

# Advanced Age Dissociates Dual Functions of the Perirhinal Cortex

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The perirhinal cortex (PRC) is proposed to both represent high-order sensory information and maintain those representations across delays. These cognitive processes are required for recognition memory, which declines during normal aging. Whether or not advanced age affects the ability of PRC principal cells to support these dual roles, however, is not known. The current experiment recorded PRC neurons as young and aged rats traversed a track. When objects were placed on the track, a subset of the neurons became active at discrete locations adjacent to objects. Importantly, the aged rats had a lower proportion of neurons that were activated by objects. Once PRC activity patterns in the presence of objects were established, however, both age groups maintained these representations across delays up to 2 h. These data support the hypothesis that age-associated deficits in stimulus recognition arise from impairments in high-order stimulus representation rather than difficulty in sustaining stable activity patterns over time.

## Introduction

The perirhinal cortex (PRC) processes, represents, and stores high-order sensory information that is critical for a wide range of behaviors (Murray and Wise, 2012). The PRC enables, for example, fear-conditioning to a stimulus (Kholodar-Smith et al., 2008) or a context (Bucci et al., 2000), and paired-associative learning (Higuchi and Miyashita, 1996). The PRC also supports an animal's ability to discriminate between novel and familiar stimuli (Málková et al., 2001; McTighe et al., 2010). A number of these cognitive functions are altered during normal aging, including observations that aged rats are more likely to incorrectly identify a novel stimulus as familiar (Burke et al., 2010) and show poorer consolidation of information in fear conditioning (Oler and Markus, 1998).

Although there is no loss of PRC neurons with age (Rapp et al., 2002), several biochemical and molecular alterations within the PRC of old animals have been identified that may contribute to cognitive deficits. First, protein composition is altered, including a reduction in the expression of the NR2A subunit of the NMDA receptor in aged compared with young rats (Liu et al., 2008). Moreover, old rats have reduced immunoreactivity for calbindin-D28k in PRC principal cells (Moyer et al., 2011), and overall glutamate levels are lower in the aged PRC (Liu et al., 2009). Finally, following object exploration,

fewer PRC neurons in old rats transcribe the activity-dependent immediate-early gene *Arc* (Burke et al., 2012b), which encodes an effector protein critical for AMPA receptor trafficking (Chowdhury et al., 2006).

In freely behaving young rats, PRC principal neurons show firing rate increases at locations adjacent to objects (Burke et al., 2012a; Deshmukh et al., 2012). This activity remains consistent as objects become familiar and across delays (Burke et al., 2012a). It is probable that this object-specific activity is a critical physiological correlate of stimulus recognition. The age-associated decline in the proportion of PRC neurons that transcribe *Arc* predicts that old rat PRC cells may show reduced object-associated spiking. Because *Arc* expression has been shown to be decoupled from neuronal activity under some conditions in which rats show learning impairments (Fletcher et al., 2006), it is also possible that old PRC neuron firing remains normal despite the fact that fewer cells show *Arc* expression during this activity. Because *Arc* expression is critical for memory maintenance (Guzowski et al., 2000), such a decline could lead to deficits in stable activity patterns across delays.

The aim of the current experiment was to determine the extent to which age-associated declines in PRC-dependent behavior arise from deficits in stimulus representation, maintenance, or both. PRC neurons were recorded as young and aged rats traversed a circular track that was empty or contained objects for two epochs separated by a delay. If object-associated neuron firing is similar between age groups, then it can be assumed that old rats have a deficit in inducing *Arc* transcription that may result in unstable activity patterns across delays. On the other hand, if there are alterations in the pattern of PRC cell activity in response to objects, then it could be inferred that advanced age results in impaired PRC-dependent stimulus representation.

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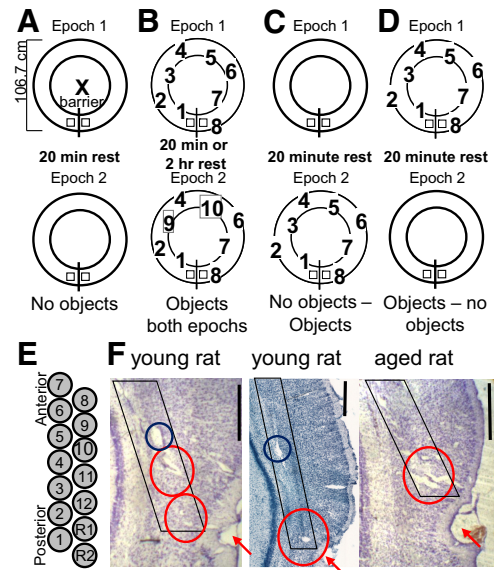
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## Materials and Methods

**Subjects and behavioral training.** All behavioral procedures were in accordance with the National Institutes of Health guidelines for rodents and protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Electrophysiological studies were conducted on six young (8–10 months old), and six aged (24–27 months old) Fisher-344 male rats. All rats participated in these experiments in pairs of one young rat and one aged rat such that a single old–young pair arrived in the colony in the same batch of animals, went through identical behavior procedures on the same days, and underwent surgical implantation within 24 h of each other. The rats were housed individually and maintained on a 12 h light/dark cycle. Before rats were implanted with the hyperdrive recording device, they were screened for spatial memory impairments and normal vision using the Morris swim task (Morris, 1984). All animals were tested over 4 d with six spatial trials on each day. Animals were then screened for visual ability with 2 d of cued visual trials (6 trials/d) in which the escape platform was above the surface of the water but the position of the platform changed between each trial. This procedure has been described in detail previously (Barnes et al., 1996). Rats' performance on the swim task was analyzed offline with either in-house software (WMAZE, M. Williams) or a commercial software application (ANY-maze). Because different release locations and differences in swimming velocity produce variability in the latency to reach the escape platform, a corrected integrated path length (CIPL) was calculated to ensure comparability of the rats' performance across different release locations (Gallagher et al., 1993). The CIPL value measures the cumulative distance over time from the escape platform corrected by an animal's swimming velocity, and is equivalent to the cumulative search error described by Gallagher and colleagues (1993). Therefore, regardless of the release location, if the rat mostly swims toward the escape platform, the CIPL value will be low. In contrast, the more time a rat spends swimming in directions away from the platform, the higher the CIPL value.

During electrophysiological recordings, the animals were food-deprived to ~85% of their *ad libitum* weight and trained to run on a circular track (~335 cm in circumference) in both the counterclockwise and clockwise directions for food reinforcement. The food reward was a mixture of rat food pellets made soft by soaking them in water, applesauce, and the diet supplement Ensure. All electrophysiological recordings took place during the dark phase of the rats' light/dark cycle. Food rewards were given in a small plastic food dish (4 × 4 cm) at two positions on the track. Both food dishes were located at the position on the track that marked the completion of one lap on opposite sides of a barrier; that is, where the rat was required to turn around and run in the opposite direction (Fig. 1A). During all electrophysiological recording sessions, rats were required to run at least 20 laps (10 in the counterclockwise direction and 10 in the clockwise direction) during two distinct episodes of behavior. Each track-running epoch was flanked by a rest period in which the rat was placed in a towel-lined pot located in a position that was central to the circumference of the track. Thus, the activity of PRC neurons was monitored during an initial rest session (before behavior), during the first epoch of track running, during a second rest session after Epoch 1 that was either 20 min or 2 h long, during a second epoch of track running, and then finally during a third rest period. Data from the rest periods were used to assess firing stability across the entire recording session.

All 12 rats participated in the same behavioral procedures. During the first procedure (Day 1), rats ran on an empty track (no objects–both epochs; Fig. 1A) for both epochs of behavior. A 20 min rest period occurred between Epoch 1 and Epoch 2. For the second procedure (Day 2), during Epoch 1, eight novel objects that varied in size, color, and texture were placed at eight different locations along the track. All the objects used in these experiments were at least 7 cm in each dimension, so as to be easily identified by the rats. The side of the track on which the objects were placed alternated between the left and the right side and the rat had to run past these objects to obtain the food reward. This also ensured that the rats had to briefly attend to the objects while traversing the track to avoid colliding with them. Following either a 20 min or a 2 h rest period, during Epoch 2, six of the same objects used in Epoch 1 remained in the



**Figure 1.** Behavioral procedures used during electrophysiological recordings. The track used for behavior during all electrophysiological recordings required rats to run 20 laps bidirectionally (10 counterclockwise, 10 clockwise) for a food reward. **A**, Under the no objects condition the track was empty during both epochs of track running. Rewards were given in two food dishes located on opposite sides of a barrier (indicated by squares), at the position where the rat was required to turn around. The “X” indicates the location of the pot that the rat was placed in during rest episodes. **B**, In the objects–both epochs condition, eight novel objects were placed at discrete locations around the track for the first epoch of behavior (top), and the rat had to run past the objects to obtain the food reward. During the second epoch of behavior (bottom), six of the eight objects used in Epoch 1 were placed on the track at the same location as in Epoch 1, while two of the eight objects were removed and substituted with two novel objects (in this case Objects 3 and 5 were replaced with Objects 9 and 10 as indicated by the gray boxes). **C, D**, All rats participated in two additional behavioral procedures: the no objects–objects (**C**) and the objects–no objects (**D**) conditions. **E**, A schematic view of bottom of the tetrode guide cannulae showing an example of the configuration of the recording probes. The numbers indicate tetrode assignment, which was used for determining the recording location of each tetrode. **F**, Coronal Nissl-stained sections of two young rat brains (left and middle), and one aged rat brain (right) showing representative tetrode tracks (black lines) and lesions (red circles) for perirhinal cortical recordings. In the young rats, the tetrodes recorded neurons in Layer V (left) and Layers II/III (left and right). In the aged rats, most perirhinal cortical neurons were recorded from Layer V. The red arrows indicate the location of the rhinal sulcus. Scale bars, ~1 mm. In some cases the tetrodes did not reach the PRC (blue circles) and cells recorded from these tetrodes were not used in the current analyses.

same location on the track and two objects that were on the track during Epoch 1 were replaced with two novel objects (objects–both epochs condition; Fig. 1B). This manipulation was included to control for any differences that object novelty could have on firing rate before it was shown that changing the objects and their relative novelty have minimal effects on PRC neuron activity (Burke et al., 2012a). Each rat completed these behavioral procedures on consecutive days, and this process was repeated a minimum of two times and a maximum of six times. Eight novel objects were always used for Epoch 1 of the 20 min and 2 h delay conditions. Yoked old–young rats pairs that underwent electrophysiological recordings on the same days were always presented with identical objects in the same location. Different aged–young rat pairs were presented with distinct novel objects such that across yoked pairs of animals, objects sets were not the same. For one aged rat only, no single-unit data were collected for the objects–both epochs condition with a 2 h delay. Following the completion of these behavioral procedures, all rats traversed the track for two additional control conditions to measure the extent to which objects modulated PRC activity within the same population of principal cells. This included a no objects–objects condition (Fig. 1C) and an objects–no objects condition (Fig. 1D) that rats participated in on different days than the conditions described above. For both of these procedures, Epochs 1 and 2 were separated by a 20 min delay.

**Table 1. Number and cell layer of PRC single units recorded from each young and aged rat over all conditions**

Rat	Age	Number (cell layer)				
		No objects	20 min delay	2 h delay	No objects–no objects	Objects–no objects
8412	Young	7 (V)	8 (V)	11 (V)	9 (V)	2 (V)
8413	Aged	62 (V)	34 (V)	45 (V)	9 (V)	13 (V)
8509	Young	7 (V)	14 (V)	12 (V)	9 (V)	4 (V)
8507	Aged	20 (V)	17 (V)		7 (V)	6 (V)
8583	Young	93 (II/III)	94 (II/III)	83 (II/III)	50 (II/III)	53 (II/III)
8615	Aged	99 (V)	56 (V)	62 (V)	55 (V)	17 (V)
8661	Young	34 and 14 (II/III and V)	33 and 12 (II/III and V)	25 and 10 (II/III and V)	1 and 26 (II/III and V)	1 and 25 (II/III and V)
8662	Aged	99 (V)	41 (V)	59 (V)	33 (V)	63 (V)
8670	Young	60 and 68 (II/III and V)	48 and 58 (II/III and V)	21 and 27 (II/III and V)	11 and 15 (II/III and V)	28 and 29 (II/III and V)
8696	Aged	39 (V)	44 (V)	27 (V)	30 (V)	44 (V)
8883	Young	35 (V)	30 (V)	12 (V)	3 (V)	2 (V)
8865	Aged	7 and 31 (II/III and V)	4 and 44 (II/III and V)	2 and 35 (II/III and V)	16 (V)	3 and 26 (II/III and V)
Total		675	537	431	274	316

For all conditions in which objects were placed on the track, the objects were fixed in place using Velcro. Thus, rats could actively explore, rear, and climb on the objects without displacing them. Additionally, during all rest periods the objects were removed from the track so that the rat could not see them during the intervening delay period.

**Surgical procedures.** Surgery was conducted according to National Institutes of Health guidelines for rodents and protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Before surgery, the rats were administered penicillin G (30,000 U, i.m., in each hindlimb) to combat infection. During surgical implantation, the rats were maintained under anesthesia with isoflurane administered at doses ranging from 0.5 to 2.5%.

All rats were implanted with a “hyperdrive” manipulator device that held an array of 14 separately moveable tetrode recording probes (Gothard et al., 1996). Each hyperdrive consisted of 14 drive screws coupled by a nut to a guide cannula. Twelve of these cannulae contained tetrodes (McNaughton et al., 1983b; Recce and O’Keefe, 1989), four-channel electrodes constructed by twisting together four strands of insulated 13  $\mu\text{m}$  nichrome wire (H.P. Reid). Two additional tetrodes had their individual wires shorted together, and the shorted tetrode with the least cellular activity was used as an indifferent reference. A full turn of the screw advanced the tetrode 318  $\mu\text{m}$  and all tetrodes were lowered between 4.0 and 6.0 mm ventral to the surface of brain. The 14 guide cannulae were arranged in two linear columns of seven each such that the configuration of tetrodes spanned  $\sim 2$  mm from the anterior to posterior position (Fig. 1E). This permitted sampling of neurons from a greater extent of the PRC and enabled more precise matching of tetrode number to track and lesion location for histological verification or recording sites (see below).

The implant was cemented in place with dental acrylic anchored by small screws. Immediately after surgery, all tetrodes were lowered  $\sim 1$  mm into the cortex, and rats were orally administered 26 mg of acetaminophen (Children’s Tylenol Elixir or ibuprofen) for analgesia. Oral administration of acetaminophen was continued for 3–5 d after surgery. Additionally, all rats were given either 25 mg of ampicillin (Bicillin, Wyeth Laboratories) or a combination of 20 mg of sulfamethoxazole and 0.4 mg of trimethoprim (Hi-Tech Pharmacal) on a 10 d on/10 d off regimen for the duration of the experiment.

In all rats, recordings were made from the middle to caudal PRC region (between 4.0 and 6.5 mm posterior, 6.0 mm lateral to bregma, and angled 14° toward the midline). Following experimental procedures, 20  $\mu\text{A}$  of direct current was administered to each tetrode. One to 2 weeks following microlesioning, rats were given a fatal dose pentobarbital and perfused with 4% paraformaldehyde (Gage et al., 2012). Brains were extracted and soaked in a 30% sucrose solution for 1 week or until they sank. Tissue was then frozen with dry ice and the area under the cranial implant was coronally sliced at 40  $\mu\text{m}$  with a cryostat. Sections were directly mounted to superfrost slides and dried overnight in a fume hood. Finally, tissue was Nissl-stained and tetrode location was verified. In most cases the tetrode tracks were not parallel to the plane of sectioning and tracks had to be followed over several adjacent slides. In these cases

the section in which the lesion was the largest was considered the approximate location. Only the units recorded from tetrodes histologically verified to be in the PRC were used in the current analyses and neurons recorded from other brain regions (e.g., ventral CA1 or area TE of the inferotemporal cortex) were excluded. The majority of tetrodes were located in Area 36 of the PRC, but in two young and two aged rats four tetrodes reached Dorsal Area 35. Moreover, in the young rats neurons were recorded from both Layer V and Layers II/III, but for the aged rats only one rat had tetrodes in Layers II/III, and all of the other single-unit recordings were from neurons in Layer V. Figure 1F shows Nissl-stained coronal sections from different young and aged rat brains with representative tetrode recording tracks and lesions within the PRC. Neurons recorded from tetrodes that produced lesions outside of the PRC were not included in any of the current analyses. In most cases these tracks were in Area TE (Fig. 1F, blue circles).

**Neurophysiology.** After surgery, tetrodes were lowered into the PRC over several weeks. The neutral reference electrode was advanced with other tetrodes and when an area of cortex was reached that did not record any unit activity, it was not moved again. The four channels of each tetrode were attached to a 50-channel unity-gain head stage (Neuralynx). A multiwire cable connected the head stage to digitally programmable amplifiers (Neuralynx). The spike signals were amplified by a factor of 1000–5000, bandpass-filtered between 600 Hz and 6 kHz, and transmitted to the Cheetah data acquisition system (Neuralynx). Signals were digitized at 32 kHz, and events that reached a predetermined threshold were recorded for a duration of 1 ms. Spikes were sorted offline on the basis of the amplitude and principal components from the four tetrode channels by means of a semiautomatic clustering algorithm (KlustaKwik, K.D. Harris). The resulting classification was corrected and refined manually with custom-written software (MClust, A.D. Redish, University of Minnesota; updated by S.L. Cowen, University of Arizona, and D.R. Euston, University of Lethbridge), resulting in a spike-train time series for each of the well isolated cells. No attempt was made to match cells from one daily session to the next. Therefore, the numbers of recorded cells reported does not take into account possible recordings from the same cells on consecutive days and the actual number of unique recorded neurons could be lower than reported in Table 1. Note that all statistics were run on the basis of pooled data for individual rats, and not with total cells as the sample size. This conservative approach was used as a means to not create a bias from oversampling the same neuron.

Putative principal neurons in the deep and superficial layers of the PRC were identified by means of their waveform characteristics and autocorrelogram features (Barthó et al., 2004). Specifically, neocortical principal cells tend to have autocorrelograms with peaks at 3–6 ms followed by an exponential decay, which is indicative of “bursting” cells, or an autocorrelogram with an exponential rise from 1 to 10s of milliseconds. These cells are considered regular-spiking neurons. In contrast, the autocorrelograms of putative interneurons are not as fast decaying or slow rising as those of pyramidal neurons (Barthó et al., 2004).

Activity from putative principal neurons was used for analysis only if their respective waveform features showed clear separation from the

spikes of other cells and from noise. This was initially determined with qualitative ratings made by experimenters. Following this rating, the extent that clusters obtained from the waveforms of neurons included in the present analyses showed separation from other cells and from noise was quantified by calculating the L ratio and isolation distance of each cluster (Schmitzer-Torbert et al., 2005). The L ratio is the degree that a cluster separates from other spikes recorded on the same tetrode normalized by the total number of spikes for a given cluster. A lower L-ratio value is indicative of better separation (Schmitzer-Torbert et al., 2005). The average L ratio was 0.15 ( $\pm 0.02$  SEM) for the young rats and 0.11 ( $\pm 0.02$  SEM) for the aged rats, which was not significantly statistically different ( $t_{(5)} = 1.22, p = 0.28$ ; paired samples). The isolation distance estimates how distant the cluster spikes are from the other spikes recorded on the same tetrode (Harris et al., 2001). Higher isolation distance therefore corresponds to better separation and a reduced probability of contamination from noise or spikes from other neurons. The mean isolation distance was 68.9 ( $\pm 20.9$  SEM) for the young rats and 55.7 ( $\pm 5.1$  SEM) for the aged rats, which was not significantly different ( $t_{(5)} = 0.58, p = 0.59$ ; paired samples). These values indicate that cluster quality was not different between the young and aged rats and that all cells used in the current analyses showed intermediate to good separation (Schmitzer-Torbert et al., 2005).

Several diodes were mounted on the head stage to allow position tracking. The position of the diode array was detected by a TV camera placed directly above the experimental apparatus and recorded with a sampling frequency of 60 Hz. The sampling resolution was such that a pixel was  $\sim 0.3$  cm. A portion of the principal data obtained from the young rats during the no objects–both epochs and objects–both epochs conditions has been published previously (Burke et al., 2012a).

**Analyses and statistics.** Spike-activity diagrams were constructed by plotting the circular trajectories of the animals on a linearized, one-dimensional scale, using a linear interpolation (Maurer et al., 2005). For each cell, the maximum firing rate, mean firing rate, and information content (spikes/bit) were calculated. Maximum and mean firing rates were obtained after normalizing spike activity by the occupancy of the animal, and information content was calculated from 161 bins of  $\sim 4.1$  cm with the following formula:  $\sum P_i(R_i/R) \log_2(R_i/R)$ , where  $P_i$  was the probability of occupancy for a bin,  $R_i$  was the firing rate of the bin, and  $R$  was the mean firing rate of the cell (Skaggs et al., 1993). The area of the track within seven bins of a food dish (28.7 cm) was excluded for the calculation of information content, because when the rats were within this area of the track, their running speed was either zero or it was changing rapidly as the rat was stopping to obtain reward or accelerating after eating. Moreover, this area of the track contained a food dish, the reward and an object during conditions with objects. Therefore, activity at this location could presumably be related to the food dish, reward, the ripple activity known to occur during behavior when animals pause (O'Neill et al., 2006), and/or the object. Because it was difficult to dissociate the relative contribution of these factors, activity in this area of the track was excluded from analysis. It is notable, however, that when a velocity filter of 10 cm/s was applied to spikes, the firing rates at the food dish regions did not significantly vary between object and no object conditions and between other regions on the track (data not shown). This suggests that activity in these areas were related to stimulus properties rather than to reward.

When the information content was calculated for all cells that showed activity on the maze (a mean firing rate  $>0.2$  spikes/bin occupancy), there was not a significant difference in mean information content between Epochs 1 and 2 ( $t_{(45)} = 0.10, p = 0.94$ ; paired-samples  $t$  test). Moreover, when all behavioral conditions were analyzed for the three rats (two young and one aged) that had tetrodes in both the deep and the superficial layers of the PRC, information content was not significantly different ( $t_{(5)} = 1.51, p = 0.19$ ; paired-samples  $t$  test) between neurons in Layer V (0.61 bits/spike) and neurons in Layers II/III (0.53 bits/spike). Therefore, the data obtained from the different epochs of track running, and from the different cortical laminae were combined for additional analysis of the effects of behavioral condition and age.

Information content was used to examine the activity correlates of PRC neurons. Specifically, it was observed that many PRC neurons show in-

creased firing rates at the location of objects (Burke et al., 2012a; Deshmukh et al., 2012). These patterns of activity were termed “object fields” (Burke et al., 2012a), and a PRC neuron was considered to have an object field if its information content was  $>0.5$  bits/spike, and the occupancy-normalized mean firing rate within a bin exceeded the mean firing rate for  $\geq 4$  consecutive bins (Burke et al., 2012a). Using the criteria described above, the mean size of object fields was 26.9 cm in the young rats and 26.1 cm in the aged rats, and object field size did not vary significantly across age group and behavioral epoch ( $F_{(3,15)} = 0.68, p > 0.58$ ; repeated measures). Moreover, the proportion of neurons with object fields was not significantly different between Epoch 1 and Epoch 2 for any of the behavioral conditions ( $F_{(1,42)} = 0.04, p = 0.85$ ; repeated-measures ANOVA). Therefore, the data were collapsed across epoch for the age comparison.

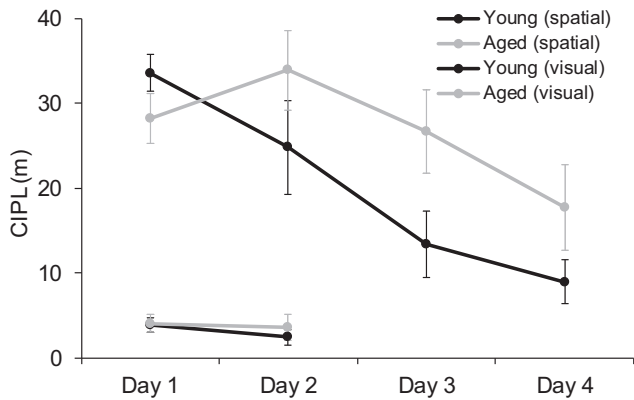
The correlated activity patterns between epochs were quantified by dividing the track into 80 bins of  $\sim 4.1$  cm, and the firing rate of an individual neuron was calculated for each bin. Laps that the rats ran in the counterclockwise direction were separated from laps where the running direction was clockwise. Therefore, the firing rate was determined for a total of 160 bins resulting in a  $160 \times 1$  firing-rate vector. The Pearson's correlation coefficient between the Epoch 1 and the Epoch 2 firing-rate vectors was then calculated for all PRC neurons across the six different behavioral conditions in the two age groups.

To examine any possible age-related differences, the mean for all cells was calculated for every rat. This reduced the chances that the analysis would be skewed because more neurons were recorded from some rats than others or because recordings were taken from the same cells over multiple days. This conservative approach also safeguarded against a bias toward finding an age difference due to the high statistical power obtained by the large number of cells. Finally, because data were always obtained from yoked pairs of young–aged rats, repeated-measures or paired statistical analyses (Spatz, 2011) were used for significance testing and  $\alpha$  was set to the 0.05 level. This analysis approach has been used previously for *in vivo* high-density electrophysiology studies comparing age groups (Insel et al., 2012; Schimanski et al., 2013).

## Results

### Recognition behavior and spatial learning

The aged rats had impaired spatial learning compared with the young animals, but were equally able to use visual cues to guide behavior, as measured by the Morris swim task (Fig. 2). Rats have a natural tendency to explore objects, or other stimuli, that are novel (Ennaceur and Delacour, 1988). Therefore, decreased running velocity during track traversals can be used as an indication of novel object detection, as animals will stop to explore new objects, while faster speeds suggest stimulus recognition. To test this idea, the rats' velocities during the first two laps were compared with the last two laps for epochs in which the track contained no objects, novel objects (Epoch 1 for the objects–both epochs conditions), and familiar objects (Epoch 2 of objects–both epochs condition; Fig. 3A). When the mean running speed of the rats for the first two laps was compared with the velocity for last laps, statistical analysis revealed that the rats ran significantly slower during the first laps relative to the last laps ( $F_{(1,20)} = 14.23, p < 0.001$ ; repeated measures). There was also a significant effect of behavioral condition on the difference in running velocity between the first and last laps ( $F_{(3,60)} = 7.59, p < 0.01$ ; repeated-measures ANOVA), which did not significantly interact with age group ( $F_{(1,20)} = 1.91, p = 0.18$ ; repeated measures). *Post hoc* analysis indicated that the difference in running speeds between the first laps and the last laps was greatest when there were novel objects on the track compared with the other conditions ( $p < 0.005$  for all comparisons, Tukey HSD). This indicates that both the young and aged animals showed the same pattern of reduced running speed when novel objects were on the track compared with when the track contained familiar objects or was empty. In



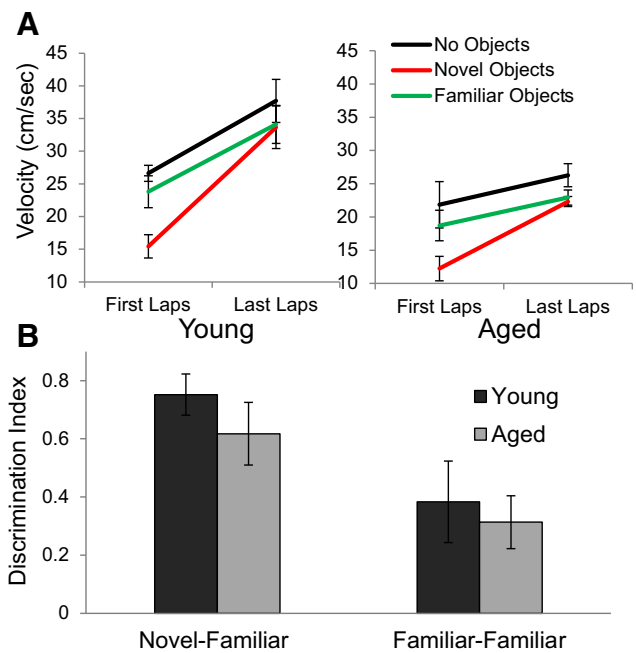
**Figure 2.** Performance on the Morris swim task. The results from the Morris swim task for the adult (black) and the aged (gray) rats. The x-axis is the day of testing and the y-axis is the mean CIPL score. Higher CIPL scores indicate longer path lengths to reach the escape platform. All rats completed 4 d of spatial trials (solid lines), in which the platform was hidden below the surface of the water, followed by 2 d of visually cued trials (dashed lines), in which the platform was visible but its location changed after every trial. During the spatial trials, the aged rats had significantly longer CIPL scores compared with the adult rats ( $F_{(1,40)} = 6.12, p < 0.02$ ; ANOVA). *Post hoc* analysis revealed that this difference was due to the aged rats having significantly longer CIPL scores on Days 3 and 4 of spatial testing ( $p < 0.05$  for all comparisons; Tukey HSD) while there was no significant difference in the path lengths between young and aged rats on Day 1 or Day 2 of spatial testing ( $p > 0.2$  for both comparisons; Tukey HSD). The CIPL scores of all rats were significantly decreased when the platform was visible, and there was no significant effect of age on the CIPL values during either Day 1 or Day 2 of the visual swim task testing ( $F_{(1,10)} = 0.07, p = 0.79$ ; repeated-measures ANOVA). Therefore, it is unlikely that any of the aged animals used in the current series of experiments had significant visual impairment relative to the young group. Error bars represent  $\pm 1$  SEM.

other words, the aged rats expressed behavior that was indicative of object recognition similar to the young rats.

To further examine whether there was a difference between novel and familiar objects on running speed, a discrimination index was calculated for epochs in which the objects were novel and became familiar during later laps (novel–familiar) and for epochs in which the objects were familiar during all laps (familiar–familiar). The discrimination index was the difference in running speed between the first two laps and the last two laps divided by the mean running speed. Figure 3B shows the discrimination index across behavioral condition for young and aged rats. There was a significant effect of novel–familiar versus familiar–familiar on the discrimination index ( $F_{(1,11)} = 5.23, p < 0.001$ ; repeated measures). This indicates that across all animals the running speeds between the first and last laps decreased more when objects were initially novel and became familiar compared with when familiar objects were on the track for the last laps. Age group, however, did not have a significant effect on the discrimination index ( $F_{(1,11)} = 1.25, p = 0.24$ ; repeated measures). Moreover, there was not a significant interaction between age and behavioral condition ( $F_{(1,11)} = 0.64, p = 0.5$ ; repeated measures). These results confirm the observation that both young and aged rats were able to recognize when objects were familiar and that this corresponded with reduced running speeds during laps in which the track contained novel objects.

### Object-related activity of perirhinal cortical neurons across the life span

The activity of 1643 PRC principal neurons was monitored in this experiment. An additional 590 neurons were recorded during the control no objects–objects and objects–no objects conditions



**Figure 3.** Running velocity of young and aged rats for the different behavioral conditions. **A**, Comparison of running speed during the first laps (Laps 1 and 2) versus the last laps segregated by epochs without objects (black), with novel objects (red), and with familiar objects (green) for young (left) and aged (right) rats. All rats ran significantly slower during the first laps relative to the last laps ( $F_{(1,20)} = 14.23, p < 0.001$ ; repeated measures). The difference in running speeds between the first laps and the last laps was greatest when there were novel objects on the track compared with the other conditions ( $p < 0.005$  for all comparisons, Tukey HSD). **B**, The discrimination index (difference in first and last lap velocity/mean velocity) for epochs in which the objects were novel and became familiar during later laps (Novel–Familiar) and epochs in which the objects were familiar (Familiar–Familiar) during all laps in young (black) and aged (gray) rats. There was a significant effect of Novel–Familiar versus Familiar–Familiar on the discrimination index ( $F_{(1,11)} = 5.23, p < 0.001$ ; repeated measures). Age group did not have a significant effect on the discrimination index ( $F_{(1,11)} = 1.25, p = 0.24$ ; repeated measures), and there was no significant interaction between age and behavioral condition ( $F_{(1,11)} = 0.64, p = 0.5$ ; repeated measures). Error bars represent  $\pm 1$  SEM.

(Table 1). The numbers of PRC neurons that were recorded during these experiments were not significantly different between the young and the aged rats ( $F_{(1,19)} = 0.03, p = 0.87$ ). Additionally, all single-unit neurons that were included in the current analyses showed stability during an entire recording session, and there was no significant difference in the mean firing rate of neurons during Rest 1 and Rest 3 ( $F_{(1,10)} = 1.01, p = 0.34$ ; repeated-measures ANOVA). Moreover, the stability of the mean firing rates between the beginning of a recording session and the end of a recording session was not significantly different between young and aged rats ( $F_{(1,10)} = 0.02, p = 0.67$ ; repeated-measures ANOVA). The mean firing rate during sleep episodes was 1.3 Hz for the young rats and 1.1 Hz for the aged rats, and these firing rates were not statistically different from each other ( $F_{(1,10)} = 2.26, p = 0.16$ ). The firing rates of neurons recorded from different cortical lamina also did not systematically differ ( $t_{(6)} = 1.23, p = 0.23$ , paired-samples *t* test), and the data were collapsed between the deep and superficial layers for subsequent analyses (Table 2). Finally, age group did have a significant effect on the mean firing rate during behavior ( $F_{(1,40)} = 22.52, p < 0.001$ ; repeated-measures ANOVA), but not on maximum firing rate ( $F_{(1,40)} = 0.31, p = 0.58$ ; repeated-measures ANOVA). Table 3 shows the *p* values for age comparisons of the mean and maximum firing rates for the different behavioral conditions and age groups.

**Table 2. Firing rates in different cortical laminae<sup>a</sup>**

Young	No objects	20 min delay	2 h delay	Aged	No objects	20 min delay	2 h delay
8412	0.8 (V)	2.7 (V)	0.9 (V)	8413	1.0 (V)	1.2 (V)	0.9 (V)
8509	0.8 (V)	1.7 (V)	1.6 (V)	8507	1.0 (V)	1.1 (V)	
8583	1.7 (II/III)	1.2 (II/III)	1.5 (II/III)	8615	1.2 (V)	1.0 (V)	0.9 (V)
8661	2.8 and 1.3 (II/III and V)	3.0 and 3.3 (II/III and V)	2.2 and 1.2 (II/III and V)	8662	1.2 (V)	1.3 (V)	1.6 (V)
8670	2.0 and 2.4 (II/III and V)	1.6 and 1.6 (II/III and V)	1.5 and 2.1 (II/III and V)	8696	1.3 (V)	1.8 (V)	0.8 (V)
8883	1.6 (V)	1.1 (V)	0.7 (V)	8865	3.3 and 1.1 (II/III and V)	3.4 and 0.8 (II/III and V)	0.7 and 0.8 (II/III and V)

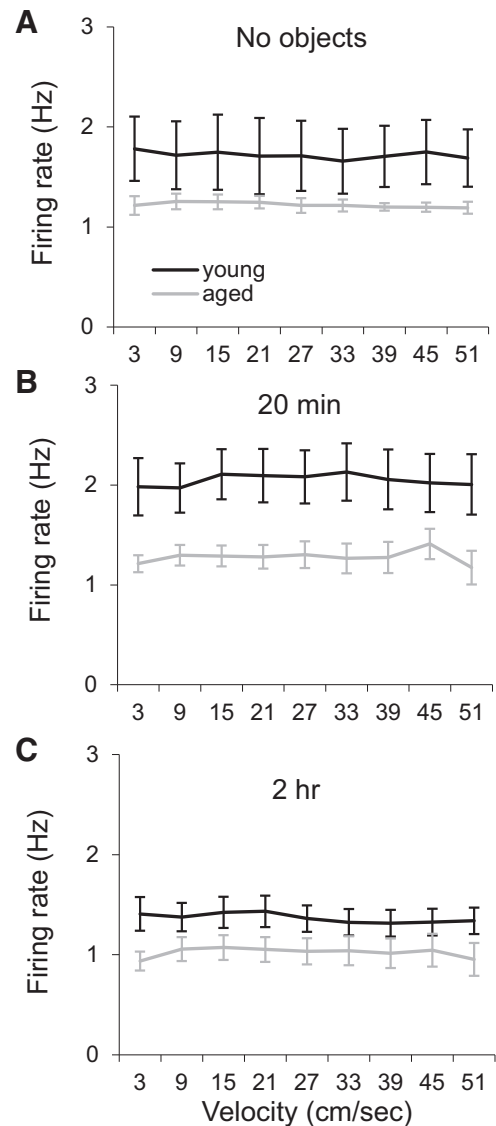
<sup>a</sup>Mean firing rate (Hz) of cells recorded from pairs of young and aged rats. Layers in parentheses.

**Table 3. Firing rate characteristics of PRC neurons recorded in young and aged rats**

	20 min delay	2 h delay	No objects	<i>p</i> value
Mean firing rate				
Young rats	1.84 ± 0.21 Hz	1.44 ± 0.20 Hz	1.59 ± 0.19 Hz	<i>p</i> = 0.33
Aged rats	1.26 ± 0.09 Hz	1.00 ± 0.11 Hz	1.21 ± 0.06 Hz	<i>p</i> = 0.16
<i>p</i> value	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	
Maximum firing rate				
Young rats	5.31 ± 0.31 Hz	5.48 ± 0.60 Hz	4.25 ± 0.33 Hz	<i>p</i> = 0.07
Aged rats	4.78 ± 0.10 Hz	4.69 ± 0.18 Hz	4.97 ± 0.24 Hz	<i>p</i> = 0.24
<i>p</i> value	<i>p</i> = 0.44	<i>p</i> = 0.83	<i>p</i> = 0.16	

In the hippocampus, the firing rate of CA1 pyramidal neurons is modulated by velocity (McNaughton et al., 1983a; Maurer et al., 2005) in both young and old animals (Shen et al., 1997). Therefore, a possible explanation for the higher mean firing rates of young compared with aged PRC neurons could be different running velocities between age groups (Fig. 3). When firing rate was compared with velocity in the aged rats for velocities between 3 and 51 cm/s, it was observed that, similar to young animals, running speed did not significantly modulate PRC neuron firing rate during any of the behavioral conditions (Fig. 4). These data suggest that the firing-rate difference between young and old rats cannot be accounted for by the aged rats' slower running speeds. Moreover, it confirms previous observations that, in contrast to neurons in the hippocampus (McNaughton et al., 1983a; Maurer et al., 2005) and medial entorhinal cortex (Sargolini et al., 2006), PRC neuron firing rates are not modulated by velocity (Burke et al., 2012a).

An alternative explanation for the decreased firing rates in aged compared with young rats could be found in the differential effect of relative novelty and familiarity on PRC neuron firing rates (Zhu and Brown, 1995; Zhu et al., 1995). Specifically, it has been hypothesized that aged rats (Robitsek et al., 2008) and humans (Daselaar et al., 2006) may have an enhanced familiarity signal in the PRC that could possibly compensate for a decline in hippocampal-dependent recollection. Recent data from young animals, however, have not observed an effect of novel versus familiar stimuli on the firing rates of PRC principal neurons (Burke et al., 2012a; Thome et al., 2012; Woloszyn and Sheinberg, 2012). The possibility remains that aged PRC principal neurons may show decremental activity patterns as stimuli go from being novel to familiar. Thus, the firing rate was measured across laps for Epochs 1 and 2 of the different behavioral conditions, and then was normalized within a cell by calculating the Z-score firing rate for each lap. The normalized firing rate change between the first and last laps within an epoch for the counterclockwise and clockwise laps was then quantified. There was no systematic change in the normalized firing rate over laps during any of the behavioral conditions for either the young or the aged rats ( $F_{(15,120)} = 0.41$ ,  $p = 0.97$ ; repeated-measures ANOVA). Moreover, the change in normalized firing rate between Lap 1 and the



**Figure 4.** Firing rate by velocity. **A–C**, Mean firing rate was not significantly modulated by velocity in either the young (black) or the aged (gray) rats for the (**A**) no objects—both epochs, (**B**) objects—both epochs with a 20 min delay, and (**C**) objects—both epochs with a 2 h delay ( $r_{(53)} < 0.1$ ,  $p > 0.5$  for all comparisons; Pearson's correlation coefficient). Error bars represent  $\pm 1$  SEM.

last lap was not significantly different between young and aged rats ( $F_{(1,10)} = 0.20$ ,  $p = 0.66$ ; repeated-measures ANOVA).

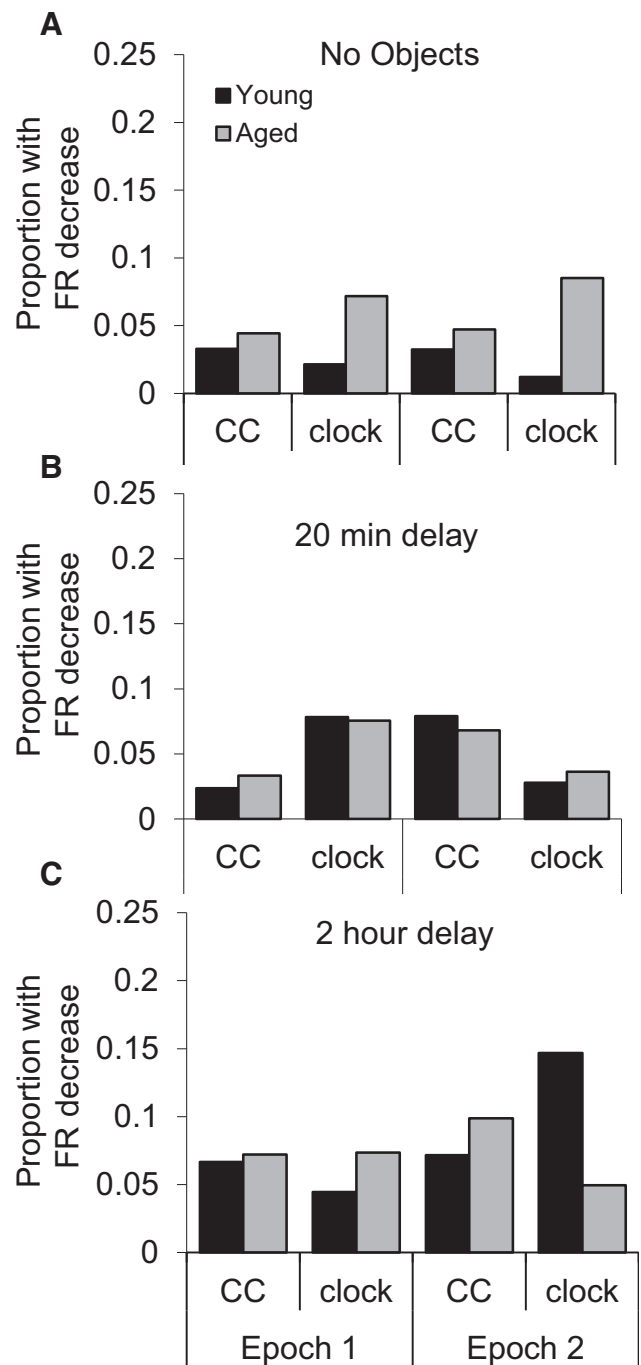
Although the population of recorded PRC neurons did not show a change in firing rate between conditions with novel objects compared with the other behavioral conditions, it is still possible that a subset of PRC neurons might show a response decrement as objects go from being novel to relatively familiar

and that this decrement was not detected when the data were collapsed across all active neurons. To examine this possibility, PRC neurons that had firing rates at least 2 SDs above mean firing rate during Lap 1 were identified. The proportions of recorded PRC neurons that met this criterion were then compared between the different behavioral conditions and epochs. Approximately 5% of all the recorded PRC neurons fit this description, but this did not vary significantly between behavioral condition ( $F_{(15,120)} = 1.51, p = 0.21$ ; repeated-measures ANOVA). Moreover, the proportion of cells that showed a response decrement between Lap 1 and the subsequent laps was not significantly different between the young and the aged rats ( $F_{(1,10)} = 3.4, p = 0.11$ , repeated-measures ANOVA). Figure 5 illustrates the mean proportion of the recorded PRC neurons in young and aged rats during Epochs 1 and 2 that had a firing rate  $\geq 2$  SDs above their average firing rate in (A) the no objects–both epochs, (B) the objects–both epochs with a 20 min delay, and (C) the objects–both epochs with a 2 h delay. Together, these data indicate no evidence of a response decrement in the activity of PRC neurons as objects went from novel to familiar in either the young or the aged rats.

Similar to previous reports in young rats (Burke et al., 2012a; Deshmukh et al., 2012), when animals traversed the track with objects on it, PRC neurons showed a selective increase in their firing rates at the locations of objects. This occurred when objects were novel (during Epoch 1 of the objects–both epochs conditions), as well as when objects were familiar (during Epoch 2 of the objects–both epochs conditions) in both age groups. Figure 6 shows representative examples of PRC neuron object field activity recorded from young (A) and aged (B) rats.

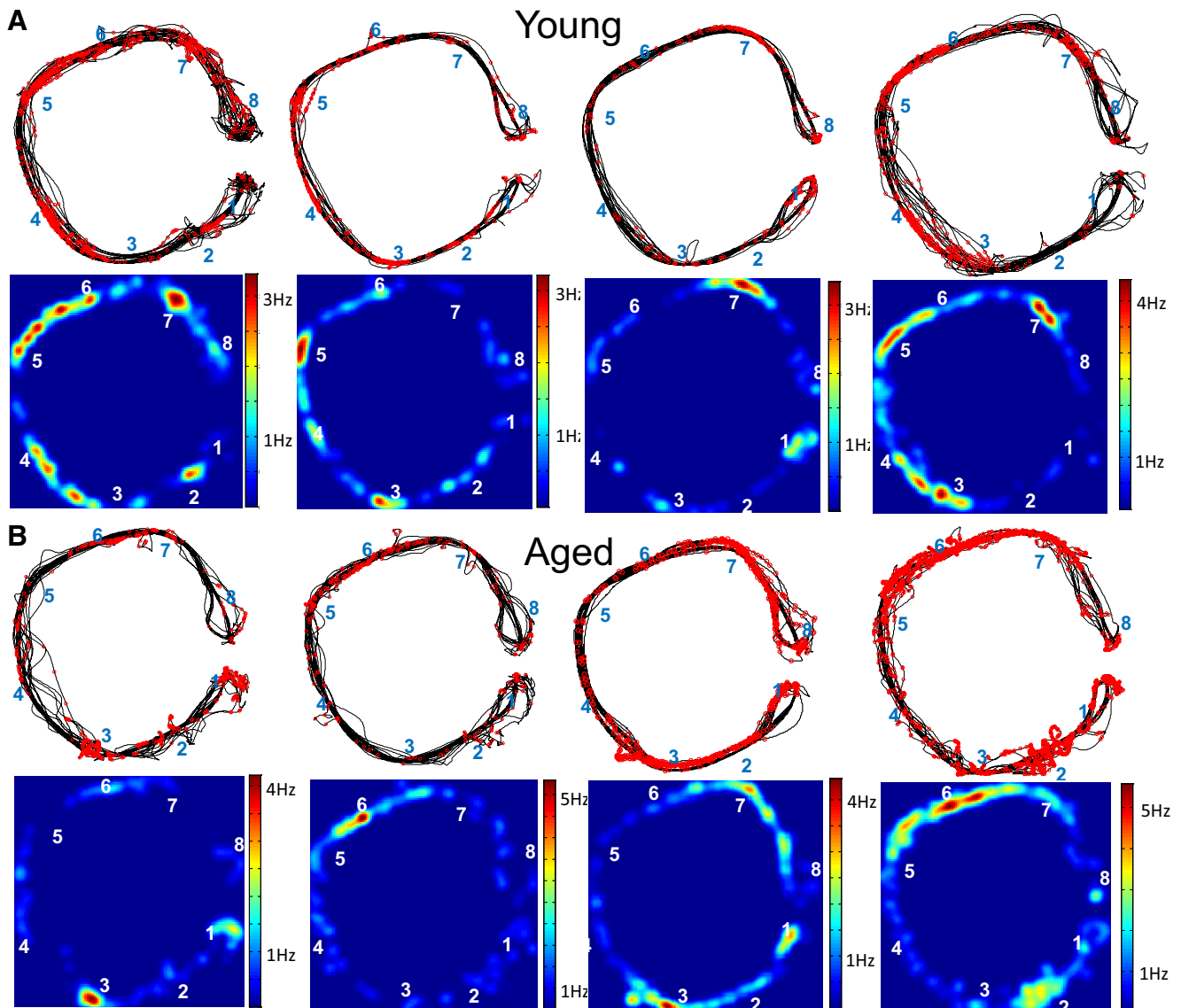
Placing objects on a track increases the information content of PRC spiking (Burke et al., 2012a), which can be used to quantify the proportion of PRC neurons that increase their firing rate at locations containing objects (see Materials and Methods). Similar to young animals, PRC neurons in both age groups had significantly higher information scores in conditions with objects relative to the no object–both epochs condition ( $F_{(3,40)} = 3.03, p < 0.05$ ; repeated-measures ANOVA). The mean information content, however, was significantly reduced in the aged compared with young rats ( $F_{(1,40)} = 8.23, p < 0.01$ ; repeated-measures ANOVA) for the conditions with objects ( $p < 0.5$ ), but not for the no objects–both epochs condition ( $p = 0.54$ ; Fig. 7). To determine whether the object-related firing properties of PRC cells were present from the very first exposure to objects on the track, the mean information content was calculated and the first behavioral experience with objects in Epoch 1 was compared with the last behavioral experience with objects in Epoch 2. In both the young and the aged rats, there was no difference in the mean information per spike between the first exposure to objects and the last ( $t_{(11)} = 0.41, p = 0.69$ , paired-samples  $t$  test). Because the information scores for the first and last exposure were virtually identical (0.58 bits/spike vs 0.63 bits/spike), it appears that objects on the track increased information content *de novo*.

The findings that aged PRC principal cell activity patterns show both reduced firing and lower information content during behavior compared with young rats suggests that in advanced age PRC neurons may be less responsive to stimuli. To test this directly, the proportion of neurons with object fields was compared across age groups and behavioral conditions. The young rats had a significantly higher proportion of PRC cells that expressed object fields compared with the aged rats ( $F_{(1,40)} = 44.53, p < 0.001$ ; repeated-measured ANOVA), and this was the case for conditions with objects ( $p < 0.05$  for all comparisons; Tukey HSD),



**Figure 5.** The proportion of recorded PRC neurons that show a response decrement. **A–C**, In both young (black) and aged (gray) rats, no significant difference was found in the proportion of cells that had a response decrement between the (A) no objects–both epochs, (B) objects–both epochs with a 20 min delay, and (C) objects–both epochs with a 2 h delay ( $F_{(15,120)} = 1.51, p = 0.21$ ; repeated-measures ANOVA). Additionally, the young and the aged rats did not differ in the proportion of cells that showed a response decrement between the first lap and subsequent laps ( $F_{(1,10)} = 3.4, p = 0.11$ , repeated-measures ANOVA). Error bars represent  $\pm 1$  SEM. CC, Counterclockwise running direction; clock, clockwise running direction.

but not for the no objects–both epochs condition ( $p = 0.12$ ). When the age groups were analyzed together, behavioral condition also had a significant effect on the proportion of PRC neurons with object fields ( $F_{(3,40)} = 10.14, p < 0.001$ ; repeated-measures ANOVA). *Post hoc* comparisons indicated that during the no objects–both epochs condition, significantly fewer neurons met the

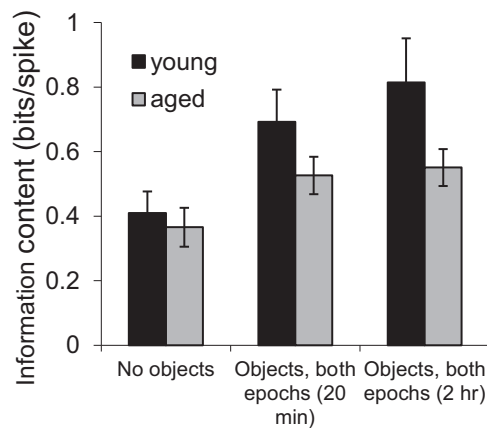


**Figure 6.** PRC neuron activity patterns in young and aged rats. **A**, The activity of four representative PRC neurons recorded from young rats under conditions with objects on the track. Top, Black trace, Path of the rats. Red spots indicate the locations of spikes. The blue numbers represent the locations of objects. Bottom, Occupancy-normalized firing-rate maps of the cells shown in **A**. **B**, Same as in **A** for four representative neurons recorded in aged rats. Note the increase in spiking at several of the locations containing objects.

criteria for having object fields relative to behavioral conditions with objects. Importantly, it did not appear that differences between the distributions of neurons recorded from deep versus superficial layers in the two age groups can account for the reduced proportion of PRC cells with object fields. Specifically, for the three rats that had tetrodes in both Layer V and Layers II/III of the PRC, there was no significant difference in the proportion of cells with object fields between the different layers of cortex ( $t_{(5)} = 0.24$ ,  $p = 0.82$ ; paired-sample  $t$  test). Figure 8A shows the mean proportion of PRC neurons that had object fields for the different behavioral conditions. These data indicate that PRC neurons in both young and aged rats show object-related spiking, but that the young rats have a higher proportion of neurons that show object fields.

The age-associated reduction in the proportion of PRC principal cells that expressed object fields cannot account for the lower firing rates of PRC neurons during the no objects–both epochs condition in old compared with young rats, because in this condition the same proportion of neurons in young and old

rats met the criteria for having an object field. An alternative explanation for the lower mean firing rates in aged compared with young rats could be an increase in the proportion of cells that fired during both Rest 1 and Rest 3 but did not fire during behavior in old animals. Consistent with this idea is the observation that the proportions of neurons that did not show any activity during an episode of track running (mean firing rate  $< 0.2$  spikes/occupancy) was significantly higher in the aged compared with the young rats ( $F_{(1,40)} = 34.80$ ,  $p < 0.001$ ; repeated-measures ANOVA; Fig. 8B). This difference was observed during all behavioral conditions ( $p < 0.05$  for all comparisons, Tukey HSD). In both young and old rats, however, there was no significant effect of condition on the proportion of inactive neurons ( $F_{(3,40)} = 0.82$ ,  $p = 0.49$ ; repeated-measures ANOVA; Fig. 8B). Because the proportion of object fields significantly increases in the object condition, and there is no difference across conditions in number of inactive cells, it does not appear that the “quiet neurons” were the cells that acquired objects fields.

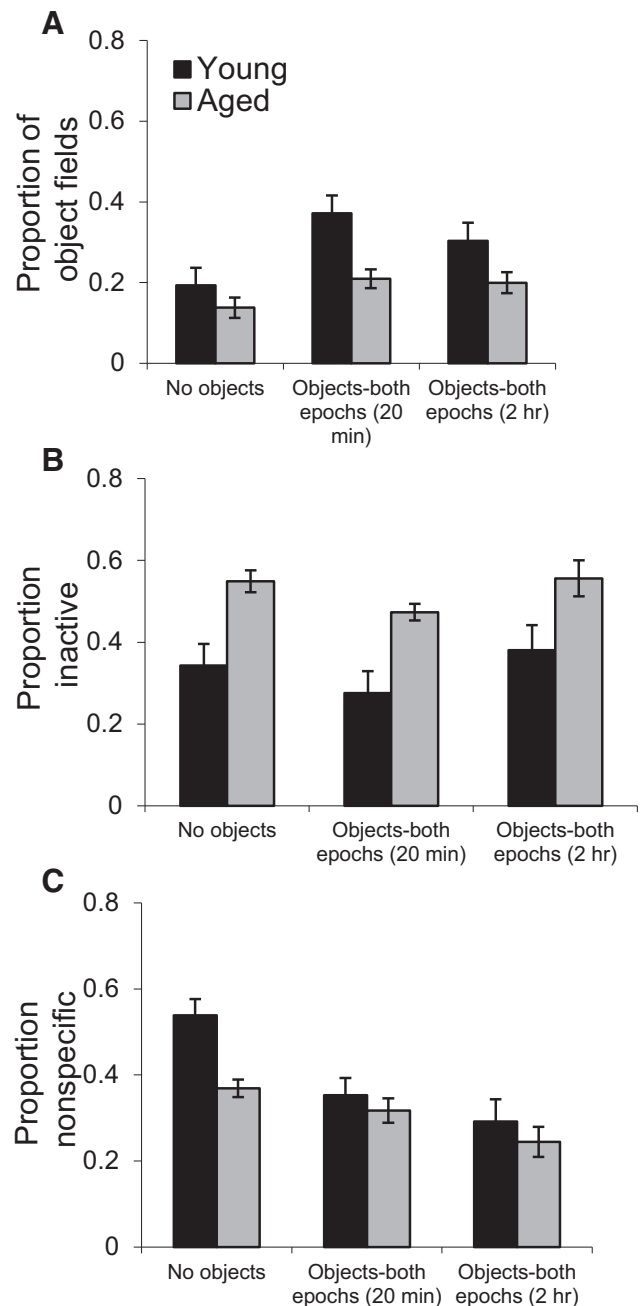


**Figure 7.** PRC neuron information content. The mean information content ( $y$ -axis) of the PRC neurons that showed activity during track running for the young (black) and aged (gray) rats in the different behavioral conditions. There was a significant effect of behavioral condition ( $F_{(3,40)} = 3.03, p < 0.05$ ; repeated-measures ANOVA) and age ( $F_{(1,40)} = 8.23, p < 0.01$ ; repeated-measures ANOVA) on the information content of PRC principal cell firing. Young and old rats did not differ in the no object condition, but young rats had significantly higher information content in the object conditions. Error bars represent  $\pm 1$  SEM.

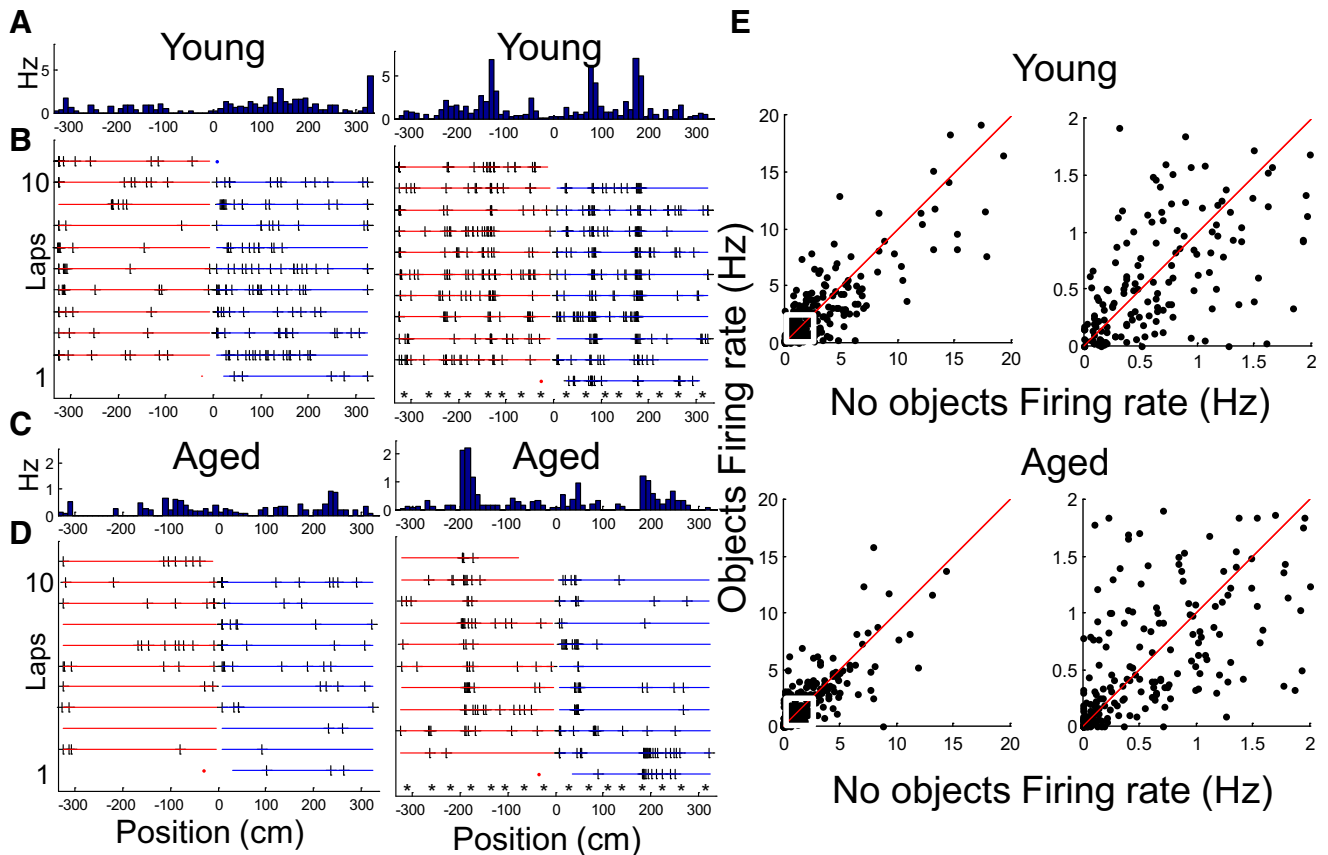
Figure 8C shows the proportions of neurons that showed nonselective activity (mean firing rate  $>0.2$  spikes/occupancy but no object field) on the track during the four different behavioral conditions. There was a significant effect of condition on the proportion of neurons with nonselective spiking on the track ( $F_{(3,40)} = 15.14, p < 0.001$ ; repeated-measures ANOVA). Specifically, in both young and aged rats, significantly more PRC neurons showed nonselective activity on the track during the no objects conditions relative to the conditions with objects ( $p < 0.5$  for all comparisons). These data indicate that it is the neurons with nonselective activity on the track that will potentially develop object fields when stimuli are added to the track. Thus, it is possible that a level of baseline activity prepares a portion of the PRC neuron population to respond when a salient feature is encountered in an environment.

Age also significantly affected the proportion of neurons with nonselective spiking on the track ( $F_{(1,40)} = 7.30, p < 0.01$ ; repeated-measures ANOVA). Specifically, the young rats had a significantly larger proportion of nonselective neurons, relative to the aged rats, during the no objects condition ( $t_{(11)} = 4.10, p < 0.01$ ; paired-samples  $t$  test), but not during conditions with objects ( $p > 0.4$  for all comparisons). Interestingly, there was an  $\sim 17\%$  reduction in the proportion of nonselective neurons between the young and aged rats. This is comparable to the  $\sim 14\%$  reduction in the proportion of neurons expressing object fields between the young and old animals. Thus, it is possible that the old rats have fewer neurons with object fields because initially, under conditions without objects, they have a smaller pool of neurons that are prepared to show selective spiking when salient features are added to an environment.

To test this idea directly, PRC neurons were recorded during two control conditions: no objects–objects and objects–no objects. This enabled the effects of objects to be measured on the same population of PRC neurons. Figure 9A–D shows a representative example of nonspecific activity of PRC neurons when no objects are on the track (left) that then express object fields when objects are on the track (right) in a young (top) and an aged rat (bottom). The patterns of firing in Figure 9A, C lend further



**Figure 8.** Proportion of PRC neurons with object fields, no firing, or nonspecific firing during behavior. **A**, The proportion of PRC neurons with object fields ( $y$ -axis) in young (black) and aged (gray) rats across the different behavior conditions ( $x$ -axis). The young rats had significantly more cells with object fields relative to the aged animals for the objects–both epochs with a 20 min delay ( $t_{(11)} = 3.50, p < 0.01$ ; paired-samples  $t$  test), and the objects–both epochs with a 2 h delay ( $t_{(9)} = 3.26, p < 0.05$ ; paired-samples  $t$  test). **B**, The proportion of PRC neurons that fired during rest, but did not show activity during behavior in young (black) and aged (gray) rats. Across all behavioral conditions, significantly more PRC neurons were inactive during track running in the aged compared with the young rats ( $F_{(1,40)} = 34.80, p < 0.001$ ; repeated-measures ANOVA). **C**, The proportions of neurons that were active on the maze but did not show selective spiking at an object location were significantly greater for the no objects condition relative to the conditions with objects ( $p < 0.05$  for all comparisons). Aged rats showed significantly fewer neurons with nonspecific activity on the track relative to the young rats during the no objects conditions ( $t_{(11)} = 4.10, p < 0.01$ ; paired-samples  $t$  test), but not during conditions with objects ( $p > 0.1$  for all comparisons). Error bars are  $\pm 1$  SEM.



**Figure 9.** Perirhinal cortical neuron activity in the no object–object condition. **A–D**, The firing patterns for a representative PRC neuron from a young (**A, B**) and an aged (**C, D**) rat during Epoch 1 without objects (left) and Epoch 2 (right) in which the track contained objects. The activity from the same cells are shown in the left and right panels. The firing-rate histogram by “linearized” position is shown in **A** for young rats and **C** for aged rats. The x-axis is positioned on the track with zero indicating the position of the barrier. Distance from the barrier is measured in centimeters. Positive numbers are for laps when the rat was running in the clockwise direction while negative numbers indicate the position when the rat was running in the counterclockwise direction. The y-axis is the occupancy-normalized firing rate of the neuron. Spike raster plots across laps are shown in **B** for young rats and in **D** for aged rats. Each horizontal line indicates a lap and blue lines are the laps in which the rat ran in the counterclockwise direction while red lines represent laps run in the clockwise direction. The asterisks indicate the position of the objects. In both the young and aged rat, there was nonspecific activity during the first epoch and significant object field firing during the second epoch. **E**, The firing rates of individual neurons during the no objects epoch plotted against the firing rates during the epoch with objects for young (top) and aged (bottom) rats. The left panels show all cells and the right panels show only those cells that have firing rates of  $< 2$  Hz (white square in left panels). Note, the higher density of low firing-rate cells in the aged rats. There was a significant correlation between firing rate across epochs with and without objects ( $R^2 = 0.79$ ,  $F_{(3,23)} = 24.32$ ,  $p < 0.001$ ). Age of the animal ( $t_{(23)} = 0.25$ ,  $p = 0.81$ ) and whether the track contained objects during the first or second epoch ( $t_{(23)} = 0.54$ ,  $p = 0.53$ ) did not significantly affect this relationship.

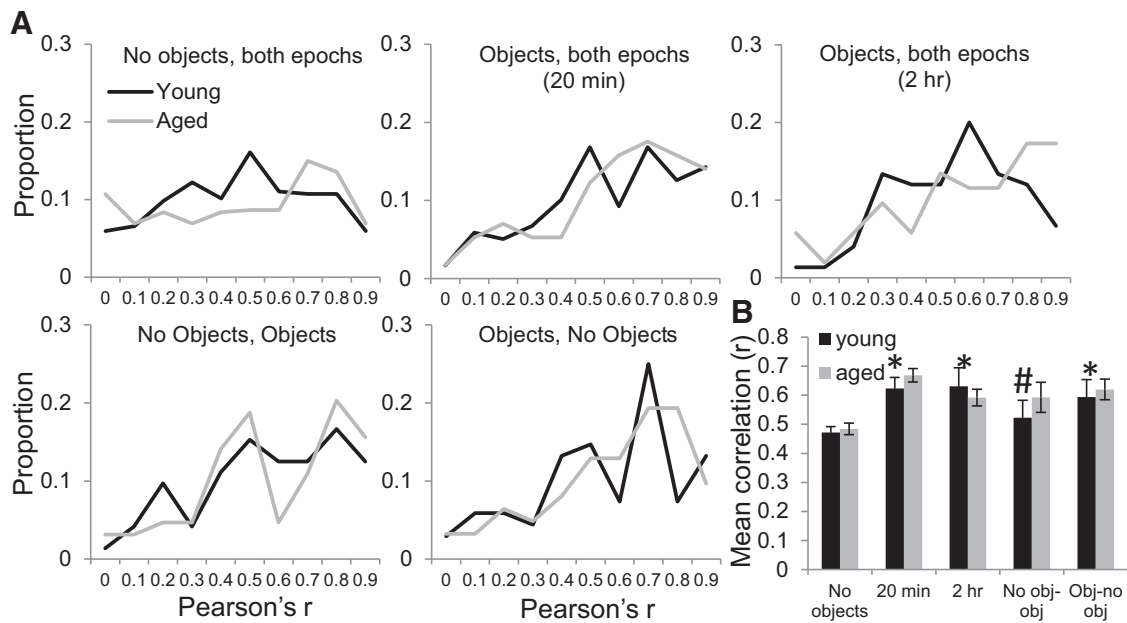
support to the hypothesis that it is the cells with nonspecific activity that are primed to express object fields. In line with this idea, within the same population of cells, placing objects on the track did not significantly affect firing rate ( $F_{(1,20)} = 0.41$ ,  $p = 0.53$ ; repeated measures) or the proportion of cells that did not fire during behavior ( $F_{(1,20)} = 2.56$ ,  $p = 0.12$ ; repeated measures). Figure 9E shows the firing rates of neurons during the no objects epochs plotted against the firing rates during the epochs with objects for young (top) and aged (bottom) rats. It is evident from Figure 9E that there was no cluster of cells above unity. Therefore, the “quiet” neurons during the no objects epoch also do not fire when objects are on the track. Moreover, there was a significant correlation between firing rates across the no object and object epochs ( $R^2 = 0.79$ ,  $F_{(3,23)} = 24.32$ ,  $p < 0.001$ ). Age of the animal did not significantly affect this relationship ( $t_{(23)} = 0.25$ ,  $p = 0.81$ ). These data lend further support to the idea that objects adjust the spike timing of PRC neurons rather than increasing overall excitability levels.

In both young and aged rats, within the same population of cells, the PRC neurons recorded during the epochs with objects had significantly higher information content ( $F_{(1,20)} = 17.78$ ,  $p <$

0.001; repeated measures) and a significantly higher proportion of cells that met the criteria for having an object field ( $F_{(1,20)} = 21.62$ ,  $p < 0.001$ ; repeated measures) compared with the epochs when the track was empty. When the aged groups were examined separately, consistent with the data collected during the no objects–both epochs and objects–both epochs conditions, the aged rats had a significantly lower proportion of PRC cells that expressed object fields when objects were on the track relative to the young animals ( $F_{(1,20)} = 4.52$ ,  $p < 0.05$ ; repeated measures). Moreover, similar to the other behavioral conditions, the mean firing rate of PRC neurons was significantly lower in the aged compared with the young rats ( $F_{(1,22)} = 5.57$ ,  $p < 0.05$ ; repeated measures). Therefore, in old rats, when three-dimensional stimuli are added to the track, there are fewer neurons primed to respond to objects, which results in a lower proportion of cells with object fields. This is consistent with the hypothesis that PRC-dependent stimulus representation is impaired in advanced age.

#### Perirhinal cortical activity across delays

To evaluate whether PRC neuron activity patterns were similar between epochs, the correlation of PRC neuron firing-rate vec-



**Figure 10.** Activity pattern correlations across epochs. **A**, The normalized frequency histograms of the Pearson's correlation coefficients of the activity during Epoch 1 and Epoch 2 for the different behavioral conditions. **B**, The mean correlation values averaged across rats. Behavioral condition had a significant effect on the correlated activity ( $F_{(5,45)} = 49.32, p < 0.001$ ; repeated measures). Planned orthogonal contrasts revealed that the mean correlation values of PRC neuron activity across epochs was significantly less for the no objects–both epochs condition relative to the conditions with objects during both epochs, and the objects–no objects control condition (indicated by \* $p < 0.01$  for all comparisons; simple contrast). The no objects–objects conditions was significantly less correlated than the 20 min delay condition (indicated by # $p < 0.05$ ; simple contrast). Age group did not have a significant effect on correlated activity patterns ( $F_{(1,9)} = 1.02, p = 0.34$ ; repeated measures).

tors between Epoch 1 and Epoch 2 was calculated for the different behavioral conditions. Figure 10A shows the normalized frequency histograms of the Pearson's correlation coefficients between Epoch 1 and Epoch 2 activity patterns for the different behavioral conditions in young (black) and aged (gray) rats, and Figure 10B shows the mean correlation values averaged across rats. Behavioral condition had a significant effect on the correlation values ( $F_{(5,45)} = 49.32, p < 0.001$ ; repeated measures) such that the PRC neuron activity correlations between epochs were significantly lower for the no objects–both epochs condition relative to the conditions with objects during both epochs (20 min and 2 h delays;  $p < 0.01$  for both comparisons; simple contrast). Together these data indicate that, contrary to object-related firing, the nonspecific activity is not spatially consistent across delays. Additionally, the between-epoch correlations for the no objects–both epochs condition was not significantly different from the no objects–objects control condition ( $p = 0.1$ ; simple contrast), but was significantly lower than the objects–no objects condition ( $p < 0.01$ ; simple contrast). This suggests that some PRC neurons may show persistent object-related activity even after the stimulus has been removed, which has been reported for a small subset of lateral entorhinal cortical neurons (Deshmukh and Knierim, 2011; Tsao et al., 2013).

Importantly, age group did not have a significant effect on the extent of correlated activity patterns ( $F_{(1,9)} = 1.02, p = 0.34$ ; repeated measures). Moreover, age group did not significantly interact with behavioral condition ( $F_{(5,45)} = 0.47, p = 0.79$ ; repeated measures), which indicates that the correlated activity patterns of PRC neurons across behavioral epochs did not vary between young and old rats as a function of experience with the objects. These data are consistent with the observation that old animals are able to identify previously experienced objects as familiar within the same environment for delays up to 24 h (Burke et al., 2010). Age also did not significantly affect rate re-

mapping (data not shown). Together these data indicate that aged rats have fewer cells that are active in the presence of objects. Once the representations of complex stimuli are established within the PRC, however, the aged rats are just as able to maintain stable activity patterns in response to familiar stimuli for delays up to 2 h.

## Discussion

### Object fields in young and aged rats

The current experiments provide evidence that old animals have selective impairments in stimulus representation rather than deficits in stimulus maintenance. Compared with the young rats, the aged rats had a lower proportion of neurons with object fields and a higher proportion of neurons that were inactive during track running. Interestingly, in both age groups, the proportion of inactive neurons were similar between conditions with and without objects. Thus, it appears that the neurons with nonselective activity were the subset of PRC cells that developed object fields. This is supported by the observed reduction in the proportion of neurons with nonselective activity in the object conditions and the lack of a significant firing rate change between object and no object conditions.

Together these data support two novel hypotheses regarding PRC function. First, it appears that when the track was relatively empty, a subset of PRC neurons express baseline activity with no obvious pattern. This activity could reflect circuit dynamics that predispose specific neurons to sharpen their tuning to track location when a salient feature is encountered. Second, with respect to aged rats, the decreased numbers of neurons showing nonselective activity on the empty track could arise from a loss of fidelity in the afferent input to the aged PRC. The PRC receives direct projections from the entire sensory cortex (Burwell and Amaral, 1998). With advanced age, there are disruptions in cortical inhibition in the auditory (de Villiers-Sidani et al., 2010), so-

matosensory (David-Jürgens and Dinse, 2010), and visual cortices (Wang et al., 2006). This has been associated with declines in signal-to-noise ratios that could feed forward to the PRC, resulting in reduced convergence of input needed to elicit spiking. Alternatively, lower firing rates in aged PRC neurons during behavior could serve as a compensatory mechanism for disrupted inhibition at earlier stages of cortical processing. Both of these hypotheses are consistent with the observation that glutamate levels are reduced in the aged PRC (Liu et al., 2009) and with the report of reduced BOLD signal in the PRC of elderly subjects (Ryan et al., 2012).

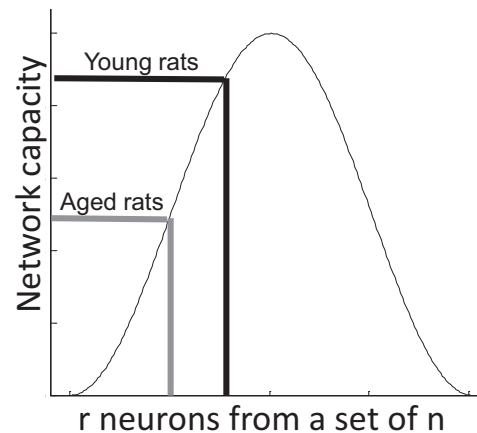
A question remains regarding the function of PRC neurons that do not show activity under the behavior conditions of the current experiment. One possibility is that these “quiet neurons” will only become active when the animal encounters a stimulus that is emotionally salient or relevant for its survival. In line with this hypothesis is the observation that more PRC neurons respond selectively to an auditory cue after it has been paired with a foot shock (Furtak et al., 2007). Under this framework, the quiet neurons are those that require extra activation, presumably through direct basolateral amygdala (Paz et al., 2006) or prefrontal cortical input (Paz et al., 2007), to be recruited into the ensemble of active PRC neurons.

#### The lack of novelty and/or familiarity modulation of perirhinal cortical neuron activity in young and aged rats

A prevailing theory regarding the neurobiology of recognition memory is that the mnemonic mechanism for this behavior is a reduction in the firing rate of PRC neurons as a stimulus goes from novel to familiar (for review, see Brown and Aggleton, 2001). The results reported in the current paper along with additional recent data, however, have been unable to provide support for this idea. Specifically, monkeys performing a passive viewing task with pictures of varying levels of novelty or familiarity do not show robust firing-rate differences in principal cell activity as a function of familiarity (Thome et al., 2012; Woloszyn and Sheinberg, 2012). Moreover, another recent investigation observed no change in the proportion of PRC neurons positive for the mRNA products of the activity-associated immediate-early gene (IEG) *Arc* as objects went from novel to familiar (Burke et al., 2012b).

Several possible explanations can account for the discrepancy between different experiments regarding the effect of stimulus familiarity on PRC activity. In terms of the effect of novelty versus familiarity on IEG expression in the PRC, it is known that IEG expression can be decoupled from neuronal activity after a massed exposure to an environment (Guzowski et al., 2006). The paired viewing procedure used in *c-fos* imaging studies involved presenting rats with the same visual stimuli many times in a day over the course of several days (Wan et al., 1999). Therefore, it remains a possibility that the PRC shows reduced *c-fos* protein levels because of a post-translational decoupling from spiking activity rather than reduced neuron activity.

One explanation of the apparently contradictory reports of reduced PRC firing rates in other electrophysiological recording experiments (Miller et al., 1991; Fahy et al., 1993; Hölscher et al., 2003) could involve the fact that in these studies acute recording techniques were used. It is possible that during acute recordings there is a sampling bias for cells with higher firing rates, and these cells may be more likely to decrease their rate over time. Investigations that have not observed novelty modulation of PRC neuron firing rates have used chronically implanted recording probes (Burke et al., 2012a; Thome et al., 2012). Another, but not mu-



**Figure 11.** Population coding in the PRC. The capacity of a hypothetical network to represent distinct stimuli based on the degree to which the population code is distributed. The  $x$ -axis is the number of neurons ( $r$ ) in a set of  $n$  neurons. Network capacity is maximal when half of the neurons are activated by a given stimulus set. In the PRC cortex, fewer neurons are activated by a stimulus set in aged (gray) compared with young (black) rats. This could have the result of decreasing the capacity of the aged PRC to represent different stimuli distinctively.

tually exclusive, explanation is that most of the initial reports of reduced PRC cell firing to familiar stimuli did not isolate interneurons from principal cells. Recent data suggest that it may be the interneurons of the inferior temporal cortex, rather than excitatory cells, that show reduced activity to familiar versus novel stimuli (Woloszyn and Sheinberg, 2012).

Lesion data are unequivocal concerning the fact the PRC is critical for stimulus recognition (Ennaceur and Aggleton, 1997; Málková et al., 2001; Winters and Bussey, 2005). In light of the current results, the notion that a simple rate code supports recognition may not be parsimonious. In fact, a rate code is vulnerable to noise and spike failures. Given that the probability of synaptic transmission between the PRC and its cortical efferents is low (Pelletier et al., 2004), the signal-to-noise ratio for relaying a “response decrement” might not be sufficient. Thus, it is more plausible that the PRC uses a population code, which relies on the joint activities of a number of neurons, each of which has a different distribution of responses over some set of inputs (Georgopoulos et al., 1986).

#### Linking physiology to age-associated recognition memory impairments

Recent data have revealed that age-associated recognition memory impairments arise from aged animals “falsely” recognizing novel stimuli (Burke et al., 2010). It has been hypothesized that this results from old animals showing an increased vulnerability to distracting stimuli encountered during long delays (Burke et al., 2010). Importantly, animals with PRC lesions show the same pattern of results, and the tendency to falsely remember a novel stimulus can be reversed by depriving lesioned animals of sensory input during the delay period (McTighe et al., 2010). These data imply that recognition memory deficits in animals with a compromised PRC are not due to the “forgetting” of previously experienced stimuli, but rather manifests from a reduced ability to discriminate novel stimuli from those that have been experienced (Burke et al., 2010; McTighe et al., 2010). This type of discrimination deficit could arise from impairments in high-level stimulus perception that arise following damage to the PRC (Murray and Bussey, 1999; Murray et al., 2007), because of a reduced ability to pattern separate between stimuli that share features. In

line with this idea is the observation that aged rats, monkeys (Burke et al., 2011), and humans (Ryan et al., 2012) have difficulty discriminating between complex stimuli that share features even when the mnemonic demands of the task are low.

In line with the behavioral observations discussed above, the physiological data reported here suggest that aged animals do not forget previously experienced stimuli. That is, the older rats' PRC neurons show the same levels of correlated activity across delays up to 2 h. Aged rats, however, appear to have a reduced pool of active PRC neurons available and prepared to represent new stimulus sets. Figure 11 shows how the capacity of a theoretical network is affected by increasing or decreasing the number of neurons that are active when a stimulus set is presented. The theoretical functional consequence of fewer neurons being activated by objects is reduced capacity to represent different objects with a unique neural code. This could explain why aged rats tend to regard novel objects as familiar (Burke et al., 2010).

An assumption regarding the cognitive consequences of normal aging is that the aspect of recognition memory supported by PRC-dependent familiarity judgments is preserved across the lifespan, while recollection is particularly vulnerable (Spencer and Raz, 1995; Daselaar et al., 2006). This hypothesis, however, may not be consistent with the observation that aged subjects show a significant increase in false recognition (Norman and Schacter, 1997; Jacoby et al., 2005; Toner et al., 2009). Moreover, other investigations have reported that aging is associated with deficits in familiarity as well as recollective processes (Davidson and Glisky, 2002; Prull et al., 2006; Toth and Parks, 2006; Duarte et al., 2010). These data, along with the current findings, call into question the notion that recollection is particularly vulnerable to advanced age in the absence of changes in familiarity. In fact it is probable that degradation of PRC-dependent stimulus representation contributes to impairments in recollection and episodic memory, both of which require the association of sensory stimuli with a spatial and temporal context.

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