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Original article

Muscarinic activity in hippocampus and entorhinal cortex is crucial for spatial and fear memory retrieval

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ABSTRACT

Background: Hippocampus and entorhinal cortex are key players of learning and memory. Despite their established role in memory processes, the contribution of muscarinic receptor activity in these brain regions during memory retrieval remains elusive. This study was aimed to assess the role of hippocampal CA1 and medial entorhinal cortex muscarinic receptors in memory retrieval.
Method: Mice were implanted with bilateral cannulas in the hippocampus CA1 and medial entorhinal cortex. After recovery they were trained for Morris water maze test, novel object recognition test and contextual fear conditioning. Scopolamine was infused 10 min prior to retrieval test.
Results: Pre-test scopolamine infusion in hippocampal CA1 and medial entorhinal cortex significantly reduced overall exploration of objects ($p < 0.001$). Similarly, pre-retrieval inactivation dorsal hippocampal CA1 and medial entorhinal cortex muscarinic activity caused significant impairment of spatial and fear memories retrieval ($p < 0.05$).
Conclusion: These findings showed vital role of muscarinic activity in retrieving hippocampal and entorhinal cortex dependent memories and suggest a possible target for treating retrograde amnesia.

Introduction

Episodic memories constitute the memories of events and personal experiences. Howard Eichenbaum and other researchers have shown the existence of human episodic like memories in rodents [1–4]. Spatial, fear and object memories in mice represent episodic like memories fulfilling what, where and when criteria of human episodic memories [1,4]. The ability to retrieve episodic memories is vital for survival of humans and animals. Inability to retrieve learned information occurs in retrograde amnesia which constitutes initial symptoms of cognitive disorders predominantly aging, mild cognitive impairment, Alzheimer's disease, Parkinson's disease and traumatic brain injury [5–7]. Alzheimer's disease is characterized by marked reduction of cholinergic signaling in brain [8], specifically in hippocampus and associated cortices. The very first brain region affected with the onset of Alzheimer's disease is entorhinal cortex, the region that connects hippocampus with rest of cortical regions. Hippocampus and entorhinal cortex are necessary for the formation and retrieval of different forms of episodic memories including spatial [9,10], object recognition [11,12] and emotional memories[13,14]. During memory recall hippocampus activity leads to replay of cortical and amygdala activity [15] *via* activation of deep layers of entorhinal cortex [16].

Central cholinergic signaling through G-protein coupled muscarinic cholinergic receptors has been implicated in learning and memory processes. Increasing cholinergic signaling by inhibiting acetylcholine hydrolysis has emerged as potential therapeutic option for treating memory disorders (amnesia) in Alzheimer's disease, Parkinson's disease, traumatic brain injury, *etc.* [17–21]. Despite the established role of muscarinic receptors in memory formation, their involvement in memory retrieval is subject to debate. Some studies have negated the involvement of muscarinic signaling during memory retrieval [22–24]. However, recent studies are reporting pretest inactivation of muscarinic receptors induced impaired spatial [24], fear memory retrieval [25,26] and object recognition memories [27]. Despite recent data supporting the role of systemic muscarinic inactivation in causing impaired recall, the involvement of hippocampal muscarinic receptors in memory retrieval is subject to debate. Rogers and Kesner's have negated the involvement of hippocampal muscarinic receptors in memory retrieval [28,29]. On the other hand Leaderbrand et al. showed co-activation of multiple muscarinic

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receptors in dorsal hippocampus is necessary for retrieval of both recent and remote memories [30].

Though there is not much data regarding the role of entorhinal cortex muscarinic receptors in memory retrieval, systemic scopolamine was shown to impair spatial navigation in rodents by reducing grid cells tuning [31] that leads to impaired spatial recall.

In our previous study we reported that pre-test systemic scopolamine administration impaired spatial and fear memory retrieval [27]. In this study we aimed to identify the contribution of dorsal hippocampus and medial entorhinal cortex in episodic memory recall. Muscarinic receptors were antagonized bilaterally in either dorsal hippocampus CA1 region or in medial entorhinal cortex and effects on spatial, object recognition and contextual fear retrieval were assessed.

Materials and methods

Animals

Naïve male Balb/c mice, 10 to 12 weeks old, were obtained from the Laboratory Animal House of Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST). After bilateral cannula implantation, mice were placed individually in polycarbonate cages with ad libitum water and food. Animals were maintained at natural 14 h light and 10 h dark cycle, and all the behavior test were carried out between 9:00 to 15:00 h during the light cycle. All the experimental protocols were approved by Internal Review Board (IRB) of ASAB, NUST and were in compliance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Experimental procedure

We studied the effect of hippocampal CA1 and medial entorhinal cortex muscarinic receptors on memory retrieval. Mice were randomly divided into two hippocampal and two entorhinal cortex groups, with 7–9 animals per group. The groups were control and scopolamine, bilaterally infused with 0.9% saline and scopolamine respectively, prior to retrieval. Experimental procedure comprised of eight days starting five to seven days after stereotaxic cannula implantation. Day 1–5 were training days. From day 1 to day 5 mice were trained for Morris water maze test. From day 3 to day 5 mice were trained for object recognition memory. Contextual fear conditioning was performed on day 5. On training days with more than one behavior test, i.e. day 3, day 4 and day 5, the sequence of behavior tests was Morris water maze training followed by object recognition training. On training day 5 contextual fear conditioning was performed after object recognition training. To minimize the possible influence of one behavior test training on the memory of other, mice were given a minimum of 30 min interval between two consecutive behavior tests. No training and treatment was performed on day 6 and 7. Retrieval memory test for three tasks were performed on the eight day, 10 min after drug infusion. The scheme of experiment is shown in Fig. 1.

Surgery and bilateral cannula implantation

Mice were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and diazepam (8 mg/kg) and secured in stereotaxic apparatus (RWD Life Science Co., LTD). Sterile stainless steel cannulas (generous gift from Dr. Daesoo Kim, KAIST, South Korea) were bilaterally implanted in hippocampus or the entorhinal cortex with following coordinates: For hippocampus: −1.7 anterioposterior (AP), ±1.25 mediolateral (ML), and −1.5 mm dorsoventral (DV) to bregma; for the medial entorhinal cortex: −4.48 mm AP, ± 3.5 mm ML, and –4.25 mm DV. The cannulas were secured to the skull with dental acrylic cement. Mice were given 5–7 days of recovery period after which behavior tests were performed. All coordinates were adjusted from The Mouse Brain in Stereotaxic Coordinates (Third Edition) of Keith B.J. Franklin and George Paxinos.

Drug

Scopolamine hydrobromide (sc-296372, Santa Cruz Biotechnology, USA) was dissolved in 0.9% saline at 30 μg/0.5 μl [32].

Intracerebral drug infusion

Bilateral intra-hippocampal and intra-entorhinal cortex infusion of saline or scopolamine was performed 10 min prior to the

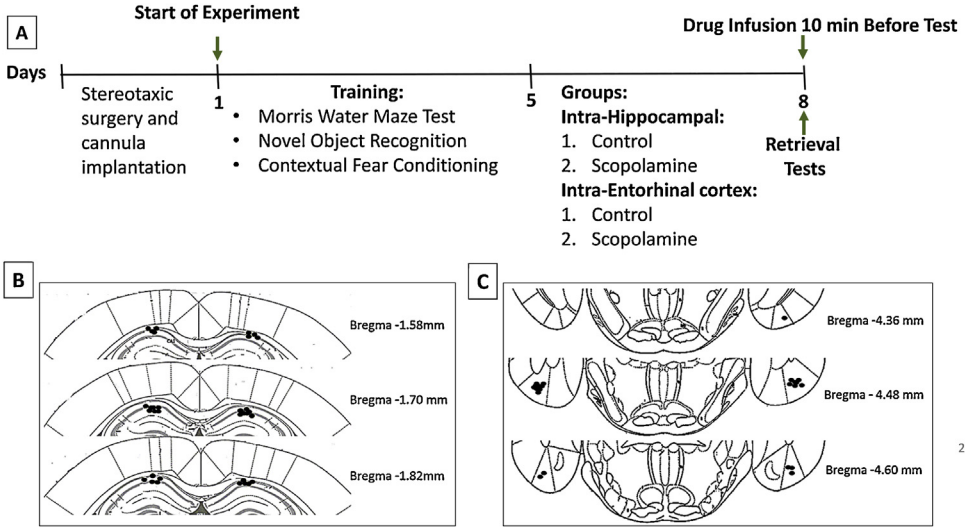

Fig. 1. Scheme of experimental design. A) Bilateral intra-hippocampal (2 groups) and intra-entorhinal cortex (2 groups) cannula implanted mice were trained for different behavior test from day 1-5. On day 8 retrieval tests were performed 10 min after saline or scopolamine infusion in the respective groups. Diagram B and C illustrating cannula placement in hippocampus and medial entorhinal cortex.

beginning of retrieval tests. 0.35 μl of saline or scopolamine [32] was infused per side through 32- guage infusion cannula, connected to 1 μl Hamilton syringe (Hamilton Company, Reno, NV, USA) through silicon tube. Drug was infused for 30 s and cannulas were remained intact for 30 s to ensure complete drug release. Drug infusion was carried out in awake mice.

Behavioral tests

Novel object recognition test

The protocol for novel object recognition described by Stackman et al., [33] was used with slight modifications. Training and test sessions were carried out in triple compartment apparatus (overall dimensions: 60 × 40 × 22 cm; dimension of individual compartment: 20 × 40 × 22 cm) training and/or test objects were placed in the side compartments while animals were released in the central compartment. Mice were trained for three training session of 5 min with two identical objects (Rubik's cubes) one session per day from day 3–5 of training. On the test day, one of the objects was replaced with a new object, the novel object (pink color plastic pencil case), and mice were allowed to explore both objects for 5 min. Training and test trials were recorded by video camera for analysis of object interaction. Object recognition memory was assessed by recording the total time spent with the presented objects during training and test sessions. Object interaction was scored when the mouse sniffed the object or touched it with its nose while looking at it. Chewing the object or climbing onto the object was not considered as interaction [34]. Discrimination index for the novel object identification was calculated by using following formula;

Discrimination Index = (Time spend with novel object / total time spend with both objects) × 100.

Apparatus was cleaned with 70% ethanol after each animal.

Morris water maze test

The protocol was same as described by Rashid et al [27]. Briefly, mice were trained from day 1 to 5 to learn the location of hidden platform (13 cm diameter and 34 cm high) submerged 1 cm below water level in a circular steel pool (120 cm diameter, 60 cm height) filled with opaque water (20 °C ± 2 °C). Pool was hypothetically divided into four quadrants. Spatial cues were provided on the walls of pool in the form of different geometric shapes. Each animal was subjected to 5 trials per day with 10 min inter trial interval. For each trial a different starting point was used. Platform was kept in same quadrant for all training trials, i.e. the target quadrant. Each animal was allowed to locate the submerged platform for maximum 90 s and was left in platform for 5 s. Animals failed to find the platform in given time were placed by experimenter on the platform for 20 s. On the test day, probe trial was conducted by removing the platform and allowing animal to swim in the pool for 90 s. Probe trial was recorded by video camera and spatial memory retrieval was assessed by manually recording following parameters: time spent in target quadrant, time spent in opposite quadrant, number of entries in target quadrant, and number of platform location crossings by each animal.

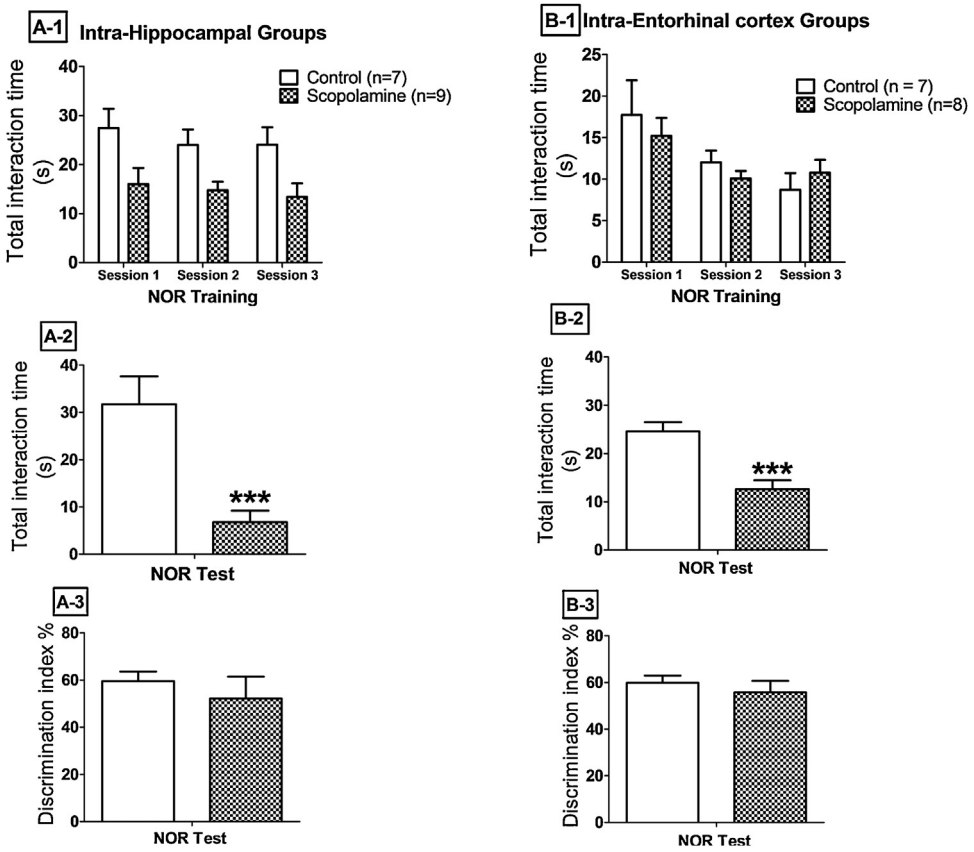

Fig. 2. Effect of hippocampal and entorhinal cortex muscarinic receptors on object recognition memory retrieval. Graph A-1 is showing the interaction time of intra-hippocampal control (n = 7) and scopolamine (n = 8) groups with similar objects during training sessions. Graph B-1: interaction time of intra-entorhinal cortex control (n = 7) and scopolamine (n = 8) groups during training. A-2 is representing total interaction time (novel object + familiar object) of intra-hippocampal control and scopolamine groups. B-2 represents total interaction time of intra-entorhinal cortex control and scopolamine groups. Scopolamine significantly reduced object interaction in all groups. A-3 and B-3 are showing percent discrimination index of intra-hippocampal and intra-entorhinal cortex groups, respectively. Percent discrimination index of all groups was above chance level. Data is presented as ± SEM. ***$p < 0.001$ comparted to time of interaction of control with novel object; NOR: novel object recognition.

Fear conditioning and context retention

Fear conditioning was performed on training day 5. The test was performed as described by Rashid et al. [27]. Briefly, mice were placed in conditioning chamber and after 5 min habituation each animal was given 5 tones (70 dB each at 3000 Hz) for 30 s co-terminated with one second 0.3 mA foot shock. Two minutes interval was given between two consecutive tones. Thirty seconds after the last tone/shock the animals were removed from conditioning chamber and placed back in their home cages. On the test day fear memory retrieval was assessed by placing the animals in the conditioning chamber for 5 min 30 s without tone or foot shock. The arena was cleaned with 70% ethanol after each animal. Freezing (as a measure of memory) was recorded by ANY-maze software. Mice that failed to show freezing response to all five tones during conditioning were considered outliers and are not shown in the results. One control and three scopolamine mice of intra-hippocampal group and one mouse from intra-entorhinal cortex scopolamine group were outliers.

Histological analysis

Upon completion of all behavior tests, mice received intraperitoneal injection of ketamine and diazepam (100 mg/kg and 8 mg/kg respectively) and were perfused trans-cardialy with 0.9% saline followed by 4% paraformaldehyde, according to the method previously described by [35]. Brains were extracted and placed in 4% paraformaldehyde at 4°C for 24 h after which they were processed for paraffin embedding and cut into 5-μm coronal sections. Sections were mounted on slides, and stained with cresyl violet. Light microscopy was used to identify cannula positions.

Statistical analysis

Data is presented as mean ± SEM. Statistical significance was determined by using paired *t* test and two way ANOVA. Significance criteria was set at $p < 0.05$. All the analysis were performed using Graph Pad Prism (Version 5.03) software.

Results

Effect of muscarinic antagonism on object recognition memory

We assessed the involvement of hippocampal and entorhinal cortex muscarinic receptors in retrieving object memories by performing novel object recognition test. Mice were trained with two identical objects (familiar objects) for three training sessions. The sum of interaction time of intra-hippocampal and intra-entorhinal groups with both objects during training sessions is shown in Fig. 2A-1 and B-1 respectively. Two-way ANOVA was used to analyze training data (intra-hippocampal: F-group = 17.14; $p = 0.0002$, F-object = 0.5151; $p = 0.60$, F-interaction = 0.065; $p = 0.937$; intra-entorhinal cortex: F-group = 0.199; $p = 0.657$, F-object = 5.158; $p = 0.01$, F-interaction = 0.625; $p = 0.54$). Except the first training session of intra hippocampal control and scopolamine groups ($p > 0.05$), Bonferroni test revealed no significant difference in object interaction time between intra hippocampal and intra entorhinal cortex control and scopolamine groups during all training sessions ($p < 0.05$). Intra- hippocampal and intra-entorhinal cortex scopolamine treated mice displayed reduced overall activity compared to their respective control groups during test session shown by total interaction time (intra-hippocampal: t = 4.28, df = 14, $p = 0.0008$; control: 31.71 ± 5.87 s, scopolamine: 6.77 ± 2.42 s, Fig. 2A-2; intra-entorhinal cortex: t = 4.49, df = 13, $p = 0.0006$; control = 24.57 ± 1.9 s, scopolamine = 12.63 ± 1.85 s, Fig. 2B-2). The percent discrimination index of intra-hippocampal control (59.51 ± 4.06%) and scopolamine (52.10 ± 9.37%) were above chance (chance = 50%) and not significantly different ($p > 0.05$, Fig. 2A-3). Similarly no significant difference was found in discrimination index of intra-entorhinal cortex control (59.73 ± 3.07%) and scopolamine (55.73 ± 4.91%) groups ($p > 0.05$, Fig. 2B-3). We also calculated the number of entries and the time spent in novel and familiar object compartments by intra-hippocampal and intra-entorhinal cortex control and scopolamine groups. No difference between number of entries or time in novel or familiar object compartment was observed among the groups (Table 1).

Effect of muscarinic antagonism on spatial memory retrieval

To assess the involvement of hippocampal and entorhinal cortex muscarinic receptors in spatial memory retrieval mice were trained for Morris water maze test. Two-way ANOVA analysis revealed that control and scopolamine mice of intra-hippocampal (Fig. 3A-1) and intra-entorhinal cortex (Fig. 3B-1) significantly learned the hidden platform location shown by shorter swim latency time on day 5 (*post-hoc* Bonferroni's test: $p > 0.05$; intra-hippocampal: control = 13.89 ± 3.15 s, scopolamine = 20.42 ± 7.11 s; intra-entorhinal cortex: control = 20.00 ± 11.82 s, scopolamine = 18.40 ± 8.11 s). Spatial memory retrieval was measured through probe trial on the test day. Pre-test scopolamine infusion in hippocampus (35.33 ± 3.14 s, n = 9, above chance, Fig. 3A-2) and entorhinal cortex (25.75 ± 4.25 s, n = 8, above chance, Fig. 3B-2) did not affect the time spent in target quadrant by the mice compared to their respective controls (intra-hippocampal: 37.57 ± 2.97 s, n = 7, t = 0.505, df =14, $p = 0.62$, Fig. 3A-2; intra-entorhinal cortex: 27.71 ± 4.04 s, n = 7, t = 0.332, df = 13, $p = 0.75$, Fig. 3B-2). However, intra-hippocampal and intra-entorhinal cortex scopolamine groups spent significantly greater time in the quadrant opposite to the target quadrant than their respective control groups (intra-hippocampal; t = 3.302, df = 14, $p = 0.005$; control: 14.37 ± 2.1 s; scopolamine: 23.04 ± 1.65 s; Fig. 3A-3; intra-entorhinal; t = 2.65, df = 13, $p = 0.02$; control = 18 ± 2.01 s; scopolamine = 24.26 ± 1.34 s; Fig. 3b-3). Scopolamine infusion caused no change in the number of entries in the target quadrants by intra-hippocampal (9.11 ± 0.63, n= 9, Fig. 3A-4) and intra-entorhinal cortex (8.75 ± 1.56, n = 8, Fig. 3B-4) groups compared to their respective controls (intra-hippocampal: 11.00 ± 0.92, n = 7, t = 1.741, df = 14, $p = 0.1$, Fig. 3A-4; and intra-entorhinal cortex: 9.14 ± 1.05, n = 7, t = 0.201, df = 13, $p = 0.84$, Fig. 3B-4). Intra-hippocampal scopolamine treated mice showed significant impairment in recalling the platform location by exhibiting significantly less crossings over the platform location compared to saline treated controls (t = 2.441, df = 14, $p = 0.02$: control: 4.71 ± 0.74, n = 7; scopolamine: 2.44 ± 0.58, n = 9) Fig. 3A-

Table 1
Mean number of entries and mean time spent in novel and familiar object compartments by intra-hippocampal and intra-entorhinal cortex groups. Data analyzed by two way ANOVA followed by *post hoc* Bonferroni's test (mean ± SEM).

Compartments		Intra- hippocampal groups			Intra- entorhinal cortex groups		
		Control	Scopolamine	*p*-value	Control	Scopolamine	*p*-value
Entries	Familiar	7.14 ± 1.28	4.11 ± 1.59	$p > 0.05$	11 ± 1.57	7.25 ± 1.09	$p > 0.05$
	Novel	9.57 ± 1.11	4.66 ± 1.89	$p > 0.05$	12.14 ± 1.77	8.0 ± 1.36	$p > 0.05$
Time	Familiar	101.9 ± 12.51 s	59.44 ± 22.46 s	$p > 0.05$	98 ± 6.64 s	93.85 ± 17.11 s	$p > 0.05$
	Novel	133 ± 11.6 s	119.88 ± 33.02 s	$p > 0.05$	104 ± 6.34 s	83.75 ± 16.97 s	$p > 0.05$

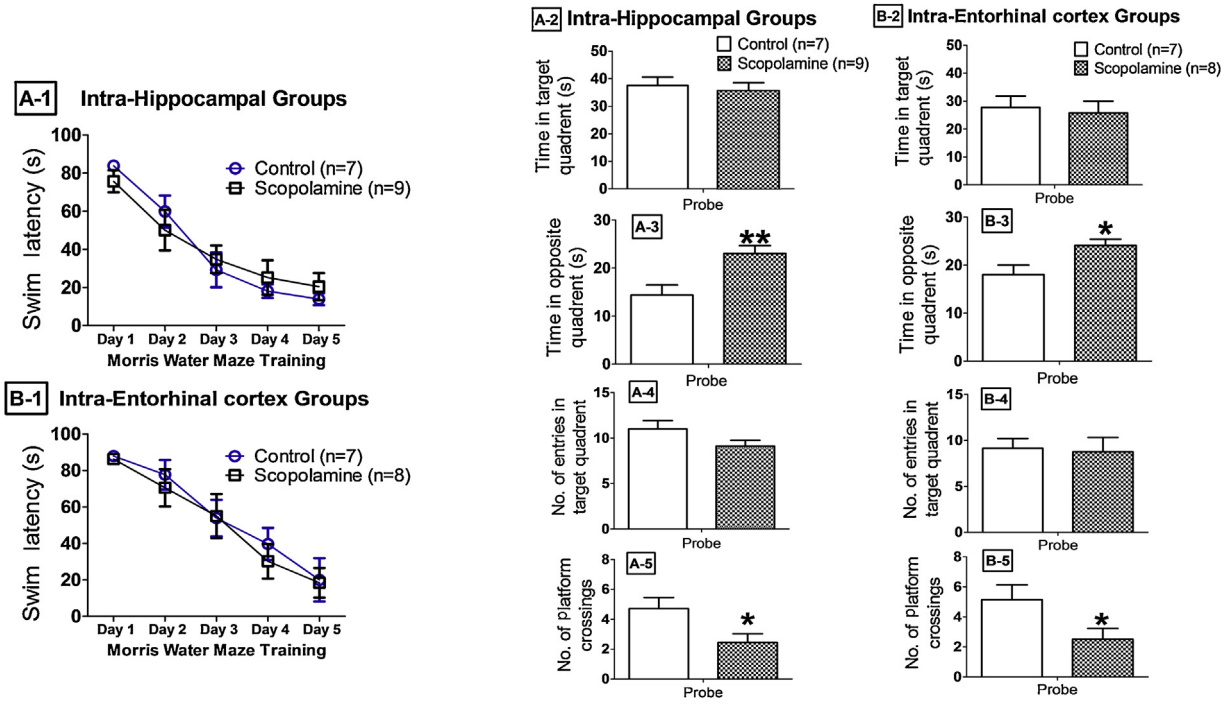

Fig. 3. Effect of hippocampal and entorhinal cortex muscarinic receptors on spatial memory retrieval. Graph A-1 and B-1 are showing Morris water maze training sessions of intra-hippocampal and intra-entorhinal cortex groups respectively. Graph A2-A5 are representing the time spent in target quadrant, time spent in opposite quadrant, number of entries in target quadrant and number of platform location crossings by intra-hippocampal control (n = 7) and scopolamine (n = 9) groups. Graph B2-B5 are representing the time spent in target quadrant, time spent in opposite quadrant, number of entries in target quadrant and number of platform location crossings by intra-entorhinal cortex control (n = 7) and scopolamine (n = 8) groups. Scopolamine administered mice spent significantly greater time in opposite quadrant and lesser number of platform location crossings compared to controls. Data is presented as ± SEM. $^{**}p < 0.005$ and $^{*}p < 0.05$.

5. Similar to the effect of intra-hippocampal groups, we found intra-entorhinal cortex scopolamine infusion significantly reduced the number of times mice crossed platform location compared to control (t = 2.18, df = 13, $p = 0.04$: control: 5.14 ± 0.98, n = 7; scopolamine: 2.50 ± 0.73, n = 8) Fig. 3B-5.

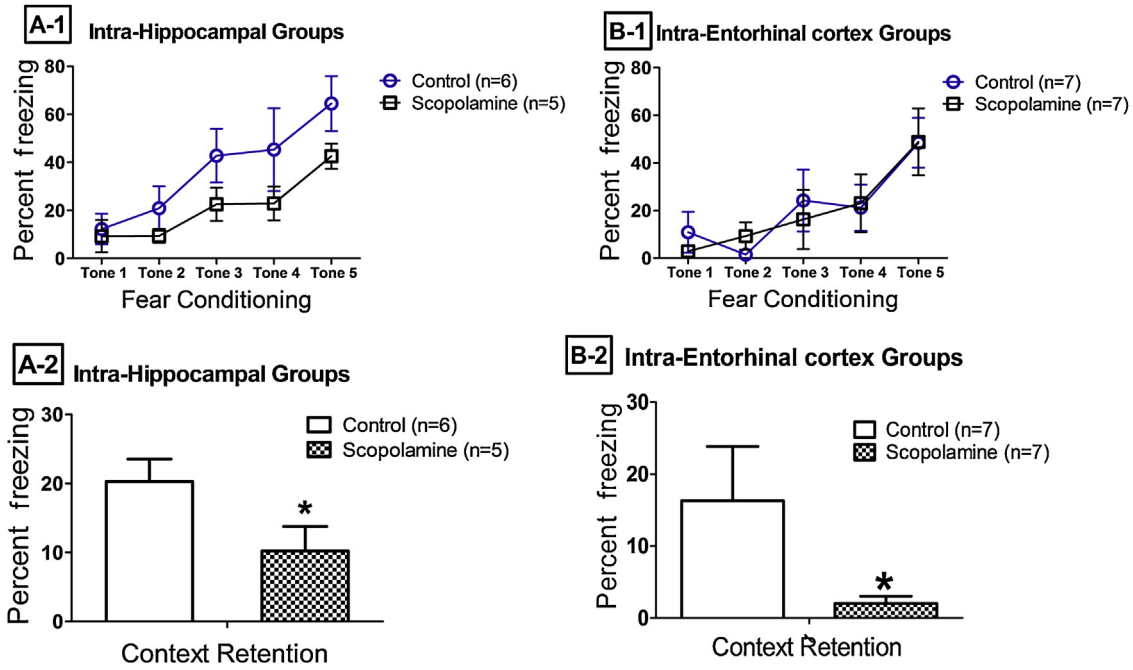

Fig. 4. Effect of hippocampal and entorhinal cortex muscarinic receptors on contextual fear retrieval. Graph A-1 and B1 are representing freezing of intra-hippocampal and intra-entorhinal cortex groups, respectively, in response to contextual fear conditioning. Graph A-2 is showing percent freezing response of intra-hippocampal control (n = 6) and scopolamine (n = 5) groups. Intra-hippocampal scopolamine impaired contextual fear memory retrieval. Graph B-2 is representing percent freezing response of intra-entorhinal cortex control (n = 7) and scopolamine (n = 7) groups. Entorhinal cortex muscarinic antagonism significantly impaired fear retrieval in conditioning context. Data is presented as ± SEM. $^{*}p < 0.05$.

Effect of muscarinic antagonism on contextual fear memory retrieval

Fig. 4A-1 and B-1 are showing the contextual fear conditioning of intra-hippocampal and intra-entorhinal cortex groups respectively. Training data of fear conditioning was analyzed by two-way ANOVA (intra-hippocampal: F-group = 7.284; p = 0.0095, F-tone = 6.809; p = 0.0002, F-interaction = 0.4118 p = 0.799; intra-entorhinal cortex: F-group = 0.0347; p = 0.8527, F-tone = 6.147; p = 0.0003, F-interaction = 0.235; p = 0.9174). All animals in of hippocampal and entorhinal cortex developed fear memory shown by increased freezing to the last training tone (*post hoc* Bonferroni test: intra-hippocampal: control = 64.48 ± 11.48%, scopolamine = 42.58 ± 5.27%, $p >$ 0.05; intra-entorhinal cortex: control = 48.43 ± 10.45%, scopolamine = 48.84 ± 14.08%, $p >$ 0.05). Though intra-hippocampal scopolamine showed relatively less percent freezing response to tone 3–5 compared to the controls, but the difference was not revealed significant by *post hoc* Bonferroni's test. Moreover, mice in both hippocampal groups showed significant association of tone with the foot shock shown by significant difference in percent freezing response to tone 1 and tone 5 (Control: t = 3.981, df = 10, tone 1 = 12.22 ± 6.36%; tone 5: 64.47 ± 11.48%; p = 0.002; Scopolamine: t = 3, df = 10, tone 1 = 9.25 ± 6.76, tone 5 = 42.58 ± 5.27, p = 0.003). On the test day we found significant involvement of hippocampal muscarinic receptors in the retrieval of contextual fear evident by the difference in freezing response between control and scopolamine groups (t = 2.086, df = 9, p = 0.03: control: 20.28 ± 3.25%, n = 6 and scopolamine: 10.22 ± 3.56%, n = 5 respectively; $p <$ 0.05, Fig. 4A-2). Muscarinic antagonism in entorhinal cortex also reduced the recall of contextual fear compared to the control group (t = 1.875, df = 12, p = 0.04: control: 16.30 ± 7.54% and scopolamine: 2.050 ± 0.97%; $p <$ 0.05, Fig. 4B-2).

Discussion

Hippocampus and entorhinal cortex constitute major brain regions involved in memory processes. In current study we assessed the involvement of muscarinic receptors in hippocampal CA1 and medial entorhinal cortex during retrieval of different forms of episodic memories. We probed the role of CA1 and medial entorhinal cortex muscarinic receptors in the recall of object recognition, spatial and contextual fear memories.

The results of our study showed that object memory retrieval was not affected by muscarinic antagonism in hippocampus and entorhinal cortex. We found over all reduced total object (novel + familiar) interaction in response to hippocampal and entorhinal cortex muscarinic antagonism. Dorsal hippocampus specifically CA1 is reported to be involved in retrieval of object recognition memory [21,23,24] but there are contrasting reports of medial entorhinal cortex involvement in object recognition memory recall [25,26]. Central and pre-frontal cortex cholinergic signaling is involved in attention during different memory tasks [27,28].

Hippocampus and entorhinal cortex are necessary for Morris water maze, fear conditioning and context retention [36,37]. Intra-hippocampal CA1 and intra-medial entorhinal cortex infusion of scopolamine lead to impaired spatial and contextual fear retrieval which is in consistence with the recently reported findings of Leaderbrand et al. [30]. According to them muscarinic receptor inactivation in dorsal hippocampus and retrosplenial cortex lead to impaired recall of contextual memories. Present results have demonstrated that muscarinic antagonism in hippocampus and media entorhinal cortex impaired the spatial and fear memories in region dependent manner (Table 2).

Memory formation and retrieval are anatomically and functionally complex process engaging participation of different brain regions in a hierarchical manner (Table 3) [38]. Brain circuitry for episodic memory is majorly conserved in mammals from rodents to humans. Hippocampus is the hub of formation and retrieval of object, spatial and fear memories [39–41]. Other brain regions, along with hippocampus are required for episodic memory formation and retrieval. Entorhinal cortex serves as interface between hippocampus and other brain regions, and is essential for spatial, object and fear memories formation and retrieval [36,42]. Perirhinal and parahippocampal cortices are implicated in novel object recognition memories [43–45]. Lesions and inactivation of reterosplenial cortex, prefrontal cortex, anterior cingulate cortex and striatum led to impaired memory functioning in Morris water maze test [46–49]. Along with hippocampus [36,50,51] and amygdala [50,52,53] frontal, ventromedial, cingulate cortices are implicated in fear memories [54,55].

Despite well-established neural mechanisms of memory formation and storage, underlying neural mechanism of memory retrieval are started to be explored recently. Medial prefrontal cortex GABA receptors mediate memory recall through ERK1/2 and phospho-CREB signaling [56]. Muscarinic activity in perirhinal cortex [32] and retrosplenial cortex [30] contribute to object and contextual memory retrieval, respectively. Hippocampal AMPA, glutamate and NMDA receptors differentially participate in retrieval of short and long term memories [57–60]. CA1 glutamate receptors retrieve long term memory through activation of PKA and MAP kinase signaling pathways [60].

Hippocampal CA1 is reciprocally connected to medial entorhinal cortex and both regions activate during memory retrieval [61]. Theta oscillations define awake and active state of animals and are crucial for encoding and retrieval phase of memory [62]. Grid cells in entorhinal cortex have great specificity for orientation, location and spatial tuning. During navigation through familiar context (retrieval) grid cells activate during trough phase of theta. Systemic scopolamine has been shown to impair grid cells spatial tuning [31] by affecting entorhinal theta oscillation [63]. Theta activity in hippocampus and medial prefrontal cortex is important for retrieval [64]. Han et al. have shown crucial role of theta synchronization between dorsal and ventral hippocampus for successful retrieval of fear memories [65]. We propose that scopolamine induced retrieval impairment observed in our study is through the modulation of theta oscillations in hippocampus and entorhinal cortex. Moreover theta oscillations are important not only for contextual memories but object recognition as well [66].

Our study provides functional evidence of muscarinic cholinergic activity during retrieval of different forms of episodic memories at the level of CA1 and medial entorhinal cortex. We suggest modulation of muscarinic receptors can be a potential

Table 2
Percent retrieval impairment induced by hippocampal and entorhinal cortex infusion of scopolamine in relation to respective controls. Control performance is considered 100%.

Memory type	% retrieval (hippocampal)	% retrieval (entorhinal cortex)
Novel object recognition	87.57%	93.18%
Platform location (spatial)	51.8%	48.68%
Context retention	50.39%	12.26%

Table 3
Brain regions involved in novel object recognition test, Morris water maze test and contextual fear conditioning.

Test	Brain regions involved	References
Novel object recognition	Hippocampus	[39–41]
	Perirhinal cortex	[43,44]
	Entorhinal cortex	[42]
	Parahippocampal cortex	[45]
Morris water maze test	Hippocampus	[36]
	Entorhinal cortex	[36]
	Retrosplenial cortex	[46]
	Anterior cingulate cortex	[47,48]
	Striatum	[47]
	Prefrontal cortex	[49]
Contextual fear conditioning	Amygdala	[50,52,53]
	Hippocampus	[36,50,51]
	Entorhinal cortex	[36]
	Frontal, ventromedial, cingulate cortex	[54,55]

strategy for treating retrograde amnesia associated cognitive disorders.

Conflict of interest

None.

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