Enhancement of object representations in primate perirhinal cortex during a visual working memory task

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Abstract

We compared single-cell activities in perirhinal cortex (PRh) as well as adjacent visual cortex (area TE) across two tasks. One task required the monkey to identify any stimulus repetition within a sequence of object stimuli. In the other task, the same stimuli were presented but the monkey didn’t have to remember them. PRh responses during the object-memory task were elevated relative to those during the second task. In TE, on the other hand, there were no significant task-related differences in responses. We did not observe task related differences related to repetition effects in either brain area. The onset of the enhanced signal in PRh during the object-memory task occurred with a latency of 80 msec following the onset of the stimulus response, suggesting that it was the result of top-down feedback.
Introduction

The inferior temporal cortex (IT) plays a central role in visual object processing. Neurons in dorsal and ventral regions of anterior IT (TEad and TEav), the last unimodal visual areas, are selective for complex object features (Desimone 1984; Rolls and Tovee 1995; Tamura and Tanaka 2001; Tanaka 1996), thus providing the capacity to perceptually identify and discriminate stimuli. Perirhinal cortex (PRh) is believed to have an involvement in the mnemonic aspect of recognition for complex objects, in addition to perhaps contributing further to perceptual processing. It is a polymodal structure medially adjacent to TEav on the ventral surface of IT. TE provides major anatomical inputs to PRh, and in return receives strong feedback projections from PRh (Lavenex et al. 2002; Saleem and Tanaka 1996; Suzuki and Amaral 1994; Van Hoesen 1982).

Perirhinal cortex in particular appears to play a multifaceted role in object processing. In the context of long-term visual memory, recognition of complex objects appears to preferentially involve the perirhinal cortex over neighboring areas. Lesions to PRh produce greater behavioral deficits for object recognition than lesions to nearby limbic structures, during delayed match to sample (DMS) or similar tasks (Alvarado and Bachevalier 2005; Baxter and Murray 2001; Meunier et al. 1993; Murray and Mishkin 1998; Zola-Morgan et al. 1989). Lesions to PRh also produce greater deficits to recognition memory than lesions to the adjacent visual cortex, area TE (Buckley et al. 1997; Buffalo et al. 1999). Moreover, disrupting cholinergic input to PRh but not TE or dentate gyrus impairs
recognition memory (Tang et al. 1997; Turchi et al. 2005). As the delay tasks in these studies used trial-unique stimuli and delays stretching into several minutes, they were most likely primarily probing long-term memory.

Long term memory has also been examined at the single-cell level in temporal cortex. Miyashita and colleagues studied associative memory between visual stimuli (Miyashita 1993; Naya et al. 2001; Takeda et al. 2005), finding a critical role for perirhinal cortex in the formation of associations. Stimulus-reward associative memory was studied by Liu and Richmond (2000) and Mogami and Tanaka (2006), who both found a larger involvement of PRh over TE. There is also an extensive literature on familiarity and recency effects on single-cell activity in TE and PRh (reviewed by Brown and Aggleton 2001), which to a large extent involve long term memory.

In addition to long term memory, working memory within temporal cortex has been the subject of monkey studies (Baylis and Rolls 1987; Davachi and Goldman-Rakic 2001; Eskandar et al. 1992; Fuster 1990; Fuster and Jervey 1981; Holscher and Rolls 2002; Miller and Desimone 1994; Miller et al. 1991; Miyashita and Chang 1988; Nakamura and Kubota 1995; Sobotka et al. 2005). Working memory is believed to be closely related to attention (Awh and Jonides 2001; Awh et al. 2006; Barnes et al. 2001; de Fockert et al. 2001; Desimone 1996). In this study, we shall be examining single-cell activity in both TE and PRh associated with the performance of a working memory task.
Working memory effects within temporal cortex are likely to be closely tied to the perceptual properties of structures within that region. While the perceptual role of area TE is clear, that of perirhinal cortex is less so. However, there is mounting evidence that, in addition to long term mnemonic functions, PRh may play a role in perceptual processing. This evidence indicates that PRh is necessary when object identification requires the conjunction of a more complex set of visual features than TE neurons are capable of handling (Buckley et al. 2001; Buckley and Gaffan 1997; Bussey et al. 2003; 2002; 2005; Eacott et al. 1994; Lee et al. 2005; Murray and Bussey 1999; Tyler et al. 2004), see also (Gaffan 2002). This perspective treats PRh, in terms of its visual perceptual properties, as the next link beyond TE in a chain of cortical areas stretching back to V1 with increasingly complex receptive fields. However, such a perceptual role for PRh remains controversial, and others have suggested that the function of PRh can be understood purely in mnemonic terms (Hampton 2005; Hampton and Murray 2002; Levy et al. 2005; Squire et al. 2004).

The primary structure associated with working memory is lateral prefrontal cortex. This association is based on lesion studies extending as far back as Jacobsen (1936) and Mishkin (1957), as well as observations of delay activity in prefrontal neurons during delayed match to sample or delayed response tasks (Fuster 1973; Fuster and Alexander 1971; Kubota and Niki 1971; Niki 1974). The portrayal of prefrontal cortex as a working memory buffer received an influential formulation by Goldman-Rakic (1987).
While there is widespread agreement that prefrontal delay activity plays an important role in working memory, there is disagreement about what that role is. On one hand there is the view that prefrontal delay activity actually represents the contents of working memory, including specific stimulus features (reviewed by Courtney 2004). By this view, representations in visual working memory do not engage the same posterior neural structures that are involved in perceptual representations of stimuli. Alternatively, there is the view that the contents of visual working memory are indeed represented in posterior sensory cortices. By this view, prefrontal delay activity serves as a control signal engaged in the attentional activation, manipulation, or monitoring of the posteriorly-held contents of working memory (Curtis and D'Esposito 2003; Lebedev et al. 2004; Petrides 2000; 1994; Postle 2006; Postle et al. 1999).

In the particular case of TE and PRh, the possibility of feedback interactions with prefrontal cortex of the sort outlined above is supported by the existence of extensive anatomical connections running both ways (Lavenex et al. 2002; Rempel-Clower and Barbas 2000; Suzuki and Amaral 1994; Ungerleider et al. 1989; Webster et al. 1994). If prefrontal cortex were indeed projecting a control or activating signal to posterior representations during a working memory task, as the later hypothesis above indicates, then PRh and TE, given their physiological properties and anatomical connections, would be likely places to look for such a signal, in the context of remembering complex objects.
Delay activity is considered the primary neural substrate of working memory in prefrontal cortex. The fact that prefrontal delay activity survives disruption by intervening visual stimuli between sample and match in a DMS task (Miller et al. 1996) reinforces that belief. However, delay activity does not survive intervening stimuli in perirhinal cortex, making it unlikely that it is the neural substrate for working memory in that structure (Miller et al. 1993). Subsequently it was suggested that a repetition enhancement effect for the match stimulus in a DMS task represented the neural substrate of working memory in PRh cortex (Miller and Desimone 1994).

In this study, we have taken a different approach to identifying activity in temporal cortex associated with working memory. Rather than comparing neural responses during different epochs within a single working memory task, we made a paired comparison across two tasks. In one task the monkey was required to keep a set of serially presented object stimuli in working memory. In the second task the monkey again viewed the same stimuli but without the memory requirement, while being kept busy encoding into memory a different set of unrelated stimuli. Instead of using a delayed match to sample task, which requires keeping just one item in working memory (the sample), we used a serial recognition task that required keeping multiple stimulus images in working memory simultaneously. If prefrontal cortex were providing a control signal to representations in temporal cortex, then it might be more visible if prefrontal cortex were keeping track of multiple items rather than just one.
While we used a working memory task, there is evidence for extensive overlap between mechanisms of working memory and attention, as will be detailed in the Discussion, and we shall consider the data within the context of both those factors.

**Methods**
Two female Japanese macaque monkeys (*M. Fuscata*) were used, weighing 7 and 9 kg. All experimental procedures conformed to NIH guidelines on the care and use of laboratory animals. Recordings were conducted on the right side of the brain in both cases, ranging over anterior-posterior positions A17-A19 in one monkey, and A14-A17 in the other (Figure 1). Chambers were angled at 10° so that electrodes slanted laterally as they advanced.

**Recordings.** There were three target structures, anterior dorsal TE (TEad), anterior ventral TE (TEav), and perirhinal cortex (PRh), each adjacent to the next as one moves down dorsoventrally along the inferotemporal cortex. TEad is in the middle temporal gyrus, extending ventrally into the lateral lip and bank of the anterior medial temporal sulcus (amts). TEav is located along the medial bank and medial lip of amts. Perirhinal cortex, or area 35/36, is located on the lateral bank and lateral lip of the rhinal sulcus (rs) extending into the medial portion of the inferior temporal gyrus (Saleem and Tanaka 1996; Suzuki and Amaral 1994).
Because these structures are located so close to each other in the mediolateral direction, electrode positions had to be determined with precision. To do this, stereotactic coordinates of the target structures were first obtained from MRI scans. Then, following each recording session, an X-ray was taken with the electrode still in place within the brain, using a portable veterinary X-ray machine. From the X-ray images, the lateral distance between the electrode guide tube tip and the brain midline was measured. This in turn allowed determination of the mediolateral position of the tip of the slanting electrode. Slight rotations of the head relative to the fronto-parallel plane of the X-ray machine were mathematically corrected using information from small (1 mm) titanium markers implanted in the skull at known stereotactic coordinates, which were visible on the X-ray images.

At the end of the experiments, the animals were sacrificed and recording sites were verified histologically through examination of electrode tracks. In a few cases, tracks fell too far medially in the rhinal sulcus, and the corresponding cells were excluded from the data.

In an attempt to reduce selection bias, all isolated cells with detectable activity, whether spontaneous or stimulated, were accepted for complete testing without informal prescreening of cells for responsiveness.

*Behavioral task and stimuli.* Stimuli consisted of a set of 110 color photographs of real-world objects, both natural and man-made (examples shown
in Figure 2). They were presented against a neutral gray background that matched the general background of the video display, set at 40 cd/m², so that the stimulus boundary box was not visible. The boundary box subtended a visual angle of 6° (full width). The video display had a resolution of 1024x768 pixels and a frame rate of 75 Hz, and was viewed from a distance of 48 cm. Eye position was monitored with an optical eye-tracker.

The monkeys were repeatedly exposed to the same set of 110 object stimuli during several months of training, so all the stimuli were highly familiar to them by the time recordings commenced.

The general testing procedure for each cell was as follows. First, a preliminary test was performed using the entire 110 object stimulus set. Based on that, ten stimuli were chosen for the main phase of the testing, in which two tasks, an object-memory task and a dot-memory distractor task, were interleaved. A serial recognition task was chosen rather than a serial delayed match to sample (sDMS) task in an attempt to increase the response difference between the two tasks. A serial recognition task requires keeping multiple stimuli in memory simultaneously, and not just a single sample stimulus as in sDMS. Thus, it provides a heavier memory load than an sDMS task.

The object-memory task and the dot-memory distractor task, as will be described below, were both essentially the same working memory task operating on different stimulus sets. The strategy was to select visual patterns for each task that stimulated different populations of neurons, taking advantage of the narrow
pattern selectivity of cells in inferotemporal cortex. As the monkey switched back and forth between remembering one set of stimuli or the other, memory related activity would be expected to switch back and forth between the two neural populations. As we would be recording from just one of those two populations (those neurons stimulated by the images for the “object-memory” task), the memory related signal would appear to switch on when the monkey had to remember the stimuli for that task, and off when the memory related signal was attached to a different population of neurons forming representations for the other class of stimuli. A “memory related signal” in this context could include an attentional aspect to it, as directing attention to a stimulus may be a condition for the stimulus to enter working memory.

During the preliminary testing phase the monkey was performing the object-memory task. Each of the 110 objects was presented a minimum of two times (mean: 2.5). Following that, stimuli were sorted in order of the firing rate elicited (effectiveness), and the top four were selected, as well as six additional stimuli evenly spaced over the remaining range of effectiveness.

In the object-memory task, which was a variant of a serial recognition task, the monkey viewed a series of object stimuli (Figure 3A). The task was to report any repetition in the series by releasing a touch sensitive bar within 800 msec of repetition onset. The trial terminated after the first repetition occurred. The stimulus series length randomly varied from 2-5 images. Each object stimulus was presented for 600 msec, with a 700 msec inter-stimulus interval
between them. A square fixation spot (0.3°) was presented at the start of each trial and during inter-stimulus intervals within the series, with no temporal gaps between presentations of the fixation spot and object stimuli.

In the second task, the dot-memory distractor task, the monkey viewed the same serially presented object stimuli, but was not required to remember them. To discourage the monkey from covertly remembering the object stimuli, the dot-memory task was performed as a distractor during each trial (Figure 3B). During this task, a round dot (0.5° diameter) whose color was randomly selected from six possibilities was displayed, starting before the first object stimulus and then afterwards during each inter-stimulus interval between object stimuli. The monkey’s task was to respond to any repetition in the color of the dot within 800 msec of stimulus onset. The object stimuli had no significance for the monkey in this task.

As was the case for the object-memory task, 2-5 object stimuli were presented during each trial, but now with the colored dot distractor displayed in the period preceding each object stimulus. The location of the colored dot was randomly placed anywhere within 2° of fixation during each presentation within a trial, in an attempt to discourage the monkey from maintaining a narrow focus of spatial attention.

For the distractor task, object stimulus presentations remained 600 msec, but the inter-stimulus intervals were increased to 900 msec. The color dot distractor stimulus was displayed during the central 500 msec of the inter-
stimulus intervals, so that there was a 200 msec gap before and after the distractor dot separating it from the object stimuli. The dot was presented on the same gray background as that used for object stimuli. A square fixation spot was displayed simultaneously with the distractor dot, in exactly the same manner that the fixation spot had been displayed during the object-memory task.

During the distractor task, 50% of the trials presented each object stimulus just once within the trial, while the other 50% included a repeat presentation of one of the objects. For this task, however, repeat presentation of an object stimulus had no significance for the monkey.

The two tasks were presented in interleaved blocks of trials, two blocks for each task for a total of four blocks. Within each block, each object stimulus was presented a minimum of 10 times (mean: 12). Trials were also included which contained only the colored dots of the distractor task, with the object stimulus periods left blank, to test sensitivity of cells to the dot-stimuli.

For both tasks, the task-relevant stimulus repetition occurred with equal likelihood at four possible time positions in the series (positions 2-5). We calculated chance performance conservatively by assuming that the monkeys had learned the basic structure of the task, so that they knew this probability of stimulus repetition at each position. In that case, if they ignore the stimuli and decide at the start of each trial to respond randomly at one of those four time periods, they would have 25% chance of correct performance.
Although the distractor task included an additional color dot in the periods between object stimuli, we believe it unlikely that this would result in a response difference between the two tasks due to temporal crowding or masking effects. The fixation spot, similar in size and shape to distractor color dot, both preceded and followed the object stimuli under both tasks, with an ISI of 0 msec. Presenting an additional color dot would likely have added little if any masking over that already produced by the fixation spot, particularly since the ISI between the color dot and object stimuli was 200 msec, and neural masking effects terminate with ISIs of 100-150 msec (Kondo and Komatsu 2000; Kovacs et al. 1995; Macknik and Livingstone 1998; Rolls and Tovee 1994; Rolls et al. 1999). Furthermore, in our data there was no perturbation to the early part of the object-stimulus response (Figure 7C), whereas suppression of the early response was a significant feature in the physiological masking studies of Macknik and Livingstone (1998) as well as Kondo and Komatsu (2000). For these reasons we believe the effects reported here reflect cognitive processing (attention, memory) rather than sensory responses to the stimuli, although the possibility of a sensory contribution cannot be completely excluded.

Besides masking, another possible effect during target detection within serial presentation of stimuli is attentional blink (Raymond et al. 1992). However our tasks proceeded at a very slow pace relative to the RSVP (rapid serial visual presentation) procedure used during attentional blink experiments, with a 1200 msec stimulus onset asynchrony (SOA) between object stimuli in our object-
memory task and 700 msec SOA between the dot stimuli and object stimuli in our dot-memory task. Therefore, we don’t think that limitations in the temporal dynamics of deploying attention were a significant factor in our experiment.

**Data analysis.** Response was defined as average firing rate over the stimulus duration shifted by latency. Latency was determined as time to half-height of the first peak in the grand average PSTH, incorporating data from all cells within a given cortical area to all object stimuli producing significant responses. Onset time for the stimulus presentation was corrected for the time it took the video electron beam to reach the center of the screen, approximately 8 msec. Estimates of response standard errors were made using Bayesian methods (Lehky 2004), and the standard errors were used to determine those stimuli producing significant responses. Spontaneous activity was determined from a 150 msec window preceding each object stimulus presentation. Cells that showed more than two spikes/sec change in responses to the colored dot distractor stimuli in trials of the distractor task with no object stimulus presentation were excluded from the analysis, regardless of the significance of that response. This was done in order to reduce the possibility that object-stimuli responses would be contaminated by aftereffects from responses to the dot-stimuli. In some cases, the activity in the excluded cells may have reflected above average variability in background firing rather than responses to the dot-stimuli *per se.*
For grand average comparisons of neural activities between the two tasks, the last stimulus presentation within each trial, which overlapped the monkeys’ behavioral responses, were omitted from the analysis. For comparisons between repeat presentations of a stimulus within single trials, all stimulus presentations were considered in the analysis, but with inclusion of only the first 300 msec of the PSTH in each case, so that there was no overlap with behavioral responses.

Visual selectivity can be measured in terms of the shape of the probability density function (pdf) of a neuron’s responses over the stimulus set (Lehky et al. 2005; Simoncelli and Olshausen 2001). Here we define selectivity $S_e$ as the change in entropy of a neural response pdf relative to the entropy of a Gaussian distribution, which has maximum entropy for a fixed variance:

$$S_e = H_G - H(r)$$  \hspace{1cm} (1)

where $H_G$ is the entropy of a Gaussian, and $H(r)$ is the entropy of a cell’s response distribution taken from the data (Lehky et al. 2005). Equating high selectivity with low entropy captures the characteristic of a highly selective cell of having little response to most stimuli and a large response to a few.

The entropy of the Gaussian reference distribution is given by (Rieke et al. 1997):

$$H_G = \frac{1}{2} \log_2(2\pi e \sigma^2) = 2.074 \text{ bits}$$  \hspace{1cm} (2)

with variance normalized to one. Entropy of the probability distribution of the data is:

$$H(r) = -\int p(r) \log_2(p(r)) dr$$  \hspace{1cm} (3)
where \( r \) is the response and \( p(r) \) is the response probability density function (pdf). For practical calculations Eq. 3 is discretized to:

\[
H(r) = -\sum_{j=1}^{M} p(r_j) \log_2(p(r_j)) \Delta r
\]

where responses from \( n \) stimulus images have been placed in \( M \) bins. Expressing the selectivity index \( S_e \) (Eq. 1) in terms of Eqs. 2 and 4, we get:

\[
S_e = 2.074 + \sum_{j=1}^{M} p(r_j) \log_2(p(r_j)) \Delta r
\]

The pdf determined from the data must be normalized to a variance of 1.0 before performing this calculation. Bin size \( \Delta r \) was set by dividing the data range in \( M \) bins equal to the square root of the number of images in the stimulus set.

To examine repetition effects, we calculated, for each trial, the ratio of the response during the second presentation of a stimulus image relative to the first presentation. The array of response ratios was then log transformed to normalize the distribution, and a t-test was conducted on the data for each cell, examining if log(ratio) was significantly different from zero. For plots of the probability distribution of response ratios, the average ratio for each cell was calculated as the geometric mean over all trials, and then response ratios were incorporated into the probability plots on a cell-by-cell basis.

**Results**

Data were analyzed from 65 cells in TEad, 181 cells in TEav, and 115 cells in PRh, collected while the macaque monkey performed two tasks, an object-
memory task (Figure 3A) and a dot-memory distractor task (Figure 3B). Histology showed that the TEad cells came from the middle temporal gyrus near the lateral lip of the anterior medial temporal sulcus (amts), TEav cells from the medial bank and medial lip of amts, and PRh cells from the lateral bank and lateral lip of the rhinal sulcus (rs) (Figure 1).

Each cell underwent preliminary testing with a large set of object images (110 images), in order to select effective stimuli for the main phase of the experiment. ANOVA analysis of this data indicated visual selectivity in 100% of TEad cells, 97% of TEav cells, and 98% of PRh cells (p<0.01).

The probability density function (pdf) of the response magnitudes of each cell to all 110 stimuli was determined using Gaussian kernel smoothing methods (Silverman 1986). Figure 4 shows the median pdf in each cortical area. They were determined by finding the median probability density value at each firing rate, over all cells. The PRh cells differ from those in TE primarily by having more symmetrical pdfs, with less of a tail of large responses extending to the right.

Visual selectivity to complex stimuli (natural images, objects) can be quantified by measures of the overall shape of the response probability density function (Lehky et al. 2005). Here we define selectivity $S_e$ as the change in entropy of a response pdf relative to a Gaussian pdf. The Gaussian is referenced as the density function for a minimally selective neuron (see Methods). Applying this selectivity measure to the pdf of each cell and taking median values, we find
$S_e=0.33$ for TEad, $S_e=0.32$ for TEav, $S_e=0.22$ for PRh, on an open-ended scale where $S_e=0$ for the low-selectivity Gaussian distribution.

Following preliminary testing of each cell with 110 object stimuli, 10 objects were selected for use in the main phase of the experiment comparing responses during the object-memory task and dot-memory distractor task.

Overall, correct performance for the object-memory task was 84% and for the dot-memory distractor task it was 74%. This was calculated on the basis of trials that were not aborted because of broken fixation. If one includes aborted trials, correct performance for the object memory-task was 74% and for the dot-memory task 68%. Poorer performance on the distractor task was seen in both monkeys.

Performance on distractor trials that included object stimulus repetition (78% correct) was close to performance during those trials that did not include repetition (85% correct). This indicates that the monkeys were indeed selectively responding to the color dot stimuli during the distractor task, and not indiscriminately to any stimulus repetition that appeared on the screen.

Behavioral statistics are presented in more detail in Figure 5. Performance declined as the length of the stimulus series increased (Figure 5A). On the other hand, performance as a function of the separation (number of items) between the first presentation (cue) and second presentation (match) of the target stimulus formed a U-shaped curve (Figure 5B). Although the distractor curves are shifted down slightly, the shapes of the curves for the two tasks are similar, and there is
no indication here that the monkeys were using different strategies for performing these two serial recognition tasks. Statistics for trials aborted because the monkeys broke fixation are also similar for the two tasks, with the abort rate slightly lower for the dot-memory task (Figure 5C). Yakovlev et al. (2004) provide a detailed consideration of behavioral statistics for monkeys performing essentially the same serial recognition task we used.

The U-shaped curves in Figure 5B are examples of well-known serial position effects that occur when a list of sequentially presented items is held in short term memory. The upturn of the curve at the left is an indication of the recency effect, as it occurs when the cue immediately precedes the match stimulus. The upturn at the right is an example of the primacy effect, as it occurs when the cue stimulus is the first item presented in the stimulus series. Primacy and recency memory effects occur in both human subjects (Atkinson and Shiffrin 1968; 1971; Glanzer and Cunitz 1966; Murdock 1962) and monkeys (Castro and Larsen 1992; Sands and Wright 1980; Wright et al. 1985).

The poorer correct identification of the match stimulus on the dot-memory distractor task may be related to lower saliency of the stimuli for that task. The stimuli in the dot-memory task were small (0.5°), simple geometric shapes that differed along only one dimension (color). On the other hand, the object-memory patterns were large (up to 6°), with each comprising a complex multidimensional mixture of shape, texture, and color. The roughly equal propensity for the monkeys to abort trials by breaking fixation under the dot-memory and object-
memory tasks suggests that the overall attentional or motivational state of the monkeys did not differ during the two tasks. Those factors are therefore not likely to account for differences in correct performance, nor the task-related differences in neural activities we observed.

We checked sensitivities of cells to the colored dot distractors presented during the distractor task. The concern was that responses to distractors might overlap responses to object stimuli. Trials were included which presented the colored dots in the normal task setting (Figure 3B), but with the “object stimuli” periods held blank. Figure 6C shows the average PSTH in PRh to the colored dots, indicating low sensitivity of this population to the distractor stimuli. TEad and TEav produced similar results (Figure 6A-B).

Despite population insensitivity to distractors, cells were examined individually and any cell whose average response showed a deviation from baseline activity of more than 2 spikes/sec (either positive or negative) was excluded from further analysis, regardless if that activity was significant or not. That left 86% (56/65) of the cells in TEad and 76% (137/181) of TEav cells remained, while 94% (108/115) of PRh cells remained. From the pool of remaining cells, we included in the analysis only those stimuli that produced activity elevation in the object-memory task that was both significant (p<0.05) and large (more than 2 spikes/sec above spontaneous). For some cells none of the 10 object stimuli met those criteria, so that following this second round of selection remaining cells amounted to 80% (52/65) in TEad, 72% (131/181) in
TEav, and 31% (36/115) in PRh, a lower percentage in PRh because of low activity levels in that area. Following these selection procedures, the total number of object stimuli out of the original 110 that survived in use, over the ensemble of recorded cells, was 87 for TEav, 108 for TEav, and 43 for PRh.

Average PSTHs to object stimuli are shown in Figure 7. Latencies from the pooled PSTHs are 69 msec in TEad, 72 msec in TEav, and 83 msec in PRh. PRh responses are markedly smaller than those in TE. PSTHs in all cortical areas show two peaks of activity. The first peak occurs with a latency of about 90 msec in both TE areas and 100 msec in PRh. The second peak occurs at 170 msec in TEad, 150 msec in TEav, and 180 msec in PRh, relative to the time of the first peak. In both TE areas, the second peak is smaller than the first, while the opposite is true in PRh. The shapes of the PRh PSTHs are sensitive to the significance level of the stimulus responses included in constructing them. As the significance level increases (smaller p), the size of the first peak diminishes relative to the second peak.

Probability distributions of the peak height ratios for the three areas are shown in Figure 8. The lack of bimodality in these curves supports the idea that the dual peaks seen in the pooled PSTHs are not due to combining responses from two single-peaked populations of neurons. However, the rightward hump in the PRh curve does leave open the possibility of a subpopulation in that area whose responses are dominated by the first peak. If one associates the first peak with feedforward sensory processes and the second peak with feedback
cognitive processes, then it is possible that the relative heights of the two peaks could depend on whether a particular task were more sensory intensive or more cognitive intensive. The double peak response in PRh may explain the extremely long 66 msec latency difference between TE and PRh reported by Liu and Richmond (2000). Such a latency difference would seem surprising given the dense direct projections between those adjacent areas, and might reflect a latency calculation procedure that tended to miss the first peak.

These data show essentially no task differences in the PSTHs for TEad or TEav. However, in PRh there is an elevation in activity during the object-memory task compared to the dot-memory distractor task. This activity increase starts after a latency of about 80 msec following the start of stimulus response, and seems to coincide with the onset of the second peak.

Histograms of average response magnitude over the stimulus duration (Figure 9A) again indicate no task effect in the two TE areas (mirroring the PSTHs in Figure 7). These included data only from cells that met our criteria for significance, described above. In contrast to TE, there is a notable elevation in PRh activity during the object-memory task. Figure 9B shows the ratios of responses during the two tasks, calculated from paired comparisons of responses to the same stimulus in the same cell. Response ratios were calculated on a cell-by-cell basis, and then the geometric mean over all cells was calculated. The ratios in TEad and TEav were 1.01 and 1.05 respectively, not significantly different from 1.0, as indicated by their Z-test scores. In PRh, however, the ratio
was 1.33 with a multiplicative SE factor of 1.05, significantly elevated above 1.0 (p<0.01). A Wilcoxon signed rank test for paired comparisons again confirmed no significant task differences in TE responses, but a significant difference in PRh (p<0.01). There were no significant serial position effects of the response ratios, performing ANOVA as a function of the position of the object image along the stimulus series.

The above ratios were calculated based on stimuli whose responses passed our significance criteria during the object memory task. If instead the selection were based on responses that met the significance criteria during both the object-memory task and the distractor dot-memory task, then the ratios in TEad and TEav were both 1.01, while the ratio in PRh was 1.21.

Also shown in Figure 9A is spontaneous activity. Spontaneous activity increased in all cases in the dot-memory distractor task relative to the object-memory task. The increase was largest in PRh (1 spike/sec) and also significant in that area (p<0.05) under a paired comparison, while being insignificant in TEav and TEad. This small increase in spontaneous activity during the dot-memory task may be a cognitive effect, perhaps reflecting increased resources (mnemonic/attentional) directed at cells during the periods between object stimuli presentations. Alternatively, it could be a sensory effect reflecting responses to the distractor dot stimuli, although this seems improbable as there is no evidence for such sensory responses in Figure 6. Because spontaneous activity was smaller during the object-memory task, a change in spontaneous
activity cannot explain the increase in activity during stimulus presentations for the object-memory task.

We examined scatterplots that compared average firing rates of individual cells during the two tasks (Figure 10). This shows again that responses in TEad during the two tasks are almost the same, as was the case in TEav, but responses in perirhinal cortex were elevated during the object-memory task. The linear regression slope in TEav was 0.98 ± 0.01, in TEad, 0.97 ± 0.01, and in PRh, 1.25 ± 0.04. Correlation coefficients between responses for the two tasks were 0.99 in both TEav and TEad, and 0.94 in PRh. Cells whose response ratios to the two tasks were significantly different from 1.0 at the p=0.01 level under a Z-test are shown in green in the figure. The proportion of such significant cells was 6% (2/52) in TEav, 4% (5/131) in TEad, and 44% (16/36) in PRh.

The ten object stimuli used during the task comparison were selected for each cell, from a preliminary set of 110, to span a range of stimulus effectiveness from high to low. To determine if stimulus selectivities are affected by the task, we examined response magnitudes over the ordered set of the ten stimuli (Figure 11). These curves were constructed by sorting the stimuli for each cell by response magnitude in the object-memory task, and then averaging over all cells. As before, there is no task effect in either TEad or TEav. In PRh, on the other hand, the curve for the object-memory task is shifted upward parallel to that of the distractor task. The parallel nature of the shift indicates that stimulus selectivity has not been changed by the task.
Under the object-memory task, repetition of a stimulus within a trial indicated a memory target. Under the distractor task, object stimulus repetition had no significance. We examined the ratio of responses of the second stimulus presentation compared to the first, determining the significance of the ratio for each cell by means of a t-test. For TEav, repetition enhancement was seen in 1% (1/131) of the units during the object-memory task and 3% (4/131) during the distractor task. TEav repetition suppression was seen in 19% (25/131) for the object-memory task and 11% (15/131) for the distractor task. For TEad, enhancement was seen in 2% (1/52) for the object-memory task and 8% (4/52) for the distractor task. Suppression occurred in 11% (6/52) for both tasks. Finally, for PRh, enhancement occurred for 0% (0/36) during the object-memory task and 6% (2/36) during the distractor task, while suppression was seen in 6% (2/36) for the object-memory task and 3% (1/29) during the distractor task. These data are broadly consistent with previous reports (Baylis and Rolls 1987; Fahy et al. 1993; Miller and Desimone 1994; Miller et al. 1993), in which repetition effects were observed in only small fractions of the recorded cells.

The probability density functions (pdfs) over all response ratios for cells that met the significance criteria described above are plotted in Figure 12. In no case is the distribution average significantly different from 1.0. The most notable difference between TE and PRh in these plots is the greater variance of the PRh distribution. That may be due to the lower activity in PRh, leading to greater uncertainty in estimating firing rates within a fixed time window. The pdfs for
the two tasks are not significantly different in any brain area, under a Kolmogorov-Smirnov test, nor are the median response ratios significantly different for the two tasks under a Wilcoxon signed rank paired comparison. Furthermore, there were no significant serial position effects on the magnitude of the ratio, performing ANOVA as a function of the number of items separating the first and second presentation of the stimulus image. Thus, we see no influence of the object-memory task on repetition effects.

Discussion

Dealing first with the perceptual aspect of perirhinal responses, our data indicate lower stimulus selectivity in PRh compared to TE. Selectivity determinations were based on calculating entropies of response probability density functions (Figure 4). The pdf of PRh lacks a rightward tail of large responses, making the distribution closer to normal than that of TE. Under an entropy measure of selectivity (as well as a kurtosis measure), that is indicative of lower selectivity. In contrast, Naya et al. (2003) report that they found essentially no difference in stimulus selectivity between PRh and TE.

Whether PRh is less selective than TE, as we observed, or equally selective as observed by Naya et al. (2003), the significant point is that PRh is not more selective than TE. Thus, these data seemingly fail to support the perceptual-mnemonic model of PRh function (Buckley et al. 2001; Bussey et al. 2005; Murray and Bussey 1999), which holds that PRh plays a perceptual role in vision by
forming more complex conjunctions of features than TE, in addition to its mnemonic role. If PRh were forming more complex conjunctions of features, one would expect it to show greater visual selectivity than TE.

Nevertheless, we shall offer two suggestions how observation of lower selectivity in PRh could still be compatible with the perceptual-mnemonic model. The first is that it is possible that the measured selectivity in PRh has been underestimated because of the limited size of the stimulus set (110 objects). To see how this might happen, imagine how the response statistics of a very highly selective cell might appear if the (rare) effective stimuli for that cell weren’t included in the stimulus set. Lacking any large responses, the cell’s pdf would likely be quasi-normally distributed around some low firing rate, rather similar to what we see in the PRh data, giving the false appearance of low selectivity. Including just one effective stimulus dramatically changes the selectivity calculations, but it is unlikely that we would by chance include something approximating that image within a small stimulus set. Because of this potential problem, the measurement here of lower selectivity in PRh relative to TE is open to question. It may take thousands of object stimuli to properly characterize neurons whose receptive fields have this level of complexity.

Alternatively, the lower selectivity observed here in PRh relative to TE may not be a sampling artifact, as suggested above, but rather may reflect the possibility that PRh is involved in visual categorization to a greater degree than TE. Object categorization requires cells to generalize across stimuli, and therefore
be less selective, contrary to the high selectivity required for object identification. Cells in inferotemporal cortex are known to be involved in visual categorization (Freedman et al. 2003; Hung et al. 2005; Sigala and Logothetis 2002; Vogels 1999), though that aspect of their properties has not been compared between PRh and TE. Therefore, under this view, the advance from TE to PRh, rather than continuing the pattern of increasing selectivity as one ascends in visual pathways, may mark the transition to a qualitatively distinct and more abstract functional mode in which object-based rather than feature-based representations predominate. Studies indicating that PRh is involved in processing more complex visual stimuli than TE (Buckley et al. 2001; Bussey et al. 2003; 2002; 2005; Eacott et al. 1994; Murray and Bussey 1999; Tyler et al. 2004), together with the observation here that PRh neurons are visually less selective than those of TE, are consistent with the idea that PRh is preferentially involved in object-based categorization rather than feature-based processing.

Moving from perception to memory, there was a significant increase in activity in perirhinal cells we recorded from during the object-memory task compared to the distractor task (Figures 7, 9, 10). Similar task-related changes in responses were not seen in the adjacent visual association areas that project directly to PRh, areas TEad and TEav. As our task involved the use of a small set (10) of highly familiar stimuli, in which every member of the set appeared repeatedly within a short period of time (several times per minute), solving the task would invoke predominantly working memory mechanisms rather than
long term mechanisms. That being the case, we would interpret the increased PRh activity during the object-memory task as reflecting some aspect of the neural activity required when visual objects are being processed by working memory.

While we observed a task-specific increase in neural responses for objects being held in working memory, we did not observe any task-specific changes in repetition effects, either repetition enhancement or repetition suppression. Thus there is no basis in these data for associating such effects with processing during our working memory task. A dissociation between performance during a working memory DMS task and repetition suppression has previously been shown by Miller and Desimone (1993). During systemic application of a cholinergic blocker, performance in the task was disrupted, but there was no change in repetition suppression in PRh. As yet, this test has not been applied to repetition enhancement or other putative working memory correlates.

The designs of the tasks used here do not allow us to associate the elevated PRh activity with specific stages within the working memory process. Unlike the delayed match to sample task, in which encoding, maintenance and retrieval are separated in time, in our serial recognition task all three processes occur concurrently, as the sequence of stimulus items are presented within each trial. In compensation for this loss of temporal separability amongst working memory sub-processes, our task offered a more complex memory load that
increased the contrast between the memory and non-memory (distractor) conditions.

Working memory effects have been detected in a number of earlier visually responsive areas (V1, V4, MT), using simple synthetic stimuli such as spots, bars and stripes (Bisley et al. 2004; Haenny et al. 1988; Maunsell et al. 1991; Motter 1994b; Pasternak and Greenlee 2005; Supèr et al. 2001). Given those observations, the question arises why the memory-related effects we saw were specific to PPr and did not include the earlier area TE. A possible explanation arises when one considers psychophysical evidence that for complex stimuli, visual working memory stores integrated objects rather than individual features (Lee and Chun 2001; Luck and Vogel 1997). It was suggested above that PPr is the site where feature clusters are integrated into more holistic object representations, while TE still maintains representations at the feature level. If that were so, then working memory effects using more naturalistic complex objects might then be preferentially associated with PPr.

Response PSTHs in all cortical areas had two peaks. The first peak most likely represents feedforward flow of responses up from the retina. The second peak appears around 150-180 msec after the first peak, and may represent recurrent input. Elevated PPr activity during the object-memory task is associated with the second peak (Figure 7C), starting about 80 msec after the onset of the stimulus response. The latency between the onset of the stimulus response and the onset of the putative memory-related activity suggests the
possibility that such activity is dependent on top-down feedback. Given the close association between working memory and lateral prefrontal cortex that was outlined earlier, the most likely source of this feedback would be prefrontal cortex, although these data cannot exclude other sources. We therefore view these data as consistent with models of working memory postulating that object representations within posterior cortices (forming the contents of working memory) are controlled or activated by feedback signals from prefrontal cortex (Curtis and D'Esposito 2003; Petrides 2000; 1994; Postle 2006; Postle et al. 1999). This prefrontal cortex signal serves to tag particular posterior visual representations as behaviorally significant for the task at hand.

Thus, under the interpretation here, as the monkey switches back and forth between the object-memory task and the dot-memory distractor task (both basically the same task, but with different stimuli), the memory-related feedback signal from prefrontal cortex switches back and forth between the neural populations forming the representations of the two sets of stimuli (Figure 13). The cells we recorded from were selected to be responsive to just one stimulus class (the complex stimuli of the object-memory task) and not the other (the simple patterns of the dot-memory distractor task). Therefore, we observed the memory related signal turning on and off depending on which neural representation was the target of prefrontal control.

As there is a close connection between working memory and attention (Awh and Jonides 2001; Awh et al. 2006; Barnes et al. 2001; de Fockert et al. 2001;
Desimone 1998), the mechanisms underlying the memory-related activation we observed in PRh could extensively overlap with mechanisms involved in attentional selection. The changes in PRh activity cannot be ascribed to shifts in spatial attention (for example see Moran and Desimone 1985), as the stimuli used in the object-memory and distractor tasks were in overlapping locations. On the other hand, it is more difficult to exclude the possibility of a contribution from object attention, if indeed working memory for objects and selective attention for objects are independent mechanisms. It is well established that attention can select features or objects non-spatially, as demonstrated psychophysically (Awh et al. 2001; Duncan 1984; Lee and Chun 2001; Vecera and Farah 1994), neurophysiologically (Chelazzi et al. 1993; Hayden and Gallant 2005; McAdams and Maunsell 2000; Motter 1994a; Sereno and Amador 2006; Treue and Trujillo 1999), and by brain imaging (Corbetta et al. 1991; Liu et al. 2003; O'Craven et al. 1999; Serences et al. 2004).

Also, given that relevant and non-relevant stimuli in our task occurred at predictable times, another possible contributor to our results is temporal attention, the ability to direct attention to particular points in time. Although not as extensively studied as object attention, temporal attention has been demonstrated with human brain imaging (Coull and Nobre 1998; Griffin et al. 2002; Nobre 2001) as well as monkey single cell recordings (Ghose and Maunsell 2002).
Again suggestive of an overlap between working memory and attentional mechanisms, an analogy may be drawn between what appears to be feedback activation of PRh during our working memory task, and feedback activation of various posterior cortices during attention (reviewed by Pessoa et al. 2003).

To summarize, in a paired comparison between two tasks we have observed activity in perirhinal cortex associated with the requirement for keeping complex objects in visual working memory. The activity did not extend into the adjacent visual cortex, area TE. There was a long latency between the onset of the stimulus response and the onset of the memory-related activation, consistent with a top-down feedback source of the activation. The nature of this feedback activation during working memory may overlap extensively with feedback activation associated with attentional selection. These results support models of visual working memory in which prefrontal cortex exerts a controlling influence on visual representations held within posterior cortices.

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Disclosures
The authors assert that they have no conflicts of interest.
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**Figure Captions**

**Figure 1.** (A) Lateral view for monkey 1, with gray band indicating anterior-posterior range of recording. (B) Coronal section for monkey 1, with gray bands indicating areas recorded from. (C-D) Lateral view and coronal section for monkey 2. rs, rhinal sulcus; amts, anterior middle temporal sulcus; sts, superior temporal sulcus.

**Figure 2.** Example stimulus images. These are a subset of the 110 images each cell was initially tested on. Based on responses during this preliminary screening, ten images were selected for further testing (a different set for each cell). Those ten images were not the ten most effective stimuli, but included a full range of effectiveness from the best to the worst.

**Figure 3.** (A) Object-memory task: monkey responded to any repetition within a series (2-5) of object stimuli. The duration of object stimuli was 600 msec and the inter-stimulus interval was 700 msec. (B) Dot-memory distractor task: monkey viewed a series of object stimuli, but was not required to remember them. To discourage the monkey from covertly retaining object stimuli in memory, a distractor task was included. The distractor involved displaying a small dot with a randomly selected color during the inter-stimulus intervals between image presentations. Each dot presentation was at a random location to discourage the monkey from maintaining a narrow focus of attention. The monkey responded to
any repetition in dot color. Duration of object stimuli was 600 msec, duration of
distractor dot stimuli was 500 msec, and the interval between object stimuli was
900 msec.

Figure 4. Probability density functions (pdfs) of neural responses to object
stimuli. Responses of each cell to 110 stimuli were used to calculate that cell’s
pdf, and then the median pdf was determined for the different cortical areas. TE
curves have heavier tails of large responses extending to the right than does the
PRh curve. Peaks of the distributions approximately coincide with spontaneous
activity. The number of units underlying these curves are TEad: 65, TEav: 181,
and PRh: 115.

Figure 5. Behavioral statistics for the two tasks. A. Percent correct as a function of
the length of the stimulus time series, which varied randomly between 2-5 in
different trials. Percentages were calculated excluding aborted trials. Chance
performance is set at 25% on the assumption that for each trial the monkey picks
at random one of the four possible time periods for the target stimulus. B.
Percent correct as a function of the difference in position along the stimulus time
series between the first presentation of a stimulus and the second presentation
(match). C. Percentage of trials that were aborted because the monkey broke
fixation, as a function of the length of the stimulus time series.
Figure 6. Population averages of response spike trains (PSTHs) of cells to the distractor colored dot displayed during the distractor task, pooled over all cells responses. The plots have been smoothed with a Gaussian kernel with $\sigma = 5$ msec.

**Figure 7.** Population averages of response spike trains (PSTHs) in TEad, TEav and PRh to object stimuli, pooled over all cell responses that met our significance criteria for object stimuli.

**Figure 8.** Probability distributions of the heights of the two peaks seen in the PSTHs (Figure 7). The ratio of the peaks was determined for each cell that met our significance criteria, and the probability density function was then generated by kernel smoothing estimation. The number of units in each brain area is the same as indicated in Figure 7.

**Figure 9.** (A) Average response magnitudes in different cortical areas under the two tasks. Spontaneous levels of activity are shown in gray. Data were pooled over stimulus duration shifted by latency. (B) Response ratios comparing object-memory and distractor tasks. Error bars indicate standard error. The number of cells in each brain area is the same as indicated in Figure 7.
Figure 10. Responses of individual cells plotted as average firing rate during the object-memory task versus average firing rate during the distractor task. In both areas of TE, responses to the two tasks were almost equal, whereas in perirhinal cortex there was greater activity during the memory task. Green points indicate cells whose response ratios for the two tasks were different than 1.0 at the p=0.01 level of significance.

Figure 11. Average response as a function of response rank for the ten stimuli presented to each cell. Each cell had a different set of ten, selected from 110 objects based on preliminary testing. The parallel shift in PRh indicates that memory processing does not change the stimulus selectivity of cells. The number of units in each brain area is the same as indicated in Figure 7.

Figure 12. Repetition suppression and repetition enhancement. These are probability density functions (pdfs) of the ratios of response magnitudes during 1st and 2nd presentations of the same stimulus within the same trial. The pdfs show that stimulus repetition effects are not significantly different for the two tasks in any of the cortical areas. The number of units in each brain area is the same as indicated in Figure 7.

Figure 13. Model of prefrontal-perirhinal interactions during visual working memory. During the object-memory task, feedback from prefrontal cortex
activated population-coded neural representations of the object stimuli in perirhinal cortex. During the dot-memory task, the prefrontal feedback signal switched to neural representations of the dot stimuli. As we recorded only from neurons that were part of the object representations, the prefrontal signal appeared to turn on and off as it was switched back and forth between the two neuronal populations.
A  Object-memory task

B  Dot-memory distractor task

Figure 3
Figure 5
Figure 6
Figure 7

Graph A: TEad
- obj-memory
- distractor
- n=52

Graph B: TEav
- obj-memory
- distractor
- n=131

Graph C: PRh
- obj-memory
- distractor
- n=36
Figure 9

A  | obj-memory  | distractor  | spont.  
---|-------------|-------------|---------
TEad | 20 ± 2.5 | 10 ± 1.2 | 5 ± 0.5 |
TEav | 22 ± 2.8 | 12 ± 1.4 | 6 ± 0.8 |
PRh  | 8 ± 1.0  | 4 ± 0.5  | 2 ± 0.2 |

B  | obj-memory/distractor  
---|------------------------
TEad | 1.4 ± 0.3 | 1.2 ± 0.1 | 1.0 ± 0.2 |
TEav | 1.3 ± 0.2 | 1.1 ± 0.1 | 1.0 ± 0.2 |
PRh  | 1.5 ± 0.3 | 1.2 ± 0.1 | 1.0 ± 0.2 |
Figure 10
Figure 12

(A) TEad

(B) TEav

(C) PRh

Probability density

Ratio 2nd/1st presentation
Figure 13