

Behavioral Neuroscience

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Online First Publication, June 4, 2018. <http://dx.doi.org/10.1037/bne0000229>

CITATION

Jiang, M. Y., DeAngeli, N. E., Bucci, D. J., & Todd, T. P. (2018, June 4). Retrosplenial Cortex Has a Time-Dependent Role in Memory for Visual Stimuli. *Behavioral Neuroscience*. Advance online publication. <http://dx.doi.org/10.1037/bne0000229>

Retrosplenial Cortex Has a Time-Dependent Role in Memory for Visual Stimuli

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Although the retrosplenial cortex (RSC) is critically involved in spatial learning and memory, it appears to have more selective contributions to learning and memory for discrete cues. For example, damage to the RSC does not impair Pavlovian delay fear conditioning to a discrete auditory cue (e.g., tone), when RSC manipulation occurs just prior to, or shortly after, conditioning. In contrast, when lesions of the RSC occur following a substantial retention interval (e.g., 28 days), the RSC is necessary for retrieval of fear to the tone. Thus, the RSC makes time-dependent contributions to memory retrieval for discrete auditory cues. The purpose of the current experiment was to assess if the time-dependent involvement of the RSC in cue-specific fear memory extended to cues of other sensory modalities. Rats firsts underwent fear conditioning to a visual stimulus, and lesions of the RSC subsequently occurred 1 or 28 days later. Lesions of the RSC impaired fear expression when made 28 days after conditioning, but not when made 1 day following conditioning. Coupled with previous findings, the current results suggest the RSC is necessary for retrieval of remotely acquired cued fear memories across multiple modalities.

Keywords: retrosplenial, remote memory, fear conditioning, visual cue

Over the past decade there has been a surge of research on the contributions of the retrosplenial cortex (RSC) to learning, memory, and behavior. Although there is still much to understand and unify regarding the function of this region and its interactions with medial temporal lobe structures, it has become clear that the RSC is especially involved in spatial navigation as well as contextual learning and memory (for reviews see Miller, Vedder, Law, & Smith, 2014; Todd & Bucci, 2015; Vann, Aggleton, & Maguire, 2009). For example, in vivo electrophysiological recording studies have demonstrated that RSC neurons process critical information related to landmarks, trajectories, and reward location (Vedder, Miller, Harrison, & Smith, 2017), as well as the overall spatial structure of complex routes (Alexander & Nitz, 2017; Clark, 2017). More so, the RSC is critically involved in contextual fear conditioning (e.g., Corcoran et al., 2011; Kwapis, Jarome, Lee, & Helmstetter, 2015; Todd, DeAngeli, Jiang, & Bucci, 2017), a process that likely involves communication between the RSC and hippocampus (Tayler, Tanaka, Reijmers, & Wiltgen, 2013). Importantly, both spatial navigation and contextual learning and memory typically require the integration of information from multiple cues in the environment.

Apart from spatial navigation and contextual learning and memory, there is renewed interest in understanding how the RSC contributes to learning and memory for discrete stimuli. While an earlier body of work by Gabriel and colleagues focused on the role of the RSC in avoidance discrimination learning involving discrete auditory stimuli (Gabriel & Sparenborg, 1987; Gabriel, Sparenborg, & Stolar, 1987), recent studies have tested the involvement of the RSC in Pavlovian fear conditioning, in which a single auditory cue is associated with mild footshock. In rodents, these studies have consistently demonstrated little, if any, contribution of the RSC to encoding or retrieval of “delay” auditory fear conditioning, in which a tone is presented for a short period of time (e.g., 10 s) and coterminates with a mild footshock (Corcoran et al., 2011; Keene & Bucci, 2008b; Kwapis, Jarome, Lee, Gilmartin, & Helmstetter, 2014, 2015). The fact that the RSC is not necessary for learning and memory for a single cue in Pavlovian delay fear conditioning procedures has led some to suggest that the RSC is specifically involved in situations that require the processing and integration of *multiple* cues (e.g., Bucci & Robinson, 2014; Keene & Bucci, 2008a), perhaps consistent with the RSC’s important role in spatial and contextual learning and memory.

There are, however, exceptions in which the RSC does contribute significantly to learning and memory for single cues in Pavlovian fear conditioning procedures. For example, insertion of a short interval between the end of a Pavlovian conditioned stimulus (CS) and presentation of shock (so called “trace” conditioning”) appears to recruit the RSC (see Kwapis et al., 2014, 2015). Additionally, and of relevance to the current set of experiments, we recently found that as cue-specific memories age, they become dependent upon the RSC for their retrieval. For example, disrupting RSC function with lesions or temporary inactivation impairs

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This work was supported by National Science Foundation Grant IOS1353137 (David J. Bucci) and National Institutes of Health Grant F32MH105125 (Travis P. Todd).

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the retrieval of conditioned fear to a tone that was acquired 28 days earlier (i.e., remote fear memory; Todd, Mehlman, Keene, DeAngeli, & Bucci, 2016), but has no effect on tone fear conditioning that occurred 1 day earlier (i.e., recent memory; Keene & Bucci, 2008b). Although the nature of this time-dependent shift is still unclear, these findings are consistent with the fact that the RSC receives direct projections from the secondary auditory cortex (Todd, Mehlman, et al., 2016; Vogt & Miller, 1983), a region necessary for the retrieval of remote, but not recently, acquired fear memories (Sacco & Sacchetti, 2010).

In addition to its connections with auditory cortex, the RSC has strong reciprocal connections with the visual cortex (van Groen, Vogt, & Wyss, 1993; van Groen & Wyss, 1990; Wyss & Van Groen, 1992). Consistent with these connections, RSC neurons are responsive to visual cues (e.g., Vedder et al., 2017), and RSC damage attenuates discrimination learning between multiple visual cues (e.g., Bussey, Muir, Everitt, & Robbins, 1996, 1997; Todd, Huszár, DeAngeli, & Bucci, 2016). Nevertheless, studies to date indicate that the RSC is not necessary for Pavlovian delay conditioning with a *single* visual cue. For example, pretraining lesions of the RSC have no impact on the acquisition of Pavlovian delay conditioning to a visual conditioned stimulus with either shock (Todd et al., 2017, Experiment 1) or food (Keene & Bucci, 2008b) as the reinforcer. However, no study has examined the involvement of the RSC in the retrieval of *remotely* acquired memory for a visual conditioned stimulus. To address this, lesions of the RSC were carried out either 1 day (Experiment 1) or 28 days (Experiment 2) after fear conditioning in which a light was paired with footshock. We hypothesized that, similar to the involvement of the RSC in auditory fear conditioning, remotely, but not recently, acquired fear memories for a visual stimulus would be RSC dependent.

Experiment 1

Methods

Subjects. The subjects were 18 naïve male Long–Evans rats (~60 days old at start of training), obtained from Envigo Laboratories, Inc. (Indianapolis, IN). Rats were housed individually and allowed at least 6 days to acclimate to the vivarium prior to surgery. Food and water were available ad libitum (Purina standard rat chow, Nestle Purina, St. Louis, MO). Throughout the study, rats were maintained on a 14:10 light–dark cycle and monitored and cared for in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines and the Dartmouth College Institutional Animal Care and Use Committee.

Surgery. Subjects were anesthetized with isoflurane gas (1.5%–3% in oxygen) and placed in a Kopf stereotaxic apparatus. The skin was retracted and holes were drilled through the skull above each of the intended lesion sites using the rat brain atlas of Paxinos and Watson (2009). RSC lesioned rats received bilateral electrolytic lesions (2.5 mA, 15 s at each site) of RSC 24 hr ($n = 10$) after behavioral training using the stereotaxic coordinates outlined in Table 1. Control rats received sham lesions ($n = 8$) consisting of a craniotomy and shallow, nonpuncturing burr holes to minimize damage to the underlying cortex 24 hr after training. All rats were allowed to recover for 10 days before testing began.

Table 1
Stereotaxic Coordinates for Restrosplenial Cortex (RSC) Lesions

AP	ML	DV
–2.0	±.3	–2.0 and –2.7
–3.5	±.4	–2.0 and –2.7
–5.0	±.4 and ±1.0	–2.0 and –2.7 (medial site) and –2.0 (lateral site)
–6.5	±.8 and ±1.5	–2.0 and –2.8 (medial site) and –3.4 (lateral site)
–8.0	±1.6 and ±2.4	–2.5 (medial site) and –3.1 (lateral site)
–9.0	±3.4	–4.0

Note. All anterior–posterior (AP), medial–lateral (ML) and dorsal–ventral (DV) measurements are derived from bregma, midline, and skull surface, respectively (measurements are in mm).

Behavioral apparatus. Two sets of four conditioning chambers served as the two contexts (counterbalanced). All chambers were of the same standard design (Med Associates, Inc., St. Albans, VT, ENV-007; 24 cm W × 30.5 cm L × 29 cm H) and each was housed in its own sound attenuation chamber (Med Associates, ENV-017M; 66 cm W × 56 cm L × 56 cm H) and outfitted with an exhaust fan to provide airflow and background noise (~68 dB). Each chamber was outfitted with a food cup, recessed in the center of the front wall, and a retractable lever (Med Associates, ENV-112CM) positioned to the right of the food cup, which remained retracted throughout the experiment. Each chamber had a panel light (Med Associates, ENV-221M) mounted approximately 16 cm above the grid floor centered over the food cup, and a house light (Med Associates, ENV-215M) mounted approximately 24 cm above the grid floor on the back wall of the chamber. A speaker (Med Associates, ENV-224AM) was located 20 cm above and to the right of the food cup. Both sets of chambers were illuminated with a 2.8 W bulb (with a red cover), mounted to the ceiling of the sound-attenuating chamber.

In one set of chambers, the side walls and ceiling were made of clear acrylic plastic and the front and rear walls were made of brushed aluminum. The grid floor was composed of stainless steel rods (5 mm diameter) spaced 1.5 cm apart (center-to-center). In the second set of chambers, the rods of the floor were staggered such that odd- and even-numbered grids were mounted in two separate planes, one 0.5 cm above the other. The staggered grid floor provided a distinct tactile feature. In these chambers, the ceiling and door were covered with laminated black and white checkerboard paper (1 cm squares) to provide distinct visual cues.

Because these two sets of chambers were located within the same room of the laboratory, in order to prevent diffusion of the olfactory cues, one olfactory cue was used for Context A sessions, and a second olfactory cue was used for Context B sessions. During Context A sessions, 3 mL of Pine-Sol (Clorox, Co., Oakland, CA) was placed in the chamber tray below the grid floor, and for Context B sessions approximately 0.5 g of Vicks Vaporub (Proctor & Gamble, Cincinnati, OH) was smeared along the chamber tray below the grid floor.

The panel light mounted to the front wall served as the visual stimulus. During CS presentation, it flashed twice per second for 10 seconds. Footshocks (1 mA, 1 s) were generated by a Med Associates shock generator (ENV-414) connected to each chamber. The apparatus was controlled by computer equipment located

in an adjacent room. Surveillance cameras located inside the sound-attenuating chambers were used to monitor the rats' behavior.

Behavioral procedures. The training session consisted of three 10-s presentations of the light coterminating with the footshock. The interval from shock to the next light (intertrial interval, [ITI]) was 64 seconds. The first trial began 3 min after the rat was placed in the chamber. Following recovery from surgery, rats were then reexposed to the original training chamber (Context A) for a single 20-min context test session during which no tones or shocks were presented. Twenty-four hours after this context test, a light test session was carried out by placing the rats in Context B and presenting the light 20 times (10 s each, 30 s ITI) beginning 3 min after the rat was placed in the chamber. Again, no shock was delivered during this test session.

Behavioral observations. Freezing served as the index of conditioned fear and was operationally defined as total motor immobility except for breathing (Blanchard & Blanchard, 1969; Fanselow, 1980). On the training day, the incidence of freezing behavior was recorded during the 64-s period prior to the first trial (baseline freezing) and during the 64-s period following each trial (postshock freezing). The rats' behavior was scored every 8 s during the 64-s epochs and the mean percent freezing across the three postshock epochs was calculated for each rat. For the context test session, each rat was scored every 8 s for the first 8 min and 32 s, yielding 64 observations for each rat (Maren, Aharonov, & Fanselow, 1997). For the light test session, freezing was recorded every 2 s during each 10-s presentation of the light. For each rat, the data was used to calculate the average freezing during the context test session and the average freezing during the light test session. The frequency of freezing behavior was converted to a percentage of total observations. A single primary observer, blind to treatment condition, scored all the behavioral data, while a second observer scored a subset of the data to assess objectivity. The observations from both observers were highly correlated ($r \geq .9$).

Lesion verification and analysis. After the behavioral procedures were completed, rats were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline for 2 min, followed by 10% buffered formalin for 6 min. Coronal brain sections (60 μ m) were collected using a freezing microtome and were Nissl-stained using thionin. Using a compound microscope (Axioskop I, Zeiss, Inc.), we identified gross tissue damage as necrosis, missing tissue, or marked thinning of the cortex. Outlines of the lesions were drawn onto digital images adapted from Paxinos and Watson (2009) using PowerPoint at 6 levels along the rostro-caudal extent of the RSC (-1.8, -3.0, -4.2, -5.4, -6.6, and -7.8 mm from bregma). At each level, area measurements were then made with ImageJ, including the total area of the target region and the area of the target region that exhibited gross tissue damage. From these measurements, we report the average percentage of RSC that was damaged. In addition, we report the average percentage of sections across the rostro-caudal plane that exhibited RSC damage (out of ~24 sections collected for each rat), the average percentage of sections with damage outside the RSC, and the number of rats with damage to regions outside the RSC. Finally, we report the average percentage of secondary visual cortex that was damaged, at points -4.2, -5.4, -6.6, and -7.8 from bregma.

Results and Discussion

Histology. Two rats were excluded from statistical analysis due to substantial extra-RSC damage. The remaining groups' sizes were sham ($n = 8$) and RSC ($n = 8$). Figure 1a shows a photomicrograph of a representative RSC lesion. In Figure 1b (Experiment 1), lesion drawings are stacked onto a single atlas image for each of the 6 coronal levels. Bilateral damage to the RSC was observed in all animals. The average area of RSC damage on each section analyzed was 70.6% ($SEM = 2.83$). Damage to the RSC was present on 90.7% ($SEM = 1.02$) of sections collected, indicating that damage extended throughout the rostro-caudal extent of the RSC. In all rats, there was minor damage to some areas outside the RSC (e.g., visual cortex, motor cortex, cingulum bundle, forceps major corpus callosum). The average area of secondary visual cortex damage was 6.4%. Damage to the secondary visual cortex was exclusively within the medial portion; there was no damage to the lateral secondary visual cortex.

Behavior. The results from Experiment 1 are presented in Figure 2. Freezing during the baseline period and the postshock period were analyzed with a 2 (Lesion: sham vs. RSC) \times 2 (Period: baseline vs. postshock) ANOVA. The analysis revealed a main effect of period, $F(1, 14) = 248.17$, $p < .001$, indicating more freezing during the postshock period than the baseline period. Neither the main effect of lesion, $F(1, 14) < 1$, $p = .95$, nor the lesion by period interaction, $F(1, 14) < 1$, $p = .95$, were significant. During the context test session, RSC-lesioned rats froze significantly less than sham lesioned rats, $F(1, 14) = 28.53$, $p < .001$. Data from the light test session are presented in the right panel of Figure 2. Freezing to the light itself is presented in 5-trial blocks. (Note: The first 5-trial block is also plotted in bar form in the left panel.) Freezing during the light test was analyzed with a 2 (Lesion: sham vs. RSC) \times 5 (Period: baseline, Block 1-4) ANOVA. This analysis revealed a main effect of period, $F(4, 56) = 39.37$, $p < .001$. Neither the main effect of lesion, $F(1, 14) < 1$, $p = .58$, nor the lesion by period interaction, $F(4, 56) < 1$, $p = .80$, were significant.

The findings from the current experiment are consistent with previous studies examining the role of the RSC in learning and memory for discrete auditory cues. As in previous studies, lesions made shortly after conditioning impaired fear expression to the context, but not the discrete cue (Keene & Bucci, 2008a). Furthermore, while lesions made shortly after conditioning have no impact on freezing to a tone conditioned stimulus (e.g., Keene & Bucci, 2008a), the current experiment demonstrates that RSC lesions, likewise, do not impair freezing to a visual conditioned stimulus. Thus, although the RSC is necessary for the retrieval of contextual fear memory, it is not necessary for retrieval of recently acquired fear to a visual conditioned stimulus.

Experiment 2

Method

Subjects and surgery. The subjects were 20 experimentally naive adult male Long Evans rats, purchased from the same vendor as those in the previous experiment and maintained under the same conditions. Surgical procedures for sham and RSC lesions were the same as Experiment 1.

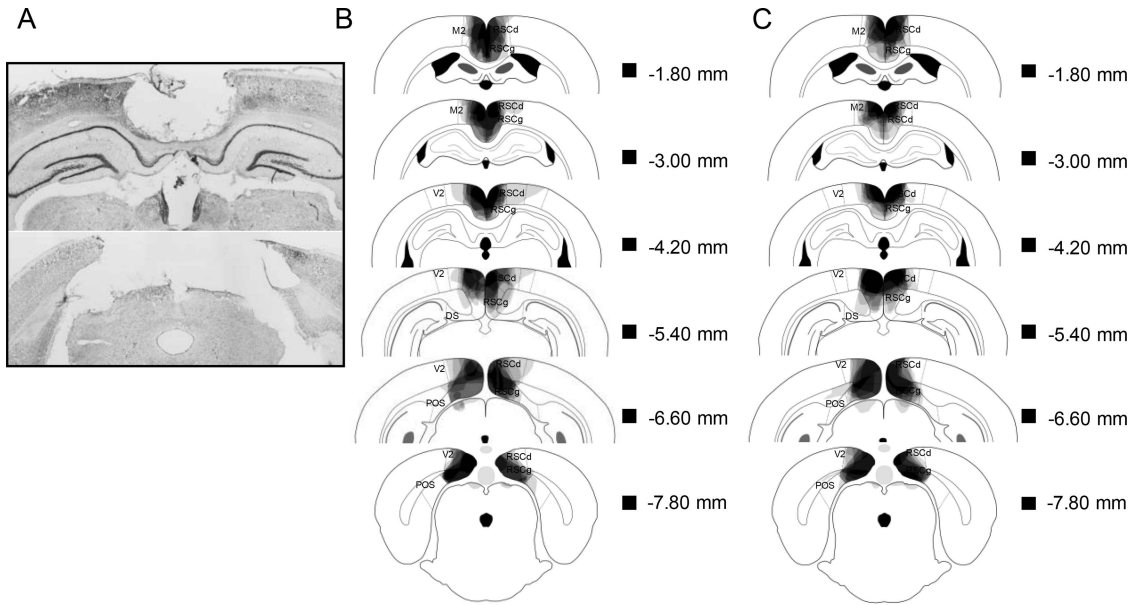


Figure 1. A: Photomicrograph of a representative RSC lesion. B: (Experiment 1) and C: (Experiment 2): drawings of lesions at six levels along the rostro-caudal extent of the RSC (-1.8 , -3.0 , -4.2 , -5.4 , -6.6 , and -7.8 mm posterior to bregma). At each level, lesion drawings were stacked onto a single image. The darkness of an area indicates the number of lesion cases that include that area. Gray boxes (next to the bregma values) represents the expected darkness for overlap from all subjects. DS = dorsal subiculum; POS = postsubiculum; M2 = secondary motor cortex; RSCd = retrosplenial dysgranular; RSCg = retrosplenial granular; V2 = secondary visual cortex.

Behavioral apparatus, procedures, and observations. The apparatus and procedures were the same as those used in Experiment 1, with the exception that all lesions occurred 28 days after behavioral training.

Lesion verification and analysis. RSC lesions were verified and analyzed using the same procedures as Experiment 1.

Results and Discussion

Histology. One rat was excluded from statistical analysis due to substantial extra-RSC damage. In addition, 1 sham and 1 RSC

lesioned rat died during surgery. The remaining groups sizes were sham ($n = 8$) and RSC ($n = 9$). In Figure 1c (Experiment 2), lesion drawings are stacked onto a single atlas image for each of the 6 coronal levels. Bilateral damage to the RSC was observed in all animals. The average area of RSC damage on each section analyzed was 65.2% ($SEM = 2.81$). Damage to the RSC was present on 87.9% ($SEM = 1.27$) of sections collected, indicating that damage extended throughout the rostro-caudal extent of the RSC and was very similar to Experiment 1. In all rats, there was minor damage outside the RSC (e.g., visual cortex, motor cortex, cingu-

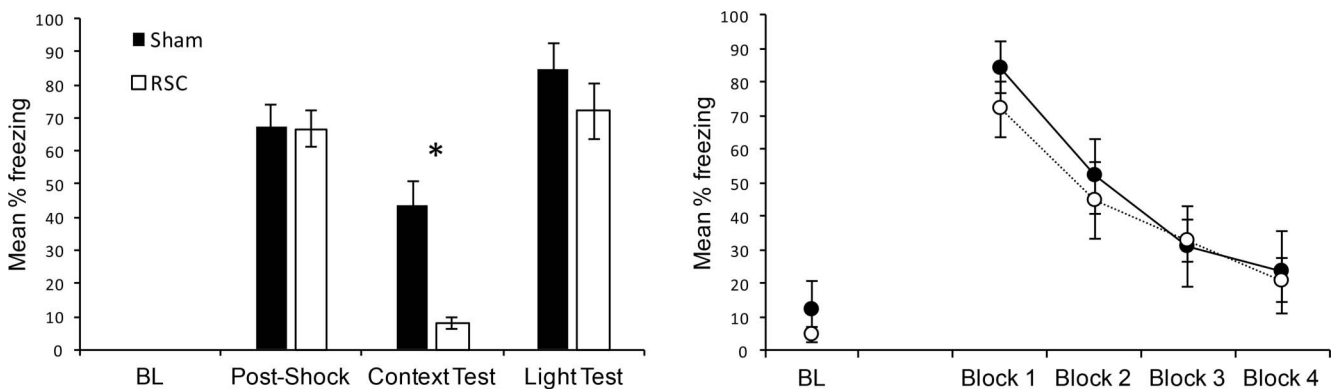


Figure 2. Left panel: results of Experiment 1. BL = freezing during the baseline period (prior to light-shock pairings) in the training session; Post-Shock = freezing during the three postshock periods of the training session; Context Test = freezing during the test session in Context A; Light Test = freezing during the first 5-trial block of the light test in Context B. Right panel: results from the Light Test session plotted in 5-trial blocks. BL: freezing during the baseline period just prior to the presentation of the first light stimulus. Sham = sham lesioned rats; RSC = retrosplenial lesioned rats. Error bars represent $\pm 1 SEM$. * $p < .05$.

lum bundle, forceps major corpus callosum). The average area of secondary visual cortex damage was 5.6%. Damage to the secondary visual cortex was exclusively within the medial portion; there was no damage to the lateral secondary visual cortex.

Behavior. The results from Experiment 2 are presented in Figure 3. Freezing during the baseline period and the postshock period were analyzed with a 2 (Lesion: sham vs. RSC) \times 2 (Period: baseline vs. postshock) ANOVA. The analysis revealed a main effect of period, $F(1, 15) = 511.90$, $p < .001$, indicating more freezing during the postshock period than the baseline period. Neither the main effect of lesion, $F(1, 15) < 1$, $p = .70$, nor the lesion by period interaction, $F(1, 15) < 1$, $p = .70$ were significant. During the context test session, RSC-lesioned rats froze significantly less than sham lesioned rats, $F(1, 15) = 39.13$, $p < .001$. Data from the light test session are presented in the right panel of Figure 3. Freezing to the light itself is presented in 5-trial blocks. (Note: The first 5-trial block is also plotted in bar form in the left panel.) Freezing during the light test was analyzed with a 2 (Lesion: sham vs. RSC) \times 5 (Period: baseline, Block 1 – 4) ANOVA. This analysis revealed a main effect of period, $F(4, 60) = 30.75$, $p < .001$. Although the main effect of lesion was not significant, $F(1, 15) = 2.16$, $p = .16$, the interaction between lesion and period approached significance, $F(4, 60) = 2.46$, $p = .055$. The effect of lesion was significant during Block 1, $F(1, 14) = 7.10$, $p = .018$, but not Block 2, Block 3, or Block 4 (all $ps > .24$). The level of freezing did not differ between groups during the baseline period of the light test $F(1, 14) = 2.51$, $p = .14$.

Lesions of the RSC impaired retrieval of remote contextual fear, consistent with previous findings (Corcoran et al., 2011; Todd, Mehlman, et al., 2016, Experiment 1). Furthermore, lesions made following a substantial retention interval (e.g., 28 days) also impaired retrieval of fear to a visual conditioned stimulus. This finding is in contrast to Experiment 1, where lesions made 1 day after conditioning had no impact on fear expression to the visual cue. Overall, these results are consistent with, and extend the results of, Todd, Mehlman, et al. (2016), demonstrating the RSC has a time-dependent role in memory retrieval for discrete cues.

Sacco and Sacchetti (2010) have previously reported that lesions of the secondary visual cortex impair remote memory for visual cues (Sacco & Sacchetti, 2010). Thus, it is notable that we observed incidental damage to the medial portion of the secondary visual cortex in all rats. We note, however, that the incidental damage to the medial secondary visual cortex was very minor ($\sim 6\%$). In addition, we note that Sacco and Sacchetti (2010) lesioned the *lateral* secondary visual cortex. There was no damage to this region in the current experiment. Moreover, there was no significant correlation between the amount of damage to the secondary visual cortex and freezing to the light ($p = .36$).

General Discussion

Our previous studies have demonstrated a role for the RSC in the retrieval of remotely, but not recently, acquired fear memories in a Pavlovian delay fear conditioning procedure with an auditory CS (Keene & Bucci, 2008a; Todd, Mehlman, et al., 2016). The purpose of the current experiment was to assess if the time-dependent involvement of the RSC in cue-specific fear memory extended to cues of other sensory modalities, or if it was unique to auditory stimuli. Lesions of the RSC made 1 day after training had no detectable impact on freezing to a visual cue (Experiment 1); however, lesions made 28 days after training did attenuate fear elicited by the visual cue (Experiment 2). These data provide evidence that the RSC is necessary specifically for the retrieval of *remotely* acquired fear memories to visual cues. In both experiments, freezing to the context was impaired, consistent with prior studies demonstrating a time-independent role for RSC in contextual fear memory (e.g., Corcoran et al., 2011; Todd, Mehlman, et al., 2016, Experiment 1).

The present findings are consistent with the notion that the neurocircuitry of remote cued fear memory differs from that of recent cued fear memory (for a review see Bergstrom, 2016). In contrast with recently acquired cued fear memories, remotely acquired cued fear memories depend upon secondary sensory cortices for retrieval (Sacco & Sacchetti, 2010). For example, posttraining lesions of the auditory cortex impair the retrieval of

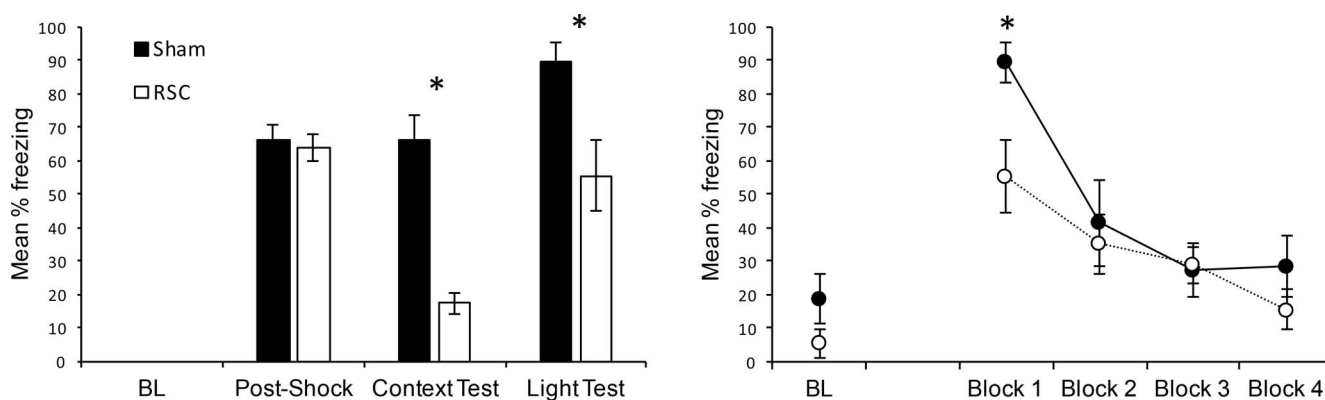


Figure 3. Left panel: results of Experiment 2. BL = freezing during the baseline period (prior to light-shock pairings) in the training session; Post-Shock = freezing during the three postshock periods of the training session; Context Test = freezing during the test session in Context A; Light Test = freezing during the first 5-trial block of the light test in Context B. Right panel: results from the Light Test session plotted in 5-trial blocks. BL = freezing during the baseline period just prior to the presentation of the first light stimulus. Sham = sham lesioned rats; RSC = retrosplenial lesioned rats. Error bars represent ± 1 SEM. * $p < .05$.

remote, but not recent, memory for auditory cues. Likewise, lesions of the piriform cortex impair remote memory retrieval for olfactory cues, and lesions of visual cortex impair remote memory retrieval for visual cues (Sacco & Sacchetti, 2010). In most cases, the observed retrieval deficits were sensory-modality specific, leading Sacco and Sacchetti (2010) to conclude that each secondary sensory cortex is involved in remote memory storage and retrieval for a *specific* sensory modality. The role of the RSC, however, appears to be more *general*. Along with the findings from Todd, Mehlman, et al. (2016), the current findings indicate the RSC is necessary for the retrieval of both visual and auditory remotely acquired cued fear memories. Thus, the RSC is necessary for retrieval of remotely acquired cued fear memories across multiple modalities.

The fact that the RSC has direct connections with both auditory cortex and visual cortex makes it well-suited to contribute to memory retrieval for cues from both modalities. It is, however, interesting to note that not all polymodal cortical areas are necessary for retrieval of remotely acquired cued fear memories. For example, although the perirhinal cortex receives both visual and auditory information (e.g., Furtak, Wei, Agster, & Burwell, 2007; Kealy & Commins, 2011), posttraining lesions of the posterior portions of the perirhinal cortex do not impact retrieval of remotely acquired fear memories (Sacco & Sacchetti, 2010). This suggests a functional dissociation between the role of the RSC and perirhinal cortex in the retrieval of remotely acquired cued fear memories. However, since the precise role of the RSC in remote memory retrieval is still unclear (see Todd, Mehlman, et al., 2016, for a discussion), future research is necessary to determine not only the exact role of the RSC, but how RSC function differs from regions such as the perirhinal cortex.

Although we observed a time-dependent effect on the retrieval of memory for a visual cue, lesions of the RSC attenuated freezing to the context at both time points. This finding is consistent with prior research demonstrating that the RSC is both active (Tyler et al., 2013) and necessary (Corcoran et al., 2011) for the retrieval of contextual fear memories at both recent and remote time points. Nevertheless, it suggests a fundamental dissociation between contextual fear memories and cue specific fear memories. On the one hand, contextual fear memories are RSC dependent at both recent and remote time points, whereas on the other hand cued fear memories are only RSC dependent at remote time points.

In the current experiments, RSC damage was produced via electrolytic lesions made either 1 or 28 days following initial Pavlovian conditioning. Although electrolytic lesions damage both cell bodies and fibers of passage, it seems unlikely that the results observed are simply due to damage of fiber pathways, since our prior studies have demonstrated that either neurotoxic or electrolytic lesions of the RSC, or temporary silencing of RSC activity, produce specific deficits in remote memory for auditory cues (Todd, Mehlman, et al., 2016).

In summary, the present findings add to a growing literature aimed at identifying the precise circumstances under which RSC is necessary for cue-specific learning and memory. For instance, the involvement of RSC in remote but not recent fear memory for visual or auditory CSs suggests a temporal dimension to its role in cue-specific memory, but it is unclear whether RSC involvement is dichotomous (i.e., recent vs. remote) or varies along a gradient. While additional studies are necessary to fully define the func-

tional contributions of RSC to learning and memory, one possibility is that the RSC serves to integrate information from multiple modalities in the service of long-term memory.

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Received August 31, 2017

Revision received November 6, 2017

Accepted December 13, 2017 ■