Functional connectivity of anterior retrosplenial cortex in object recognition memory

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Keywords:
Retrosplenial cortex
Disconnection
Object recognition memory
Transient inactivation
Memory consolidation
Muscimol

A B S T R A C T

Recognition memory can rely on three components: "what", "where" and "when". Recently we demonstrated that the anterior retrosplenial cortex (aRSC), like the perirhinal cortex (PRH) and unlike the hippocampus (HP), is required for consolidation of the "what" component. Here, we aimed at studying which brain structures interact with the aRSC to process object recognition (OR) memory in rats. We studied the interaction of six brain structures that are connected to the aRSC during OR memory processing: PRH, medial prefrontal cortex (mPFC), anteromedial thalamic nuclei (AM), medial entorhinal cortex (MEC), anterior cingulate cortex (ACC) and the dorsal HP (dHP). We previously described the role of the PRH and dHP, so we first studied the participation of the mPFC, AM, MEC and ACC in OR memory consolidation by bilateral microinfusions of the $GABA_A$ receptor agonist muscimol. We observed an impairment in OR long-term memory (LTM) when inactivating the mPFC, the AM and the MEC, but not the ACC. Then, we studied the functional connections by unilateral inactivation of the aRSC and each one of the six structures in the same (ipsilateral) or the opposite (contralateral) hemisphere. Our results showed an amnesic LTM effect in rats with ipsilateral inactivations of aRSC-PRH, aRSC-mPFC, aRSC-AM, or aRSC-MEC. On the other hand, we observed memory impairment when aRSC-ACC were inactivated in opposite hemispheres, and no effect when the aRSC-dHP connection was inactivated. Thus, our ipsilateral inactivation findings reveal that the aRSC and, at least one brain region required in OR LTM processing are essential to consolidate OR memory. In conclusion, our results show that several cortico-cortical and cortico-thalamic pathways are important for OR memory consolidation.

1. Introduction

The retrosplenial cortex (RSC) is involved in episodic and spatial memory (Fournier, Eddy, DeAngeli, Huszár, & Bucci, 2019; Milczarek & Vann, 2020; Todd & Bucci, 2015; Todd, DeAngeli, Jiang, & Bucci, 2017; Vann, Aggleton, & Maguire, 2009). Most of the RSC functions are related to its sensorial inputs, as the RSC receives information from the visual and auditory cortex (Shibata, Honda, Sasaki, & Naito, 2009; Sugar, Witter, van Strien, & Cappaert, 2011; Todd, Mehlman, Keene, De Angeli, & Bucci, 2016; Vogt & Miller, 1983). In addition, the role of the RSC in memory is related to its main connections with regions of the medial temporal lobe such as the hippocampus (HP), perirhinal cortex (PRH) and entorhinal cortex. It has been observed that the anterior RSC (aRSC) projects to HP via subiculum (Shibata, 1994) and receives hippocampal information from the subiculum and CA1 (Miyashita & Rockland, 2007; Sugar et al., 2011). Moreover, the aRSC presents reciprocal connections with the PRH (Jones & Witter, 2007; Shibata, 1994; Sugar et al., 2011) and the medial entorhinal cortex (MEC) (Jones & Witter, 2007; Kerr, Agster, Furtak, & Burwell, 2007; Sugar et al., 2011). Also, the aRSC is connected to other brain structures relevant to memory, presenting reciprocal connections with the anteromedial thalamic nuclei (AM) (Van Groen, Kadish, & Wyss, 1999; Wright, Vann, Erichsen, O'Mara, &

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Aggleton, 2013) and the anterior cingulate cortex (ACC) (Shibata, Kondo, & Naito, 2004; Shibata & Naito, 2008), and projecting to the prefrontal cortex (Shibata et al., 2004; van Eden, Lamme, & Uylings, 1992). The connections between aRSC and the above mentioned brain structures are mainly ipsilateral, however there are sparse contralateral connections (Jones & Witter, 2007; Mathiasen, Dillingham, Kinnavane, Powell, & Aggleton, 2017; Shibata et al., 2004; Shibata & Naito, 2008; van Groen & Michael Wyss, 1990).

Memory has different stages; acquisition, consolidation and retrieval. After the acquisition of novel information memory is thought to be stored through a consolidation process during which is susceptible to disruption. Retrieval is the recall of the stored memory. Recognition memory is important to our daily routine. It allows us to distinguish already experienced stimuli from new ones. This type of memory can rely on three components: "what", "where" and "when" (Ennaceur, 2010). It has been proposed that different functional connectivity pathways encode the different recognition components (Diana, Yonelinas, & Ranganath, 2007; Eichenbaum, Yonelinas, & Ranganath, 2007). Previous works demonstrated the involvement of the PRH and medial prefrontal cortex (mPFC) in object recognition (OR) memory, i.e. the "what" pathway (Akirav & Maroun, 2006; Miranda & Bekinschtein, 2018; Olarte-Sánchez, Amin, Warburton, Aggleton, & Dalley, 2015; Rossato et al., 2013; Tuscher, Taxier, Fortress, & Frick, 2018; Winters, Forwood, Cowell, Saksida, & Bussey, 2004); while dorsal HP (dHP) role in OR memory remains controversial (Cohen & Stackman, 2015). On the other hand, the HP, mPFC, MEC, ACC and AM are usually related to spatial recognition memories (Hales et al., 2018; Jankowski et al., 2013; Teixeira, Pomedli, Maei, Kee, & Frankland, 2006; Tuscher et al., 2018; Warburton & Brown, 2015), i.e. the "where" pathway.

Recently, we showed the requirement of the aRSC for OR memory consolidation (de Landeta, Pereyra, Medina, & Katche, 2020). However, memory relies on more than one brain structure, therefore we propose that the aRSC and connected brain structures participate altogether in OR memory consolidation. Thus, the aim of this work was to study the requirement of the functional connectivity between the aRSC and six brain structures, based on their connections, (PRH, PFC, AM, MEC, dHP and ACC) for OR memory consolidation.

2. Methods

2.1. Subjects

We used 296 2.5 month-old male Wistar rats (Facultad de Ciencias Exactas y Naturales, UBA, Argentina) weighing about 220–300 g. Animals were housed in groups of three per cage and maintained under 12 h light/dark cycle (lights on at 7:00 am) at 21–23 °C with water and food *ad libitum*. Experimental procedures followed the guidelines of the USA National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the University Buenos Aires (CICUAL).

2.2. Surgery

Rats were implanted bilaterally under deep ketamine/xylazine anesthesia (40 and 2 mg/kg, respectively) with 22G guide cannula in the aRSC at AP −3.9, L ± 0.5, DV −1.8 (Fig. 1A), PRH at AP −5.5, L ±6.6, DV −7.0 (Fig. 1B), mPFC (prelimbic and infralimbic cortexes) at AP +3.2, L ±0.75, DV −3.2 (Fig. 1C), MEC at AP −6.5, L ±4.3, DV −6.5 (Fig. 1D), AM at AP −1.55, L ±0.8, DV −5.8 (Fig. 1E), caudal ACC at AP +2.2, L ±0.75, DV −2.4 (Fig. 1F) and dHP at AP −3.9, L ±3.0, DV −3.0 (Fig. 1G), coordinates in mm from Bregma according to the atlas of Paxinos and Watson (Paxinos & Watson, 2007). The cannulas were fixed to the skull with dental acrylic. Obturators were then inserted into the cannula to prevent blockage. After four or five days of recovery from surgery, the animals were gently handled once a day for 2 days and then trained in the object recognition task.

2.3. Drug infusion

We infused the $GABA_A$ receptor agonist muscimol (Sigma Aldrich, USA) at a dose of 0.1 μg per side into the aRSC, PRH, mPFC, MEC, AM, ACC or dHP immediately after the sample phase to study memory consolidation (Martin, 1991). Drug was dissolved in sterile saline. Infusions were bilateral or unilateral and had a volume of 1 μl into aRSC, ACC and dHP, and 0.5 μl into PRH, mPFC, MEC and AM. The entire infusion procedure took around 4 min, the infusion rate was 1 μl/min. Injectors were left in place for an additional minute following infusion before they were removed carefully to avoid backflow.

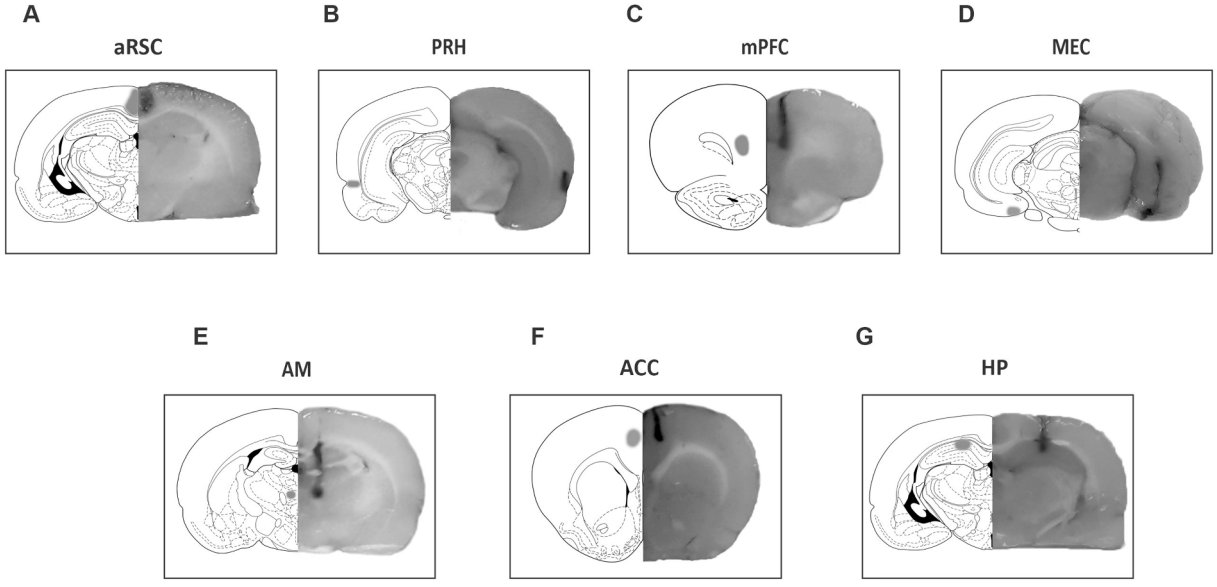

Fig. 1. Representation of the infusion area for the different structures. Gray area represents the mean area reached by the infusion into (A) aRSC, (B) PRH, (C) mPFC, (D) MEC, (E) AM, (F) ACC and (G) dHP. Pictures show the methylene blue infusion area (black).

2.4. Cannula placement

Cannula placement was verified after the end of the behavioral procedures by infusions of 1 μl or 0.5 μl of 4% methylene blue in saline. Histological examination of cannula placements was performed. Only the behavioral data from animals with the cannula located in the intended site were included in the final analysis (33 animals were excluded from the analysis).

2.5. Y-shape object recognition

Object recognition was conducted in a Y-shaped acrylic maze (Winters et al., 2004). Each arm of the maze was 27 cm length and 10 cm wide, with white walls 40 cm high, preventing the animal to visualize any external cue. The arms in which the objects were placed were shortened to 8.5 cm by guillotine doors. The used of this maze reduced the internal and external spatial cues and focuses the animals' exploration on the objects. In the habituation session rats were allowed to explore the empty maze during 10 min for one day. One day after the habituation there was a sample phase, during which rats were placed in the start arm and led explored for 5 min two identical objects made of glass, metal or plastic, placed in each arm of the apparatus. Choice phase was performed 24 h after sample phase, and consisted in letting the rat explore two different objects for 3 min, one familiar (from the sample phase) and the other novel.

In both sample and choice phases we used manual timers to score the time the rodent spent exploring (sniffing or touching) the objects. We calculated the novel object discrimination index as exploration time of the novel object minus the exploration time of the familiar object divided by the total exploration time. Indexes significantly greater than

Table 1
Total exploration times for each manipulation. Mean ± SD exploration time for each experiment during sample phase and choice phase. Results of the two-tailed student's *t*-test for the different groups' exploration in each experiment.

			Sample Phase			Choice Phase			
Fig.	Inactivation/s	Group	expl time (s)	p-value	t-value	expl time (s)	p-value	t-value	dF
2a	mPFC			0.24	1.22		0.30	1.07	13
		Veh	83.5 ± 16.1			49.6 ± 25.6			
		Mus	74.6 ± 11.4			28.7 ± 8.7			
2b	MEC			0.76	0.32		0.94	0.08	15
		Veh	93.3 ± 25.1			45.0 ± 16.6			
		Mus	97.4 ± 27.4			45.6 ± 16.6			
2c	AM			0.21	1.31		0.75	0.33	15
		Veh	63.0 ± 21.2			40.6 ± 25.0			
		Mus	75.5 ± 18.6			37.4 ± 12.4			
2d	ACC			0.51	0.67		0.92	0.10	14
		Veh	79.3 ± 19.1			42.5 ± 17.8			
		Mus	85.9 ± 20.2			41.8 ± 12.4			
3a	aRSC			0.91	0.12		0.81	0.25	8
		Veh	70.4 ± 36.4			25.8 ± 17.9			
		Mus	72.4 ± 7.50			23.6 ± 8.02			
3b	PRH			0.31	1.12		0.24	1.31	6
		Veh	55.3 ± 6.7			31.0 ± 5.7			
		Mus	67.3 ± 20.5			40.8 ± 13.8			
3c	mPFC			0.24	1.27		0.57	0.60	8
		Veh	61.8 ± 11.2			31.8 ± 12.0			
		Mus	71.2 ± 12.2			37.0 ± 15.3			
3d	MEC			0.67	0.44		0.73	0.36	8
		Veh	82.8 ± 34.5			34.3 ± 14.9			
		Mus	73.3 ± 32.6			31.0 ± 13.4			
3e	AM			0.28	1.15		0.42	0.85	8
		Veh	36.8 ± 17.2			30.0 ± 13.9			
		Mus	48.0 ± 13.2			37.8 ± 15.0			
4a	aRSC-PRH			0.96	0.05		0.73	0.36	11
		Contra	63.1 ± 18.4			40.0 ± 20.1			
		Ipsi	43.7 ± 21.4			36.2 ± 17.9			
4b	aRSC-mPFC			0.93	0.10		0.37	0.94	11
		Contra	85.3 ± 21.8			41.7 ± 16.5			
		Ipsi	86.7 ± 30.4			33.3 ± 15.3			
4c	aRSC-MEC			0.02	2.71		0.81	0.97	11
		Contra	70.1 ± 15.3			29.0 ± 10.1			
		Ipsi	89.3 ± 9.1			30.5 ± 11.5			
4d	aRSC-AM			0.73	0.35		0.62	0.51	15
		Contra	81.8 ± 23.1			36.9 ± 11.7			
		Ipsi	77.8 ± 24.7			39.9 ± 12.3			
4e	aRSC-ACC			0.69	0.41		0.84	0.21	18
		Contra	78.3 ± 23.5			51.7 ± 15.3			
		Ipsi	73.9 ± 24.6			53.2 ± 16.7			
4f	aRSC-dHP			0.23	1.24		0.97	0.04	15
		Contra	65.0 ± 23.6			41.7 ± 21.0			
		Ipsi	78.1 ± 19.9			41.4 ± 13.7			

zero were indicators of memory. Previous to the beginning of the experiments the objects were set up to discard any object preference. We analyzed data from animals that had a minimum exploration time of 15 s/per object during the sample phase showing no preference for any of the sampled objects, and that explored more than 10 s during the choice phase (57 animals were excluded from the analysis). Total exploration times for each experiment and manipulation are shown in Table 1. The objects and apparatus were cleaned with a solution of soap, alcohol and water before being presented to each animal.

2.6. Data analysis

Behavioral data were analyzed by unpaired Student's *t* test between groups or the theoretical value 0. We used Graph Pad Prism 8 (Graphpad, USA). In all cases α level was set at 0.05. All data are presented as mean ± SEM.

3. Results

Based on aRSC connections, we studied the interaction between the aRSC and six brain structures: PRH, mPFC, MEC, AM, ACC and dHP. Our previous work confirmed the requirement of PRH for OR memory consolidation and the lack of participation of the dHP in this memory (de Landeta et al., 2020). Therefore, we first assessed whether the other four of these brain regions were required for OR memory (Fig. 2). We transiently inactivated these structures by bilaterally infusing muscimol immediately after the sample phase into the mPFC, MEC, AM, or ACC. Inactivation of the mPFC (Fig. 2A), MEC (Fig. 2B) or the AM (Fig. 2C) blocked memory consolidation. In contrast, the inactivation of the ACC had no effect on this process (Fig. 2D). Statistics are shown in Table 2. These results demonstrate the requirement of MEC and AM, but not ACC, during OR memory consolidation and support the involvement of the mPFC in OR memory consolidation (Akirav & Maroun, 2006; Rossato et al., 2013; Tanimizu, Kono, & Kida, 2018; Tuscher et al., 2018).

Then, we studied the interaction between the six different structures and the aRSC. We performed ipsilateral and contralateral muscimol infusions immediately after the sample phase; we always inactivated the aRSC in one hemisphere and one of the target brain regions in the same (ipsilateral) or opposite (contralateral) hemisphere. These inactivations allowed us to discriminate whether the functional connectivity between the aRSC and each of the six brain structures was contralateral or ipsilateral. Before performing the contralateral or ipsilateral inactivation, we checked for an effect of unilateral muscimol infusion in each brain structure (Fig. 3). Memory remained intact after unilateral infusion of muscimol immediately after the sample phase into the aRSC (Fig. 3A, PRH (Fig. 3B, mPFC (Fig. 3C), MEC (Fig. 3D) and AM (Fig. 3E). Thus, we performed the ipsilateral or contralateral inactivations (Fig. 4). We observed memory impairment after ipsilateral inactivation of the aRSC-PRH pair (Fig. 4A), the aRSC-mPFC pair (Fig. 4B), the aRSC-MEC pair (Fig. 4C) or the aRSC-AM pair (Fig. 4D) and the contralateral inactivation of the aRSC-ACC pair (Fig. 4E). Conversely, we observed no difference between ipsilateral or contralateral inactivation of the aRSC-dHP pair (Fig. 4F). Statistics for all experiments are shown in Table 2.

4. Discussion

Our results demonstrate the participation of the AM and MEC in OR long-term memory (LTM) consolidation. We demonstrate that the "what" circuit is, at least, composed by the aRSC, PRH, mPFC, AM and MEC; however the information flow between the aRSC and the other

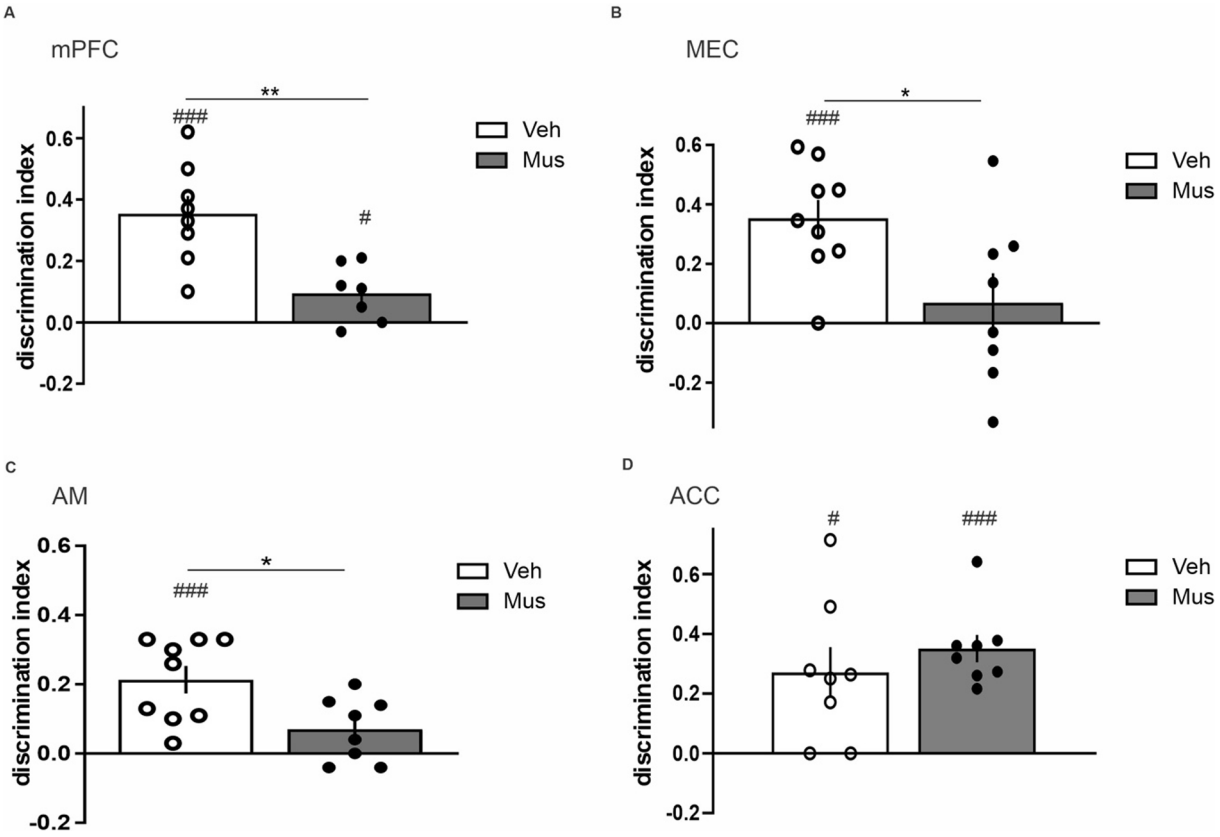

Fig. 2. Inactivation of mPFC, AM and MEC but not ACC impairs OR memory. Animals were infused with vehicle (Veh, white bar) or muscimol (Mus, 0.1 μg per side, gray bar) immediately after the sample phase. The choice phase was performed 24 h after the sample phase. (A,B,C,D) The discrimination index from animals infused into (A) mPFC, (B) MEC, (C) AM and (D) ACC. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$. Veh vs. Mus, two-tailed student's *t*-test. #$p < 0.05$, ###$p < 0.001$. Group vs. 0, two-tailed student's *t*-test. (A) n = 7–8, (B) n = 8–9, (C) n = 8–9, (D) n = 8.

Table 2
Statistical analysis for each experiment. Results of the two-tailed student's *t*-test between groups and from the mean discrimination index of each group vs. zero.

Fig.	Inactivation/s	Group	n	*t*-test	p-value	t-value	dF	*t*-test	p-value	t-value	dF
2a	mPFC	Veh	8	Veh vs. Mus	0.0026	3.712	13	Veh vs. 0	0.0005	6.148	7
		Mus	7					Mus vs. 0	0.0363	2.686	6
2b	MEC	Veh	9	Veh vs. Mus	0.0249	2.493	15	Veh vs. 0	0.0005	5.693	8
		Mus	8					Mus vs. 0	0.5072	0.699	7
2c	AM	Veh	9	Veh vs. Mus	0.0153	2.736	15	Veh vs. 0	0.0007	5.347	8
		Mus	8					Mus vs. 0	0.0694	2.142	7
2d	ACC	Veh	8	Veh vs. Mus	0.4212	0.829	14	Veh vs. 0	0.0152	3.195	7
		Mus	8					Mus vs. 0	0.0001	7.619	7
3a	aRSC	Veh	5	Veh vs. Mus	0.9520	0.062	8	Veh vs. 0	0.0229	3.595	4
		Mus	5					Mus vs. 0	0.0090	4.744	4
3b	PRH	Veh	4	Veh vs. Mus	0.5588	0.619	6	Veh vs. 0	0.0150	5.747	3
		Mus	4					Mus vs. 0	0.0051	7.416	3
3c	mPFC	Veh	5	Veh vs. Mus	0.2963	1.117	8	Veh vs. 0	0.0344	3.152	4
		Mus	5					Mus vs. 0	0.0214	3.668	4
3d	MEC	Veh	4	Veh vs. Mus	0.3725	0.945	8	Veh vs. 0	0.0752	2.677	3
		Mus	6					Mus vs. 0	0.0003	8.671	5
3e	AM	Veh	5	Veh vs. Mus	0.0924	1.911	8	Veh vs. 0	0.0024	6.801	4
		Mus	5					Mus vs. 0	0.0002	12.92	4
4a	aRSC-PRH	Contra	7	Contra vs. Ipsi	0.0010	4.430	11	Contra vs. 0	0.0007	6.294	6
		Ipsi	6					Ipsi vs. 0	0.2506	1.299	5
4b	aRSC-mPFC	Contra	7	Contra vs. Ipsi	0.0004	5.079	11	Contra vs. 0	<0.0001	10.25	6
		Ipsi	6					Ipsi vs. 0	0.1624	1.638	5
4c	aRSC-MEC	Contra	7	Contra vs. Ipsi	0.0280	2.530	11	Contra vs. 0	0.0034	4.685	6
		Ipsi	6					Ipsi vs. 0	0.0988	2.024	5
4d	aRSC-AM	Contra	9	Contra vs. Ipsi	0.0315	2.372	15	Contra vs. 0	0.0011	4.965	8
		Ipsi	8					Ipsi vs. 0	0.3832	0.9303	7
4e	aRSC-ACC	Contra	10	Contra vs. Ipsi	0.0015	3.731	18	Contra vs. 0	0.0303	2.568	9
		Ipsi	10					Ipsi vs. 0	<0.0001	10.95	9
4f	aRSC-dHP	Contra	7	Contra vs. Ipsi	0.8832	0.150	15	Contra vs. 0	0.0036	4.634	6
		Ipsi	10					Ipsi vs. 0	0.0068	3.491	9

structures (i.e. OR memory network) is dynamic and flexible and that, during memory consolidation, the network can adapt (within a certain range) to successfully store memory. We observed that the unilateral transient inactivation of any of the brain regions that integrates the "what" circuit can be compensated, although the ipsilateral inactivation of the aRSC and one of the other structures tested disrupts the "what" circuit in a way that cannot be compensated by the remaining structures or the intact hemisphere. This suggests that different brain regions are encoding different features of the "what" component of recognition memory.

Many studies showed the involvement of the PRH in OR LTM (Brown, Barker, Aggleton, & Warburton, 2012; Kim, Kim, Lee, Park, & Ryu, 2014; Morillas, Gómez-Chacón, & Gallo, 2017; Winters et al., 2004; Winters, Saksida, & Bussey, 2008). While there is agreement on the participation of the PRH in OR memory, the role of HP remains controversial. Differences in the methodology used may generate different results, for example, the type of experimental subjects, the complexity of the task and the degree of injury or inactivation produced (Broadbent, Squire, & Clark, 2004; Cohen & Stackman, 2015). Regarding the mPFC, previous works from different groups showed that this region is consistently required for OR memory (Akirav & Maroun, 2006; Pezze, Marshall, Fone, & Cassaday, 2015; Rossato et al., 2013; Tanimizu et al., 2018; Tuscher et al., 2018, but see Barker, Wong, Uney, & Warburton, 2020). In this particular study, we showed that bilateral inactivation of the mPFC during memory consolidation, impaired memory retention at 24 h. The role of the AM and MEC in memory was mostly described for spatial memories (Hales et al., 2014, 2018; Igarashi, 2016; Jankowski et al., 2013; Kinnavane, Amin, Aggleton, & Nelson, 2019; Mitchell & Dalrymple-Alford, 2005). However, AM connectivity with the PRH and visual cortex (Jankowski et al., 2013) and the connectivity between the MEC and the visual cortex (Kerr et al., 2007), suggests that these structures could also participate in OR memory processing. Our results support this premise, since transient inactivation of both structures produce an amnesic effect at 24 h, thus both the AM and MEC are required for OR LTM consolidation.

Our results also showed that the ACC is not required for OR memory consolidation. This is in agreement with previous results from Cassaday group showing that transient inactivation of the ACC before the sample phase had no effect in OR LTM, but they did observed an amnesic effect when inactivating ACC before retrieval at 24 h (Pezze, Marshall, Fone, & Cassaday, 2017). Together these results suggest that, during the first stages of memory consolidation, object information is not processed by the ACC, but that it may be recruited by other structures during other memory phases like retrieval.

Usually, disconnections studies show that unilateral inactivation or lesions of two brain areas in both hemispheres affects memory, while ipsilateral treatments do not impair memory (Hernandez et al., 2017; Holland, 2007; Keefer & Petrovich, 2020; Nasser, Lafferty, Lesser, Bacharach, & Calu, 2018; Warburton, Baird, Morgan, Muir, & Aggleton, 2001). However, some functional connectivity studies observed impaired memory when transiently inactivating both contralaterally and ipsilaterally (Baker, Rao, Rivera, Garcia, & Mizumori, 2019; Gilmartin, Kwapis, & Helmstetter, 2012; Mathis et al., 2017; Scott et al., 2020). Our functional disconnection results show an ipsilateral effect of the inactivation between the aRSC and the other brain structures involved in OR memory consolidation. We consider three possible explanations to our results: 1. the inactivation of a pair of structures required for OR memory consolidation in the same hemisphere cannot

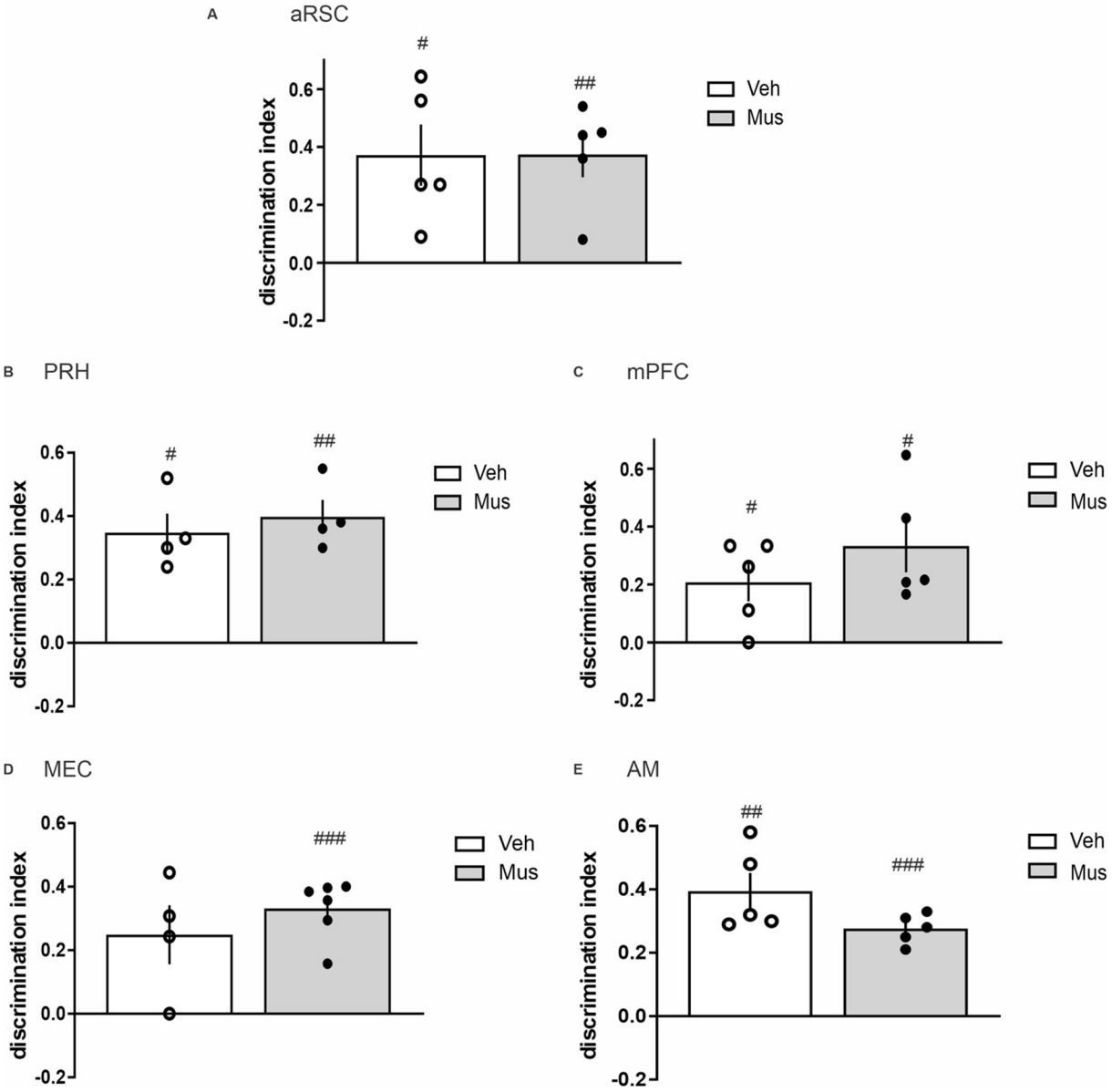

Fig. 3. Unilateral inactivation of aRSC, PRH, mPFC, MEC and AM had no effect on memory consolidation. Rats were bilaterally infused with vehicle (Veh, white bar) or unilaterally infused with vehicle into one side and musimol into the opposite side (Mus, 0.1 μg, light gray bar) immediately after sample phase. Choice phase was performed 24 h after sample phase. (A, B, C, D, E) Discrimination index from animals infused into (A) aRSC, (B) PRH, (C) mPFC, (D) MEC and (E) AM. Data are expressed as mean ± SEM. Veh vs. Mus, two-tailed student's *t*-test. #p < 0.05, ## p < 0.01, ###p < 0.001. Group vs. 0, two-tailed student's *t*-test. (A) n = 5, (B), n = 4, (C) n = 5, (D) n = 4–6 and (E) n = 5.

be compensated by the remaining ipsilateral structures or by the rest of the structures of the intact hemisphere. In agreement, contralateral inactivations had no effect on memory consolidation, i.e. unilateral inactivation of two structures in different brain hemispheres was compensated by the same structure in the opposite hemisphere or the remaining active structures of the OR network. This is consistent with the results obtained after the unilateral inactivation of single brain structures. The Fanselow group previously proposed a dynamic-system view of memory in which the pathway that dominates memory processing is always the most efficient, and plasticity in alternative structures is capable of compensating the inactivity of other brain regions required for LTM consolidation (Poulos, Ponnusamy, Dong, & Fanselow, 2010; Zelikowsky et al., 2013). Moreover, it has been proposed that the homeostatic regulation of neuronal dynamics contributes to functional recovery after brain injury (Otchy et al., 2015). In addition, it was previously observed that in some cases, transient inactivation can triggered a compensation effect from other brain structures that belong to the memory network (Goshen et al., 2011). Then, a possible explanation for our findings is that each brain region is encoding different features and that altogether creates the "what" component of memory; nevertheless, we cannot discard some overlap in the processing of the objects features. As we observed that the unilateral inactivation of one structure was not sufficient to disrupt OR memory, we suggest that this inactivation could be compensated by the intact hemisphere or that the dynamics of the system are able to compensate the lack of only one feature per hemisphere, yet the lack of more than one feature in the same hemisphere disrupts the system in a way that cannot be compensated by the remaining structures. 2. There is a hub structure that collects the information from the brain regions of one hemisphere and crosses it between hemispheres, i.e. contralateral, but not ipsilateral, information flow is essential for OR memory consolidation. 3. The sparse contralateral connections between the aRSC and the brain structures involved in

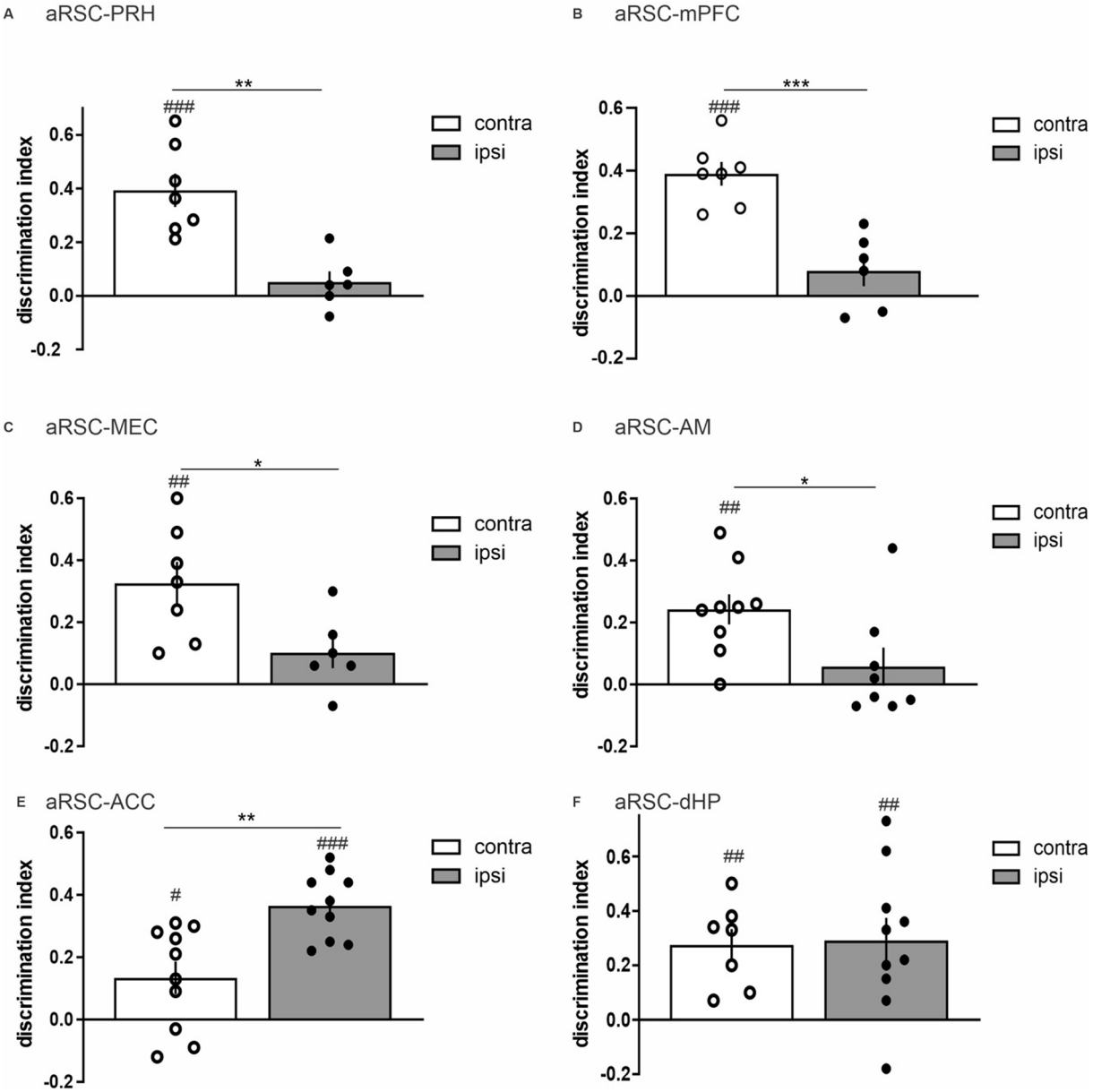

Fig. 4. Functional connectivity between the aRSC and PRH, mPFC, AM, MEC, ACC and dHP. Animals were unilaterally infused with muscimol (0.1 μg per structure) immediately after sample phase. Infusions were made into aRSC and one of the other brain structures in the opposite hemisphere (contralateral, contra, white bar) or same hemisphere (ipsilateral, ipsi, gray bar).Vehicle was infused in the opposite side of the muscimol infusion into each structure. Choice phase was performed 24 h after sample phase. (A, B, C, D, E, F) Discrimination index from animals infused into (A) aRSC-PRH, (B) aRSC-mPFC, (C) aRSC-MEC, (D) aRSC-AM, (E) aRSC-ACC and (F) aRSC-dHP. Bars represent mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Contra vs. Ipsi, two-tailed student's *t*-test. #$p < 0.05$, ## $p < 0.01$, ###$p < 0.001$. Group vs. 0, two-tailed student's *t*-test. (A) n = 6–7, (B) n = 6–7, (C) n = 6–7, (D) n = 8–9, (E) n = 10 per group, (F) n = 7–10.

this study, which are minor in comparison to the ipsilateral connections, might be crucial for OR memory processing and the behavioral output related to this memory. These three explanations might be possible, we will need to further analyze all the possibilities to unravel the reason behind our results and increase our knowledge about OR memory processing.

5. Conclusions

The main findings of the present study are: 1- in addition to the role of aRSC, PRH and mPFC in OR memory consolidation, we found a requirement of MEC and AM; 2- the ipsilateral inactivation of the aRSC and another brain structure involved in OR memory consolidation had an amnesic effect, while contralateral inactivation had no effect in OR-LTM. 3- The ACC itself is not required for OR memory consolidation, however the information flow between ACC and aRSC appears to be required for it.

CRediT authorship contribution statement

Ana Belén de Landeta: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Magdalena Pereyra:** Conceptualization, Investigation, Formal analysis. **Magdalena Miranda:** Conceptualization, Investigation. **Pedro Bekinschtein:** Conceptualization, Methodology, Writing – review & editing. **Jorge H. Medina:** Conceptualization, Methodology, Resources, Funding acquisition, Writing – review & editing. **Cynthia Katche:** Conceptualization, Methodology, Investigation, Formal analysis,

Resources, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Funding

This work was supported by grants from National Agency of Scientific and Technological Promotion of Argentina (ANPCyT, Argentina) to J.H.M. (grant number 2016–0034) and C.K. (grant number 2015–1552) and the Argentinian National Research Council (CONICET).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Previously reported data (bilateral inactivation of aRSC, dHP and PRH) were used to support this study and are available at https://doi.org/10.1038/s41598-020-60937-z.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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