Behavioural Brain Research

Hippocampus and cortex are involved in the retrieval of a spatial memory under full and partial cue availability

--Manuscript Draft--

Manuscript Number:	BBRES-D-21-00118R1
Article Type:	Research Paper
Keywords:	Retrieval; allocentric strategy; cue modification; spatial memory; cytochrome c oxidase, brain metabolism,
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Abstract:	Retaking routes after a period of time usually occurs in an environment which has suffered from spatial configuration modifications. Thus, the original visual stimuli that allowed us to establish cognitive mapping using an allocentric strategy during the acquisition phase may not remain physically identical at the time of retrieval. However, in the standard experimental paradigms the cues are typically maintained constant. In this study, we explored memory retrieval with spatial modifications from learning in the Morris Water Maze. We trained rats on a reference memory protocol with five cues placed on black curtains that surrounded the pool, and seven days later, we tested memory retrieval under different conditions: maintenance of the five cues, removal of two and four of them, and the addition of three extra ones. Under full-cue and partial cue-conditions, rats showed successful memory retrieval, whereas adding extra cues resulted in impaired retrieval. Furthermore, we assessed brain oxidative metabolism through cytochrome c oxidase (CCO) histochemistry and found that, under full- and partial-cue conditions, there is an enhancement of the hippocampal, prefrontal, retrosplenial, parietal, and rhinal cortex metabolism. Rats that failed to retrieve spatial information in the extra cues condition showed similar or lower CCO activity than controls across many limbic areas. It is suggested that the presence of a partial portion of visual stimuli from learning makes it possible to reactivate the entire memory trace, but extra spatial information hinders retrieval, making it difficult to disengage the novel information from the older knowledge and establish a contextual generalization.

Highlights

- Full- and partial-cue availability from acquisition lead to successful retrieval
- Adding extra cues from learning disrupts spatial retrieval in healthy rats
- Fewer, but not extra, cues from learning allow to reactivate a prior memory trace
- Retrieval needs an enhancement of hippocampal and cortex metabolic activity
- CA1 and the retrosplenial cortex show increased activity when extra cues are added

Abstract

Retaking routes after a period of time usually occurs in an environment which has suffered from spatial configuration modifications. Thus, the original visual stimuli that allowed us to establish cognitive mapping using an allocentric strategy during the acquisition phase may not remain physically identical at the time of retrieval. However, in the standard experimental paradigms the cues are typically maintained constant. In this study, we explored memory retrieval with spatial modifications from learning in the Morris Water Maze. We trained rats on a reference memory protocol with five cues placed on black curtains that surrounded the pool, and seven days later, we tested memory +retrieval under different conditions: maintenance of the five cues, removal of two and four of them, and the addition of three extra ones. Under full-cue and partial cue-conditions, rats showed successful memory retrieval, whereas adding extra cues resulted in impaired retrieval. Furthermore, we assessed brain oxidative metabolism through cytochrome c oxidase (CCO) histochemistry and found that, under full- and partial-cue conditions, there is an enhancement of the hippocampal, prefrontal, retrosplenial, parietal, and rhinal cortex metabolism. Rats that failed to retrieve spatial information in the extra cues condition showed similar or lower CCO activity than controls across many limbic areas. It is suggested that the presence of a partial portion of visual stimuli from learning makes it possible to reactivate the entire memory trace, but extra spatial information hinders retrieval, making it difficult to disengage the novel information from the older knowledge and establish a contextual generalization.

Keywords: Retrieval, allocentric strategy, cue modification, spatial memory, cytochrome c oxidase, brain metabolism.

1. Introduction

Spatial navigation is an indispensable cognitive function without which we would lose the ability to find a path to reach a desired location [1]. For this purpose, it is necessary to learn, memorize, and remember the position and spatial relationship between certain geographical landmarks, that is, to shape the mental representations [2,3]. This search strategy -known as allocentric navigation- is based on visual information from the surrounding environment [4].

Spatial configuration can suffer from some variations as a result of human activities or the passage of time, leading to modifications [5]. This means that the original stimuli present during the acquisition of spatial learning and, consequently, used to create a cognitive mapping of the environmental space, may not remain physically identical at the time of retrieval [6]. Thus, recovering spatial memories in a different environmental configuration from the one where the memory was initially formed may influence both behavior and the brain mechanisms underlying the recovery process. However, most of the time, the presence of some of the original stimuli that were present during memory formation seems to make it possible to recover spatial memory traces [6,7]. The successful retrieval of a certain memory from degraded or partial-cue conditions could correspond to a pattern completion process, namely, when an element can be recalled from fragmented stimuli or the altered availability of familiar cues [8–10].

What brain structures are involved in the aforementioned conditions? The pivotal role of the dorsal hippocampus and medial prefrontal cortex has been highlighted. In particular, the CA3 subfield of the hippocampus seems to have an active role in pattern completion; consequently, targeted-lesion studies result in worse behavioral performance [8,11]. In addition, CA3 NMDA receptor- knockout mice show an impaired water maze response, suggesting that synaptic plasticity mechanisms within this area are critical [9]. In order to perform a pattern completion process that relies on the hippocampus, μ opioid receptors may also be essential, highlighting the relevance of mossy fiber opioid-dependent pathways [12]. In addition, it has been pointed out that the medial prefrontal cortex is related to the retrieval of a spatial memory trace that was initially encoded in an environmental configuration that has experienced changes, as well as suggesting the action of NMDA receptors when allocentric spatial information

is degraded [6]. Moreover, it is plausible that pattern completion requires an interaction between hippocampus and prefrontal cortex [7].

Greater knowledge about the brain areas involved in the aforementioned circumstances could make it possible to understand spatial cognition -and specifically, spatial memory retrieval- in situations that are similar to real life. Hence, in the present study, we aimed to analyze the brain areas underlying spatial retrieval in slightly different environmental conditions from those linked to the acquisition phase, with varying degrees of difficulty. To do this, we trained male rats on an allocentric spatial reference memory protocol in the Morris Water Maze (MWM) [13] with five visual distal cues on five consecutive days. Seven days later, we explored their memory retrieval with the same cues, subtracting two or four cues, and adding three cues. Then, to explore the brain-related function, we assessed oxidative metabolism through cytochrome c oxidase (CCO) histochemistry. CCO is a mitochondrial enzyme involved in the oxidative phosphorylation process that generates ATP [14]. This technique is a marker of brain energy demands and reflects changes in metabolic activity in relation to energy consumption of the nervous system [14,15], and in particular it labels both neurons and glia, being neurons the primary site of CCO [16]. Thus, it has been widely used to discern how different brain areas modify their metabolic demands in response to rats' performance across several cognitive tasks, including those linked to spatial learning [17–19].

2. Material and methods

2.1.Subjects

A total of 48 male Wistar rats (266.38 ± 7.62 g. at the beginning of the experiment) were used and maintained at controlled room temperature (20 ± 2 ºC) and humidity (65-70%) under an artificial light-dark cycle of 12 h (08:00-20:00h on/20:00-08:00h off). They had access to food and tap water *ad libitum*. All the experimental procedures were performed according to the European Communities Council Directive 2010/63/UE and the Spanish legislation related to the protection of animals used for experimentation and other scientific purposes (Royal Decree 53/2013). The local committee for animal studies at Oviedo University approved the study. Prior to the behavioral procedure, all the rats were handled daily for seven days. Behavioral tests were performed between 8:00 and 14:00 h.

2.2.Experimental procedure

Five groups of male rats were trained on an allocentric spatial reference memory task with five geometric visual cues with different volumes and color patterns. Seven days after the end of the last session, their memory retrieval linked to modifications in the number of available cues was explored. Rats were randomly divided into the following groups: maintenance of the original five cues (5C, *n=8*), maintenance of one original cue (1C, *n=10*), maintenance of three original cues (3C, *n=10*), maintenance of the original five cues and aggregation of three extra cues (8C, *n=10*), and a learning control group that performed the spatial reference memory task with the five cues and was sacrificed seven days later without performing the retrieval task (C, *n=10*).

2.3.Behavioral procedure

2.3.1. Apparatus

Spatial reference memory and memory retrieval under different conditions were evaluated in the MWM [13]. The maze consisted of a cylindrical fiberglass tank 150 cm in diameter and 40 cm high, supported by a 35 cm high platform. The pool was filled with tap water with a temperature of 22 ± 2 °C, and the level of the water was 30 cm. The MWM was in the center of a 16 m^2 room illuminated by an indirect 4000 lx light. The pool was divided into four imaginary quadrants, three of them non-reinforced and one in which a hidden platform allows rats to escape from the water during learning, in other words, the reinforced quadrant. The escape platform consisted of a cylinder 10 cm in diameter and 28 cm high, hidden 2 cm below the surface of the water. The pool was surrounded by black panels located 30 cm away from the maze. On the panels, five geometric visual cues with different shapes and color patterns were placed, acting as allocentric cues during the learning phase. The selection and arrangement of cues varied during the retrieval phase according to the experimental design. The experimenter was located behind the panels. The rats' behavior was recorded *(*V88E, *Sony, Spain)* using a computerized video-tracking system (Ethovision XT 14.0, *Noldus Information Technologies, Wageningen, The Netherlands*).

2.3.2. Spatial reference learning and memory retrieval task

One day before conducting the spatial reference learning task, rats were habituated to the testing contingencies of the MWM. The 5C, 1C, 3C, 8C, and C groups were subjected to four trials in which they had to reach a visible platform located in the center of the pool. Rats were released from each quadrant facing the pool wall in a pseudo-

randomized sequence. On the following five consecutive days, the rats performed six trials each day, with an inter-trial interval of 30 s. The first four were the acquisition trials, in which rats had to reach the hidden platform located in the reinforced quadrant (in these experiments, quadrant D), after being released from each quadrant facing the pool wall in a pseudo-randomized sequence. Once the rat had found the platform, it remained in the reinforced place for 15 s. If the rat failed to reach the platform after 60 s, it was placed on it for 15 s. After completing the four daily training trials, a 60 s learning probe trial was carried out in which the escape platform was removed and the rat was introduced from the opposite quadrant to where the platform had been located in previous trials. Finally, rats received an additional trial, equal to the four acquisition trials, with the hidden platform located in the usual position to avoid possible extinction of learning. Seven days after the last learning session, groups 5C, 1C, 3C, and 8C were submitted to a memory retrieval test in a single 60 s retrieval probe trial, under the different environmental conditions explained in section 2.2. The C group was euthanatized without performing the retrieval probe trial to establish control of spatial learning. After the once-daily session ended, rats were carefully dried and returned to their home cage. The amount of time in each quadrant during the learning and retrieval probe test was recorded.

2.4.Sacrifice and tissue processing

Groups that performed retrieval, 5C, 1C, 3C and 8C were decapitated 90 minutes after completing the retrieval probe test. C rats underwent the same procedure seven days after the last acquisition session. The encephalon was removed, frozen in N-methyl butane (*Sigma-Aldrich, Germany*), and stored at -40 ° C to make coronal sections 30 µm thick in a cryostat at -20 ° C (*Leica CM1900, Germany*) for CCO histochemistry. The regions of interest and their distances in mm counted from bregma were: +3.24 mm for the cingulate (CG), infralimbic (IL), and prelimbic cortex (PL); -3.48 mm for the CA1 and CA3 subfields of the dorsal hippocampus, dentate gyrus (DG), granular retrosplenial (RSG), agranular retrosplenial (RSA), and parietal cortex (PAR); and −4.68 mm for the entorhinal (ENT) and perirhinal (PHr) cortices, according to Paxinos and Watson's atlas [20] (Figure 1).

2.5.Cytochrome c oxidase histochemistry and optical density quantification

The procedure carried out for tissue treatment was the previously described [21], based on the method [15]. Briefly, tissue sections were fixed in 0.1 phosphate buffer (PB) with 10% (w/v) sucrose and 0.5 (v/v) glutaraldehyde. Next, three baths of 0.1 M phosphate buffer with 10% w/v sucrose were given for 5 min each, and one bath of 0.05M Tris buffer with 275 mg/l cobalt chloride, sucrose, and 0.5 (v/v) dimethyl-sulfoxide. Then, sections were incubated in 0.0075 % cytochrome-c (w/v), 0.002 % catalase (w/v), 5 % sucrose (w/v), 0.25 % dimethylsulfoxide (v/v), and 0.05% diaminobenzidine tetrahydrochloride at 37ºC. Next, the tissue was fixed in buffered formalin with 0.1 M PB with 10% (w/v) sucrose and 37% v/v formalin. Finally, the tissue was dehydrated with alcohol, cleared with xylene, and cover-slipped with Entellan. Sets of tissue homogenate standards from Wistar rat brains were cut at different thicknesses (10, 30, 40, and 60 μm) and included with each bath of slides to control possible variations in staining intensity across different baths. The CCO histochemical staining intensity was quantified by optical densitometry using a computer-assisted image analysis workstation (*MCID, Interfocus Imaging Ltd., Linton, England*) consisting of a high precision illuminator, a digital camera, and a computer with specific image analysis software. The mean optical density values were transformed into CCO activity units, determined by the enzymatic activity of the standards measured [15].

2.6.Statistical analysis

All behavioral and CCO activity data were analyzed using the SigmaStat 12.5 program (*Systat, Richmond, USA*). Shapiro-Wilk's test was employed to evaluate the normality assumption, and the Levene test was used to assess homoscedasticity. When the data fit a normal distribution and the variances were equally distributed ($P > .05$), one-way ANOVA was used; otherwise, Kruskal-Wallis One Way Analysis of Variance on Ranks was selected. In the case of the behavioral data, the time spent in the reinforced quadrant (D) was compared to the time spent in the non-reinforced quadrants (A, B, and C). A statistically significant difference between D and the remaining three quadrants was considered a learning and retrieval criterion. With regard to CCO activity data, a separate analysis was performed in each brain region. Statistical differences were considered significant at the .05 level. Then, the Holm-Sidak post-hoc method was applied with parametric tests, and Dunn's Method with non-parametric procedures. Data

(expressed as mean ± SEM) were graphically represented with the SigmaPlot 12.5 program (*Systat, Richmond, USA*).

3. Results

3.1.Behavioral results

Time spent in each pool's quadrant of the 5C group revealed differences between them from the first day (D1: $F_{(3, 28)}$= 29.731, *P*< .001; D2: $F_{(3, 28)}$= 9.519, *P*< .001; D3: $F_{(3, 28)}$= 14.675, *P*< .001; D4: $F_{(3, 28)}$= 29.574, *P*< .001; D5: $F_{(3, 28)}$= 81.387, *P*< .001), and post-hoc analysis showed differences between quadrant D and the rest of the quadrants every day of the test (*P*< .05), in addition to differences between quadrants C and A (*P*= .044) and B (*P*< .001) and between A and B (*P*= .031) on day one. Thus, rats reached the learning criteria from the second day. In the case of the retrieval probe test, the 5C group showed differences between quadrants ($F_{(3, 28)}$= 6.225, *P*= .002), with the Holm-Sidak method revealing more time spent in the reinforced quadrant than in the non-reinforced quadrants (*P*< .05). Consequently, 5C rats achieved the retrieval criteria (Figure 2A).

The 1C group showed successful learning from the third day (D1: $F_{(3, 36)}$= 11.324, *P*< .001; D2: $F_{(3, 36)}$= 44.461, *P*< .001; D3: $F_{(3, 36)}$= 42.062, *P*< .001; D4: $F_{(3, 36)}$= 42.752, *P*< .001; D5: $F_{(3, 36)}$= 80.740, *P*< .001) because post-hoc procedures revealed differences between quadrant D and the rest of the quadrants every day of the test (*P*< .05), whereas differences between quadrants A and B were found on the first day (*P*= .025), and between A and B (*P*= .013) and C (*P*= .010) on the second day. Finally, the 1C group showed differences between quadrants on the retrieval day ($F_{(3, 36)}$= 6.225, *P*= .002), revealing differences between the target quadrant and the rest of the quadrants (*P*< .05) (Figure 2B).

The 3C group also showed differences between quadrants from the first day of the test (D1: $H_{(3)}$= 14.046, *P*= .003; D2: $F_{(3, 36)}$= 29.153, *P*< .001; D3: $F_{(3, 36)}$= 104.556, *P*< .001; D4: $F_{(3, 36)}$= 51.083, *P*< .001; D5: $F_{(3, 36)}$= 85.638, *P*< .001), with post-hoc analysis revealing that the learning criterion was achieved on the second, fourth, and fifth days, given that differences were found between quadrant D and the rest of the quadrants (*P*< .001). On the first day, there was a difference between quadrant B and quadrants A and D (*P*< .05), and on the third day, apart from the target quadrant, when comparing A, B, and C, there was a difference between A (*P*= .001) and C (*P*= .002). With regard to the

retrieval criteria, the 3C group reached it ($F_{(3, 36)}$= 7.259, P< .001), showing differences between the reinforced-quadrant and the non-reinforced quadrants (P< .05) (Figure 2C).

The 8C group also displayed differences between quadrants (D1: $F_{(3, 36)}$= 14.877, P< .001; D2: $F_{(3, 36)}$= 18.775, P< .001; D3: $F_{(3, 36)}$= 74.156, P< .001; D4: $H_{(3)}$= 27.434, P< .001; D5: $H_{(3)}$= 30.339, P< .001), revealing successful learning on day one and from the third day to the end of the test, due to differences between the target quadrant and the non-target quadrants (P< .05). Moreover, on the second day, rats showed a difference between A and B (P= .048) and C (P= .05). However, when seven days had elapsed and three cues were added, the rats did not show differences between quadrants (P= .161) (Figure 2D).

Finally, the C group achieved the learning criteria from day three (D1: $F_{(3, 32)}$= 59.293, P< .001; D2: $F_{(3, 33)}$= 38.822, P< .001; D3: $H_{(3)}$= 21.368, P< .001; D4: $F_{(3, 32)}$= 56.133, P< .001; D5: $F_{(3, 32)}$= 55.937, P< .001) because they showed differences between quadrant D and the rest of the quadrants. Moreover, on day one, the C group displayed differences between A compared to B and C (P< .001) on day one, and on day two, differences were found between A and C (P= .010) (Figure 2E).

3.2.CCO activity results

Analysis of CCO activity showed differences between groups across all the brain areas measured (CG: $F_{4, 42}$ = 13.484, P< .001; PL: $F_{4, 42}$ = 13.041, P< .001; IL: $F_{2, 42}$ = 14.25, P< .001; CA1: $F_{4, 41}$ = 16.914, P< .001; CA3: $F_{4, 42}$ = 12.138, P< .001; DG: $F_{4, 41}$ = 8.816, P< .001; RSG: $H_{(4)}$= 29.886, P< .001; RSA: $F_{5, 42}$ = 11.739, P< .001; PAR: $H_{(4)}$= 23.289, P< .001; PRh: $F_{4, 39}$ = 10.154, P< .001; ENT: $F_{4, 39}$ = 9.603, P< .001). In particular, CG, PL, IL, CA1, CA3, RSG, RSA, PAR, PRh and ENT showed higher CCO activity in the 5C, 1C, and 3C groups, in comparison with the C group (P< .05), whereas this difference with respect to the C group was limited to the 5C and 1C groups in DG (P< .05). Furthermore, the 5C, 1C, 3C, and C groups also showed an enhancement of CCO activity in CG, PL, PRh, and ENT when compared to the 8C group (P< .05). In the case of IL, CA1, and CA3, the difference compared to the 8C group was noted in the 5C and 1C groups (P< .05). Finally, CA1 and RSA areas showed a statistically significant difference between the 8C and C groups, revealing higher CCO activity in the 8C group (Figure 3).

4. Discussion

In the present study, we aimed to determine the recall of an allocentric MWM spatial memory after performing modifications in the distal cues, in relation to the learning phase one-week earlier, with various degrees of difficulty, along with the underlying metabolism of hippocampal and cortical brain areas. Rats subjected to the removal of some of the original visual distal cues during retrieval achieved adequate retrieval, whereas those exposed to extra cues, in addition to those from the acquisition phase, failed to retrieve the spatial memory. Overall, brain metabolism results showed increased CCO activity in groups that successfully retrieved the spatial memory, particularly within the hippocampus and the prefrontal, retrosplenial, parietal, and rhinal cortices, compared to controls. Moreover, there was an enhancement of CCO activity in the hippocampus and the prefrontal and rhinal cortices in the majority of the successful-retrieval groups in comparison with the group that failed to solve the task.

The spatial configuration of certain places may change due to the passage of time [5]. Consequently, in everyday life, the retrieval of spatial memories does not always occur in an environment with the same stimuli configuration where the memory was primarily formed. Indeed, when we return to a specific location, it is rare to find the same sets of cues that were available during memory formation [6,7]. However, in the majority of the experimental paradigms the cues are typically maintained constant, leading to a lack of knowledge about real-life scenario. The animal studies related to this topic have shown that, under cue modification, it is possible to navigate successfully and reach the goal location [6,7,9,22], probably because the presence of some of the original cues, or a partial version of them, allows the animal to recover the entire memory [6]. Moreover, as often occurs in humans [23], rodents are able to detect contextual changes, leading to a spatial discrimination cognitive ability that could help under cue modification paradigms [8,24].

In order to achieve the task, rats need to use an allocentric navigational strategy; that is, they need to learn the location and establish a spatial relationship between visual cues, leading to a complex cognitive map formation [3,25]. As expected, all the groups (5C, 3C, 1C, 8C, and C) were able to learn the task from day two or three. Seven days after the last learning session, we assessed spatial retrieval under four different circumstances: with the availability of the five cues present in the spatial learning (5C),

by subtracting two (3C) or four (1C) cues, and by adding three extra cues (8C). Spatial retrieval studies with room configuration alterations have been performed after short time intervals, such as 10 minutes [24], two [6] or three hours [7], or one day after training [9,22,26,27]. However, longer periods of time have not been depicted yet. It should be highlighted that, depending on the remoteness of the event, we can refer to a recent or remote memory, which is accompanied by behavioral and brain activity distinctions [28]. Considering that the passage of time leads to a decline in spatial memories [17,29], we have chosen to study relatively recent recall in order to avoid confounding effects. Across different spatial tasks, several authors have shown that a time interval of six or seven days is long enough to trigger robust memory retrieval [29–32]. Thus, we can claim that the retrieval difficulties in our experiment are a consequence of the modification of the extra-maze cues, rather than the remoteness of the spatial retrieval probe.

On the one hand, we have shown that subtracting allocentric cues triggers successful retrieval because groups 3C and 1C achieved the retrieval criteria by spending more time swimming in the target quadrant. These results indicate that the degradation of cues does not impair remembering, in agreement with previous MWM studies in healthy animals [6,7,9,22,26,27], suggesting that a partial portion of landmarks are sufficient to maintain a spatial memory [27]. Rats are able to detect mild contextual changes, as reported Seib et al. [24]. Interestingly, if subjects are exposed to a differential room configuration thorough the distal cue novelty task, which consists on the same room configurational setting in five consecutive trials, and locating a black curtain that covers part of the room on the sixth trial, there are information-gathering behaviors in response to the room modification, proposing that rats are able to discriminate contextual changes [24]. Furthermore, studies have revealed that, after removing cues on an exploratory task, rats maintain their preference to stay adjacent to the place where the cues were previously located [33]. In the present study, we cannot ensure that the 3C and 1C groups were able to note contextual changes because we did not study this specifically, for example, by exploring the proximity of their swimming to a previous cue location during retrieval. However, we can robustly state that, despite the diminished spatial information, they were able to orient themselves like the 5C group, which were exposed to the same spatial setting one week later. This may be because they shift their attention across the entire set of cues during learning [6], and the

subsequent visualization of a partial portion allows them to reactivate the cognitive mapping.

On the other hand, the 8C group failed to solve the task, with no target quadrant preference, showing that the addition of several cues impairs memory retrieval, in accordance to Fenton et al. [27], which observed a similar disruption of the spatial MWM performance. Opposite results have been also found. Thus, Jo and Choi [6] revealed successful retrieval by adding three cues in relation to the acquisition phase. However, our study differs from this in the time-interval between learning and retrieval, creating a more difficult procedure in which spatial details can be weakened. The passage of time has been shown to trigger a more schematic spatial representation at the expense of a more detailed one [34]. With a one-week interval, the addition of extra cues could challenge the brain spatial-schema. Our results suggest that the 8C group is not capable of disengaging the novel spatial information from the older information, thus failing to establish a contextual generalization, *i.e.* extracting the variance in an item and applying it to a new instance [35], as a result of an interference process [27]. It is worth noting that generalization in contextual cueing can be facilitated by implementing cue variability during the learning phase [35]. The 8C group results are also supported by a recent study conducted in pigeons and humans, which showed that, on a spatial change detention task, the sensory memory system is less accurate in recognizing visual changes when more items are used [23]. Moreover, when cues are placed in unpredictable locations, it is possible to find a behavioral perturbation due to an overshadowing of the environmental stimuli [36]. This disruptive effect of distraction can also be found across different cognitive tasks, such as on the contextual fear conditioning, where a reactivation session in the presence of distractor stimuli leads to a reduction in the freezing response [37].

Lastly, for the behavioral results, it is important to note that across all the experimental groups, the cue that was closer to the platform location, that is, the green pentagon (see Figure 1), remained fixed during retrieval. Therefore, the rats could be employing a cue-guide strategy in order to solve the task, where the availability of only one cue can act as a beacon [38]. Nevertheless, the presence of the green pentagon in the retrieval phase of the 8C group does not prevent failed retrieval of the platform location, which indicates that rats are employing the allocentric strategy to guide their behavior. This hypothesis is supported by a previous study in which two out of four cues were removed

in the retrieval phase, and the successfully MWM performance was the same regardless of the set of cues deleted [27].

According to systems consolidation, the maintenance of a certain memory trace relies on the reorganization of the related brain networks [39]. It is postulated that hippocampal-dependent memories, such as spatio-temporal events, become less dependent on the hippocampus in order to be stored in neocortical networks, thus establishing a functional hippocampal-cortical dialogue [39,40]. However, it has also been suggested that the hippocampus is always needed during retrieval, especially to remember fine spatial details [7,9,41]. Conversely, there is little research on the brain mechanisms underlying the retrieval of a past event that does not occur in exactly the same configuration setting as when it was originally formed.

Hence, in this study, we examined the brain metabolism underlying the subtraction or addition of cues during spatial MWM retrieval. Specifically, CCO activity was assessed within the prefrontal cortex (CG, PL, IL), hippocampus (CA1, CA3, DG), and retrosplenial (RSG, RSA), parietal, and rinhal (PRh, ENT) cortices. Controls were used to isolate the brain metabolic activity linked to the learning that occurred seven days earlier. Thus, control rats were trained in the same conditions, but they were not subjected to the spatial retrieval task.

Regarding the prefrontal data, our results showed that there was an increase in the CCO activity within the CG and PL areas when rats achieved the retrieval criteria (5C, 1C, and 3C groups), in comparison with those that failed to solve the task (8C group) and the C group. The IL showed similar results, except that the 3C group did not show metabolic differences with respect to the 8C group. In the 8C group, when the rats were not capable of remembering the spatial task due to the extra cue information, their prefrontal brain metabolism remained similar to controls. Overall, these results suggest the involvement of prefrontal areas when total cues are available, but also in degraded cue conditions, in agreement with previous studies also focused on metabolic demands [22]. Nevertheless, it is important to highlight that Arias et al. [22] trained rats in a partial-cue environment after performing four days of spatial training under full-cue conditions, without the time-interval of one-week we used to test retrieval. Jo et al. [7] revealed that prefrontal lesions trigger spatial memory deficits under partial cue-conditions, and further analysis revealed the key role of N-methyl-D-aspartate receptors

(NMDARs) [6]. NMDAR antagonists led to impairments when recalling a spatial trace memory with the majority of the extra-maze cues removed, suggesting their role in the reactivation of the complete cognitive map associated with partial retrieval stimuli [6].

With regard to hippocampal energy consumption, we noticed an enhancement on the CA1 and CA3 subfields in the 5C, 1C and 3C groups, in comparison with the C group, in addition to higher CA1 metabolic activity in the 8C rats compared to controls. Also, the 5C and 1C group showed higher CA1 and CA3 metabolic activity when compared to the group that failed to solve the task. The DG showed higher metabolism in the 5C and 1C groups in comparison with the 8C group and controls. In addition, there were differences in DG metabolism between the group with complete cue availability and the one with the subtraction of two extra-maze cues, suggesting a role of the DG in detecting fine spatial details [42]. The recall of an entire memory from its fragments is known as pattern completion [9,10,43], which takes place by identifying a prior similar experience [44], and it relies on excitatory connections from the CA3 hippocampal cells [9,11] to the CA1 and to the ENT [44]. Moreover, retrieval can be hippocampal-dependent and may reinstate neocortical areas linked to the initial memory storage [45]. The ENT establishes projections to the DG through the perforant path and via mossy fibers the DG projects to the CA3. Then, the CA3 neurons interact with the CA1 regions via Schaffer collaterals (for details, see [45]. Most pattern completion research has focused on deciphering the pivotal hippocampal role, suggesting its importance in extracting differences in spatial stimuli [8–11]. The CA1 and CA3 metabolic results obtained in the present study coincide with Arias et al. [22], and add evidence about hippocampal metabolism recruitment during spatial retrieval in both partial-cue and full-cue conditions. It has been shown that CA3 lesions triggers to impaired memory retrieval under partial-cue conditions [7], specifically requiring the functionality of the CA3 neurotransmitter NMDARs [9], but not if there is a lack of CA1 NMDARs limited to the recall stage under full-cue and degraded-cue conditions [26]. However, the NMDARs gene ablation in the CA3 pyramidal cells leads to a reduction in the activity of CA1 pyramidal neurons upon partial cue removal, suggesting that a diminished CA3 drive to CA1 can alter the coding properties of CA1 places cells, and as a result, make more difficult to retrieve spatial memories under cue removal conditions [9]. Furthermore, similar to what occurs in prefrontal areas, hippocampal CCO activity in the 8C group remains at control levels, with the CA1 exception mentioned above. We

hypothesize that, because the CA1 subfield seems to be essential in retrieving fine details [44], and its functionality is indispensable for memory consolidation [46] and reconsolidation [37], the slight brain metabolism difference could indicate that the rats are trying to refresh the cognitive map, which is a challenging task.

Regarding the PAR and retrosplenial cortices, we found that the group with full-cue availability and both cue-removal groups showed higher CCO activity than controls. Moreover, comparable results to those in CA1 were found within RSA because adding extra cues also resulted in higher activity in comparison with the C group. PAR CCO activity data are in accordance with Arias et al. [22]. However, no research has focused on deciphering the retrosplenial involvement in retrieval with several degrees of difficulty. Moreover, the retrosplenial cortex supports allocentric navigation [47]; as a result, its functional projections between limbic regions essential for memory acquisition and storage, including the hippocampus and other cortical structures such as the prefrontal and PAR [48], can play a pivotal role during spatial recall [29]. Moreover, the RSA, but not the RSG, is densely connected to visual areas [49]. Thus, it has a key function in spatial discrimination [50], which could partly explain the RSA CCO results when adding extra cue-information. As for the rhinal metabolic demands, there is increased CCO activity linked to successful retrieval because differences were found compared to the group that failed the task and controls in both rhinal cortices. The ENT can play a functional role during the pattern completion process, due to its projections to the hippocampus [45], and its active function during retrieval has been described in animals [30] and humans [51]. Finally, a targeted inactivation of PRh leads to spatial cognition deficits, specifically within the retrieval –and not during consolidation– phase, proposing that this area is differentially involved in the recall of allocentric spatial information [52]. Therefore, we suggest that all these cortical areas are essential, not only during spatial retrieval under full-cue conditions, but also when the environmental stimuli are slightly different from the acquisition phase.

Future research is needed to more closely examine the brain basis responsible of noticing distal environmental changes, and delve into the related behavioral response. Thus, as it has been reported, rats are able to retrieve a previously learned spatial location under partial cue availability with a time-interval up to one week, it can be interesting to study whether this memory is maintained for longer periods of time, adding information about what happens in terms allocentric cue alterations in remote

memories. Moreover, we have noticed an impaired spatial retrieval when adding distractor stimuli, but we do not know if the higher amounts of cues surrounding the pool rather than the addition of extra ones are the determinant factors of the impaired behavioral response. Therefore, this issue may be addressed by training rats with only one or two cues, and add in the retrieval phase the extra ones. Finally, given the fact that sex differences have been depicted in spatial learning and memory [53–55], it could be fruitful to perform the reported experiments in females, as its behavioral and underlying brain response may be different.

5. Conclusions

Our study shows that healthy male rats are able to retrieve a one-week spatial MWM memory with accessibility to the entire set of cues inherent to the prior acquisition, but also under partial-cue conditions, leading to an enhancement of hippocampal, prefrontal, retrosplenial, parietal, and rhinal cortex metabolism. However, the addition of extra cue information triggers impaired memory recall, with the subsequent decrease in CCO activity across many limbic areas.

Financial Support

This study was funded by Project grants of Secretaría de Estado de Investigación, Desarrollo e Innovación del Gobierno de España (PSI2017-83893-R and PSI2017-90806-REDT) and Programa "Severo Ochoa" de Ayudas Predoctorales de la Consejería de Cultura y Deporte del Principado de Asturias (PA-18-PF-BP17-011) to C.Z.

Acknowledgements

We thank the AINDACE Foundation (Ayuda a la Investigación del Daño y Enfermedades Cerebrales).

Declarations of interest: none.

References

[1] C. V. Vorhees, M.T. Williams, Assessing spatial learning and memory in rodents, ILAR J. 55 (2014) 310–332. https://doi.org/10.1093/ilar/ilu013.

[2] R.A. Epstein, E.Z. Patai, J.B. Julian, H.J. Spiers, The cognitive map in humans:

Spatial navigation and beyond, Nat. Neurosci. 20 (2017) 1504–1513. https://doi.org/10.1038/nn.4656.

[3] E.C. Tolman, Cognitive maps in rats and men, Psychol. Rev. 55 (1948) 189–208. https://doi.org/10.1037/h0061626.

[4] T.D. Ferguson, S.A. Livingstone-Lee, R.W. Skelton, Incidental learning of allocentric and egocentric strategies by both men and women in a dual-strategy virtual Morris Water Maze, Behav. Brain Res. 364 (2019) 281–295. https://doi.org/10.1016/j.bbr.2019.02.032.

[5] L. Chu, X.R. Zhang, T.W. Wang, Z.X. Li, C.F. Cai, Spatial-temporal evolution and prediction of urban landscape pattern and habitat quality based on CA-Markov and InVEST model, Chinese J. Appl. Ecol. 29 (2018) 4106–4118. https://doi.org/10.13287/j.1001-9332.201812.013.

[6] Y.S. Jo, J.S. Choi, Memory retrieval in response to partial cues requires NMDA receptor-dependent neurotransmission in the medial prefrontal cortex, Neurobiol. Learn. Mem. 109 (2014) 20–26. https://doi.org/10.1016/j.nlm.2013.11.004.

[7] Y. Sang Jo, H.P. Eun, H.K. Il, K.P. Soon, H. Kim, T.K. Hyun, J.S. Choi, The medial prefrontal cortex is involved in spatial memory retrieval under partial-cue conditions, J. Neurosci. 27 (2007) 13567–13578. https://doi.org/10.1523/JNEUROSCI.3589-07.2007.

[8] R.P. Kesner, R.A. Kirk, Z. Yu, C. Polansky, N.D. Musso, Dentate gyrus supports slope recognition memory, shades of grey-context pattern separation and recognition memory, and CA3 supports pattern completion for object memory, Neurobiol. Learn. Mem. 129 (2016) 29–37. https://doi.org/10.1016/j.nlm.2015.08.010.

[9] K. Nakazawa, M.C. Quirk, R.A. Chitwood, M. Watanabe, M.F. Yeckel, L.D. Sun, A. Kato, C.A. Carr, M.A. Wilson, S. Tonegawa, Requirement for hippocampal CA3 NMDA receptors in associative memory recall, Science (80-.). 297 (2002) 211–218. https://doi.org/10.1126/science.1071795.Requirement.

[10] A. Treves, E.T. Rolls, Computational analysis of the role of the hippocampus in memory, Hippocampus. 4 (1994) 374–391. https://doi.org/10.1002/hipo.450040319.

[11] A.E. Gold, R.P. Kesner, The role of the CA3 subregion of the dorsal hippocampus in spatial pattern completion in the rat, Hippocampus. 15 (2005) 808–814. https://doi.org/10.1002/hipo.20103.

[12] R.P. Kesner, D.K. Warthen, Implications of CA3 NMDA and opiate receptors for spatial pattern completion in rats, Hippocampus. 20 (2010) 550–557. https://doi.org/10.1002/hipo.20676.

[13] R.G. Morris, Developments of a water-maze procedure for studying spatial learning in the rat, J. Neurosci. Methods. 11 (1984) 47–60. https://doi.org/10.1016/0165-0270(84)90007-4.

[14] M.T. Wong-Riley, Cytochrome oxidase: an endogenous metabolic marker for neuronal activity., Trends Neurosci. 12 (1989) 94–101.

[15] F. Gonzalez-Lima, A. Cada, Cytochrome oxidase activity in the auditory system of the mouse: A qualitative and quantitative histochemical study, Neuroscience. 63 (1994) 559–578. https://doi.org/10.1016/0306-4522(94)90550-9.

[16] F. Gonzalez-Lima, Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease, Plenum Press, New York, 1998.

[17] C. Zorzo, J.L. Arias, M. Méndez, Retrieval of allocentric spatial memories is preserved up to thirty days and does not require higher brain metabolic demands, Neurobiol. Learn. Mem. 175 (2020) 1–12. https://doi.org/10.1016/j.nlm.2020.107312.

[18] M. Méndez-López, M. Méndez, P. Sampedro-Piquero, J.L. Arias, Spatial learning-related changes in metabolic activity of limbic structures at different posttask delays, J. Neurosci. Res. 91 (2013) 151–159. https://doi.org/10.1002/jnr.23134.

[19] C. Zancada-Menendez, P. Alvarez-Suarez, P. Sampedro-Piquero, M. Cuesta, A. Begega, Requiring collaboration: Hippocampal-prefrontal networks needed in spatial working memory and ageing. A multivariate analysis approach, Neurobiol. Learn. Mem. 140 (2017) 33–42. https://doi.org/10.1016/j.nlm.2017.02.008.

[20] G. Paxinos, C. Watson, The rat brain in stereotaxic coordinates, Elsevier

Academic Press, 2005.

[21] C. Zorzo, M. Méndez-López, M. Méndez, J.L. Arias, Adult social isolation leads to anxiety and spatial memory impairment: Brain activity pattern of COx and c-Fos, Behav. Brain Res. 365 (2019) 170–177. https://doi.org/10.1016/j.bbr.2019.03.011.

[22] N. Arias, M. Méndez, G. Vallejo, J.L. Arias, Finding the place without the whole: Timeline involvement of brain regions, Brain Res. 1625 (2015) 18–28. https://doi.org/10.1016/j.brainres.2015.08.021.

[23] K. Leising, J. Jacqmain, C. Elliott, J. Wolf, J. Taylor, L. Cleland, R. Lee, J.F. Magnotti, A.A. Wright, Sensory and working memory in a spatial change-detection task by pigeons and humans, Behav. Processes. 169 (2019) 103957. https://doi.org/10.1016/j.beproc.2019.103957.

[24] D.R. Seib, E. Chahley, O. Princz-Lebel, J. Scott Snyder, Intact memory for local and distal cues in male and female rats that lack adult neurogenesis, PLoS One. 13 (2018) 1–15. https://doi.org/10.1371/journal.pone.0197869.

[25] J. Rogers, L. Churilov, A.J. Hannan, T. Renoir, Search strategy selection in the Morris water maze indicates allocentric map formation during learning that underpins spatial memory formation, Neurobiol. Learn. Mem. 139 (2017) 37–49. https://doi.org/10.1016/j.nlm.2016.12.007.

[26] B. Mei, F. Li, Y. Gu, Z. Cui, J.Z. Tsien, Nmda receptors are not required for pattern completion during associative memory recall, PLoS One. 6 (2011). https://doi.org/10.1371/journal.pone.0019326.

[27] A.A. Fenton, M.P. Arolfo, J. Bures, Place navigation in the morris water maze under minimum and redundant extra-maze cue conditions, Behav. Neural Biol. 62 (1994) 178–189. https://doi.org/10.1016/S0163-1047(05)80016-0.

[28] A. Doron, I. Goshen, Investigating the transition from recent to remote memory using advanced tools, Brain Res. Bull. 141 (2018) 35–43. https://doi.org/10.1016/j.brainresbull.2017.09.005.

[29] M.M. Milczarek, S.D. Vann, F. Sengpiel, Spatial Memory Engram in the Mouse Retrosplenial Cortex, Curr. Biol. 28 (2018) 1975-1980.e6.

https://doi.org/10.1016/j.cub.2018.05.002.

[30] D.N. Barry, A.N. Coogan, S. Commins, The time course of systems consolidation of spatial memory from recent to remote retention: A comparison of the Immediate Early Genes Zif268, c-Fos and Arc, Neurobiol. Learn. Mem. 128 (2016) 46–55. https://doi.org/10.1016/j.nlm.2015.12.010.

[31] M. Mendez-Couz, N.M. Conejo, H. Gonzalez-Pardo, J.L. Arias, Functional interactions between dentate gyrus, striatum and anterior thalamic nuclei on spatial memory retrieval, Brain Res. 1605 (2015) 59–69. https://doi.org/10.1016/j.brainres.2015.02.005.

[32] V. Ramíarez-Amaya, I. Balderas, J. Sandoval, M.L. Escobar, F. Bermúdez-Rattoni, Spatial long-term memory is related to mossy fiber synaptogenesis, J. Neurosci. 21 (2001) 7340–7348. https://doi.org/10.1523/jneurosci.21-18-07340.2001.

[33] H. Lehmann, B.. Clark, I.Q. Whishaw, Similar Development of Cued and Learned Home Bases in Control and Hippocampal-Damaged Rats in an Open Field Exploratory Task, Hippocampus. 17 (2007) 370–380. https://doi.org/10.1002/hipo.

[34] J.M.J. Ramos, Is spatial memory transformed during the consolidation process? Effect of reminding, Acta Neurobiol. Exp. (Wars). 69 (2009) 545–551.

[35] Y. Higuchi, Y. Ueda, K. Shibata, J. Saiki, Spatial Variability Induces Generalization in Contextual Cueing, J. Exp. Psychol. Learn. Mem. Cogn. (2019). https://doi.org/10.1037/xlm0000796.

[36] C. Brandner, Perinatal choline treatment modifies the effects of a visuo-spatial attractive cue upon spatial memory in naive adult rats, Brain Res. 928 (2002) 85–95. https://doi.org/10.1016/S0006-8993(01)03363-7.

[37] A.P. Crestani, F. Zacouteguy Boos, J. Haubrich, R. Ordoñez Sierra, F. Santana, J.M.D. Molina, L.D.F. Cassini, L.D.O. Alvares, J.A. Quillfeldt, Memory reconsolidation may be disrupted by a distractor stimulus presented during reactivation, Sci. Rep. 5 (2015) 1–9. https://doi.org/10.1038/srep13633.

[38] D.R. Harvey, L. Brant, S. Commins, Differences in cue-dependent spatial

navigation may be revealed by in-depth swimming analysis, Behav. Processes. 82 (2009) 190–197. https://doi.org/10.1016/j.beproc.2009.06.008.

[39] P.W. Frankland, B. Bontempi, The organization of recent and remote memories, Nat. Rev. Neurosci. 6 (2005) 119–130. https://doi.org/10.1038/nrn1607.

[40] L.R. Squire, P. Alvarez, Retrograde amnesia and memory consolidation:a neurobiological perspective, Curr. Opin. Neurobiol. PG - 178. 5 (1995) 183.

[41] L. Nadel, M. Moscovitch, Memory consolidation, retrograde amnesia and the hippocampal complex, Curr. Opin. Neurobiol. 7 (1997) 217–227. https://doi.org/10.1016/S0959-4388(97)80010-4.

[42] M.R. Hunsaker, J.S. Rosenberg, R.P. Kesner, The role of the dentate gyrus, CA3a,b, and CA3c for detecting spatial and environmental novelty, Hippocampus. 18 (2008) 1064–73. https://doi.org/10.1002/hipo.20464.

[43] E.T. Rolls, The storage and recall of memories in the hippocampo-cortical system, Cell Tissue Res. 373 (2018) 577–604. https://doi.org/10.1007/s00441-017-2744-3.

[44] D. Poli, B.C. Wheeler, T.B. DeMarse, G.J. Brewe, Pattern separation and completion of distinct axonal inputs transmitted via micro-tunnels between co-cultured hippocampal dentate, CA3, CA1 and entorhinal cortex networks Daniele, J. Neural Eng. 15 (2018). https://doi.org/10.1088/1741-2552/aabc20.Pattern.

[45] E.T. Rolls, The mechanisms for pattern completion and pattern separation in the hippocampus, Front. Syst. Neurosci. 7 (2013) 1–21. https://doi.org/10.3389/fnsys.2013.00074.

[46] L. Parsaei, A. Torkaman-Boutorabi, F. Asadi, M.R. Zarrindast, Interaction between dorsal hippocampal NMDA receptors and lithium on spatial learning consolidation in rats, Brain Res. Bull. 127 (2016) 1–10. https://doi.org/10.1016/j.brainresbull.2016.07.007.

[47] T.P. Todd, D.I. Fournier, D.J. Bucci, Retrosplenial cortex and its role in cue-specific learning and memory, Neurosci. Biobehav. Rev. 107 (2019) 713–728. https://doi.org/10.1016/j.neubiorev.2019.04.016.

[48] A.P. Miller, L.C. Vedder, M.L. Law, D.M. Smith, Cues, context, and long-term memory: The role of the retrosplenial cortex in spatial cognition, Front. Hum. Neurosci. 8 (2014) 1–15. https://doi.org/10.3389/fnhum.2014.00586.

[49] Q. Wang, O. Sporns, A. Burkhalter, Network analysis of corticocortical connections reveals ventral and dorsal processing streams in mouse visual cortex, J. Neurosci. 32 (2012) 4386–4399. https://doi.org/10.1523/JNEUROSCI.6063-11.2012.

[50] E.L. Hindley, A.J.D. Nelson, J.P. Aggleton, S.D. Vann, The rat retrosplenial cortex is required when visual cues are used flexibly to determine location, Behav. Brain Res. 263 (2014) 98–107. https://doi.org/10.1016/j.bbr.2014.01.028.

[51] S.E. Qasim, J. Miller, C.S. Inman, R.E. Gross, J.T. Willie, B. Lega, J. Lin, A. Sharan, C. Wu, M.R. Sperling, S.A. Sheth, G.M. Mckhann, E.H. Smith, C. Schevon, J.M. Stein, J. Jacobs, Memory retrieval modulates spatial tuning of single neurons in the human entorhinal cortex, Nat. Neurosci. 22 (2019) 2078–2086. https://doi.org/10.1038/s41593-019-0523-z.Memory.

[52] J.M.J. Ramos, Perirhinal cortex lesions produce retrograde amnesia for spatial information in rats: Consolidation or retrieval?, Learn. Mem. 15 (2008) 587–596. https://doi.org/10.1101/lm.1036308.

[53] C. Chow, J.R. Epp, S.E. Lieblich, C.K. Barha, L.A.M. Galea, Sex differences in neurogenesis and activation of new neurons in response to spatial learning and memory, Psychoneuroendocrinology. 38 (2013) 1236–1250. https://doi.org/10.1016/j.psyneuen.2012.11.007.

[54] Z. Jonasson, Meta-analysis of sex differences in rodent models of learning and memory: A review of behavioral and biological data, Neurosci. Biobehav. Rev. 28 (2005) 811–825. https://doi.org/10.1016/j.neubiorev.2004.10.006.

[55] A.K. Munion, J.K. Stefanucci, E. Rovira, P. Squire, M. Hendricks, Gender differences in spatial navigation: Characterizing wayfinding behaviors, Psychon. Bull. Rev. 26 (2019) 1933–1940. https://doi.org/10.3758/s13423-019-01659-w.

Figure legends

Figure 1. Sampling frames of CCO histochemistry. CG= Cingulate cortex, PL= Prelimbic cortex, IL=Infralimbic cortex, DG= Dentate Gyrus, RSG= Granular retrosplenial cortex, RSA= Agranular retrosplenial cortex, PAR= Parietal cortex, PRh= Perirhinal cortex, ENT= Entorhinal cortex.

Figure 2. Spatial reference learning and memory retrieval results in the MWM. Time spent in reinforced and non-reinforced quadrants during the learning and retrieval probe tests. The x-axis shows the days. (A) The 5C group reached the learning criteria from day two and revealed successful retrieval. (B) The 1C group reached the learning criteria from day three and revealed successful retrieval. (C) The 3C group reached the learning criteria on days two, four, and five and showed successful retrieval. (D) The 8C group reached the learning criteria from day three and showed impaired retrieval. (E) The C group reached the learning criteria from day three. DR= Day of Retrieval. Statistical differences were considered significant if $*P \leq .05$.). Data are expressed as mean ± standard error of mean (SEM).

Figure 3. CCO values (mean ± SEM) in 5C, 1C, 3C, 8C, SC, and C groups. CG= Cingulate cortex, PL= Prelimbic cortex, IL=Infralimbic cortex, DG= Dentate Gyrus, RSG= Granular retrosplenial cortex, RSA= Agranular retrosplenial cortex, PAR= Parietal cortex, PRh= Perirhinal cortex, ENT= Entorhinal cortex. Differences with C ($*P \leq .05$). Differences with 8C ($+P \leq .05$). Differences with 3C ($\#P \leq .05$). Data areexpressed as mean ± standard error of mean (SEM).

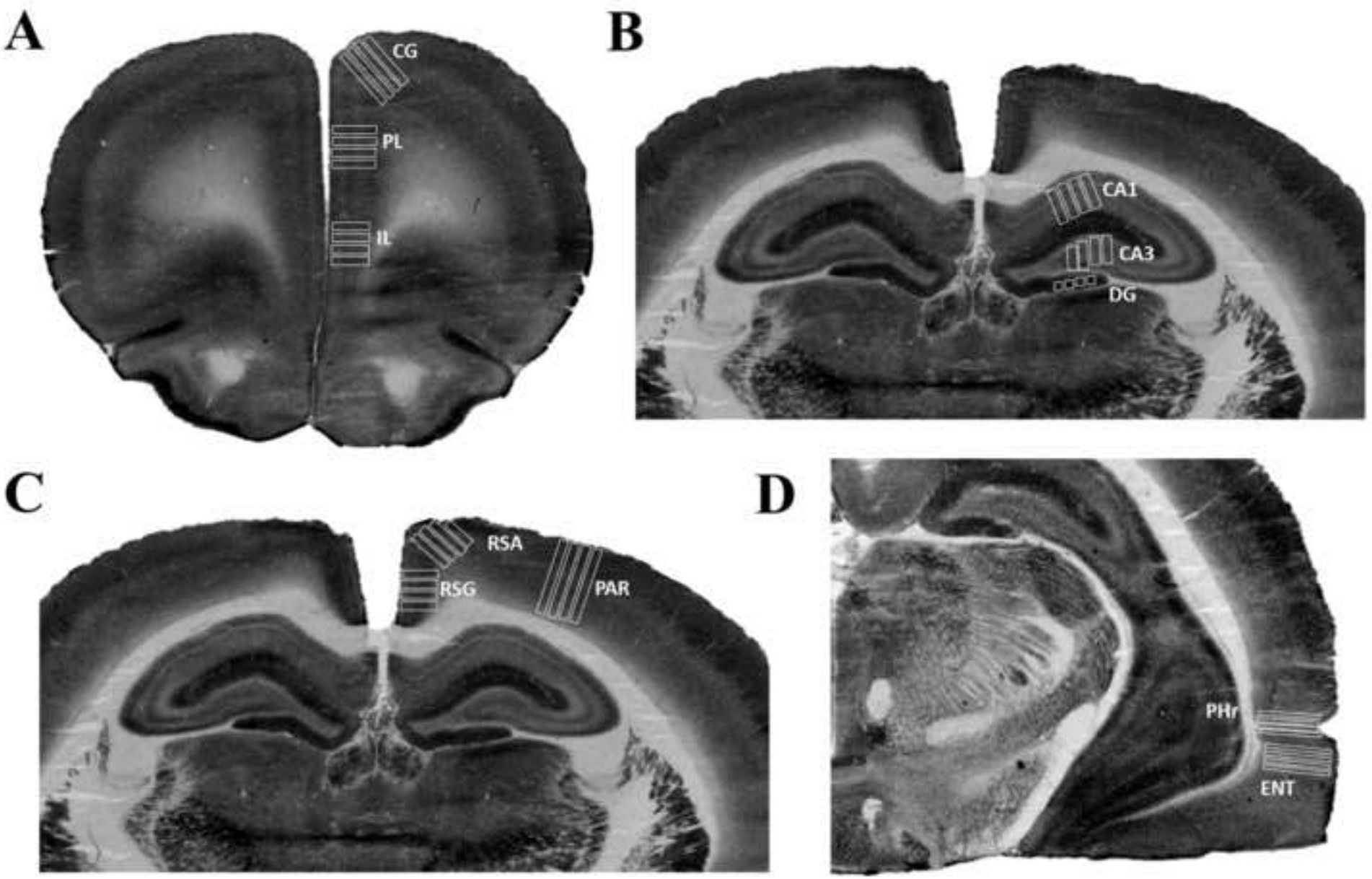

Figure2

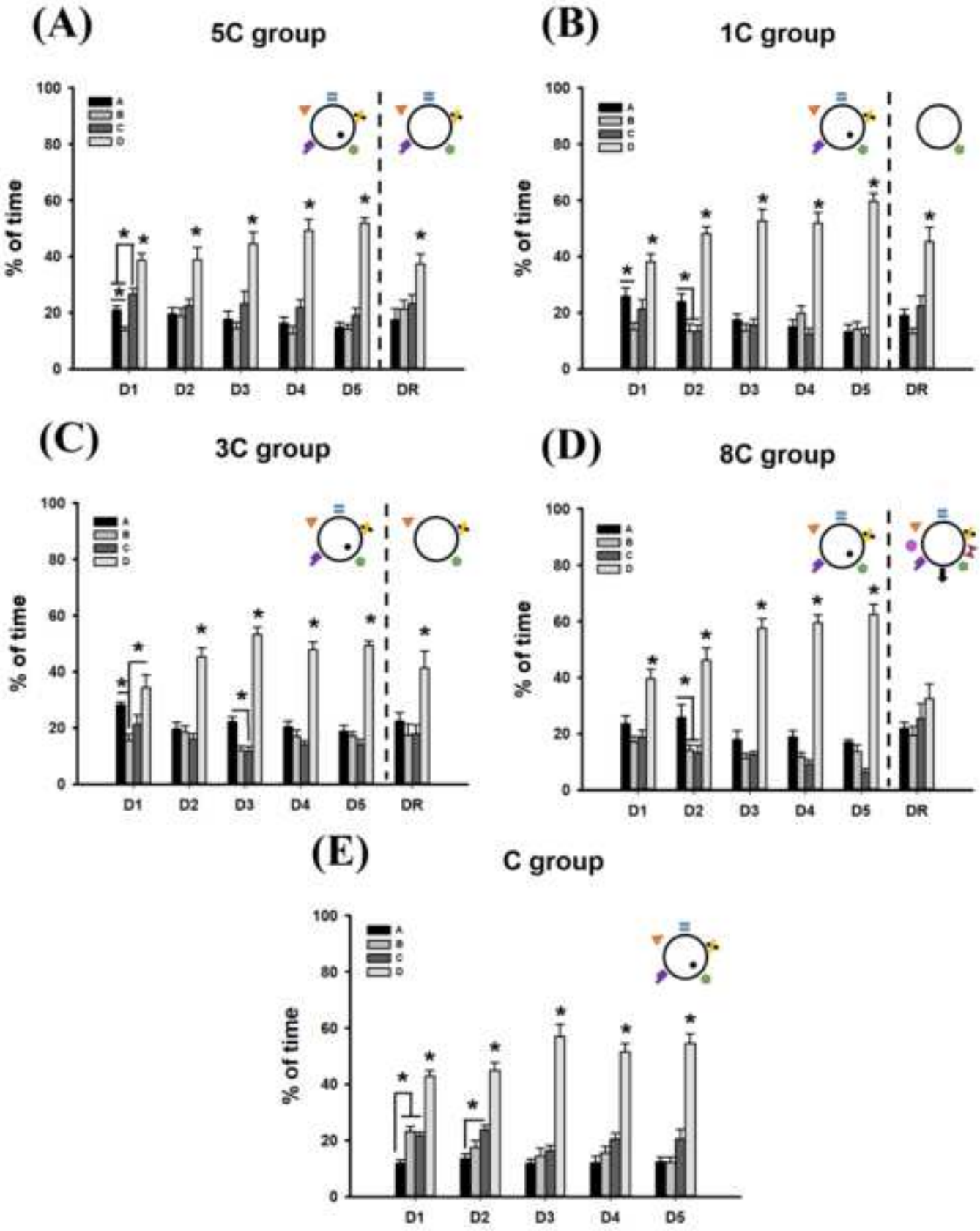

Figure3

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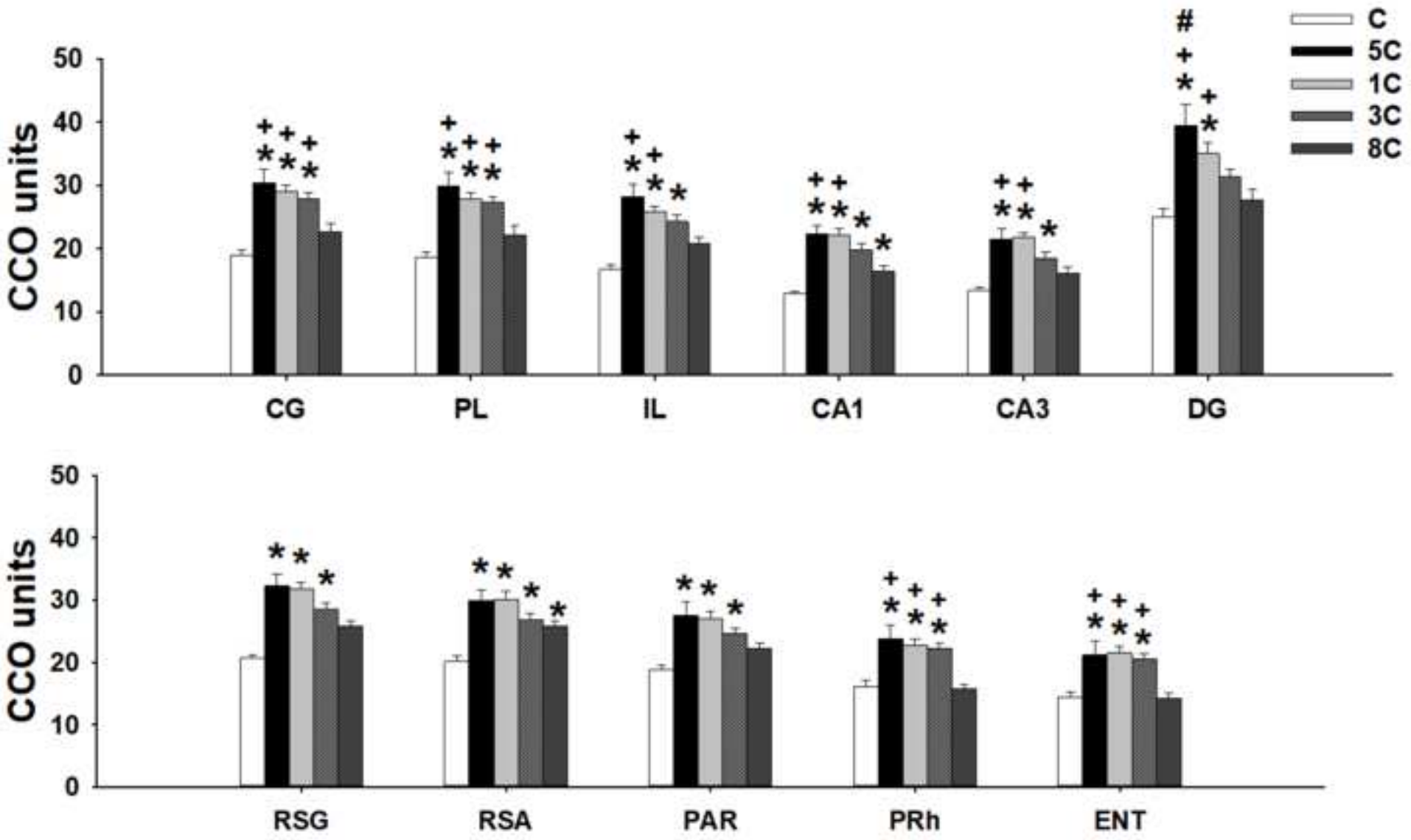

CRediT authorship contribution statement

Candela Zorzo: Investigation, Formal analysis, Writing – original draft, Visualization.

Jorge L. Arias: Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Resources, Funding acquisition.

Marta Méndez: Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.