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Research report

Acetylcholine efflux from retrosplenial areas and hippocampal sectors during maze exploration

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ABSTRACT

Both the retrosplenial cortex (RSC) and the hippocampus are important for spatial learning across species. Although hippocampal acetylcholine (ACh) release has been associated with learning on a number of spatial tasks, relatively little is understood about the functional role of ACh release in the RSC. In the present study, spatial exploration was assessed in rats using a plus maze spontaneous alternation task. ACh efflux was assessed simultaneously in the hippocampus and two sub-regions of the RSC (areas 29ab and 30) before, during and after maze exploration. Results demonstrated that there was a significant rise in ACh efflux in RSC area 29ab and the hippocampus during maze traversal. The rise in ACh efflux across these two regions was correlated. There were no significant behaviorally driven changes in ACh efflux in RSC area 30. While both the hippocampal sectors and area 29ab displayed increases in ACh efflux during maze exploration, the percent ACh rise in area 29ab was higher than that observed in the hippocampus and persisted into the post-baseline period. Joint efflux analyses demonstrated a key functional role for ACh release in area 29ab during spatial processing.

1. Introduction

The retrosplenial cortex (RSC) is important for spatial processing and navigation through space [14,61,72,80]. The neuroanatomical connectivity of the RSC includes extensive reciprocal connections with areas integral for spatial cognition—including the hippocampal formation, anterior nuclei of the thalamus, and various neocortical sites mediating perceptual processes that assist in spatial coding and accurate navigation [57,67,79]. As such, the RSC may serve as a crucial junction of information flow that holds the capacity to not only mediate the transmission of signals between the hippocampal formation and neocortical sites, but to also participate in the functional integration of varying types of spatial information including visual and self-motion cues.

Electrophysiological investigations of cell activity within the RSC have revealed the presence of cells displaying strong spatial and movement-related correlates [10–12]. There are head direction (HD) cells and place cells within the RSC, as well as other neurons that demonstrate response patterns to a mixture of place, angular velocity and running speed [12]. Furthermore, Cooper and Mizumori [15] found that temporary inactivation of the RSC lead to transient shifts in hippocampal place cell responsivity that resulted in place cells firing outside of their preferred location during a radial arm maze. This demonstrates a direct functional interrelationship between signals derived from the RSC and localization processes in the hippocampus. Therefore, the RSC provides key information to the hippocampal formation about distal sensory information and self-motion cues that are necessary for certain mnemonically guided navigational strategies, such as path integration and allocentric memory processes [13,14,41,61,72].

The RSC is composed of four cytoarchitectonical areas: granular areas 29a–c and dysgranular area 30 [79]. Furthermore, these areas display unique patterns of connectivity including dense and reciprocal connections with the visual cortex that may underlie different facets of cognitive function [57,67,70,74,79,83]. Spatial memory performance was found to be differentially affected as a function of selective lesions to either retrosplenial granular b (area 29b) or retrosplenial granular a (area 29a). Damage confined to area 29b, but not 29a, resulted in select spatial deficits on an open field water maze task, although concomitant destruction of both regions tends to result in greater impairment [71]. This finding concurs with anatomical studies demonstrating higher connectivity between area 29a and structures integral for spatial coding including the parietal cortex, dorsal subiculum, and various thalamic nuclei [82,83]. Additionally, ablation of area 30 increased reliance upon egocentric cues during a radial arm maze task, suggesting impaired utilization of distal visual cues and allocentric memory processes [73]. Thus, the organization of the RSC may reflect a diver-

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sity of function, with each area uniquely contributing to spatial cognition.

The RSC, like the hippocampus, receives its primary cholinergic input from the basal forebrain. Cholinergic cells in the rat forebrain are organized into four subdivisions (Ch1–4) that have different innervation patterns: Ch1 (medial septal [MS] area) and Ch2 (diagonal band [DB]) innervate the hippocampus and surrounding cortical areas; Ch3 innervates the olfactory bulb, whereas Ch4 innervates the neocortex and amygdala [16]. Amaral and Kurz [3] further noted three distinct pathways within the MS/DB cholinergic projection system (dorsal, ventral, and intermediate). The dorsal group innervates the ventral hippocampus, whereas the ventral group innervates the dorsal hippocampus. In contrast, the intermediate group innervates the cingulate cortex—without providing hippocampal connections. Recent evidence suggests that the RSC receives greater innervation from Ch2 over Ch1 [27]. In addition, the RSC receives abundant cholinergic innervations from the nucleus basalis of Meynert [5,17,24,46,56].

To date the evidence for the functional involvement of acetylcholine (ACh) in the RSC comes from lesion and pharmacological studies. These studies found that disruption of the septo-retrosplenial cholinergic pathway, via transection of the fornix and cingulate pathway, led to a nearly complete loss of choline acetyltransferase (ChAT) positive nerve terminals and major reductions of high affinity choline uptake (HACU) levels in the RSC that corresponded with significant impairments on several spatial memory-related tasks [37–39]. Subsequent transplantations of fetal septal neurons into the RSC attenuated the spatial deficits that initially followed retrosplenial deafferentation [39]. Furthermore, it has been demonstrated that intracerebral infusions of scopolamine, a muscarinic ACh antagonist, into the RSC before behavioral training impaired spatial learning during a water maze task [51]. These observations suggest that cholinergic activity in the RSC is critical for spatial memory function and navigational skills.

Despite growing evidence for a functional role of ACh in the RSC, surprisingly little is known about dynamic fluctuations in ACh release in the RSC when spatial information is being processed. In contrast, numerous studies have demonstrated that changes in hippocampal ACh efflux are related to spatial learning and navigation [19,20,25,54]. *In vivo* ACh efflux has proven to be a neurochemical marker of regional activation and is associated with attention and memory-related processes [25,53–55]. Thus, if ACh is critical for processing spatial information within the RSC circuit, changes as a function of spatial demands should be observed.

The goal of the present study was to assess, compare, and relate the profiles of ACh efflux in both areas 29ab and 30 to hippocampal ACh efflux during a spontaneous alternation task. However, spontaneous alternation is not a direct measure of spatial memory per se; but instead is related to spatial memory ability [34,35]. Appropriate alternation requires a rat to discriminate between arms on the basis of which arms were most recently visited. Such alternation behavior has been described to be a memory-dependent response to the environment driven by a natural tendency toward novel locations [34]. Furthermore, spontaneous alternation is affected by brain manipulations that alter memory performance on other tasks (lesions of the hippocampus, medial septum, cholinergic drugs, etc.—see [34,35]) and by environmental manipulations of cues within and external to the maze [36]. Given that the RSC has multiple sources of cholinergic innervation and greater connectivity with areas processing visual and perceptual spatial information, we expected that the RSC—in particular area 29ab—would display greater changes in ACh efflux relative to the hippocampus during spatial exploration. Such data would support the idea the RSC plays a significant role in spatial navigation.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (3–4 months; 250–300 g) were used as subjects in this study. They were housed one per cage with unlimited access to water and Purina rodent chow in a colony room with a 12 h/12 h light-dark cycle (onset at 7:00 am). Each subject was implanted with two plastic cannulae (CMA/11 mm, Carnegie Medicine Associates, Chelmsford, MA) aimed at the hippocampus or the RSC via a stereotaxic apparatus (David Kopf Instruments, USA). Half the rats had a cortical cannulae aimed to transect area 29ab and the other half had cortical cannulae aimed at area 30. Prior to surgery, animals were anesthetized with an i.p. injection (0.1 ml/kg) of a ketamine (83 mg/kg)/Xylazine (17 mg/kg) mixture. Hemispheric placement (L, R) of cannulae was counterbalanced across subjects. One cannula (CMA/11) was lowered into either the left or right hippocampus (5.0 mm posterior to Bregma, 5.0 mm lateral to the midline, and 4.2 mm DV) and another guide cannula was lowered into either area 29ab (6.3 mm AP, 1.0 mm lateral to the midline, and 1.25 mm DV) or area 30 (6.7 mm AP, 1.2 mm lateral to the midline, and 1.20 mm DV) of the retrosplenial cortex in the left or right RSC according to the atlas of Paxinos and Watson [48]. After surgery animals were allowed a 4-day recovery period followed by 5 days of handling (5 min/day) prior to behavioral testing. All rats were fasted the night before behavioral testing.

2.2. In vivo microdialysis and behavioral testing

Nine days following surgery two microdialysis probes (CMA/11, 3 mm for the hippocampus, CMA/11, 2 mm for area 29ab of the RSC or CMA/11, 1 mm for area 30 of the retrosplenial cortex) were inserted into the guide cannulae. The depth of the structure determined the length of the probe used in the region. The probes were connected to plastic tubing and driven by a microinfusion system (CMA/100 pump). The dialysis probes were perfused continuously at a rate of 2.0 μl/min with artificial CSF (in mM: 128 NaCl, 2.5 KCl, 1.3 CaCl, 2.1 MgCl, NaHPO, and 1.0 glucose, brought to a pH of 7.4), which contained the acetylcholinesterase inhibitor neostigmine (500 nM). Prior to maze testing, the microdialysis probes were inserted into the hippocampal and retrosplenial cannulae (either area 29ab or 30) and the animal was placed into the holding cage (41 cm × 30 cm × 35 cm) located in the testing room. After 60 min of stabilization, dialysis samples (sample volume 12 μl) were collected every 6 min for a period of 18 min in the holding cage to determine basal levels of ACh in awake rats. During this initial baseline phase, the animal was free to move about the holding cage. After initial exploration, most animals sat in a corner and occasionally groomed. After three baseline samples were collected, the rat was gently picked up and placed on the center of the maze. The plus maze used for behavioral testing was made of wood with clear Plexiglas sidewalls (12 cm high) and a painted black floor with four arms of equal distance (55 cm). It was elevated 80 cm from the floor. The rat was allowed to transverse the maze freely for an 18 min period, the number and sequence of arms entered were recorded to determine alternation scores.

An alternation score was determined by recording the number of different arms (minimum = 1, maximum = 4) entered during a four-choice sequence, dividing the number of different arms entered by 4 and multiplying that fraction by 100. The averaged probability of making certain behavioral choices is listed in Table 1. The maze testing room contained various extramaze cues (posters, doors, tables, etc.). Upon completion of 18 min of maze testing, rats were transferred back to the holding cage for an additional 18 min and dialysate samples were collected throughout this period (post-baseline).

Dialysate samples were assayed for ACh using high-performance liquid chromatography (HPLC) with electrochemical detection (Bio Analytic Systems, West Lafayette, IN). The system included an ion-exchange microbore analytical column, a microbore ACh/Ch immobilized enzyme reactor containing acetylcholinesterase and choline oxidase, and perioxidase wired working electrode. The detection limit of this system is 10 fm. ACh peaks were quantified by comparison to peak heights of standard solutions (ACh [Sigma–Aldrich, St. Louis, MO] at 100 and 20 nM) and corrected for *in vitro* recovery of the probe.

Table 1
The random probability of making certain alternation patterns in a four-choice sequence.

Response pattern	Number of permutations	Probability
1111	24	0.093
0211	144	0.56
0022	72	0.28
0031	12	0.46
0004	4	0.015

The number 0 in the response pattern column denotes that the rat did not an arm.