Contribution of the Retrosplenial Cortex to Temporal Discrimination Learning

Travis P. Todd,* Heidi C. Meyer, and David J. Bucci

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 whether 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 non-reinforced 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.

KEY WORDS: retrosplenial; temporal context; episodic memory

The retrosplenial cortex (RSC) is situated at the interface between primary cortical sensory areas and parahippocampal and hippocampal regions (van Strein et al., 2009; Sugar et al., 2011). Specifically, the 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, the RSC is well positioned to influence learning and memory processes that are mediated by medial temporal lobe structures.

Behaviorally, the RSC has an important role in contextual learning and memory (for a review see Bucci and Robinson, in press). Permanent lesions of the RSC disrupt contextual fear conditioning regardless of whether those lesions are made prior to, or after, fear conditioning (e.g., Keene and Bucci, 2008). Furthermore, temporary blockade of NMDA receptors in the 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 the 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 the 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 and García-Gutiérrez, 2006; Todd et al., 2010; Bouton and Hendrix, 2011). While there is evidence that the hippocampus is sometimes involved in processing temporal information (e.g., Iordanova et al., 2009; Campese and 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, ~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 and Eichenbaum, 2006), which was an important factor in this study given the close proximity of the RSC to related cortico-hippocampal regions (e.g., posterior parietal cortex, postsubiculum; Burwell and 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...
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).

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 and 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 (1,500 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 sec (RT 30s) schedule resulting in 60 pellets being delivered on average. For the next 20 consecutive days, rats received a single daily session (~85 min) in which the tone conditioned stimulus (CS) was presented eight times. For all rats, the tone co-terminated with delivery of the unconditioned stimulus (US) when it followed a 16-min intertrial interval (ITI), but not when it followed the 4-min ITI. Both groups received four reinforced (R) and four non-reinforced (N) trials every session. Rats demonstrate successful discrimination in this procedure by responding more to the tone after a 16-min ITI relative to a 4-min ITI. There were two, double alternating sequences of trials. On odd days the trials were RNRNRRNN and on even days the trials were NRRNNRRN (e.g., Bouton and Hendrix, 2011).

The computer recorded the amount of time spent in the food cup during three 10-sec periods: the 10-sec period prior to CS presentation of each trial (pre-CS), the 10-sec period during CS presentation, and the 10-sec 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 and 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 the RSC damaged on each of the five sections analyzed from each rat was 68 ± 14%. A representative lesion is illustrated in Figure 1a. Damage to the RSC was present on 98 ± 4% of the sections collected from each subject, indicating that damage extended throughout the rostro-caudal extent of the RSC (Fig. 1b). Minor damage to cortical regions outside of the 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 ± 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 the 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: RSC vs. Control) × 2 (Trial Type: R vs. N) × 10 (Session) ANOVA. For the first half of training (sessions 1–10), this analysis revealed a main effect of session, \( F(9, 126) = 5.161, P < 0.01 \), and trial type, \( F(1, 14) = 8.82, P < 0.05 \), as well as an interaction between session and trial type, \( F(9, 126) = 6.64, P < 0.01 \). Importantly, there was a significant interaction between group and trial type, \( F(1, 14) = 4.55, P = 0.05 \). Neither the main effect of group, nor any other interactions were significant, largest \( F(9, 126) = 1.7, P = 0.1 \). Simple effect analyses revealed that the effect of trial type was significant for control rats, \( F(1, 14) = 13.02, P < 0.01 \), 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 \( F(1, 14) = 1.38, P = 0.26 \), 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, \( F(1, 14) = 25.86, P < 0.01 \), and an interaction between trial type and session, \( F(9, 126) = 3.82, P < 0.01 \). No other main effects or interactions were significant, largest \( F(9, 126) = 1.42, P = .2 \). 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 largest \( F(1, 14) = 1.9, P = .2 \). Averaged over sessions 1–10, control rats spent 0.41 ± 0.10 (SEM) and 0.35 ± 0.08 sec in the food

### TABLE 1. Stereotaxic Coordinates for Restrosplenial Cortex (RSC) Lesions

<table>
<thead>
<tr>
<th>AP</th>
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<tr>
<td>−2.0</td>
<td>± 0.3</td>
<td>−2.0 and −2.7</td>
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<td>−3.5</td>
<td>± 0.4</td>
<td>−2.0 and −2.7</td>
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<tr>
<td>−5.0</td>
<td>± 0.4 and ± 0.1</td>
<td>−2.0 and −2.7 (medial site) and −2.0 (lateral site)</td>
</tr>
<tr>
<td>−6.5</td>
<td>± 0.8 and ± 1.5</td>
<td>−2.0 and −2.8 (medial site) and −3.4 (lateral site)</td>
</tr>
<tr>
<td>−8.0</td>
<td>± 1.6 and ± 2.4</td>
<td>−2.5 (medial site) and −3.1 (lateral site)</td>
</tr>
<tr>
<td>−9.0</td>
<td>± 3.4</td>
<td>−4.0</td>
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Hippocampus
cup R and N trials, respectively, and RSC-lesioned rats spent 0.27 ± 0.07 and 0.40 ± 0.09 sec in the food cup for R and N trials respectively. Averaged over sessions 11–20, control rats spent 0.43 ± 0.11 and 0.35 ± 0.10 sec in the food cup R and N trials, respectively, and RSC-lesioned rats spent 0.23 ± 0.15 and 0.16 ± 0.09 sec 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 (RSC vs. Control) differences during the first or second half of training. For sessions 1–10, there was a main effect of session, $F(9, 126) = 8.02, P < 0.01$, trial type, $F(1, 14) = 147.39, P < 0.01$ and an interaction between session and trial type, $F(9,$

FIGURE 1. (a) Photomicrograph of a representative RSC lesion at 3.0 mm posterior to bregma. The dotted line indicates the boundaries of the RSC. (b) Schematic diagram indicating the rostro-caudal extent and the largest (gray) and smallest (black) lesions of the RSC. Abbreviations: RSC, retrosplenial cortex; M2, secondary motor cortex; V2, secondary visual cortex; fmj, forceps major corpus callosum. Figure 1(b) is adapted from *The Rat Brain in Stereotaxic Coordinates* (6th edition), G. Paxinos and C. Watson, 2007. Copyright 2007, with permission from Elsevier.
For sessions 11–20 there was a main effect of trial type, \(F(1, 14) = 576.01, P < 0.01\). No other main effects or interactions were significant during either half of training, largest \(F(9, 126) = 5.167, p = .10\). Averaged over sessions 1–10, control rats spent 5.13 ± 0.62 (SEM) and 1.63 ± 0.45 sec in the food cup R and N trials, respectively, and RSC-lesioned rats spent 4.36 ± 0.43 and 1.34 ± 0.45 sec in the food cup for R and N trials respectively. Averaged over sessions 11–20, control rats spent 6.11 ± 0.58 and 1.66 ± 0.34 sec in the food cup R and N trials, respectively, and RSC-lesioned rats spent 5.53 ± 0.72 and 1.78 ± 0.51 sec in the food cup for R and N trials respectively.

In summary, permanent lesions of the RSC attenuated, but did not completely abolish, temporal discrimination learning. Importantly, the deficit produced by the 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 (RSC vs. Control) in baseline behavior (pre-CS) or food related consumption behavior (post-CS). Furthermore, although in the first half of training the 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 the 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 and 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 the RSC in processing the temporal context, in addition to the physical context. This is consistent with the notion that the RSC is part of the “where/when” pathway (Bucci and Robinson, in press). Until now, the RSC’s role in temporal processing has never been demonstrated.

It is also relevant to note the implications of these findings for a role of the 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, 2001; Tulving and 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 the RSC’s role in temporal processing. In the current study, rats were required to discriminate between two intervals: 16 and 4 min. Future experiments might investigate whether the 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 the 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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