of the responses to each non-target stimulus, and the average of those responses is indicated by horizontal broken line.

B: Ranking of the responses to the target stimuli. There were 40 stimulus conditions: 4 targets and 16 non-targets presented in 2 attended orientations. Neuronal responses were sorted in order of response magnitude. White bars indicate the responses to the target stimuli in the valid condition.

Panels C and D show the rank analysis of the responses of the Nr neuron depicted in Fig. 3D-F.
Figure 8

Distribution of the ranks of the responses to the target stimuli in the valid condition for the two classes of neurons.

The ranks of the responses to each target stimulus within the entire stimulus set were determined across the 40 responses of each neuron. A histogram was then generated by counting the occurrences of each rank across the recorded neurons. The height of the bars indicate the number of occurrences of each rank. The stimulus that induced the strongest response was ranked #1. Panels A and B depict the distributions of the ranks for Br and Nr neurons, respectively. A triangle indicates the median of the ranks for each population. The horizontal broken line indicates the number of responses that would be expected if there were no bias in the distribution of the ranks, and the gray area represents the 95% confidence interval for the median under that assumption computed using a permutation test.

Note that there were four different targets, so each neuron was counted four times when making the histogram.
A Target Orientation

Contrast
- White
- Black

Non-target

C Attended orientation

Target orientation

Horizontal Vertical
- Valid
- Invalid

Horizontal Vertical
- Invalid
- Valid

B

Fixation

Pre-stimulus period: 800 ms

No-target: 500 ms

ISI: 300 ms

Target: 500 ms

Reward
Attention modulation index

Br neurons

Nr neurons

Numbers of neurons

Attention modulation index
Response modulation for horizontal target (spk/s)

Br neurons

Nr neurons

Response modulation for vertical target (spk/s)
A
Br neurons

B
Nr neurons

Numbers of responses

Response rank