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Contribution of the retrosplenial cortex to temporal discrimination learning

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Abstract

The retrosplenial cortex (RSC) has an important role in contextual learning and memory. While the majority of experiments have focused on the physical context, the present study asked if the RSC is involved in processing the temporal context. Rats were trained in a temporal discrimination procedure where the duration of the intertrial interval (ITI) signaled whether or not the next tone conditioned stimulus would be paired with food pellet reinforcement. When the tone was presented after a 16-min ITI it was reinforced, but when it was presented after a 4-min ITI it was not. Rats demonstrated successful discrimination in this procedure by responding more to the tone on reinforced trials than on nonreinforced trials. Pre-training electrolytic lesions of the RSC attenuated acquisition of the temporal discrimination. The results are the first to demonstrate a role for the RSC in processing temporal information and in turn extend the role of the RSC beyond the physical context to now include the temporal context.

Keywords

retrosplenial; temporal context; episodic memory

The retrosplenial cortex (RSC) is situated at the interface between primary cortical sensory areas and parahippocampal and hippocampal regions (Sugar, Witter, van Strien, & Cappaert, 2011; van Strein, Cappaert, & Witter, 2009). Specifically, RSC receives visuo-spatial input and has strong reciprocal connections with the postrhinal cortex, postsubiculum, medial entorhinal cortex, as well as direct connections with the hippocampus. Thus, RSC is well positioned to influence learning and memory processes that are mediated by medial temporal lobe structures.

Behaviorally, RSC has an important role in contextual learning and memory (for a review see Bucci & Robinson, 2014). Permanent lesions of RSC disrupt contextual fear conditioning regardless of whether those lesions are made prior to, or after, fear conditioning (e.g., Keene & Bucci, 2008). Furthermore, temporary blockade of NMDA receptors in RSC impairs retrieval of contextual fear memories (Corcoran et al., 2011). In these experiments, “context” typically refers to the background stimuli provided by the physical apparatus (e.g.,

operant chamber). Thus, these findings indicate that RSC is involved in processing the *physical* context.

Both external and internal cues can act as contexts (Bouton, 2002; 2010). Just as physical features of the environment (external) can provide a source of contextual information, so too can the passage of time (internal). In the present study, we tested the hypothesis that the role of RSC in processing contexts extends beyond physical stimuli. We used a temporal discrimination learning paradigm to determine the involvement of the RSC in processing temporal context (Bouton & García-Gutiérrez, 2006; Bouton & Hendrix, 2011; Todd, Winterbauer & Bouton, 2010). While there is evidence that the hippocampus is sometimes involved in processing temporal information (e.g., Iordanova et al., 2009; Campese & Delamater, 2014; cf. Kyd et al., 2008) to our knowledge this has never been studied in the RSC or other parahippocampal regions.

Seventeen adult male Long Evans rats, approximately 60 days old, were obtained from Harlan Laboratories (Indianapolis, IN). Rats were housed individually and allowed at least 6 days to acclimate to the vivarium prior to surgery with food available ad libitum (Purina standard rat chow; Nestle Purina, St. Louis, MO). All surgeries took place over the course of a four-day period that immediately followed the acclimation period. Eight rats received bilateral electrolytic lesions (2.5 mA, 15 sec at each site) of the RSC (see Table 1 for coordinates) prior to the behavioral training using surgical procedures previously described (e.g., Robinson et al., 2011). Electrolytic lesions were chosen to provide a high degree of control over the extent of damage (Ross & Eichenbaum, 2006), which was an important factor in this study given the close proximity of RSC to related cortico-hippocampal regions (e.g., posterior parietal cortex, postsubiculum; Burwell & Amaral, 1998). Control rats ($n = 8$) received sham lesions consisting of a craniotomy and shallow, non-puncturing burr holes to minimize damage to underlying cortex. Rats were allowed to recover for at least 2 weeks before beginning behavioral training. During that period they were allowed to return to pre-surgical weight before being subsequently food-restricted to 85% of their baseline weight. One sham lesioned rat developed a head-tilt after surgery and was removed from the experiment. This rat was replaced with an un-operated control rat from the same shipment.

Following recovery, rats were trained in a temporal discrimination procedure (Bouton & Hendrix, 2011). The behavioral procedures were carried out in standard conditioning chambers (Med Associates) previously described (Robinson et al., 2011). The chambers were illuminated by a house light that was mounted 15 cm above the grid floor on the back wall of the chamber. A speaker was located 15 cm above and to the right of the food cup (recessed in the center of the front wall) and was used to present the auditory stimulus (1500 Hz, 78 dB). A pair of infrared photocells was mounted just inside the food cup to detect head entries into the cup. The unconditioned stimulus was the presentation of two 45-mg grain-based rodent food pellets (Bio-Serv).

On the first day of the experiment, all rats received a single 30-min session of magazine training during which food pellets were delivered freely on a random time 30 s (RT 30s) schedule resulting in 60 pellets being delivered on average. For the next 20 consecutive days, rats received a single daily session (~90 min) in which the tone stimulus was presented

8 times. For all rats, the tone co-terminated with delivery of the US when it followed a 16-minute intertrial interval (ITI), but not when it followed the 4-minute ITI. Both groups received 4 reinforced (R) and 4 nonreinforced (N) trials every session. Rats demonstrate successful discrimination in this procedure by responding more to the tone after a 16-minute ITI relative to a 4-minute ITI. There were two, double alternating sequences of trials. On odd days the trials were RRNNRRNN and on even days the trials were NNRRNNRR (e.g., Bouton & Hendrix, 2011).

The computer recorded the amount of time spent in the food cup during three 10-s periods: the 10-s period prior to CS presentation of each trial (pre-CS), the 10-s period during CS presentation, and the 10-s period immediately following CS presentation (post-CS).

After the behavioral procedures were completed, lesions were verified and analyzed using methods previously described (e.g., Keene & Bucci, 2008; Robinson et al., 2011). The results are presented in Figure 1. Bilateral damage of the RSC was observed in all rats and the average area of RSC damaged on each of the 5 sections analyzed from each rat was $68 \pm 14\%$. A representative lesion is illustrated in Figure 1a. Damage to the RSC was present on $98 \pm 4\%$ of the sections collected from each subject, indicating that damage extended throughout the rostro-caudal extent of RSC (Figure 1b). Minor damage to cortical regions outside of RSC (anterior cingulate, secondary motor cortex, secondary visual cortex and forceps major corpus callosum) was observed in all of the rats. However, extra-RSC damage was present on only $39 \pm 14\%$ of the sections analyzed. In addition, 5 of the 8 rats exhibited minor damage to the cingulum bundle.

Acquisition of the temporal discrimination is presented in Figure 2. The time spent in the food cup during the CS on R (16-min ITI) and N (4-min ITI) trials is presented for both control (upper panel) and RSC-lesioned (lower panel) rats. Overall, RSC-lesioned rats were impaired in this form of temporal discrimination learning. At the completion of the first half of training (10 sessions), control rats demonstrated a clear discrimination, responding more to the tone on R trials relative to the N trials. In contrast, RSC-lesioned rats responded non-differentially to both trial types. However, with continued training, the RSC-lesioned rats were indeed capable of discriminating trial types. To confirm these impressions, each 10-session block was analyzed with a 2 (Group: Lesion vs. Control) $\times$ 2 (Trial Type: R vs. N) $\times$ 10 (Session) ANOVA. For the first half of training (sessions 1–10), this analysis revealed a main effect of session, $F(9, 126) = 5.161, p < .01$, and trial type, $F(1, 14) = 8.82, p < .05$, as well as an interaction between session and trial type, $F(9, 126) = 6.64, p < .01$. Importantly, there was a significant interaction between group and trial type, $F(1, 14) = 4.55, p = .05$. The three-way interaction between group, trial type, and session was not significant, $F(9, 126) = 1.7, p = .1$. Simple effect analyses revealed that the effect of trial type was significant for control rats, $F(1, 14) = 13.02, p < .01$, but not RSC-lesioned rats, $F < 1$. Thus, after the first 10 sessions control rats had acquired the temporal discrimination but RSC-lesioned rats had not. More so, the effect of group was not significant within either trial type, largest $F(1, 14) = 1.38, p = .26$, suggesting that the overall level of responding between groups was not different during this period.

Over the second half of training, both groups correctly discriminated trial types. For sessions 11–20 there was a main effect of trial type, $F(1, 14) = 25.86, p < .01$, and an interaction between trial type and session, $F(9, 126) = 3.82, p < .01$. The lack of a significant main effect of group or interaction between group and other factors indicates that RSC-lesioned and control rats were not significantly different during the second half of training.

There were no substantial differences in pre-CS (i.e., baseline) behavior. Separate ANOVAs on session 1–10 and 11–20 did not reveal any significant main effects or interactions. Averaged over sessions 1–10, control rats spent $0.41 \pm .10$ (SEM) and $0.35 \pm .08$ s in the food cup R and N trials, respectively, and RSC-lesioned rats spent $0.27 \pm .07$ and $0.40 \pm .09$ s in the food cup for R and N trials respectively. Averaged over sessions 11–20, control rats spent $0.43 \pm .11$ and $0.35 \pm .10$ s in the food cup R and N trials, respectively, and RSC-lesioned rats spent $0.23 \pm .15$ and $0.16 \pm .09$ s in the food cup for R and N trials respectively.

As training progressed, both groups responded more in the post-CS period after R trials (due to the presence of food) than N trials. However, there were no systematic group (Lesion vs. Control) differences during the first or second half of training. For sessions 1–10, there was a main effect of session, $F(9, 126) = 8.02, p < .01$, trial type, $F(1, 14) = 147.39, p < .01$ and an interaction between session and trial type, $F(9, 126) = 4.55, p < .01$. For sessions 11–20 there was a main effect of trial type, $F(1, 14) = 76.01, p < .01$. Averaged over sessions 1–10, control rats spent $5.13 \pm .62$ (SEM) and $1.63 \pm .45$ s in the food cup R and N trials, respectively, and RSC-lesioned rats spent $4.36 \pm .43$ and $1.34 \pm .45$ s in the food cup for R and N trials respectively. Averaged over sessions 11–20, control rats spent $6.11 \pm .58$ and $1.66 \pm .34$ s in the food cup R and N trials, respectively, and RSC-lesioned rats spent $5.53 \pm .72$ and $1.78 \pm .51$ s in the food cup for R and N trials respectively.

In summary, permanent lesions of RSC attenuated, but did not completely abolish, temporal discrimination learning. Importantly, the deficit produced by RSC lesions was specific to discriminating between trial types (i.e., temporal intervals); lesions did not produce a general inability to respond. For example, there were no group differences (Lesion vs. Control) in baseline behavior (pre-CS) or food related consumption behavior (post-CS). Furthermore, although in the first half of training RSC lesioned rats did not discriminate between trial types, the *overall* level of responding did not differ between lesions and controls. Taken together, these findings suggest that damage to RSC produces a specific deficit in the ability to differentiate between temporal intervals.

It is already known that the RSC is involved in processing *physical* contexts (e.g., Keene & Bucci, 2008; Corcoran et al., 2011). However, interoceptive cues, such as the passage of time can also act as contexts (e.g., Bouton, 2010). The fact that lesions of RSC disrupt temporal discrimination learning suggests a potential role for RSC in processing the temporal context, in addition to the physical context. This is consistent with the notion that RSC is part of the “where/when” pathway (Bucci & Robinson, 2014). Until now, the RSC’s role in temporal processing has never before been demonstrated.

It is also relevant to note the implications of these findings for a role of RSC in episodic memory. Episodic memory is thought to include “what”, “where”, and “when” information and is mediated by medial temporal lobe structures (e.g., Eichenbaum, 2000, Tulving & Markowitsch, 1998). Studies of temporal sequence learning have demonstrated an important role for the hippocampus in “when” processing (e.g., Fortin et al., 2002; Lehn et al., 2009). However, the present findings extend beyond the hippocampus, and demonstrate a role for the RSC as well. It is possible that the RSC contributes “when” information to episodic memories.

It remains to be determined the full extent of RSC’s role in temporal processing. In the current study, rats were required to discriminate between two intervals: 16 and 4 m. Future experiments might investigate if RSC is involved in processing temporal information that is shorter and/or longer in duration than the present intervals.

Nevertheless, the present data are the first to demonstrate a role for RSC in temporal processing and thus extend the role of the RSC beyond processing the physical context to now include the temporal context.

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Figure 1.

(a) Photomicrograph of a representative RSC lesion at 3.0 mm posterior to bregma. The dotted line indicates the boundaries of RSC. (b) Schematic diagram indicating the rostro-caudal extent and the largest (gray) and smallest (black) lesions of RSC. Abbreviations: RSC, retrosplenial cortex; M2, secondary motor cortex; V2, secondary visual cortex; fmj, forceps major corpus callosum. Figure 1b is adapted from *The Rat Brain in Stereotaxic Coordinates* (6th edition), G. Paxinos and C. Watson, 2007. Copyright 2007, with permission from Elsevier.

Figure 2.

Responding during the tone over 20 sessions for controls (upper panel) and RSC lesions (lower panel). R = reinforced (following 16 m ITI), N = nonreinforced (following 4 m ITI). Dashed vertical line indicates first vs. second half of training. Data are mean $\pm$ standard error.

Table 1

Stereotaxic coordinates for retrosplenial cortex (RSC) lesions.

AP	ML	DV
-2.0	± 0.3	-2.0 and -2.7
-3.5	± 0.4	-2.0 and -2.7
-5.0	± 0.4 and ± 0.1	-2.0 and -2.7 (medial site) and -2.0 (lateral site)
-6.5	± 0.8 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).