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Review Article

The Retrosplenial Cortical Role in Encoding Behaviorally Significant Cues

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Abstract

The retrosplenial cortex (RSC) has recently begun to gain widespread interest due to its anatomical connectivity with other well-known memory structures such as the hippocampus and anterior thalamus and its role in spatial, contextual and episodic memory. Although much of the current work on the RSC is focused on spatial cognition, there is also an extensive literature which shows that the RSC plays a critical role in a variety of conditioning tasks that have no obvious spatial component. Many of these studies suggest that the RSC is involved in identifying and encoding behaviorally significant cues, particularly those cues that predict reinforcement or the need for a behavioral response. Consistent with this idea, recent studies have shown that RSC neurons also encode cues in spatial navigation tasks. In this article, we review these findings and suggest that the encoding of cues is an important component of the RSC contribution to many forms of learning.

Key Words: retrosplenial, hippocampus, learning, context, navigation, memory

The role of the retrosplenial cortex (RSC) has been a subject of study since the 1970s but it has only recently begun to gain widespread interest from the neurobiology of learning and memory community. Much of the current interest arises from the observation that the RSC shares functional similarities and anatomical interconnections with the more well-studied hippocampus. The RSC is reciprocally interconnected with the hippocampal system via the subicular complex, anterior thalamus and entorhinal cortex (for reviews see Aggleton et al., 2010; Bucci & Robinson, 2014), and RSC lesions impair spatial navigation (Harker & Whishaw, 2002; Keene & Bucci, 2009; Sutherland, Whishaw, & Kolb, 1988; Vann & Aggleton, 2002), contextual memory (Keene & Bucci, 2008b; Kwapis, Jarome, Lee, & Helmstetter, 2015; Robinson, Poorman, Marder, & Bucci, 2012), and episodic memory (Bowers, Verfaellie, Valenstein, & Heilman, 1988; Valenstein et al., 1987). There is also a growing body of rodent neurophysiology and human fMRI data on the RSC role in spatial navigation (Alexander & Nitz, 2015, 2017; Cho & Sharp, 2001; Maguire, 2001; Miller, Vedder, Law, & Smith, 2014), and the RSC plays a central role in the default mode network, which supports constructive memory processes (Hassabis, Kumaran, Vann, & Maguire, 2007; Schacter & Addis, 2007). Given current interest in the RSC role in navigation and memory, including current work in our laboratory, this literature will surely continue to grow. However, there is also an extensive but largely separate body of data indicating that the RSC plays a critical role in processing behaviorally significant cues, even in tasks that have no obvious spatial component. We believe these findings will be critical for understanding the underlying contribution of the RSC to a wide variety of learning and memory situations, including spatial and contextual memory. In this article, we

review these findings and discuss new data on how this cue processing function might fit into a broader account of RSC functions.

Findings from Discriminative Avoidance and Approach Model Systems. Perhaps the most extensive and systematic research on the role of the RSC was done by Michael Gabriel and colleagues using an instrumental discriminative avoidance model system (for review see Gabriel, 1993). In this task, subjects (rabbits) were placed in a large activity wheel and two different pure tone auditory stimuli (0.5 sec) were delivered: one tone, the CS+, was followed 5 sec later by a footshock which could be avoided by taking a step in the wheel, while the other tone, the CS-, predicted no reinforcement. Using this model system, and a formally similar water-rewarded discriminative approach task, Gabriel and colleagues performed numerous lesion and neuronal recording studies to identify the neural circuitry supporting this form of instrumental learning, which includes the anterior cingulate cortex, RSC and their interconnected thalamic nuclei the mediodorsal thalamus and anterior nuclear group respectively. Unfortunately, much of this literature does not appear in search results because the phrase 'posterior cingulate cortex' was used to describe the target area (Brodmann's areas 29b, c and d) rather than 'retrosplenial cortex' which is more commonly used in the rodent literature.

These studies typically involved recording tone-evoked multi-unit activity in control and lesion subjects throughout learning, including recordings during the naïve state prior to learning, during each of the daily training sessions, and during asymptotic performance after the task has been well-learned. With learning, multi-unit activity in these regions begins to discriminate between the cues, with greater firing in response to the CS+ than the CS- (Fig. 1). These studies identified a highly plastic, rapid learning

system that encoded the predictive value of the auditory tone cues during the earliest stages of learning, including the medial geniculate nucleus, the basolateral amygdala, medial dorsal thalamus and the anterior cingulate cortex (Poremba & Gabriel, 1997a, 1997b). Multi-unit activity in these regions began to preferentially encode the CS+ immediately at the outset of training, even before subjects exhibited significant behavioral evidence of learning. In contrast, preferential encoding of the CS+ generally developed more slowly in the RSC and the interconnected anterior thalamic nuclei and only appeared after many training trials (Gabriel & Orona, 1982). The temporal characteristics of this plasticity led Gabriel to characterize these as rapid and slow learning systems, centered on the anterior cingulate cortex and the RSC respectively. The idea is that rapid plasticity in the anterior system could promote highly flexible fast learning, particularly in emergency conditions such as aversive learning, while the slower plasticity of the RSC system was specialized for encoding the reliable regularities of the environment which only become apparent through repeated experiences. The effects of lesion studies was consistent with this idea: damage to components of the rapid learning system impaired performance during the initial stages of learning but allowed subjects to eventually reach normal levels of asymptotic performance (Gabriel, Kubota, Sparenborg, Straube, & Vogt, 1991; Gabriel, Sparenborg, & Kubota, 1989). In contrast, lesions of the slow learning system had no effect on the initial acquisition of the avoidance response: subjects learned normally but performance dropped off after the subjects reached asymptote (Gabriel, Sparenborg, & Stolar, 1987). Interestingly, the RSC is probably not the final repository for the associative memory since lesions of the anterior thalamic component of this circuit

made after extensive overtraining do not impair behavior (Hart, Poremba, & Gabriel, 1997).

Gabriel used the term 'significance coding' to describe the fact that neurons within this region were highly sensitive to the behavioral relevance of the cues. That is, the neural responses did not seem to encode the hedonic value of the cues, nor did they encode the reinforcing stimulus or the specific behavioral response that was required. Instead, the neurons seemed to be sensitive the cues as predictors of reinforcement and the need for a behavioral response. For example, subjects could be trained to perform appetitive and aversive versions of the task on alternating days. In that situation, RSC neurons preferentially responded to a cue that predicted an impending foot shock and the need for a locomotor response during the avoidance task and on alternating days they also preferentially responded to a cue that predicted water reward for licking a drinking spout (Fig 1B, Freeman, Cuppernell, Flannery, & Gabriel, 1996). RSC neurons also responded to other task events that had predictive value, such as the insertion of a drinking spout that signaled the opportunity to respond in the appetitive task (Smith, Freeman, Nicholson, & Gabriel, 2002).

Another component of the significance coding idea is that neural responses should amplify sensory cues that signal important outcomes, such as pain or danger. Consistent with this, RSC firing was sensitive to changes in the salience and probability of the tone cues. After training with a standard 500 millisecond tone cue, a change to either a shorter (200 msec) or longer (5000 msec) tone induced a heightened response in the RSC (Sparenborg & Gabriel, 1990), an effect that was particularly prominent in the anterior cingulate cortex. Heightened responses also occurred during test sessions

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when the predictive tone was infrequent (20% of trials compared to 50% of trials during training, Stolar, Sparenborg, Donchin, & Gabriel, 1989). Thus, manipulations that altered the salience or probability of these cues evoked a heightened neural response. Overall, these findings clearly point to an RSC role in encoding cues on the basis of their predictive value. Less is known about the RSC role in behavioral response output. However, RSC neuronal firing has been shown to ramp up to the time when subjects initiate an avoidance or approach response, which may function as a 'go' signal sent to the motor systems of the brain (Kubota, Wolske, Poremba, Kang, & Gabriel, 1996; Smith et al., 2002).

Findings from Other Learning Tasks. In the time since the Gabriel research program, an extensive body of work has accumulated on the RSC role in a variety of conditioning tasks. An exhaustive review of this work is beyond the scope of this article, but many of the findings have reinforced the idea that the RSC is critical for processing behaviorally significant cues. Overall, the data suggest that the RSC is not needed for simpler forms of learning, but it becomes engaged when the task becomes more complex and cue-outcome relationships become more difficult for subjects to discern. For example, the RSC and related thalamic structures do not appear to be necessary for Pavlovian eyeblink or fear conditioning when the CS and US overlap in time (i.e. delay conditioning, Gabriel et al., 1996; Kwapis et al., 2015), but the RSC is needed for the acquisition and extinction fear conditioning with a trace interval between the CS and US (i.e. trace conditioning, Kwapis et al., 2015). Trace conditioning is thought to rely on explicit memory whereas delay conditioning does not (Weike, Schupp, & Hamm, 2007), so this finding may suggest an RSC role in explicit memory for cue-outcome

associations (Kwapis, Jarome, Lee, Gilmartin, & Helmstetter, 2014). However, one study has failed to find an RSC role in trace eyeblink conditioning (Weible, McEchron, & Disterhoft, 2000) and another has shown that the RSC is needed for the retrieval of a remote delay-conditioned fear memory (Todd, Mehlman, Keene, DeAngeli, & Bucci, 2016).

In trace-conditioning tasks, the trace interval creates added difficulty in discerning cue-outcome relationships. The RSC can also be engaged by tasks where cue-outcome relationships are difficult to discern due to the presence of two or more potentially important cues. For example, the RSC is required for reversal of a dual cue discriminative eyeblink response, even without a trace interval (Berger, Weikart, Bassett, & Orr, 1986). RSC lesions also impair feature negative discrimination learning (Keene & Bucci, 2008a; Robinson, Keene, Iaccarino, Duan, & Bucci, 2011), where subjects must learn that a given cue (e.g. a tone) predicts reinforcement when it is presented alone but that same cue predicts no reinforcement when presented along with a second cue (e.g. a light). Similarly, RSC lesions impaired the ability to respond appropriately to compound cues formed by combining elemental cues that individually require incongruent behavioral responses (Nelson, Hindley, Haddon, Vann, & Aggleton, 2014). The discriminative avoidance task described above also fits this general framework since it involves two cues that must be disambiguated. Consistent with this, RSC lesions impaired a visuospatial conditional discrimination involving two different cues, each requiring a different response (go-left or go-right, Bussey, Muir, Everitt, & Robbins, 1997).

While the above findings highlight the RSC sensitivity to the relationship between cues and reinforcement or behavioral response requirements (i.e. cue-outcome and cue-response contingencies), another body of work has shown that the RSC is needed for the ability to spontaneously form associations among neutral stimuli. Specifically, lesions or DREADD inactivation of the RSC impair sensory preconditioning (Robinson, Adelman, Mogul, Ihle, & Davino, 2018; Robinson et al., 2014), where subjects are initially exposed to repeated pairing of two neutral stimuli (e.g. a tone and a light) without any reinforcement. During the subsequent conditioning phase, one of the cues (e.g. the light) is paired with reinforcement. The subjects are then tested with the second cue (the tone, which was never paired with reinforcement): responding to the tone indicates that subjects must have formed an association between the light and tone during the initial preconditioning phase. On the basis of these findings and the observation that the RSC is critical for contextual memory processes (Keene & Bucci, 2008b; Kwapis et al., 2015; Robinson et al., 2012), Bucci and colleagues have proposed a theoretical account in which a key contribution of the RSC is to generate stimulus-stimulus associations between neutral stimuli, resulting in the formation and storage of a configural representation of the context (Bucci & Robinson, 2014; Todd & Bucci, 2015). The observation that contextual fear memory is prevented in subjects that do not have sufficient time to explore the context prior to delivery of the shock (the immediate shock deficit) is consistent with this idea (Todd, DeAngeli, Jiang, & Bucci, 2017), as is the apparent tendency for the RSC to become engaged when subjects must deal with multiple cues as described above. However, it is worth noting that much of the currently available data is agnostic as to whether the RSC generates coherent

configural representations or simply encodes individual cues that are strongly

associated with a particular context (Bar & Aminoff, 2003). Preliminary

neurophysiological evidence from our laboratory suggests that RSC ensembles do

encode contexts (Miller, Serrichio, Tse, Shi, & Smith, 2017), but careful manipulation of

contextual variables will be needed to fully resolve this issue.

Recent Findings on the RSC Role in Processing Navigational Cues. Conditioning

tasks involving explicit cues are not the only domain where the RSC is involved. The

RSC has also been implicated in processing navigational cues, and much of this

literature has come from studies of human subjects (for review see Miller et al., 2014).

RSC lesions frequently cause impairments in spatial navigation and a striking feature of

this deficit is the inability to use landmarks to construct routes to goal locations (Ino et

al., 2007; Kim, Aminoff, Kastner, & Behrmann, 2015; Maguire, 2001; Takahashi,

Kawamura, Shiota, Kasahata, & Hirayama, 1997). fMRI studies in healthy subjects

show RSC involvement when subjects view visual scenes containing prominent

landmarks (Morgan, Macevoy, Aguirre, & Epstein, 2011), especially when subjects were

asked to make spatial judgments about the scenes (Epstein, Parker, & Feiler, 2007;

Wolbers, Weiller, & Buchel, 2004). The RSC even appears to preferentially encode

permanent features of the environment rather than temporary moveable objects, which

would be less useful for navigation (Auger, Mullally, & Maguire, 2012; Auger, Zeidman,

& Maguire, 2015). RSC lesions are known to impair navigation in rodents (Harker &

Whishaw, 2002; Keene & Bucci, 2009; Sutherland et al., 1988; Vann & Aggleton, 2002)

and as with humans, some observations suggest an RSC role in the use of navigational

cues. For example, in some maze studies rats with RSC lesions were only impaired

when inconsistencies between intra- and extramaze cues were introduced to the task (Nelson, Powell, Holmes, Vann, & Aggleton, 2015; Pothuizen, Aggleton, & Vann, 2008; Vann & Aggleton, 2004). Others have found that RSC inactivation had little effect on radial maze performance until access to visual cues was removed by testing the rats in darkness (Cooper, Manka, & Mizumori, 2001; Cooper & Mizumori, 1999). The RSC is known to contain head direction cells (Cho & Sharp, 2001) and a recent study found that some RSC neurons simultaneously exhibit two opposing directional preferences in an environment with two distinctive orienting cues, suggesting a unique RSC sensitivity to landmarks (Jacob et al., 2017).

Findings from our laboratory have also provided indirect support for the idea that RSC neurons encode navigational cues (Smith, Barredo, & Mizumori, 2012, Fig. 2). We trained rats on a blocked alternation task, in which they approached one location for chocolate milk reward for the first half of the each daily training session and then they switched to a different location for the second half. Rats learn this task, reaching an asymptote of ~84% correct in 6.7 sessions on average. In solving this task, rats invariably employed a 'win-stay' strategy: even after reaching asymptotic performance they repeatedly went to the first location as long as rewards continued to be dispensed there and they only switched to the new location after failing to get a reward at the initial location (Fig. 2A). Thus, in addition to being a reinforcing stimulus the rats used the reward as an important navigational cue that instructs the rat to return to the same location on the next trial. Consistent with this idea, a large number of RSC neurons (~45%) selectively responded to either the east or west reward. These responses were not simply a response to the chocolate milk reward per se (because they were selective

for one reward location or the other) nor were they purely spatial (large changes in firing rate occurred at the time of the reward even when the rat's position did not change). Instead, these responses were driven by the co-occurrence of the reward and the specific location where it was obtained. We have seen these responses throughout the rostro-caudal extent of the RSC granular b region, suggesting that this is a very large-scale signal involving many neurons. Remarkably, this phenomenon emerged on the very first day of training (Fig. 2C) before the rats had shown clear behavioral evidence of learning (performance was not statistically different from chance performance). This suggests that encoding the reward location is a primary concern for the RSC at the outset of learning and the win-stay approach that rats took suggests this could be due to the fact that the reward location is potentially useful as a cue. However, this finding was serendipitous and our interpretation is hypothetical since this task was not designed to test hypotheses about navigational cues and we could not isolate the dual roles of the reward as a navigational cue and a reinforcing stimulus.

In order to conclusively determine whether RSC neurons encode navigational cues, we developed a T-maze task in which the reward location was explicitly cued by a flashing light which served as a beacon that the rats learned to approach for a chocolate milk reward (Fig. 3, Vedder, Miller, Harrison, & Smith, 2017). At the start of each trial, the rat was placed on the stem of the maze facing away from the choice point. As soon as the rat turned around to approach the choice point, the experimenter turned on one of the two flashing light cues to indicate the reward location for that trial (right or left). Right and left trials were randomly intermixed and the light remained on until the rat arrived at the reward location and consumed the reward. We chose to use a beacon,

rather than one or more landmarks, because the light has an unambiguous onset time and we could be confident that the rats would attend to this highly salient cue. Additionally, onset of the light cue occurred several seconds before the rat arrived at the reward so we could distinguish responses to the navigational cue and the reward.

Nearly 30% of RSC neurons responded to the light cue even before it had acquired any predictive value (i.e. during a pre-training session in which the light was randomly presented at the choice point on half the trials). This large initial response was likely due to high salience of the flashing light cue. However, the percentage of light-responsive neurons more than doubled to 63% on the very first day of training and remained high for the rest of the training sessions (Fig. 3C). Thus, the RSC emitted a massive response to the critical predictive cue at the very earliest stages of learning, similar to the reward location responses of the previous experiment. Interestingly, these neuronal responses did not distinguish between the left and right light positions, but instead responded equally to both. This was unexpected given the RSC role in the discrimination tasks and the reasons for this are not known. However, this may have occurred because the left and right lights were equally predictive of reinforcement and both required the same kind of locomotor approach response, unlike the cues in a discrimination task which predict different outcomes and require different behavioral responses. Nevertheless, these results clearly confirm that RSC neurons encode navigational cues, consistent with the findings of fMRI and lesion studies reviewed above.

Interestingly, we also found that RSC neurons exhibited the same kind of responses to the reward locations that we observed in the previous study (Fig. 4B).

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However, unlike the previous study, these responses did not suddenly emerge on the first day of training. Instead, they gradually increased in prevalence throughout learning (Fig 4A). This difference is particularly striking since the stimuli that evoked the response (0.2 ml of chocolate milk delivered to a metal cup at the end of the maze arm) were the same in both experiments. This comparison suggests that whenever a particular stimulus can serve as a navigational cue, regardless of whether it is an explicit cue such as the light or a more abstract cue such as the reward location in a win-stay task, it evokes a large and rapid response in the RSC. When the same stimuli are not useful as navigational cues, the RSC may still encode them but not as rapidly or robustly (Fig 4C).

Lastly, we found that individual RSC neurons frequently exhibited discrete responses to several different stimuli and task events. For example, the neuron illustrated in figure 4D emitted a burst of spikes at the start of the trial, when the rat was placed on the stem of the maze, and another burst after the cue light was illuminated and a third burst as the rat arrived at the reward location (with the last burst selective for the right reward location). We only used one explicit cue in this task (the light), but this tendency toward 'multi-responsivity' was quite common, with ~67% of RSC neurons exhibiting at least two separately identifiable responses. This may suggest that RSC neurons would be predisposed to encode multiple cues, consistent with the RSC role in encoding contexts (Bucci & Robinson, 2014; Todd & Bucci, 2015). In the instrumental discrimination studies described above RSC neurons responded to both the predictive and non-predictive tones and the discrimination resulted from the fact that firing was increased in response to the predictive tone (Fig 1A).

Remaining Questions and Concluding Remarks. Together, the results reviewed

here suggest that the RSC plays a particular role in encoding behaviorally significant

cues. This role is apparent in spatial tasks where the RSC may uniquely encode

navigational cues such as landmarks and beacons, and in non-spatial conditioning

tasks where the RSC encodes important predictive cues and cues that may be

important components of the context. The specific factors that make a given task

critically dependent on the RSC are not fully understood, but the need to process

multiple cues or complex contingencies is a common feature of RSC-dependent

conditioning tasks and the capacity to cope with multiple cues is also important for most

spatial navigation and contextual memory tasks. This is notably reminiscent of

discussions about the factors that lead to hippocampal involvement in various memory,

navigation and non-spatial conditioning tasks (e.g. Fanselow, 2000; McEchron &

Disterhoft, 1999; Rudy & Sutherland, 1995). Nevertheless, the tendency of RSC

neurons to produce large scale responses to any task relevant discrete cue is a

remarkably consistent finding, suggesting that the encoding of cues reflects an

important component of RSC function.

However, some important questions remain. Several findings suggest that the

temporal characteristics of RSC involvement may be quite important, but they remain

poorly understood. Findings from the discriminative avoidance model suggest that RSC

involvement is limited to the late stages of learning, after subjects reach asymptotic

performance, and preliminary data from our laboratory suggest a similar late-stage

impairment in spatial alternation (Miller & Smith, 2012). Selective involvement in

asymptotic performance is consistent with an RSC role in consolidation of long-term

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memories, as several findings have suggested (Cowansage et al., 2014; Czajkowski et al., 2014; Freeman & Gabriel, 1999; Katche et al., 2013). However, we have also seen that some RSC neural responses emerge at the very earliest stages of learning (e.g. Fig. 3), suggesting a role in rapidly identifying and encoding important cues. Even in the discriminative avoidance model where overall RSC responses tended to develop slowly over the course of learning, some RSC cortical layers and their interconnected anterior thalamic nuclei exhibited rapidly developing plasticity (Gabriel & Orona, 1982). Similarly mixed fast and slow responses were seen in our cued spatial tasks (Fig 4C). Another unresolved issue is that RSC responses appear to be driven by the predictive relationship with reinforcement and the need for a behavioral response in many tasks. However, sensory preconditioning studies convincingly demonstrate an RSC role in encoding neutral cues that have no reinforcement-predictive value at the time of encoding, and the RSC role in contextual memory, where the association of contextual stimuli occurs spontaneously without the need for reinforcement, is well established.

These two apparent contradictions, rapid vs slow engagement and reinforcement-predictive vs neutral cue processing, may be related to each other. Rapid responses may be driven by reinforcement contingencies while slow responses might reflect the accumulation and consolidation of reliable regularities in the environment that do not involve explicit reinforcement. If so, this suggests a dual role of the RSC that is reflective of the fact that the RSC is both an input structure that carries important sensory information to other limbic memory regions such as the hippocampus (Cooper & Mizumori, 2001) and a likely target for the consolidation of hippocampal-dependent memories (Cowansage et al., 2014; Czajkowski et al., 2014; Katche et al., 2013).

Initially, the RSC may rapidly identify and encode cues that have clear reinforcement contingencies and pass this information to the hippocampus, where such contingencies have been shown to influence hippocampal representations (Smith & Mizumori, 2006; Yeshenko, Guazzelli, & Mizumori, 2004). In contrast, the processing of neutral cues may reflect the slower accumulation and consolidation of neutral stimulus-stimulus associations such as those that define contexts. A comprehensive account of the RSC contribution to memory will need to account for these temporal characteristics as well as the factors that drive the encoding of various kinds of sensory cues.

Figure Captions

Figure 1. Neuronal responses to auditory cues during discriminative approach and avoidance learning. Plot A) Tone-evoked multi-unit neuronal responses to the auditory cues are shown in the form of z-scores normalized to pre-tone baseline, with 400 msec of firing data shown in 10 msec time bins (Smith et al., 2002). Before learning, RSC neurons respond equally to auditory cues of different tonal frequencies (left). After learning, neuronal firing in response to the predictive tone (CS+, black bars) is significantly greater than to the non-predictive tone (CS-, white bars). Plot B) RSC neuronal responses are shown for subjects trained to perform the approach and avoidance tasks on alternating days (Freeman et al., 1996). Note that RSC neurons preferentially respond to the reinforcement-predictive auditory cue in both tasks, regardless of differences in tonal frequency, hedonic value (appetitive vs aversive) and response requirements (approach or avoidance).

Figure 2. RSC responses during blocked alternation. Plot A) Rats were trained to approach the east arm for reward for the first 15 trials of each daily training session and the west arm for the next 15 trials. Each day, the rats were given a 30 sec lights-out period after trial 15 to indicate that the reward location was about to shift to the west arm. However, even in well-trained subjects (>80% correct overall), the rats never learned to use the 'lights-out' cue to shift their responding. Instead, they invariably went to the old (east) reward location until no reward was found there and only then did they shift to the west arm. Plot B) Average behavioral performance is shown for a baseline recording session prior to learning (pretraining, PT), the first training session (Day1), the session midway through acquisition (Mid) and during asymptotic performance (Asymp).

Plot C) The percentage of RSC neurons that exhibited a significant response that was selective for both the receipt of the reward and the location (east or west) at each of the same training stages as in plot B. Plots D and E) Two examples of RSC neurons that selectively responded to one reward location are shown in the form of peri-event time histograms showing 10 seconds of firing data before and after the receipt of the reward at time zero, with trial by trial rasters below each histogram. The neuron in plot D fired selectively for rewards received at on the east arm while the neuron in plot E fired on the west arm.

Figure 3. RSC responses during a cued T-maze task. Plot A) Rats were trained to approach a flashing light cue positioned over the right or left reward location (left reward trial is illustrated). The rat was placed on the stem of the maze facing away from the choice point and the light was illuminated as soon as the rat turned around and took a step forward (dashed line). Before regular training sessions began, we recorded baseline responses to a light cue positioned at the choice point (dashed circle) which was illuminated during half of the trials in a random manner. This light was only used during this pretraining session and it did not predict the reward locations, which were also randomly selected. Plot B) Rats learned this task in 6.4 training sessions on average, and reached an asymptote of 94% correct. Plot C) The percentage of RSC neurons that exhibited a significant response to the light cue are shown for each training stage (same as in plot 1B). Plot D) An example RSC neuron that responded to the onset of the light cue (time zero) is shown in the form of a peri-event time histogram along with a raster display. From Vedder, L. C., Miller, A. M. P., Harrison, M. B., & Smith, D. M. (2017). Retrosplenial Cortical Neurons Encode Navigational Cues,

Trajectories and Reward Locations During Goal Directed Navigation. Cerebral Cortex, 27(7), pp 3716, 3717. Adapted with permission.

Figure 4. Other RSC responses from the cued T-maze task. Plot A) The percentage of RSC neurons that exhibited a significant response that was selective for both the receipt of the reward and the location (left or right) is shown for each training stage (same as in plot 1B). Note that these responses did not emerge immediately on the first day of training. Plot B) An example neuron with a reward-location response is shown in the form of a peri-event time histogram with a raster display. Trials with the reward on the right are shown in blue while trials with the reward on the left are shown in red. Left and right trials were randomly intermixed and they are only separated in the raster for illustration. Plot C) The percentage of RSC neurons that encode an explicit navigational cue, such as the light cue, increases dramatically on the first day of training (solid line). In the blocked alternation study where rats used a win-stay strategy (Fig 2), the reward and its location may have served as an important navigational cue and similarly rapid encoding was seen (fine dashed line). In contrast, the reward location could not be used as a cue in the light cued T-maze study because the current reward did not indicate the location for the upcoming reward which randomly switched from one trial to the next. Under these conditions, the responses did not show a sudden increase early in training (coarse dashed line). Data are expressed as the increase in the percentage of neurons at each stage of training, relative to the pre-training baseline. Plot D) An example neuron that exhibited significant responses to three separate task events is shown in the form of a peri-event time histogram aligned to receipt of the reward (time zero). Additional events are indicated by arrows (S=trial start, L=light onset and R=reward).

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From Vedder, L. C., Miller, A. M. P., Harrison, M. B., & Smith, D. M. (2017). Retrosplenial Cortical Neurons Encode Navigational Cues, Trajectories and Reward Locations During Goal Directed Navigation. Cerebral Cortex, 27(7), pp 3716, 3717. Adapted with permission.

Figure 5. Theoretical model of RSC interactions with the hippocampus. Early in learning, visuospatial and other sensory information arrives at the RSC where behaviorally significant cues are identified and encoded. Large scale RSC responses to these predictive cues emerge rapidly (e.g. Fig. 3) and may play an important role in establishing or orienting hippocampal representations (blue pathway). In contrast, RSC spatial and contextual representations, which do not rely on reinforcement contingencies, emerge slowly and may reflect consolidation of information from the hippocampus, where such representations are known to emerge rapidly (red pathway). These interactions could arise from relatively direct anatomical pathways via the subiculum or CA1 projections to the RSC (direct arrows) or through more indirect routes involving the anterior thalamus and entorhinal cortex.

References

Aggleton, J. P., O'Mara, S. M., Vann, S. D., Wright, N. F., Tsanov, M., & Erichsen, J. T.
(2010). Hippocampal-anterior thalamic pathways for memory: uncovering a
network of direct and indirect actions. *Eur J Neurosci, 31*(12), 2292-2307. doi:
10.1111/j.1460-9568.2010.07251.x

Alexander, A. S., & Nitz, D. A. (2015). Retrosplenial cortex maps the conjunction of
internal and external spaces. *Nat Neurosci, 18*(8), 1143-1151. doi:
10.1038/nn.4058

Alexander, A. S., & Nitz, D. A. (2017). Spatially Periodic Activation Patterns of
Retrosplenial Cortex Encode Route Sub-spaces and Distance Traveled. *Curr
Biol, 27*(11), 1551-1560 e1554. doi: 10.1016/j.cub.2017.04.036

Auger, S. D., Mullally, S. L., & Maguire, E. A. (2012). Retrosplenial cortex codes for
permanent landmarks. *PLoS One, 7*(8), e43620. doi:
10.1371/journal.pone.0043620

Auger, S. D., Zeidman, P., & Maguire, E. A. (2015). A central role for the retrosplenial
cortex in de novo environmental learning. *Elife, 4*. doi: 10.7554/eLife.09031

Bar, M., & Aminoff, E. (2003). Cortical analysis of visual context. *Neuron, 38*(2), 347-
358.

Berger, T. W., Weikart, C. L., Bassett, J. L., & Orr, W. B. (1986). Lesions of the
retrosplenial cortex produce deficits in reversal learning of the rabbit nictitating
membrane response: implications for potential interactions between hippocampal
and cerebellar brain systems. *Behav Neurosci, 100*(6), 802-809.

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Bowers, D., Verfaellie, M., Valenstein, E., & Heilman, K. M. (1988). Impaired acquisition of temporal information in retrosplenial amnesia. *Brain Cogn, 8*(1), 47-66.

Bucci, D. J., & Robinson, S. (2014). Toward a conceptualization of retrohippocampal contributions to learning and memory. *Neurobiol Learn Mem, 116*, 197-207. doi: 10.1016/j.nlm.2014.05.007

Bussey, T. J., Muir, J. L., Everitt, B. J., & Robbins, T. W. (1997). Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. *Behavioral Neuroscience, 111*(5), 920-936.

Cho, J., & Sharp, P. E. (2001). Head direction, place, and movement correlates for cells in the rat retrosplenial cortex. *Behavioral Neuroscience, 115*(1), 3-25.

Cooper, B. G., Manka, T. F., & Mizumori, S. J. (2001). Finding your way in the dark: the retrosplenial cortex contributes to spatial memory and navigation without visual cues. *Behavioral Neuroscience, 115*(5), 1012-1028.

Cooper, B. G., & Mizumori, S. J. (1999). Retrosplenial cortex inactivation selectively impairs navigation in darkness. *Neuroreport, 10*(3), 625-630.

Cooper, B. G., & Mizumori, S. J. (2001). Temporary inactivation of the retrosplenial cortex causes a transient reorganization of spatial coding in the hippocampus. *Journal of Neuroscience, 21*(11), 3986-4001.

Cowansage, K. K., Shuman, T., Dillingham, B. C., Chang, A., Golshani, P., & Mayford, M. (2014). Direct reactivation of a coherent neocortical memory of context. *Neuron, 84*(2), 432-441. doi: 10.1016/j.neuron.2014.09.022

23

Czajkowski, R., Jayaprakash, B., Wiltgen, B., Rogerson, T., Guzman-Karlsson, M. C., Barth, A. L., . . . Silva, A. J. (2014). Encoding and storage of spatial information in the retrosplenial cortex. *Proc Natl Acad Sci U S A, 111*(23), 8661-8666. doi: 10.1073/pnas.1313222111

Epstein, R. A., Parker, W. E., & Feiler, A. M. (2007). Where am I now? Distinct roles for parahippocampal and retrosplenial cortices in place recognition. *J Neurosci, 27*(23), 6141-6149. doi: 10.1523/JNEUROSCI.0799-07.2007

Fanselow, M. S. (2000). Contextual fear, gestalt memories, and the hippocampus. *Behav Brain Res, 110*(1-2), 73-81.

Freeman, J. H., Jr., Cuppernell, C., Flannery, K., & Gabriel, M. (1996). Context-specific multi-site cingulate cortical, limbic thalamic, and hippocampal neuronal activity during concurrent discriminative approach and avoidance training in rabbits. *Journal of Neuroscience, 16*(4), 1538-1549.

Freeman, J. H., Jr., & Gabriel, M. (1999). Changes of cingulothalamic topographic excitation patterns and avoidance response incubation over time following initial discriminative conditioning in rabbits. *Neurobiology of Learning & Memory, 72*(3), 259-272.

Gabriel, M. (1993). Discriminative avoidance learning: A model system. In B. A. Vogt & M. Gabriel (Eds.), *Neurobiology of cingulate cortex and limbic thalamus* (pp. 478-523). Boston: Birkhauser.

Gabriel, M., Kang, E., Poremba, A., Kubota, Y., Allen, M. T., Miller, D. P., & Steinmetz, J. E. (1996). Neural substrates of discriminative avoidance learning and classical

eyeblink conditioning in rabbits: a double dissociation. *Behavioural Brain Research, 82*(1), 23-30.

Gabriel, M., Kubota, Y., Sparenborg, S., Straube, K., & Vogt, B. A. (1991). Effects of cingulate cortical lesions on avoidance learning and training-induced unit activity in rabbits. *Experimental Brain Research, 86*(3), 585-600.

Gabriel, M., & Orona, E. (1982). Parallel and serial processes of the prefrontal and cingulate cortical systems during behavioral learning. *Brain Research Bulletin, 8*(6), 781-785.

Gabriel, M., Sparenborg, S., & Kubota, Y. (1989). Anterior and medial thalamic lesions, discriminative avoidance learning, and cingulate cortical neuronal activity in rabbits. *Experimental Brain Research, 76*(2), 441-457.

Gabriel, M., Sparenborg, S. P., & Stolar, N. (1987). Hippocampal control of cingulate cortical and anterior thalamic information processing during learning in rabbits. *Experimental Brain Research, 67*(1), 131-152.

Harker, K. T., & Whishaw, I. Q. (2002). Impaired Spatial Performance in Rats with Retrosplenial Lesions: Importance of the Spatial Problem and the Rat Strain in Identifying Lesion Effects in a Swimming Pool. *Journal of Neuroscience, 22*, 1155-1164.

Hart, M., Poremba, A., & Gabriel, M. (1997). The nomadic engram: overtraining eliminates the impairment of discriminative avoidance behavior produced by limbic thalamic lesions. *Behavioural Brain Research, 82*(2), 169-177.

Hassabis, D., Kumaran, D., Vann, S. D., & Maguire, E. A. (2007). Patients with hippocampal amnesia cannot imagine new experiences. *Proc Natl Acad Sci U S A, 104*(5), 1726-1731.

Ino, T., Doi, T., Hirose, S., Kimura, T., Ito, J., & Fukuyama, H. (2007). Directional disorientation following left retrosplenial hemorrhage: a case report with fMRI studies. *Cortex, 43*(2), 248-254.

Jacob, P. Y., Casali, G., Spieser, L., Page, H., Overington, D., & Jeffery, K. (2017). An independent, landmark-dominated head-direction signal in dysgranular retrosplenial cortex. *Nat Neurosci, 20*(2), 173-175. doi: 10.1038/nn.4465

Katche, C., Dorman, G., Gonzalez, C., Kramar, C. P., Slipczuk, L., Rossato, J. I., . . . Medina, J. H. (2013). On the role of retrosplenial cortex in long-lasting memory storage. *Hippocampus, 23*(4), 295-302. doi: 10.1002/hipo.22092

Keene, C. S., & Bucci, D. J. (2008a). Involvement of the retrosplenial cortex in processing multiple conditioned stimuli. *Behav Neurosci, 122*(3), 651-658.

Keene, C. S., & Bucci, D. J. (2008b). Neurotoxic lesions of retrosplenial cortex disrupt signaled and unsignaled contextual fear conditioning. *Behav Neurosci, 122*(5), 1070-1077.

Keene, C. S., & Bucci, D. J. (2009). Damage to the retrosplenial cortex produces specific impairments in spatial working memory. *Neurobiol Learn Mem, 91*(4), 408-414. doi: 10.1016/j.nlm.2008.10.009

Kim, J. G., Aminoff, E. M., Kastner, S., & Behrmann, M. (2015). A Neural Basis for Developmental Topographic Disorientation. *J Neurosci, 35*(37), 12954-12969. doi: 10.1523/JNEUROSCI.0640-15.2015

Kubota, Y., Wolske, M., Poremba, A., Kang, E., & Gabriel, M. (1996). Stimulus-related

    and movement-related single-unit activity in rabbit cingulate cortex and limbic

    thalamus during performance of discriminative avoidance behavior. *Brain*

    Research, 721(1-2), 22-38.

Kwapis, J. L., Jarome, T. J, Lee, J. L, & Helmstetter, F. J. (2015). The retrosplenial

    cortex is involved in the formation of memory for context and trace fear

    conditioning. *Neurobiol Learn Mem, 123*, 110-116.

Kwapis, J. L., Jarome, T. J., Lee, J. L., Gilmartin, M. R., & Helmstetter, F. J. (2014).

    Extinguishing trace fear engages the retrosplenial cortex rather than the

    amygdala. *Neurobiol Learn Mem, 113*, 41-54. doi: 10.1016/j.nlm.2013.09.007

Maguire, E. A. (2001). The retrosplenial contribution to human navigation: a review of

    lesion and neuroimaging findings. *Scand J Psychol, 42*(3), 225-238.

McEchron, M. D., & Disterhoft, J. F. (1999). Hippocampal encoding of non-spatial trace

    conditioning. *Hippocampus, 9*(4), 385-396. doi: 10.1002/(SICI)1098-

    1063(1999)9:4<385::AID-HIPO5>3.0.CO;2-K

Miller, A. M., Serrichio, A. C., Tse, A. L., Shi, C., & Smith, D. M. (2017). Retrosplenial

    ensembles encode spatial and temporal context. *Society for Neuroscience*

    Abstracts, Program Number 84.26.

Miller, A. M., & Smith, D. M. (2012). The Retrosplenial Cortex is Critical for Delayed

    Spatial Alternation on a Continuous T-Maze. *Society for Neuroscience Abstracts,*

    Program Number 706.06.

Miller, A. M., Vedder, L. C., Law, L. M., & Smith, D. M. (2014). Cues, context, and long-term memory: the role of the retrosplenial cortex in spatial cognition. *Front Hum Neurosci, 8*, 586. doi: 10.3389/fnhum.2014.00586

Morgan, L. K., Macevoy, S. P., Aguirre, G. K., & Epstein, R. A. (2011). Distances between real-world locations are represented in the human hippocampus. *J Neurosci, 31*(4), 1238-1245. doi: 10.1523/JNEUROSCI.4667-10.2011

Nelson, A. J., Hindley, E. L., Haddon, J. E., Vann, S. D., & Aggleton, J. P. (2014). A novel role for the rat retrosplenial cortex in cognitive control. *Learn Mem, 21*(2), 90-97. doi: 10.1101/lm.032136.113

Nelson, A. J., Powell, A. L., Holmes, J. D., Vann, S. D., & Aggleton, J. P. (2015). What does spatial alternation tell us about retrosplenial cortex function? *Front Behav Neurosci, 9*, 126. doi: 10.3389/fnbeh.2015.00126

Poremba, A., & Gabriel, M. (1997a). Amygdalar lesions block discriminative avoidance learning and cingulothalamic training-induced neuronal plasticity in rabbits. *Journal of Neuroscience, 17*(13), 5237-5244.

Poremba, A., & Gabriel, M. (1997b). Medial geniculate lesions block amygdalar and cingulothalamic learning-related neuronal activity. *Journal of Neuroscience, 17*(21), 8645-8655.

Pothuizen, H. H., Aggleton, J. P., & Vann, S. D. (2008). Do rats with retrosplenial cortex lesions lack direction? *Eur J Neurosci, 28*(12), 2486-2498. doi: 10.1111/j.1460-9568.2008.06550.x

Robinson, S., Adelman, J. S., Mogul, A. S., Ihle, P. C. J., & Davino, G. M. (2018). Putting fear in context: Elucidating the role of the retrosplenial cortex in context

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discrimination in rats. *Neurobiol Learn Mem, 148*, 50-59. doi: 10.1016/j.nlm.2017.12.009

Robinson, S., Keene, C. S., Iaccarino, H. F., Duan, D., & Bucci, D. J. (2011). Involvement of retrosplenial cortex in forming associations between multiple sensory stimuli. *Behav Neurosci, 125*(4), 578-587. doi: 10.1037/a0024262

Robinson, S., Poorman, C. E., Marder, T. J., & Bucci, D. J. (2012). Identification of functional circuitry between retrosplenial and postrhinal cortices during fear conditioning. *J Neurosci, 32*(35), 12076-12086. doi: 10.1523/JNEUROSCI.2814-12.2012

Robinson, S., Todd, T. P., Pasternak, A. R., Luikart, B. W., Skelton, P. D., Urban, D. J., & Bucci, D. J. (2014). Chemogenetic silencing of neurons in retrosplenial cortex disrupts sensory preconditioning. *J Neurosci, 34*(33), 10982-10988. doi: 10.1523/JNEUROSCI.1349-14.2014

Rudy, J. W., & Sutherland, R. J. (1995). Configural association theory and the hippocampal formation: an appraisal and reconfiguration. *Hippocampus, 5*(5), 375-389.

Schacter, D. L., & Addis, D. R. (2007). The cognitive neuroscience of constructive memory: remembering the past and imagining the future. *Philos Trans R Soc Lond B Biol Sci, 362*(1481), 773-786. doi: 10.1098/rstb.2007.2087

Smith, D. M., Barredo, J., & Mizumori, S. J. (2012). Complimentary Roles of the Hippocampus and Retrosplenial Cortex in Behavioral Context Discrimination. *Hippocampus, 22*, 1121-1133.

Smith, D. M., Freeman, J. H., Jr., Nicholson, D., & Gabriel, M. (2002). Limbic Thalamic

Lesions, Appetitively-Motivated Discrimination Learning and Training-Induced

Neuronal Activity In Rabbits. *Journal of Neuroscience, 22(18)*, 8212-8221.

Smith, D. M., & Mizumori, S. J. Y. (2006). Learning-Related Development of Context-

Specific Neuronal Responses to Places and Events: The Hippocampal Role in

Context Processing. *Journal of Neuroscience, 26*(12), 3154-3163.

Sparenborg, S., & Gabriel, M. (1990). Neuronal encoding of conditional stimulus

duration in the cingulate cortex and the limbic thalamus of rabbits. *Behavioral

Neuroscience, 104*(6), 919-933.

Stolar, N., Sparenborg, S., Donchin, E., & Gabriel, M. (1989). Conditional stimulus

probability and activity of hippocampal, cingulate cortical, and limbic thalamic

neurons during avoidance conditioning in rabbits. *Behavioral Neuroscience,

103*(5), 919-934.

Sutherland, R. J., Whishaw, I. Q., & Kolb, B. (1988). Contributions of cingulate cortex to

two forms of spatial learning and memory. *Journal of Neuroscience, 8*(6), 1863-

1872.

Takahashi, N., Kawamura, M., Shiota, J., Kasahata, N., & Hirayama, K. (1997). Pure

topographic disorientation due to right retrosplenial lesion. *Neurology, 49*(2), 464-

469.

Todd, T. P., & Bucci, D. J. (2015). Retrosplenial Cortex and Long-Term Memory:

Molecules to Behavior. *Neural Plast, 2015*, 414173. doi: 10.1155/2015/414173

Todd, T. P., DeAngeli, N. E., Jiang, M. Y., & Bucci, D. J. (2017). Retrograde amnesia of

    contextual fear conditioning: Evidence for retrosplenial cortex involvement in

    configural processing. *Behav Neurosci, 131*(1), 46-54. doi: 10.1037/bne0000183

Todd, T. P., Mehlman, M. L., Keene, C. S., DeAngeli, N. E., & Bucci, D. J. (2016).

    Retrosplenial cortex is required for the retrieval of remote memory for auditory

    cues. *Learn Mem, 23*(6), 278-288. doi: 10.1101/lm.041822.116

Valenstein, E., Bowers, D., Verfaellie, M., Heilman, K. M., Day, A., & Watson, R. T.

    (1987). Retrosplenial amnesia. *Brain, 110*(Pt 6), 1631-1646.

Vann, S. D., & Aggleton, J. P. (2004). Testing the importance of the retrosplenial

    giudance system: effects of different sized retrosplenial cortex lesions on heading

    direction and spatial working memory. *Behavavioral Brain Research, 155*, 97-

    108.

Vann, S. D., & Aggleton, J. P. (2002). Extensive cytotoxic lesions of the rat retrosplenial

    cortex reveal consistent deficits on tasks that tax allocentric spatial memory.

    Behav Neurosci, 116(1), 85-94.

Vedder, L. C., Miller, A. M. P., Harrison, M. B., & Smith, D. M. (2017). Retrosplenial

    Cortical Neurons Encode Navigational Cues, Trajectories and Reward Locations

    During Goal Directed Navigation. *Cereb Cortex, 27*(7), 3713-3723. doi:

    10.1093/cercor/bhw192

Weible, A. P., McEchron, M. D., & Disterhoft, J. F. (2000). Cortical involvement in

    acquisition and extinction of trace eyeblink conditioning. *Behav Neurosci, 114*(6),

    1058-1067.

Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness

    in trace but not delay conditioning. *Psychophysiology, 44*(1), 170-180. doi:

    10.1111/j.1469-8986.2006.00469.x

Wolbers, T., Weiller, C., & Buchel, C. (2004). Neural foundations of emerging route

    knowledge in complex spatial environments. *Brain Res Cogn Brain Res, 21*(3),

    401-411. doi: 10.1016/j.cogbrainres.2004.06.013

Yeshenko, O., Guazzelli, A., & Mizumori, S. J. (2004). Context-dependent

    reorganization of spatial and movement representations by simultaneously

    recorded hippocampal and striatal neurons during performance of allocentric and

    egocentric tasks. *Behav Neurosci, 118*(4), 751-769.