Systems/Circuits

Functional Reorganization of a Prefrontal Cortical Network Mediating Consolidation of Trace Eyeblink Conditioning

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The medial prefrontal cortex (mPFC) has been studied for its role in various cognitive functions, but the roles of its subregions remain unclear. We performed tetrode recordings simultaneously from prelimbic (PL) and rostral (rACC) and caudal (cACC) anterior cingulate subregions of the rabbit mPFC to understand their interactions during learning and tests of remote memory retention for whisker-signaled trace eyeblink conditioning. cACC neurons exhibited an innate response to the conditioning stimulus (CS) that rapidly decreased across sessions, suggesting an attentional role for facilitating CS–US associations. rACC neurons from conditioned rabbits exhibited robust responses to the CS that decreased within each session, possibly evaluating its emotional salience. PL neurons exhibited robust persistent activity during the trace interval during tests of remote memory retention, suggesting its involvement in retrieval and execution of a consolidated response. Mechanistically, conditioning was associated with a greater percentage of persistently responsive neurons than neurons from pseudoconditioned control rabbits, and responses differed significantly between trials with and without conditioned responses. Collectively, these responses reflect a functional reorganization of neural activity within the prefrontal network from an attentional mode to one that orchestrates the retrieval and execution of the learned response.

Key words: anterior cingulate cortex; hippocampus; medial prefrontal cortex; memory acquisition; persistent firing; prelimbic cortex

Introduction

The medial prefrontal cortex (mPFC) has been investigated widely for its roles in various cognitive functions, including decision making, error detection, executive control, and memory (Euston et al., 2012). With respect to mnemonic processes, what is known has come mainly from examination of human amnesics and animal lesion studies. These studies have led to two general views regarding the longitudinal function of the mPFC during memory processes: (1) the mPFC becomes selectively responsive for memories that were initially mediated by the hippocampus and is essential for the retrieval of remotely but not recently acquired memories (Takehara et al., 2003; Frankland and Bontempi, 2006; Takehara-Nishiuchi and McNaughton, 2008); and (2) the mPFC plays a global role in the encoding, consolidation, and retrieval of remotely acquired memories and thus is involved during both recently and remotely acquired memories (Blum et al., 2006; Corcoran and Quirk, 2007; Lee and Solivan, 2008; Quinn et al., 2008; Churchwell et al., 2010). Specifically, studies

have suggested that the mPFC is essential for the assimilation of new memories into preexisting networks of knowledge, known as schemas, through cognitive and/or strategic control over memory processes within other brain areas during memory acquisition and consolidation (Moscovitch, 1992; Buckner and Wheeler, 2001; Miller and Cohen, 2001; Dobbins et al., 2002; Postle, 2006; Preston and Eichenbaum, 2013). This process may be mediated by various functions, such as working memory, attentional modulation, and evaluative mechanisms that are designated to specific regions within the mPFC.

A paradigm used to examine neural substrates for associative memory and that has contributed support for these views is trace eyeblink conditioning (EBC; Christian and Thompson, 2005; Kalmbach et al., 2009; Weiss and Disterhoft, 2011). It pairs an initially neutral conditioning stimulus (CS) with an aversive unconditioned stimulus (US) to the eye or periorbital region after a stimulus-free trace interval. Repeated noncontiguous pairings of the two stimuli elicit a conditioned blink response (CR), the behavioral assay for memory. Some mPFC lesion studies using trace EBC have provided evidence for mPFC involvement during remote memory retrieval, whereas others contend involvement during both recent and remote time points. The disparate findings are likely attributable to a functional segregation among the mPFC subregions, including the prelimbic (PL) and rostral (rACC) and caudal (cACC) anterior cingulate cortices, and the time at which data were examined relative to the time of learning.

Specifically, trace EBC acquisition was impaired by permanent or reversible pretraining lesions of the cACC (Kronforst-Collins and Disterhoft, 1998; Weible et al., 2000; Kalmbach et al., 2009), and the physiological responses were most robust during the initial trials and sessions of conditioning (Weible et al., 2003),

leading to views that the cACC may play a role in modulation of attention. Conversely, retention of remotely acquired trace EBC was impaired by PL lesions performed 1 week but not immediately after learning (Oswald et al., 2008, 2010), suggesting its role in remote memory storage and expression. Pretraining lesions of the rACC impaired extinction of trace EBC (Weible et al., 2000) but did not affect acquisition, suggesting an evaluative role for the rACC.

These studies suggest a functional segregation of mPFC subdivisions that would be accompanied by subregion-specific changes in neuronal activity as learning evolves and memory consolidates, a process we will hereafter refer to as a functional reorganization of neuronal activity within the mPFC network. However, no study has examined neuronal activity within the subregions of the mPFC network simultaneously and longitudinally during recent and remote time points and in comparison to the neuronal activity of control animals. Thus, whether differential activation patterns of distinct mPFC areas support the proposed functions of these mPFC regions during successive stages of learning and memory remain unclear.

Here we report multiple single-neuron activity recorded simultaneously from the rabbit PL, rACC, and cACC during acquisition of whisker-signaled trace EBC and during retention sessions administered after a 1 month training-free hiatus. Results provide evidence for a learning and memory-dependent functional reorganization of neuronal activity within the mPFC network.

Materials and Methods

Subjects. Subjects were 14 young adult, female New Zealand White rabbits, 3–6 months of age (seven conditioned, seven pseudoconditioned). All subjects were individually housed and maintained on a 12 h light/dark cycle.

Surgical procedure. All surgeries were performed using sterile procedures approved by the Northwestern University Institutional Animal Care and Use Committee. Rabbits were prepared as described previously (Flores and Disterhoft, 2013). Burr holes were drilled for skull screws to anchor a restraining head bolt and a 96-channel microdrive containing 24 independently moveable tetrodes and three reference tetrodes. A 4 × 8 mm craniotomy was performed over the intended locations of recording sites into the PFC, and the dura was slit and reflected. The microdrive was then stereotaxically positioned onto the surface of the brain, and the craniotomy around the guide tubes was filled with Kwik-Sil (WPI). A screw connected to a ground wire from the electrode interface board (EIB) on the microdrive was then implanted into the skull. Dental acrylic was used to cement the microdrive and head bolt to the skull screws. Rabbits were administered 0.2 mg/kg Metacam (intramuscularly) postoperatively and the following day for analgesia.

Behavioral training. Rabbits were given at least 7 d of postoperative recovery and were then brought to the laboratory for acclimation to the conditioning environment inside an Industrial Acoustics sound and light attenuating chamber. During acclimation sessions, tetrodes were gradually lowered to their intended targets. Rabbits were prepared for training (Das et al., 2001), and conditioning sessions were done as reported previously (Galvez et al., 2006). The CS consisted of 250 ms whisker vibrations delivered at 60 Hz using a vibrotactile transducer. The trace interval consisted of a 500 ms stimulus-free interval. The US consisted of a 150 ms corneal air puff delivered at 3 psi. An adaptive CR was defined as a blink with a voltage amplitude exceeding the mean baseline amplitude (250 ms pre-CS) by 4 SDs for 15 ms minimum within the 20 ms interval before US onset. Pseudoconditioned animals were yoked for the number of sessions with the conditioned animals and were presented with explicitly unpaired CS and US trials in random order.

Single-neuron recording. A custom-designed microdrive equipped with 24 independently movable tetrodes was used to acquire single-neuron activity simultaneously from the PL cortex (A6–A8), rACC (A5–A7), and cACC (A2–A5). Tetrodes were fabricated by twisting four strands of 12.7-μm-diameter Stablohm 800 wire together (RO-800; Cal-

ifornia Fine Wire). Twisted wires were briefly heated to enhance rigidity. Tetrode tips were gold-plated to achieve an impedance of 200–500 kΩ at 1 kHz. During recording sessions, the drive assembly was connected to a 96-channel Cheetah data acquisition system via EIB-36 boards (Neuralynx). Neuronal signals were referenced to a common skull screw or reference tetrode. A threshold voltage was adjusted for each wire of each tetrode so that a spike exceeding the threshold triggered collection of the activity on all four wires of the tetrode for that time window. Cheetah acquisition software (Neuralynx) was used to collect neuronal data, blink amplitudes, and timestamps of CS and US presentations.

Single-neuron isolation. Activity of single neurons was separated offline using Klustakwik in the Neuralynx SpikeSort 3D program. Sorting was based on a number of different waveform parameters, including peak/valley amplitudes, height, and spike widths. Custom scripts written in Igor (WaveMetrics) were used to calculate the ratio of signal-to-noise (S/N) of individual clusters to separate well isolated from noisy clusters. For each cluster, the waveform of each spike was divided into 32 points with the peak forced to the eighth point. The mean waveform and variance waveform were then computed for each neuron. Noise, N, was defined as follows: $N = \sqrt{\frac{1}{n}\Sigma_{i=1}^{n}\sigma_i^2}$, where $n = 32$ points and σ^2 is the variance waveform. The signal, S, was defined as the difference between the peak and the trough in the mean waveform. The quotient of S and N yielded the S/N ratio.

Neuronal clusters with S/N ratio of at least 6.5:1 were included for subsequent analyses. Tetrode placement was changed only if spikes were not detected. However, given the possibility of tetrode drift and loss of signal or appearance of new neuronal spikes, neuronal clusters from each recording session were regarded as representing the activity of unique neurons for each session. The distribution of spike widths (peak-valley) and baseline firing rate for all neurons was examined to distinguish presumptive pyramidal neurons from presumptive interneurons. Results indicated a wide range of baseline firing rates with a large proportion of neurons having a rate <15 Hz. Although the percentage of neurons with rates >15 Hz was relatively low, we found that the response profile of the population for a given region changed drastically if those neurons were included. For this reason, we excluded those neurons and focused our analyses on presumptive pyramidal neurons.

Data analyses. The total number of neurons from the current study includes the number of neurons identified for 13 sessions, including the first day of training (D1), seven sessions centered around the day of criterion (C−3, C−2, C−1, C, C+1, C+2, C+3), and five sessions of retention testing that were given after a 30 d training-free hiatus. Neurons from the first training session of each rabbit were analyzed to examine the innate responsiveness to novel stimuli and changes attributable to subsequent learning. The first training session was day C−3 for three rabbits from the conditioned group; those neurons were included in both the D1 and C−3 sessions.

All analyses were conducted using custom scripts written in MATLAB (MathWorks). For each neuron, a nonparametric Mann–Whitney U test was used to compare the mean baseline firing rate with the neuronal activity during the subsequent 250 ms CS and 500 ms trace periods (Flores and Disterhoft, 2013). Rate-increasing (RI) and rate-decreasing (RD) neurons exhibited, respectively, significant increases or decreases in firing rate during the CS–trace period after the baseline period. The proportions of responsive neurons between the conditioned and pseudoconditioned groups were compared using a χ^2 analysis. Neuronal activity was also compared between the groups by converting raw firing rates into z-scores (firing rates normalized to 2 s pre-CS baseline interval). Perievent time histograms (PETHs) were generated for the time interval between the 1 s pre-CS and 2 s post-CS windows using 10 ms bins. Both the raw firing rate and normalized z-scores were calculated to compare the response profiles during the trial. Statistical comparisons of changes in firing rate between the groups were performed using two-way ANOVA with training group (conditioned vs pseudoconditioned) and session as factors.

Persistent responses during a trial for each neuron were computed by first converting rasters into continuous spike density functions (SDFs). Individual spike trains were convolved using a Gaussian kernel with an SD of 10 ms (equivalent to putting a confidence interval on the spike

times; Richmond et al., 1987). Neurons in which the absolute amplitude of the SDF exceeded 2.575 SDs (99% confidence) of the mean baseline SDF for 70% of the trace interval were regarded as persistently responsive. Persistently responsive neurons were further classified into RI and RD depending on the sign of the responses.

Response onset for each neuron among the subregions were determined from PETHs using a method reported previously (Kiani et al., 2005). PETHs with 1 ms bins were generated and then convolved with a 3 ms SD Gaussian kernel. The first of 10 consecutive bins that exceeded 1.96 SDs above the mean baseline activity defined response onset for RI neurons. For RD neurons, the threshold for determining response onset was set at ¼ SD below the mean baseline activity (to avoid a floor effect).

Histology. Marking lesions were made to locate the placement of the recording sites by passing current through individual tetrode wires (+50 μA, 5 s) after completion of behavioral testing. Rabbits were then administered an intravenous dose of ketamine/xylazine (50 and 25 mg/kg, respectively) for deep anesthesia and were perfused transcardially with 0.9% saline, followed by 10% buffered Formalin. All tetrodes were then raised to the surface of the brain, and the brain was extracted out of the skull for 24 h postfixation in 10% Formalin. Subsequently, the brain was prepared for detecting marking lesions (Weiss et al., 1996). Recording sites were estimated by calculating any advancement made at the end of recording sessions with the location of the marking lesion. Any activity recorded from positions outside the area of interest was excluded from additional analyses.

Results

Behavior

Conditioned rabbits ($n = 7$) reached the behavioral learning criterion of at least eight adaptive CRs during a span of any 10 consecutive trials with an average of 4.3 sessions. Pseudoconditioned control rabbits ($n = 7$) were matched for the number of sessions with the conditioned animals and received explicitly unpaired CS-only and US-only trials in pseudorandom order.
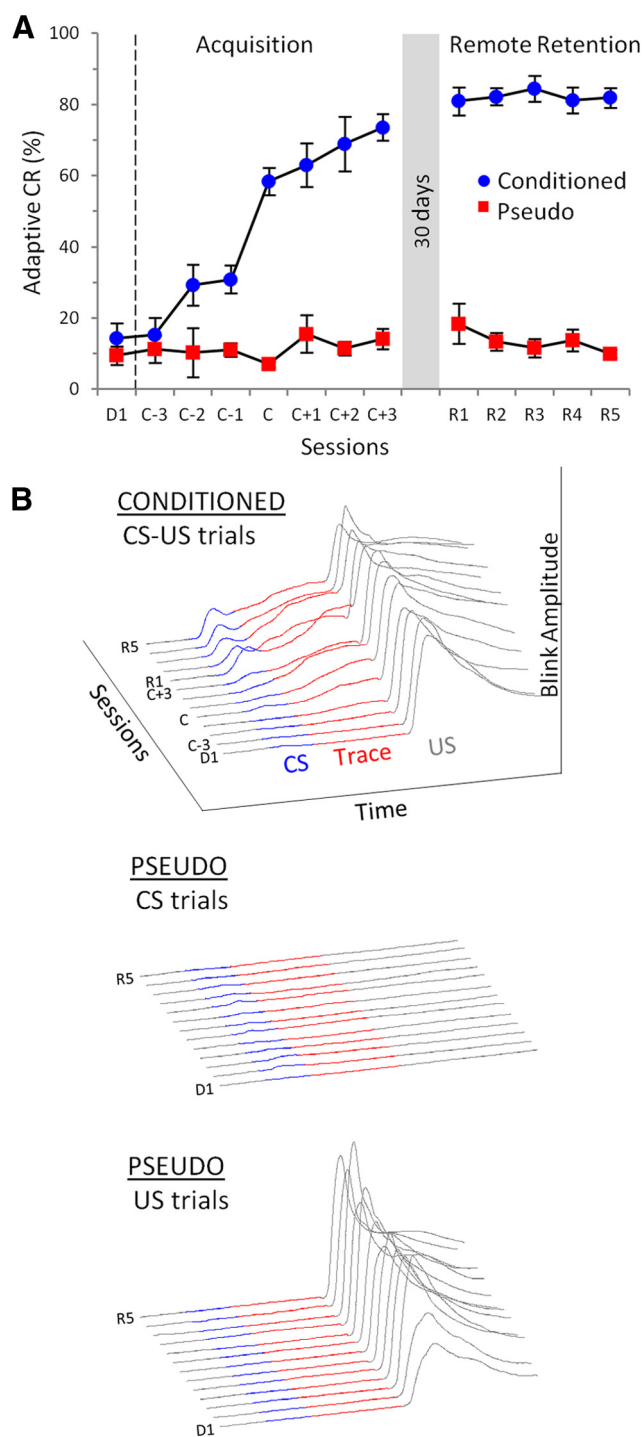
The percentage of adaptive CRs across training sessions for both groups is shown in Figure 1A. This figure shows the data for the first day of training and other days after synchronizing the data to the day that behavioral criterion was achieved (day C). It also shows the performance of the rabbits during retention testing after they stayed in their home cages for 30 d. Note that the initial six rabbits (three conditioned, three pseudoconditioned) in the study did not undergo retention testing. However, their behavioral performance during acquisition did not differ from rabbits that did receive retention testing.

A repeated-measures ANOVA on the percentage of trials with CRs indicated a significant interaction of group and session ($F_{(7,77)} = 13.8, p < 0.0001$) because of the significant increase for the conditioned group that occurred as of session C−2 ($p = 0.056$) and C−1 ($p = 0.0007$) according to *post hoc* Fisher's tests; the pseudoconditioned group did not exhibit any significant change in CRs across sessions. The difference between the groups was maintained during testing for remote retention (82 vs 13%) according to another ANOVA ($F_{(1,6)} = 750, p < 0.0001$), and both groups had a plateau level of performance because neither group had a significant change in performance across the five retention testing sessions.

The average increase in the amplitude of the CRs across training sessions can be seen for the conditioned group in Figure 1B. Note that there are minimal blink responses to the CS for pseudoconditioned rabbits.

Neuronal population and histology

Only neurons histologically verified to be within the medial PFC and that had an S/N ratio of at least 6.5:1 were included for analysis (see Materials and Methods). The activity of 2201 neurons from

Figure 1. Paired CS–US stimuli lead to an increase in CRs. *A*, The mean percentage of trials with adaptive CRs (those that are present within 20 ms of US onset) are plotted as a function of training sessions during acquisition and retention testing (D1, first day of training; C, day of criterion; R, retention). Data are shown for conditioned rabbits (those that received paired whisker-puff trials) and CS-alone trials from control rabbits (those that received explicitly unpaired CS and US presentations). *B*, "Waterfall" behavioral plots showing the time course amplitude of the average blink for each session for the conditioned group (top), CS-alone trials of the pseudoconditioned group (middle), and US-alone trials of the pseudoconditioned group (bottom). Blue portions represent the time during which the CS was presented (250 ms). Red portions represent the trace interval between CS offset and US onset (500 ms). Note the increase in amplitude and the shortening in onset latency across sessions for the conditioned group. Note that data from the pseudoconditioned group have been sorted into CS-alone and US-alone trials that were presented pseudorandomly during training.
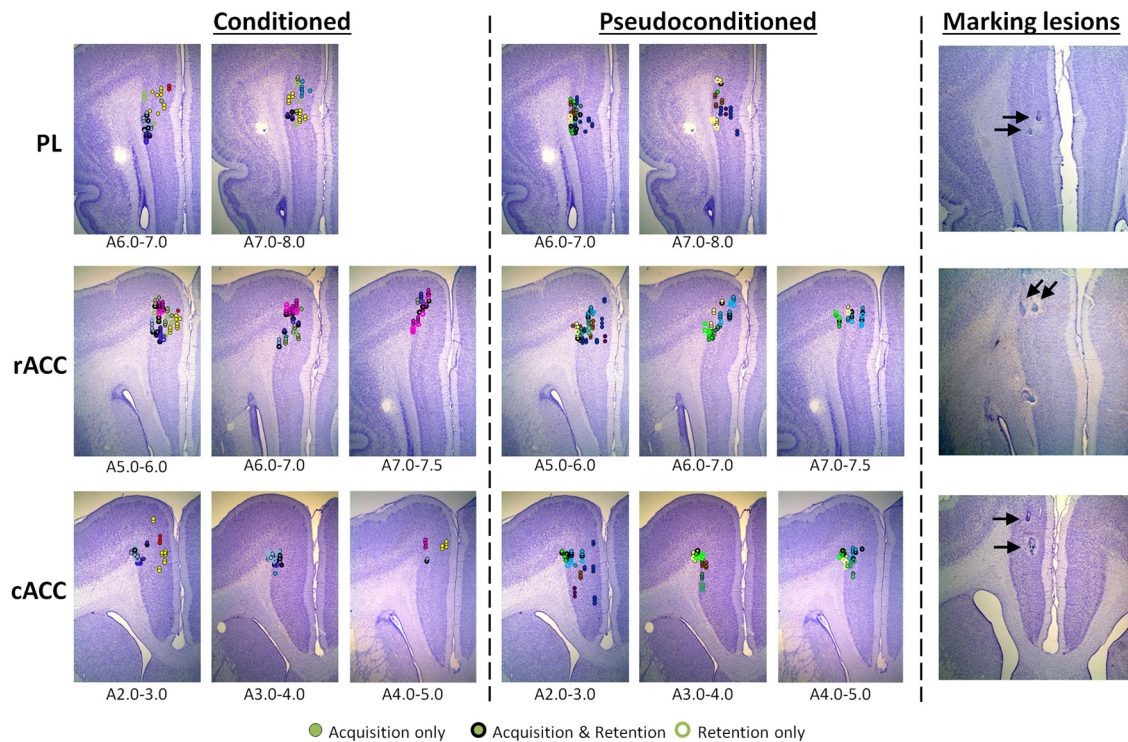
Figure 2. Histological localization of all recording sites for conditioned and pseudoconditioned control rabbits. Reconstruction of recording sites are shown segregated by region (PL, rACC, cACC) and group (conditioned, pseudoconditioned). Different marking colors represent the different rabbits used in the study. The different symbols indicate whether data were recorded during acquisition, retention, or both types of sessions. Numbers under each panel indicate approximate distance (millimeters) rostral to bregma. Three panels on the right show representative marking lesions (arrows) indicating recording sites from three respective PFC subregions.

conditioned rabbits and 2092 neurons from pseudoconditioned control rabbits were analyzed for acquisition sessions. During sessions to test retention of remote memory, we analyzed the activity of 998 neurons from conditioned rabbits and 612 neurons from pseudoconditioned rabbits. Histologically verified locations of neurons within different subregions of the PFC are shown in Figure 2. A breakdown of the number of neurons recorded by group, region, and phase of training is shown in Figure 3A. The results indicate that recordings were stable for the duration of the experiment.

A comparison of baseline firing rate and spike width was made for all the recorded neurons to distinguish pyramidal neurons from interneurons (Ranck, 1973; Weiss et al., 1996; Bruno and Simons, 2002). The population was clearly skewed toward neurons with low baseline firing rates (Fig. 3B). A threshold of 15 Hz was used to discriminate pyramidal neurons from interneurons. Results are reported after eliminating responses from presumptive interneurons.

Pyramidal neurons were separated into those that exhibited a significant stimulus-induced increase in activity (RI neurons) from those that exhibited a significant decrease in activity (RD neurons) according to a Mann–Whitney U test. Less than 10% of the population exhibited a mixed response; neurons with that profile were included in both groups. Both RI and RD neurons were found at each recording site. Sites were located in different cortical layers of all three subregions. This indicates that there is no obvious regional or layer specificity for these two general classes of neurons.

**Analysis of activity by PFC subregions and response type**
PETHs spanning 1 s before and 2 s after CS onset were generated for all neurons for both raw (hertz) and normalized data (z-scores). Individual histograms of RI and RD response types within a region and belonging to a particular session were averaged to generate population responses. Average population re-

sponses for all RI (Fig. 4) and all RD (Fig. 5) neurons are shown in both hertz and z-scores across sessions. A three-way ANOVA with region (PL, rACC, cACC) and sessions as factors and intervals (CS vs Trace) as repeated measures revealed a significant interval × region interaction ($F_{(2,1403)} = 8.81$, $p = 0.0002$), indicating that the neural activity varied differentially across trial phases and regions. Subsequently, a two-way ANOVA of mean CS or trace interval z-score activity was performed for each brain region with group (conditioned vs pseudoconditioned) and session (D1, pre-C, C, and post-C) as factors to examine significant changes in the population response among acquisition sessions and between groups. An additional two-way ANOVA that included only the post-C and retention sessions was performed to determine any differences in population activity as a result of the 30 d hiatus.

PL cortex
RI neurons
Activity of RI neurons over acquisition sessions for both conditioned and pseudoconditioned groups exhibited a gradual decline in the mean trace interval z-scores ($F_{(3,347)} = 4.2$, $p < 0.01$; Fig. 4A). However, neurons from the conditioned group did respond with statistically greater magnitude than neurons from the pseudoconditioned group ($F_{(1,347)} = 10.67$, $p = 0.001$). Notably, a comparison of trace interval z-scores between post-C and retention sessions, when consolidation should be well established, revealed a significant and robust increase in responding from the conditioned group (session × group interaction, $F_{(1,170)} = 7.24$, $p < 0.003$); this was not observed for activity recorded from pseudoconditioned rabbits during CS-alone presentations. Subsequent *post hoc* tests confirmed that neurons from conditioned rabbits had a significant increase in z-scores during the trace period of retention sessions compared with post-C sessions (0.64 for post-C vs 1.53 for retention; one-way ANOVA,
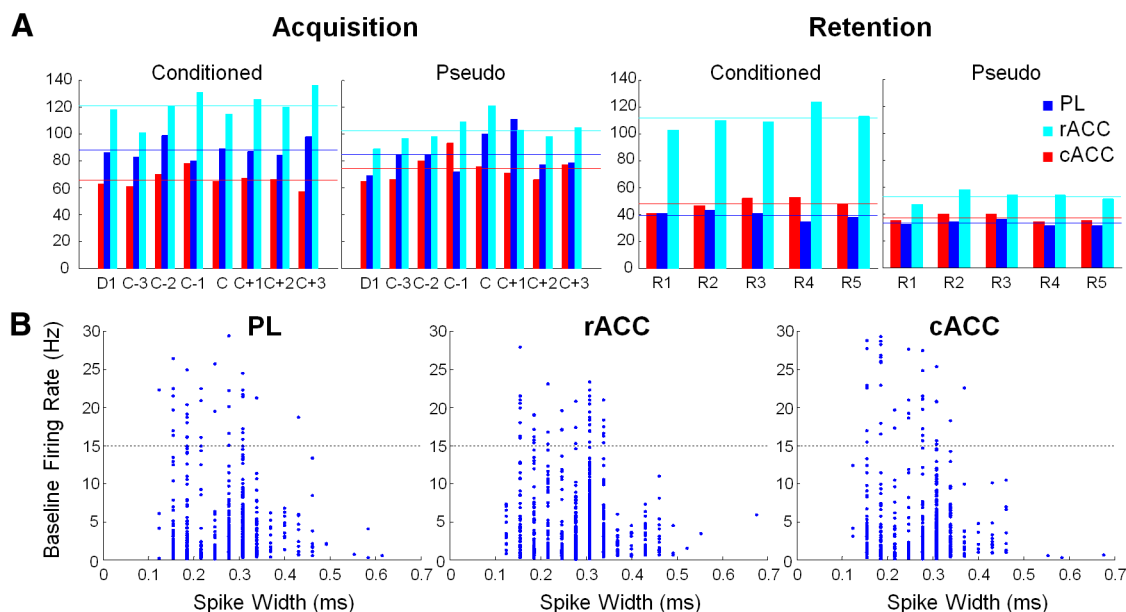
Figure 3. Recorded neurons and classification of cell types. **A**, The total number of neurons recorded per region plotted across days for acquisition and retention sessions for both groups. Red, cACC; blue, PL; cyan, rACC. Horizontal lines represent average number of cells recorded across days for each region in each group. ANOVA revealed no significant effect of sessions and no significant interactions of sessions with group or regions. Note that the acquisition groups included three more rabbits than the retention groups, which accounts for the greater number of neurons recorded during acquisition. **B**, Clustering within the distribution of spike width and baseline firing rate (2 s before CS onset) for all neurons recorded from each region that passed our S/N criteria (see Materials and Methods) was examined to extracellularly classify recorded neurons into cell types (i.e., pyramidal vs interneuron). A baseline firing rate of 15 Hz was used to separate putative pyramidal neurons from interneurons (dotted horizontal line).

$F_{(1,103)} = 16.8$, $p < 0.0001$); the change for neurons from pseudo-conditioned rabbits was not significantly different between sessions (0.41 for post-C vs 0.30 for retention). In contrast, activity during the CS interval did not show any significant differences across sessions or between groups. Last, the baseline firing rate across sessions did not change for either group but did increase for both groups during retention sessions relative to the post-C session ($F_{(1,170)} = 7.75$, $p = 0.006$).

In addition to examining changes in activity of conditioned animals relative to control animals, we further examined within conditioned animals changes in activity specific to the CS and trace intervals across sessions. A significant interval (CS vs trace) × session interaction was observed during acquisition sessions ($F_{(3,196)} = 2.61$, $p = 0.05$) in which on D1, trace interval activity was of higher magnitude than the CS interval (0.54 ± 0.21 for CS vs 1.09 ± 0.15 for trace, $F_{(1,26)} = 4.438$, $p = 0.04$). However, on subsequent sessions and also during retention sessions, no significant differences were observed between CS and trace interval mean z-scores.

RD neurons
CS and trace interval z-score activity of PL RD neurons (Fig. 5A) did not differ between conditioned and pseudoconditioned groups and did not exhibit changes across days. However, RD neurons of the conditioned group exhibited overall a significantly lower baseline firing rate than neurons from the pseudoconditioned group during acquisition sessions (2.37 ± 0.17 vs 3.00 ± 0.27 Hz, $F_{(3,423)} = 5.97$, $p = 0.01$) and during retention sessions as well (1.83 ± 0.20 vs 2.93 ± 0.43 Hz, $F_{(1,268)} = 6.34$, $p = 0.01$).

cACC
RI neurons
Neurons throughout the extent of the cACC of conditioned rabbits exhibited an obvious, stimulus-locked response to the CS and US (Fig. 4C), especially on the first day of training. In general, significant differences in activity between groups and sessions

were revealed when comparing the responses during the trace interval but not the CS interval. During acquisition sessions, mean trace interval z-scores of both groups exhibited a gradual decline over sessions ($F_{(3,265)} = 4.85$, $p = 0.003$), but the conditioned group activity had a greater overall magnitude than that of the pseudoconditioned group ($F_{(1,265)} = 7.39$, $p = 0.007$). Interestingly, a comparison of mean trace interval z-score activity during post-C and retention sessions revealed a rebound in activity during retention sessions for both groups combined ($F_{(1,156)} = 6.37$, $p = 0.01$), indicating a re-engagement of the cACC after the 30 day hiatus. Comparisons of activity during the CS interval did not reveal any significant differences.
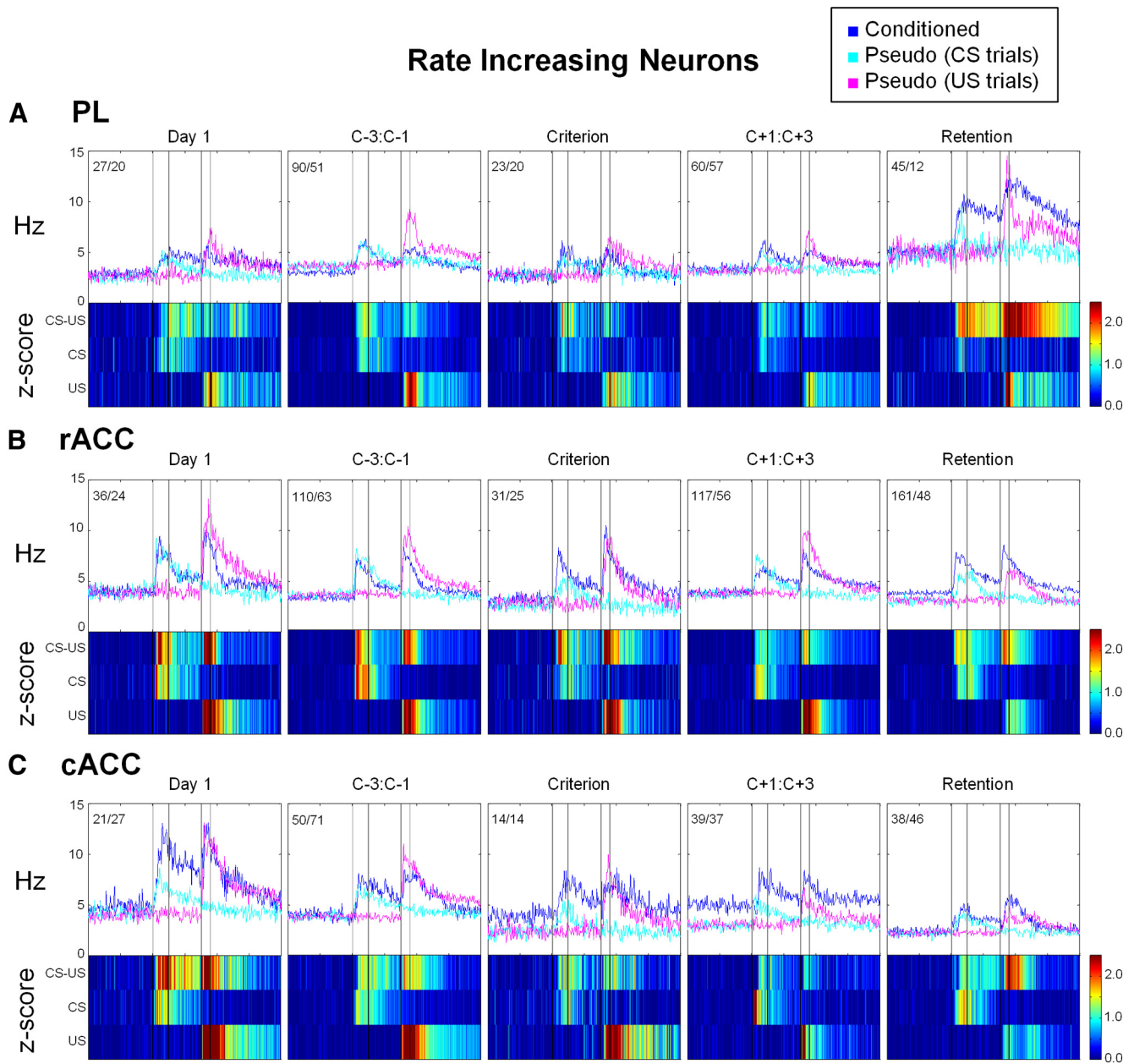
RD neurons
Similar to RI neurons, RD neurons (Fig. 5C) did not exhibit significant differences in CS interval activity either between groups or across acquisition sessions. However, decreases during the trace interval were found to be significantly greater for the conditioned than for the pseudoconditioned group for the post-C and retention sessions (−0.57 ± 0.03 vs −0.41 ± 0.04, $F_{(1,335)} = 11.17$, $p < 0.001$). In fact, comparison of CS activity to trace activity of conditioned animals indicated a significant interval × session interaction ($F_{(1,198)} = 6.03$, $p = 0.01$) in which the magnitude of rate decrease was significantly greater during the trace interval than the CS interval (−0.35 ± 0.03 for CS vs −0.55 ± 0.02 for trace, $F_{(1,199)} = 23.67$, $p < 0.0001$).

rACC
RI neurons
Mean trace interval activity of RI rACC neurons (Fig. 4B) exhibited a gradual decline across acquisition sessions with both groups combined ($F_{(3,454)} = 2.71$, $p = 0.045$); there was no difference between groups and no interaction of group and session. During retention sessions, there was a generalized significant increase in activity from post-C to retention sessions ($F_{(1,378)} =$
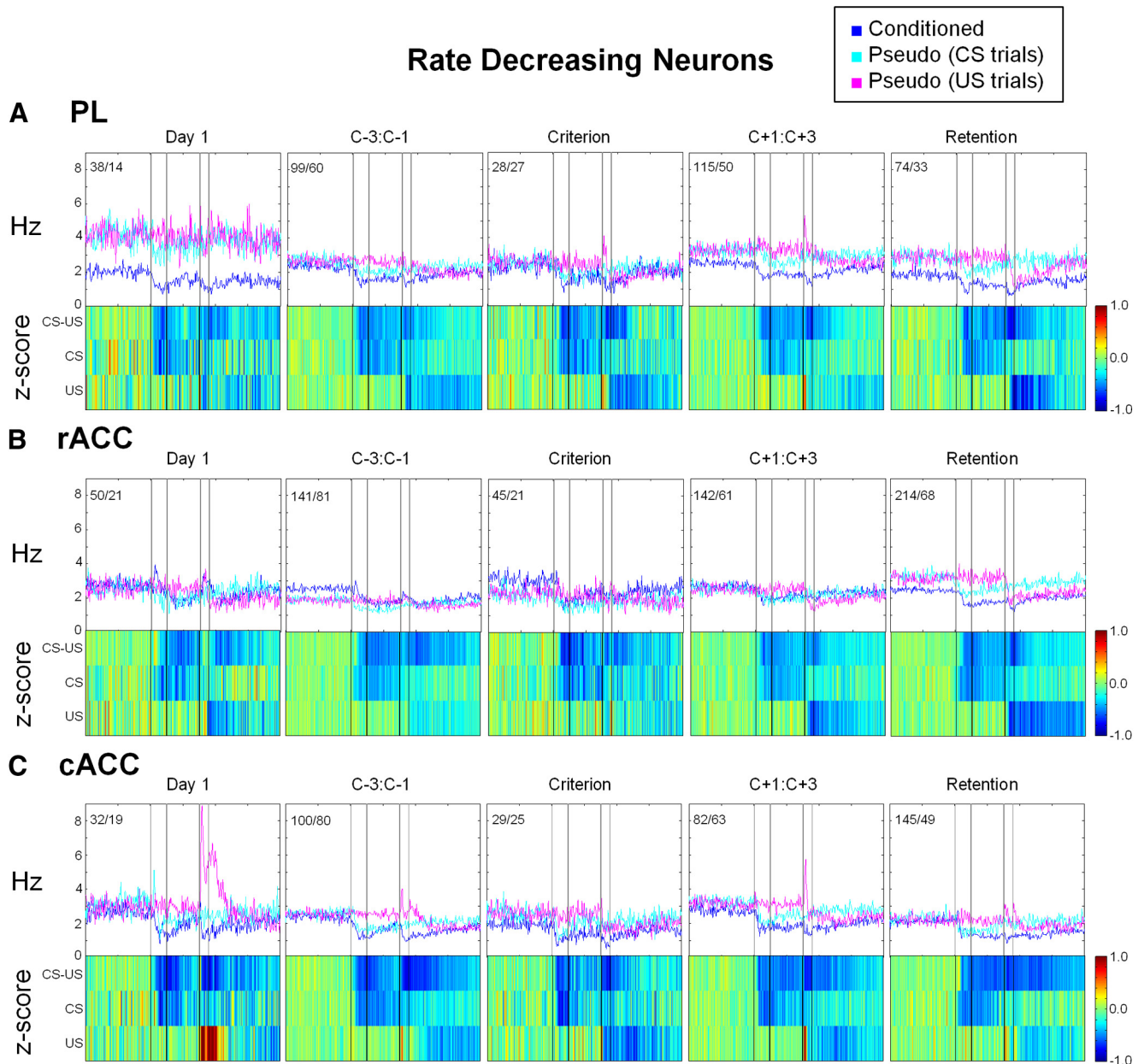
Figure 4. Activity of RI neurons differs across sessions and among regions. *A*, PL; *B*, rACC; *C*, cACC. Top panels of each section show PETHs of neuronal activity (hertz) for the population of neurons for the first day of training, the average of the three sessions before the criterion session (C−3:C−1), the criterion session, the average of the three sessions after the criterion session (C+1:C+3), and the average of five sessions to test retention at a point 30 d after the end of acquisition training. Bottom panels show the population data as *z*-scores normalized to the baseline period. Values are color coded to indicate the magnitude of the *z*-score. Vertical lines indicate CS and US intervals. Values at top left of each rate histogram show number of RI neurons for the two groups (Conditioned, Pseudo).

5.48, $p = 0.020$), and neurons from the conditioned group exhibited an overall greater magnitude of response than those from the pseudoconditioned group ($F_{(1,378)} = 5.13$, $p = 0.024$); there was no significant interaction of group and session. In comparison, CS interval activity did not exhibit any significant differences during acquisition sessions. However, during retention sessions, a significant group × session interaction was observed ($F_{(1,378)} = 4.6$, $p = 0.033$), and follow-up ANOVA confirmed that the activity of neurons from the conditioned group increased from post-C to retention sessions (0.877 ± 0.138 vs 1.27 ± 0.127, $F_{(1,276)} = 4.327$, $p = 0.038$), whereas the activity of neurons from the pseudoconditioned group exhibited a decrease in responsivity during the CS period.

In conditioned animals, comparison of mean *z*-scores during the CS and trace intervals revealed a significant interval effect during both acquisition ($F_{(1,196)} = 8.23$, $p = 0.0046$) and retention sessions ($F_{(1,198)} = 12.79$, $p = 0.0004$). Subsequent ANOVAs confirmed that, at both time points, CS interval activity was of much greater amplitude than that during the trace interval (acquisition: 1.33 ± 0.14 for CS vs 0.76 ± 0.06 for trace, $F_{(1,199)} = 15.12$, $p = 0.0001$; retention: 1.18 ± 0.12 for CS vs 0.76 ± 0.05 for trace, $F_{(1,199)} = 11.11$, $p = 0.001$), suggesting stimulus-specific responses of rACC neurons.

Previous examinations of the rACC have led to the suggestion that the rACC is involved in error detection, conflict monitoring, attention, or evaluative mechanisms that monitor emotional sa-

Rate Decreasing Neurons

Figure 5. Activity of RD neurons differs across sessions and among regions. *A*, Prelimbic cortex (PL). *B*, Rostral anterior cingulate cortex (rACC). *C*, Caudal anterior cingulate cortex (cACC). For more detail, see legend to Figure 4.

lience of stimuli (Totah et al., 2009). To determine whether this premise is evident based on the history of trials, we plotted the mean firing rate of RI neurons during the CS–trace interval for all trials of all sessions. Results from conditioned animals (Fig. 6A) indicate that RI rACC neurons exhibited a decrease in firing rate across trials within each session. For each session, a linear regression was performed to highlight this decrease in activity. A plot of the linear regression slopes across all sessions shows consistently negative slopes for the conditioned group. In contrast, RI rACC neurons from pseudoconditioned rabbits show negative slopes for the first three sessions, but thereafter, the remaining slopes appear to be variable in terms of direction (positive/negative). These results appear to be unique to the rACC subregion (Fig. 6) and suggest that the rACC is involved in evaluating the emotional salience of stimuli (Phan et al., 2003). Although the mean firing rate of RI PL neurons from pseudoconditioned rabbits also ap-

pears to decrease within each retention testing session (Fig. 6C), that result is based on the population with the fewest number of neurons (one to four neurons per session; Fig. 4B, PL) and neurons that were much less likely to exhibit a persistent change in firing rate (Fig. 7, R1:R5). Thus, the variability in CS–trace interval responding during retention sessions accounts for a poor fit to the linear model as measured by the root mean square error (RMSE) of the linear regression, i.e., the RMSE during retention sessions is greater than during acquisition sessions (2.61 ± 0.35 vs 0.91 ± 0.07, mean ± SEM). As such, the resultant slopes for the retention sessions may be spurious when analyzing the data on a trial-by-trial basis as opposed to analyzing the session mean for each animal.

RD neurons

Comparisons of CS interval activity of RD rACC neurons (Fig. 5B) did not reveal any significant differences between groups or
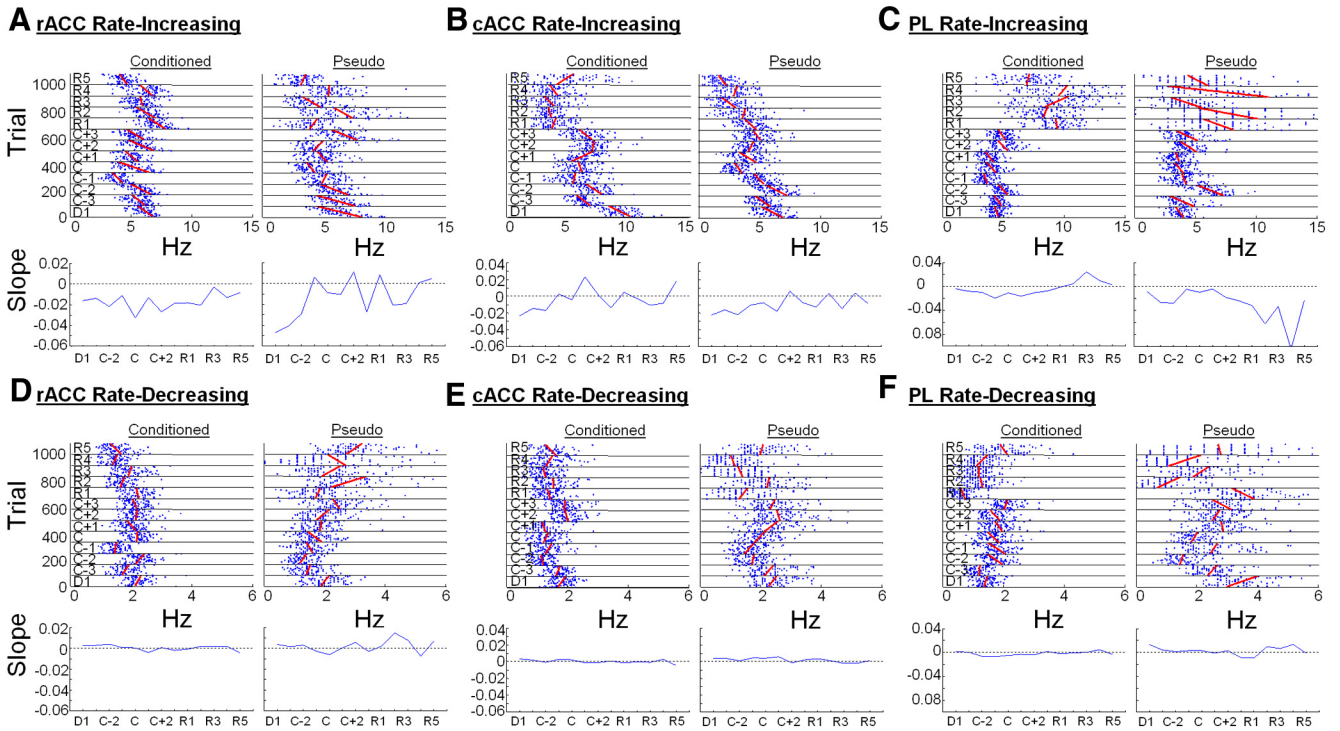
Figure 6. rACC neurons exhibit within-session decreases in responsivity to stimuli. Mean CS−trace interval firing rate in hertz (x-axis) of RI (**A–C**) and RD (**D–F**) neurons from the rACC (**A**, **D**), cACC (**B**, **E**), and PL (**C**, **F**) of conditioned and pseudoconditioned rabbits plotted over all trials (y-axis) from first day of acquisition (D1; bottom) through last day of retention testing (R5; top). Horizontal black lines separate individual training sessions during acquisition and retention (80 trials per session). The red lines denote linear regression of firing rates of all trials within each session, demonstrating the change in firing rate from early trials to later trials within the session. Below each raster type display is a graph plotting the slope of the linear regression fits across training sessions. Note the consistently negative slopes (a shift from high to low firing rates within each session) for data from the rACC of conditioned rabbits as opposed to data from pseudoconditioned rabbits and other brain regions.
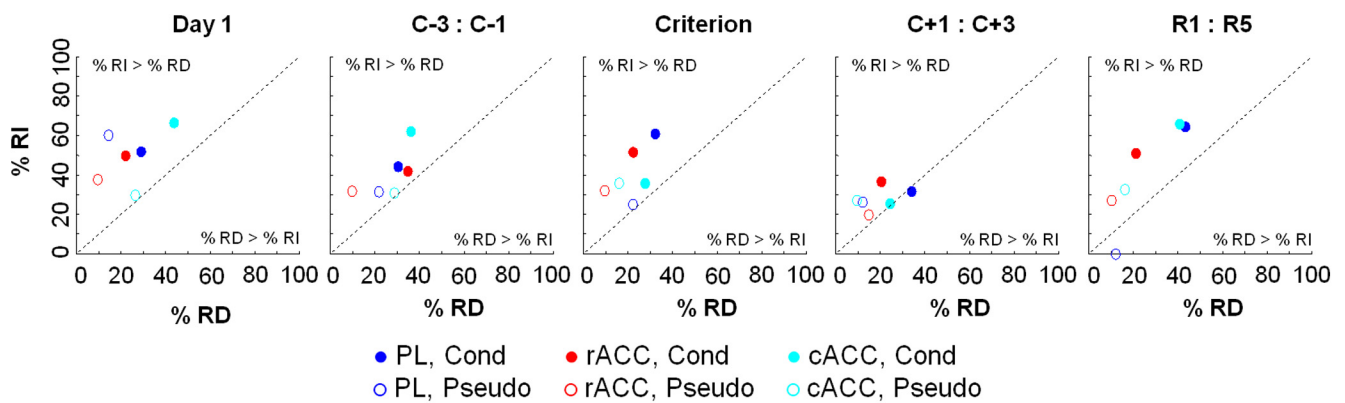
Figure 7. Percentage of persistently responsive neurons is greater for conditioned than pseudoconditioned rabbits. The percentage of persistently responsive RI neurons are plotted against the percentage of persistently responsive RD neurons for day 1, the three pre-criterion sessions (C−3:C−1), the criterion session (C), the three post-criterion sessions (C+1:C+3), and the five retention sessions (R1:R5). Data are separated by region (blue, PL; red, rACC; cyan, cACC) and group (filled circles, conditioned; open circles, pseudoconditioned) according to color and symbols. Diagonal line represents unity, i.e., an equal percentage of persistently responsive RI and RD neurons. Notice that, in most cases, persistently responsive RI neurons are more frequent than persistently responsive RD neurons, and conditioned rabbits exhibit more persistently responsive neurons than pseudoconditioned control rabbits.

among acquisition sessions. However, activity during the trace interval revealed a significant group difference during acquisition sessions ($F_{(1,554)}$ = 4.2, p = 0.04), with neurons of the conditioned group exhibiting larger magnitude rate decreases than neurons from the pseudoconditioned group. No such trend was apparent for the transition from post-C to retention sessions. Within conditioned rabbits, trace interval activity exhibited a significantly larger magnitude of rate decrease than the CS interval (-0.25 ± 0.06 for CS vs -0.51 ± 0.02 for trace, $F_{(1,199)}$ = 15.76, p = 0.0001) during acquisition sessions but not during retention sessions.

Changes in activity across PFC subregions

A three-way ANOVA comparing trace interval z-score activity for interactions of session (D1, pre-C, C, post-C, retention), group (conditioned vs pseudoconditioned), and brain region (PL, rACC, cACC) was used to determine differences among subregions across sessions. Results indicated a three-way interaction that approached statistical significance for RI neurons ($F_{(8,1403)}$ = 1.82, p = 0.06). This is seen clearly in Figure 4 where there is noticeable RI activity in the cACC during early acquisition periods that shifts to the PL during retention sessions. Subsequent ANOVAs revealed that this three-way interaction is driven by

neuronal activity in the conditioned group, which demonstrated a significant session × region interaction ($F_{(8,847)} = 3.0$, $p = 0.003$), whereas neuronal activity obtained from CS-only trials of the pseudoconditioned group did not. Only the PL ($F_{(4,240)} = 8.10$, $p < 0.0001$) and cACC ($F_{(4,157)} = 3.56$, $p < 0.01$) regions were found to change across sessions during conditioning. ANOVAs conducted separately for each time point revealed that the three regions exhibited significant differences in activity especially during retention sessions (one-way ANOVA, $F_{(2,347)} = 6.32$, $p = 0.002$) in which PL activity was significantly different from cACC activity (*post hoc* Fisher's PLSD test, $p = 0.0033$) and rACC activity (*post hoc* Fisher's PLSD test, $p = 0.0006$). These changes are evidence of the functional reorganization of neuronal activity that occurs in the PFC as memory is stored.

Conversely, RD activity did not exhibit a significant three-way interaction among group, session, and brain region. However, an overall group difference was observed ($F_{(1,1976)} = 7.76$, $p = 0.005$), with neurons from the conditioned group having greater negative z-scores compared with neurons from the pseudoconditioned group (*post hoc* Fisher's PLSD, −0.46 vs −0.40, $p < 0.001$).

To examine changes in activity relative to distinct events within the trial, we further performed within conditioned animals an interval × region × session three-way ANOVA with CS and trace intervals as repeated measures. Results revealed that, for RI neurons, activity between the CS and trace intervals significantly differed among regions during both acquisition ($F_{(2,606)} = 6.50$, $p = 0.001$) and retention ($F_{(1,454)} = 4.17$, $p = 0.01$) sessions. Specifically, activity of RI rACC neurons during the CS interval was significantly greater than activity during the trace interval during both acquisition and retention (acquisition: 1.34 ± 0.15 for CS vs 0.76 ± 0.06 for trace, $F_{(1,199)} = 15.12$, $p = 0.0001$; retention: 1.18 ± 0.12 for CS vs 0.76 ± 0.05 for trace, $F_{(1,199)} = 11.11$, $p = 0.001$). These results suggest that RI rACC activity is specific to the CS interval as evident in the population histogram in Figure 4B. In comparison, other regions (PL and cACC) did not exhibit this trend, suggesting that CS-driven responses generally continue into the trace interval.

For RD neurons, a significant interval × region × session interaction was observed for acquisition sessions ($F_{(6,889)} = 2.22$, $p = 0.04$), indicating that the activity differed between the intervals among regions and over learning. Specifically, ANOVAs revealed interval × region interactions on D1 ($F_{(2,117)} = 3.28$, $p = 0.04$) and pre-C ($F_{(2,337)} = 4.18$, $p = 0.02$) in which rACC neurons exhibited a greater magnitude of rate decrease during the trace interval than the CS interval (D1: -0.16 ± 0.14 for CS vs -0.50 ± 0.04 for trace, $p = 0.026$; pre-C: -0.27 ± 0.07 for CS vs -0.50 ± 0.03 for trace, $p = 0.003$). CS and trace interval RD neuron activity did not differ significantly on C and post-C sessions. During retention sessions, a significant interval × region interaction ($F_{(2,766)} = 3.35$, $p = 0.03$) and interval × session interaction ($F_{(1,766)} = 3.78$, $p = 0.05$) was observed but no interaction. Overall, the trace interval exhibited a greater magnitude of rate decrease than the CS interval (-0.35 ± 0.03 for CS vs -0.55 ± 0.02 for trace, $F_{(1,226)} = 27.438$, $p < 0.0001$) in which the difference was driven primarily by cACC activity ($F_{(1,226)} = 27.438$, $p < 0.0001$).

We further examined differences in the latency of responses of each neuron among the subregions relative to CS onset. After binning the activity of each neuron at 1 ms resolution, onset latencies were determined using a method inspired by Kiani et al. (2005) (see Materials and Methods). Results indicated that onset times of RI neurons differed significantly among the regions dur-

ing acquisition sessions ($F_{(2,625)} = 3.25$, $p = 0.04$), with cACC neurons exhibiting earlier onset latencies relative to those of the PL and rACC neurons. Specifically, cACC neurons preceded PL activity by 64 ms (cACC, 180.74 ± 13.83 ms; PL, 245.41 ± 17.82 ms, $p = 0.017$) and preceded rACC activity by 54 ms (rACC, 234.63 ± 14.65 ms, $p = 0.035$). In contrast, onset latencies among the three regions did not differ significantly during retention sessions (cACC, 208.13 ± 19.59 ms; PL, 230.66 ± 24.62 ms; rACC, 228.43 ± 15.88 ms). Similarly, onset latencies of RD neurons were not significantly different during acquisition (cACC, 25.18 ± 1.92 ms; PL, 28.85 ± 2.52 ms; rACC, 31.57 ± 2.28 ms) or retention (cACC, 36.14 ± 2.98 ms; PL, 29.52 ± 2.74 ms; rACC, 31.72 ± 2.08 ms) sessions but were generally much earlier than RI neurons.

Persistent responses span the stimulus-free trace interval

We examined neurons for a significant stimulus-induced persistent response during the stimulus-free trace interval. We used continuous SDFs to minimize artifacts for spikes that occur near the edge of a time bin (Richmond et al., 1987; Szucs, 1998). Neurons with persistent activity were operationally defined as those with activity that exceeded the mean baseline period SDF by 2.575 SDs ($p = 0.01$) for a minimum of 70% of the 500 ms trace interval, i.e., $\geq$350 ms. Figure 7 shows plots of the percentage of RI and RD neurons that are persistently responsive for each of the three subregions. Note that neurons from conditioned rabbits exhibit a greater percentage of persistent neurons than the percentage observed from pseudoconditioned rabbits for most regions during most sessions and that the majority of points are above the line of unity, which indicates that the percentage of persistently responsive RI neurons is greater than the percentage of persistently responsive RD neurons, although both cell types were observed. On day 1, both conditioned and pseudoconditioned groups exhibited persistent responses, suggesting an innate responsiveness to the CS. During retention testing (R1–R5), the difference in persistent activity between conditioned and control rabbits becomes more robust in all three PFC regions. These results indicate that memory for the CS–US association during remote retention is associated with persistent responses that span the stimulus-free trace interval and act to express the CR.

CR vs no-CR trials

If neurons in a region of the PFC are driving expression of CRs, there should be a reflection of that activity during CR trials and not during trials in which no CRs were elicited (no-CR trials). As shown in Figure 8, all three PFC subregions exhibited a greater magnitude of change during no-CR trials during sessions before criterion (D1, C−3:C−1), i.e., a greater increase for RI neurons and greater decrease for RD neurons. This pattern reversed after the day of criterion, showing a greater change in activity during CR trials than no-CR trials in post-criterion sessions. This trend was of even greater magnitude at the 30 d retention test [mixed-design ANOVA with trial type (CR/no-CR) as a repeated measure and session as factors; RI PL: $F_{(4,240)} = 52.72$, $p < 0.001$; RI rACC: $F_{(4,450)} = 42.53$, $p < 0.001$; RI cACC: $F_{(4,157)} = 17.04$, $p < 0.001$; RD PL: $F_{(4,240)} = 50.26$, $p < 0.001$; RD rACC: $F_{(4,450)} = 76.08$, $p < 0.001$; RD cACC: $F_{(4,383)} = 64.45$, $p < 0.001$]. These results, combined with the observation that neuronal recruitment does not appear to change across sessions (see below, Changes in neuronal recruitment; Fig. 9), suggests that the mPFC likely codes learning and memory through rate modulation of innately responsive neurons rather than by the recruitment of neurons that were previously unresponsive.
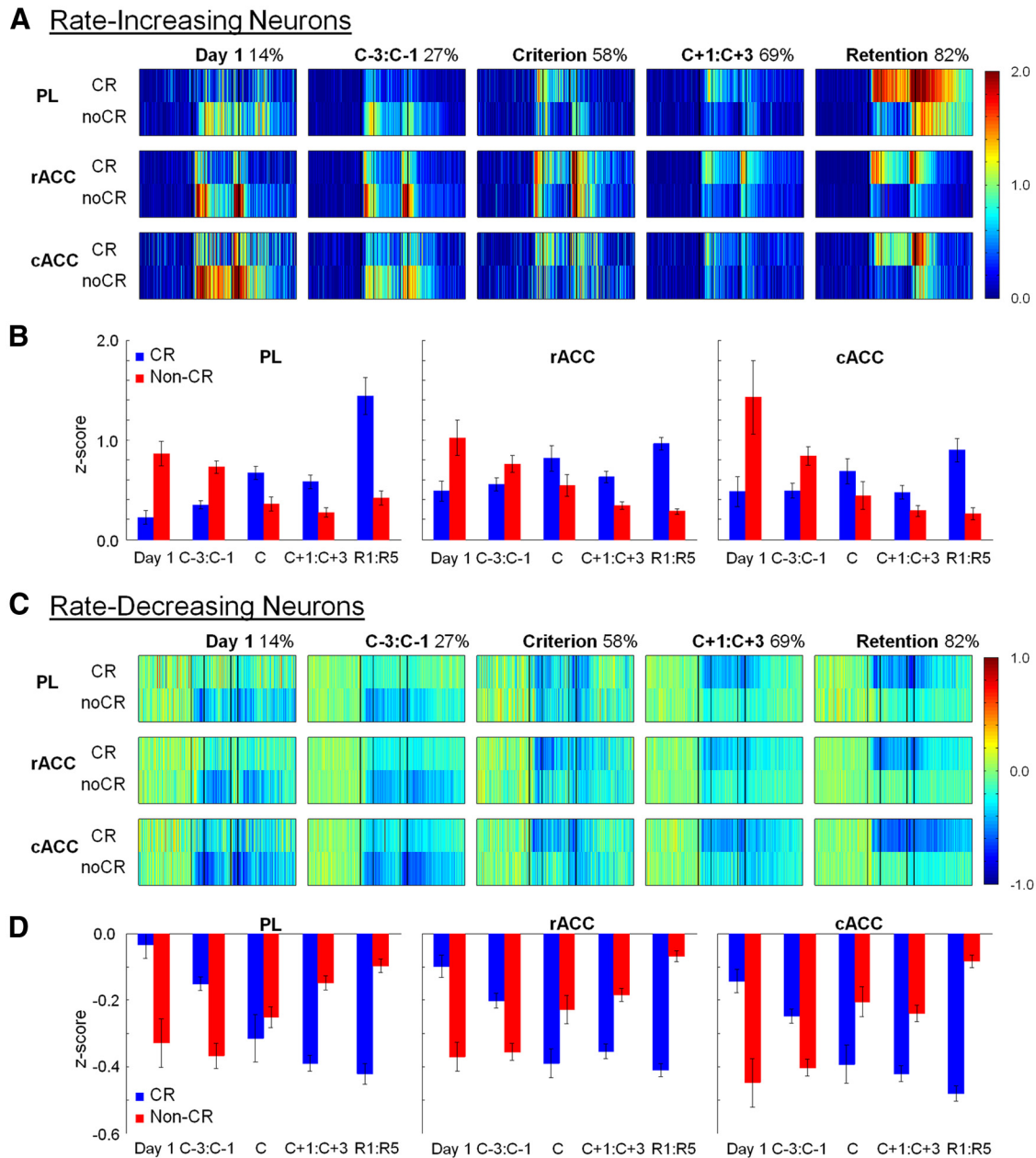
Figure 8. Neuronal activity is significantly different between CR and non-CR trials. PETHs of z-scores expressed in pseudocolor (**A**, **C**) and the mean z-score during the CS–trace interval across sessions (**B**, **D**) after separating the data into trials with (CR) or without (no-CR) a conditioned response for each of the three PFC subregions. Note that both RI and RD neurons are more responsive during no-CR trials of pre-criterion sessions and during CR trials of post-criterion sessions.

Changes in neuronal recruitment

In addition to rate modulation, plasticity can also be encoded by changes in the number of responsive neurons (although this can also be considered a change in rate modulation from subthreshold to suprathreshold activation). The percentage of responsive neurons was compared between conditioned and pseudoconditioned rabbits for individual training sessions using χ^2 analyses (Fig. 9). Overall, the percentage was greater for conditioned than for pseudoconditioned rabbits (69 vs 46%), and the difference between the two groups was larger for RD neurons (42% for conditioned vs 25% for pseudoconditioned) than for RI neurons (27% for conditioned vs 21% for pseudoconditioned), especially in the rACC (39 vs 23%) and cACC (51 vs 30%). Note that, although the conditioned group exhibits more responsive neurons than the pseudoconditioned group, there is relatively little

change in the percentage of responsive neurons from the first day of training through the last day of retention testing. This suggests that the PFC codes learning by rate modulation of innately responsive neurons rather than by the recruitment of neurons that were previously unresponsive.

Discussion

The present study aimed to characterize how neuronal activity within three subregions of the rabbit mPFC changes over time as an associative memory is formed, consolidated, and remotely retrieved. The present results provide electrophysiological support for a longitudinal, subregional functional segregation and reorganization of the rACC, cACC, and PL network during mnemonic processes. We propose, based on review of existing literature and results of the current study, that depending on the cognitive require-

ments to complete the task, the mPFC as a whole is flexible and involved in attention, stimulus valuation, and expression of behavior evoked by remotely acquired memories (Fig. 10).

Compared with acquisition sessions, a striking increase in neuronal activity was observed in the PL during remote trace EBC retention of conditioned animals but not of control animals (Fig. 4A). Furthermore, the activity started during the CS period and persisted throughout the stimulus-free trace period for conditioned animals but not control animals (Fig. 7), thus indicating that the persistent response is specific to learning rather than mere exposure to the CS. These results suggest that the CS–US association is gradually conferred to the PL such that the PL mediates the retrieval/expression of remotely acquired memories as learning progresses and is consolidated (Fig. 10). A similar trend was reported previously in contextual trace EBC (Takehara-Nishiuchi and McNaughton, 2008), although we show that the responsivity is exhibited by putative pyramidal neurons. Lesion studies also support this view because studies have shown transient impairments in remote trace EBC memory recall but not in acquisition after selective damage to the PL (Oswald et al., 2008, 2009, 2010). Fear conditioning studies have also suggested a role for the PL in remote storage/expression of learned fears but not acquisition of fear responses (Runyan et al., 2004; Corcoran and Quirk, 2007; Quinn et al., 2008; Choi et al., 2010). Thus, the response patterns we report in the PL supports the idea that the PL mediates retrieval/expression of remotely acquired associations.

The large responses observed in cACC during early sessions of trace EBC acquisition, but not during tests of remote retention, suggest that the cACC mediates an attentional role to enable the formation of a CS–US association by encoding behaviorally relevant stimuli (Ng et al., 2007), perhaps with feedback from the cerebellum (Weiss and Disterhoft, 1996, 2011; Weiss et al., 2006; Siegel and Mauk, 2013). This view is consistent with cACC lesion studies that have demonstrated impaired trace EBC acquisition (Weible et al., 2007). Furthermore, the activity of RI cACC neurons we report is similar to those reported by Weible et al. (2003) in that cACC activity decreased across trace EBC acquisition sessions. However, that study did not examine activity after consolidation, so our results, obtained longitudinally during recent and remote time points, revealed that the decrease in activity stabilizes by the day of criterion and cACC activity rebounds in responsiveness during retention testing. These results suggest that the cACC shows an innate responsiveness to novel stimuli that decreases if there is no behavioral significance as in the case of pseudoconditioned trials and a reactivation when the significance of the stimuli needs to be reinstantiated. The spatial distribution and responsivity of persistently firing neurons in cACC was similar to that reported by Siegel et al. (2012), although they did not evaluate such activity during pseudoconditioning, or after a conditioning-free hiatus to examine the effects of consolidation.
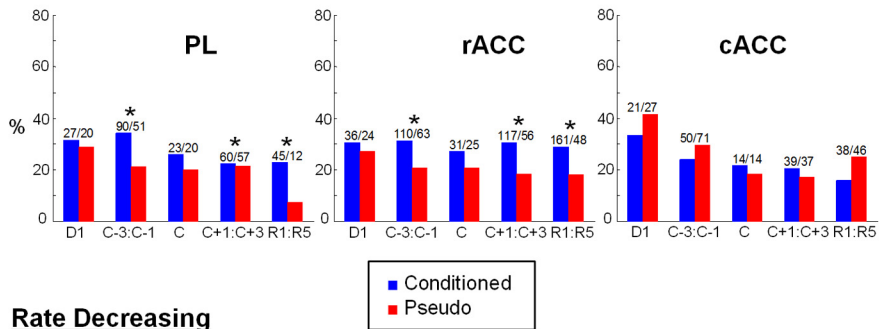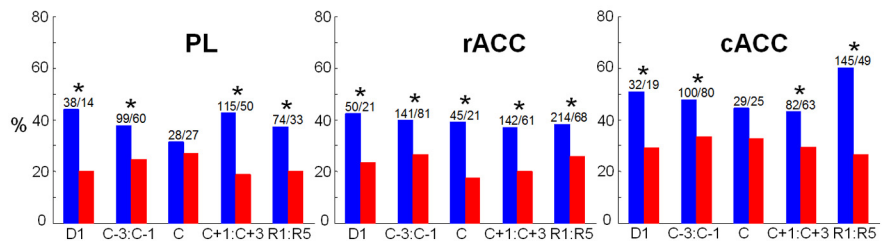

Figure 9. Neuronal recruitment is greater for conditioned than pseudoconditioned rabbits in most regions of PFC. The percentage of responsive neurons that exhibited either a significant increase (RI) or decrease (RD) in firing rate during the 250 ms CS and/or the 500 ms trace interval was computed across sessions for both conditioned and pseudoconditioned groups. Asterisks indicate a significant difference in the proportion of responsive neurons between the groups (χ^2 analysis). Counts of responsive neurons from conditioned (Cond) and pseudoconditioned (Pseudo) rabbits are shown on top of each bar.
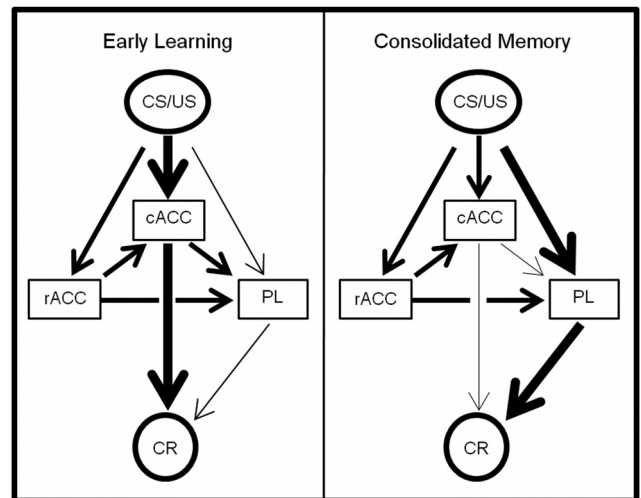
Figure 10. Proposed functional connectivity among mPFC subregions during acquisition and consolidation of CRs. During acquisition of EBC, the cACC may mediate an attentional role to enable the formation of a CS–US association. Thus, connectivity among regions involved in the association of the CS and US and the areas involved in the production of CRs would become strengthened (bold lines). As learning progresses and consolidates, the CS–US association may gradually be conferred to the PL cortex such that, during remote memory recall, the PL cortex mediates expression of remotely acquired CRs. The cACC and PL together may form a cognitive component of the mPFC that enables the formation and consolidation of the CS–US association. In contrast, the rACC, given its phasic changes in activity during each session (Fig. 4), may form an affective component of the mPFC that evaluates the emotional salience of incoming stimuli and modulates the activity of the other regions. Arrows do not necessarily represent monosynaptic connections.

Animal studies using other paradigms have also reported that cACC lesions affect the early stages of learning, i.e., animals take longer to achieve above-chance performance levels but eventually do achieve similar levels of accuracy as controls (Gabriel, 1990; Bussey et al., 1996). In addition, various human studies

have demonstrated cACC involvement in the modulation of attention or stimulus selection (Bush et al., 1998, 1999; Posner and DiGirolamo, 1998; Carter et al., 1999). In fact, some argue, based on human and animal cytoarchitectural, lesion, and electrophysiological studies, that the ACC comprises two major subdivisions that serve distinct functions: the rostral "affective division" and the caudal "cognitive division" (Vogt et al., 1992; Devinsky et al., 1995; Bush et al., 2000). Anatomically, the caudal cognitive division maintains reciprocal connections with the lateral prefrontal, parietal, premotor, and supplementary motor cortices (Devinsky et al., 1995), areas all thought to subserve higher-order, cognitive control. Therefore, the cACC is a prime candidate for modulating attentional mechanisms.

Although our results, in conjunction with empirical literature, make a strong case for an attentional role for the cACC, other functions have been ascribed for the cACC, including error detection, working memory, response selection, complex motor control, motivation, and conflict monitoring (Gemba et al., 1986; Picard and Strick, 1996; Drevets and Raichle, 1998; Posner and DiGirolamo, 1998; Carter et al., 1999). In fact, some studies even suggest that the ACC acts as a memory "storage site" and is preferentially involved in the expression of remote contextual fear and spatial memory (Frankland et al., 2004; Maviel et al., 2004; Teixeira et al., 2006; Weible et al., 2012). However, it can be argued that remote memory expression is accompanied by attentional processing because it has been reported that awareness or knowledge of stimulus contingencies may be an important contributor to mnemonic processes (Clark and Squire, 1998; Knuttinen et al., 2001; Manns et al., 2001). For example, an *in vivo* recording study for spatial object memory demonstrated that some cACC neurons respond to locations where an object was encountered previously 1 month ago and that animals preferentially explored the location of the absent object (Weible et al., 2012). Although these results certainly indicate long-term memory for object locations, it could also be accompanied by the animal actively searching for and attending to the area to find the missing object. Similarly, remote memory retrieval in other paradigms, such as fear conditioning, requires animals to attend to cues that initiate the expression of remotely acquired memories (e.g., tone or context). Such behavior would manifest in the neural activity of brain regions involved in attentional processing. Thus, dissociating between these functions is difficult, and additional studies are required to fully elucidate the role(s) of the cACC. However, what is common to all the proposed functions of the cACC is that each is contingent on a cognitively demanding task. It has been shown, at least in trace EBC, that the use of different US stimuli can alter the cognitive demand placed on the mPFC. In particular, airpuff US places a greater cognitive demand than periorbital shock US (Oswald et al., 2009). Thus, although our experimental aim was not to explore every proposed function of the cACC per se, by use of airpuff US to place maximal demands on the mPFC, our results nonetheless underscore the importance of the cACC in the formation of CS–US associations in a cognitively demanding associative memory task.

Unlike the other subregions, rACC neurons of conditioned rabbits exhibited phasic decreases in activity during each session, whereas rACC neurons of pseudoconditioned rabbits did not. This pattern of activity may reflect habituation to the stimuli and code the emotional salience of the CS within each training session, i.e., the paired stimuli are less novel and aversive with each successive presentation. This view is consistent with literature that contend that the rostral affective division (Vogt et al., 1992;

Devinsky et al., 1995; Bush et al., 2000) of the ACC is involved in the evaluation of emotional salience in concert with limbic areas, such as the amygdala, nucleus accumbens, hypothalamus, anterior insula, and orbitofrontal cortex (Devinsky et al., 1995; Whalen et al., 1998; Bush et al., 2000; Phan et al., 2002, 2003). For example, human fMRI studies have shown consistent rACC activation in affect-related tasks (Whalen et al., 1998; Bush et al., 2000) and even habituation effects to affective stimuli (Phan et al., 2003), a trend very much similar to the ones we report. Cytoarchitectural and connection studies have further demonstrated that the rACC projects to autonomic, visceromotor, and endocrine systems, such as the basal forebrain cholinergic system and locus ceruleus noradrenergic system, which project back to mPFC areas (Vogt et al., 1992; Devinsky et al., 1995; Drevets and Raichle, 1998; Whalen et al., 1998), thus corroborating an affective role for the rACC. By assessing the emotional salience of the CS, the rACC may, via direct or indirect neuromodulatory inputs to other brain regions including the cACC and PL, allocate attentional resources to emotionally arousing stimuli and adapt/strengthen existing memory networks (Fig. 10). In particular, evidence exists that rACC lesions do not affect acquisition but impair extinction of trace EBC (Weible et al., 2000). The rACC, by influencing arousal states and activity of other regions (e.g., cACC), may modulate sensitivity to behaviorally relevant stimuli and orient the functional state of the mPFC into a mode appropriate for the task at hand. As the emotional value of the stimulus decreases on repeated presentations within each session, there is less need for the rACC to maintain a constant drive, thus a decrease in activity as we and others have observed. Consequently, the loss of rACC functioning may lead to an inability to orient and modulate existing mPFC networks, thus accounting for the inability of conditioned animals to extinguish CS–US associations in response to CS-alone presentations.

Other interpretations for this phasic activity observed in the rACC are possible. For example, this pattern of activity may reflect a decrease in attention within a session. However, if this activity was correlated with attention, it should also be reflected in behavioral performance, i.e., animals should exhibit phasic decreases in trials in which CRs are elicited. Given that animals continue to exhibit CRs once they learn the CS–US association and do not exhibit phasic decreases in CRs within sessions, this interpretation is unlikely. Nonetheless, other interpretations are possible, and future studies of the rACC may provide more insight on these possibilities.

Although the present results were obtained and examined within the context of mnemonic processes, we believe that our results apply to other proposed functions of the mPFC. Holistically, the mPFC has been implicated for various roles besides mnemonic processes, including decision making, conflict monitoring, error detection, and executive control (Gemba et al., 1986; Picard and Strick, 1996; Drevets and Raichle, 1998; Posner and DiGirolamo, 1998; Carter et al., 1999; Euston et al., 2012). To reconcile these disparate views, Euston et al. (2012) have proposed a unifying theory that posits that the role of the mPFC is to integrate inputs regarding current context and events and output the most adaptive response based on past experience. We believe that our findings are in agreement with this view and provide electrophysiological support for this model. The functional reorganization we observed as manifested by the changes in activity within the three mPFC subregions as learning and memory consolidation proceeds underscores the roles of the individual mPFC subregions. They provide insight into how these subdivisions in-

teract and work together to mediate the integration of inputs and modulation of other brain regions.

In summary, the present study provides, for the first time, longitudinal insight into the neurophysiological organization of three subdivisions of the mPFC during a cognitively demanding associative memory task. These data help to elucidate the normal response patterns occurring within the mPFC during acquisition and execution of remotely acquired behaviors. Future research can build on these findings, possibly through simultaneous mPFC subdivision recordings during other cognitively demanding tasks.

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