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Inactivation of muscarinic receptors impairs place and response learning: Implications for multiple memory systems

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ABSTRACT

Extensive research has shown that the hippocampus and striatum have dissociable roles in memory and are necessary for place and response learning, respectively. Additional evidence indicates that muscarinic cholinergic receptors in the hippocampus and striatum exert an important role in the modulation of these memory systems. In our experiments, we assessed whether intact hippocampal and striatal muscarinic cholinergic transmission may be essential and/or necessary for place and response learning. We addressed these questions using administration of the muscarinic receptor antagonist, scopolamine, on both place and response learning in a food-rewarded T-maze task. The administration of scopolamine (15 μg or 30 μg) directly into the dorsal hippocampus impaired the performance of rats subjected to both place and cue-rich response version of the task, but did not affect the response version, when the task was performed under cue-poor conditions. However, the administration of scopolamine in the dorsolateral striatum impaired the cue-poor response version of the T-maze task without interfering with the place version or cue-rich response version. Taken together, these results indicate that activation of muscarinic cholinergic receptors in the hippocampus and striatum facilitate the use of different strategies of learning, thus strengthening the hypothesis of multiple memory systems. Additionally, these results emphasize the importance of the environmental conditions under which tasks are performed.

1. Introduction

Extensive evidence from human and other animal studies indicates the existence of multiple memory systems (Squire and Zola-Morgan, 1988; White and Mcdonald, 2002; Doeller et al., 2008). In rats, lesions of the hippocampus or anatomically related structures impair the learning of tasks that require information about the place or are based on the use of extra-maze cues (i.e., place learning). However, striatal damage generally impairs the performance of tasks that involve associations between discrete cues and behavioral responses (i.e., cued or response learning) (Morris et al., 1982; Packard and McGaugh, 1996; Xavier et al., 1999; Lee et al., 2008; Miyoshi et al., 2012; but see Oliveira et al., 1997; Chang and Gold, 2004).

Several studies support the view that acetylcholine (ACh) modulates learning and memory processes in these multiple neural systems (for review, see Gold, 2003; Hasselmo, 2006; Havekes et al., 2011; Deiana et al., 2011). In the hippocampus and striatum, as well as in other brain areas, the effects of ACh are mediated primarily by activation of different subtypes of muscarinic receptors (Hersch et al., 1994; Levey et al., 1995; Yan et al., 2001). In general, studies using muscarinic antagonists injected directly into the hippocampus (Riekkinen and Riekkinen, 1997; Herrera-Morales et al., 2007; Mikami et al., 2007; Olson and Cero, 2010) or striatum (Prado-Alcala et al., 1985; Diaz del Guante et al., 1991; Ragozzino et al., 2002; Legault et al., 2006) impair learning and memory tasks related to the particular neural system. Furthermore, some experiments indicate that the activation of muscarinic receptors in these regions is required for the induction of LTP (long-term potentiation), a form of synaptic plasticity that is widely thought to underlie learning and memory processes (Segal and Auerbach, 1997; Suzuki et al., 2001; Ghiglieri et al., 2011).

The hippocampus and the striatum have high concentrations of ACh. While hippocampal cholinergic inputs arise from basal forebrain structures (Lewis et al., 1967; Mesulam et al., 1983; Dutar et al., 1995), the high amount of ACh in the striatum is due to the presence of cholinergic interneurons (Lynch et al., 1972; Bolam

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et al., 1984; Calabresi et al., 2000). This distinct pattern of cholinergic innervation suggests that the cholinergic system may play different roles in modulating behavioral tasks mediated by these two structures. Studies using *in vivo* microdialysis methods to measure ACh release in these brain areas have shown that rats trained in a dual-solution task – a food-rewarded T-maze that can be learned using either place or response strategies – show different ACh efflux between the hippocampus and striatum (Chang and Gold, 2003b). Furthermore, the use of a place strategy at the beginning of training was accompanied by early increases of ACh release in the hippocampus, and the use of a response strategy later in training was associated with increases in ACh in the striatum. Similar results were observed in experiments using other versions of the task, designed to require the use of only one of these two strategies. In the place version of the T-maze task, rats were trained to find food at a particular spatial location (e.g., the arm pointing west). In the response version of the task, the goal arm was always the arm to the right (or left) of the start arm, regardless of the start position. For the response version, rats were trained to find food by repeating the same body turns. Of related interest, the ACh release in the hippocampus increased during training for both place and response versions of the task. These findings might reflect the use of spatial information to solve the place task as well as the response task, possibly because of the availability of extra-maze cues. To test this hypothesis, rats were trained in a response version of a maze under either "cue-rich" or "cue-poor" environmental conditions. The results indicate that a similar increase of ACh release in the hippocampus was present for both cue conditions, but a decrease of ACh release was observed during training in the cue-poor condition (Pych et al., 2005). These findings suggest that the hippocampus remained activated throughout training when extra-maze cues were available but not when the cues were minimized. Consequently, if intact hippocampal ACh function is necessary for place and response learning, it is possible that blocking cholinergic function in the hippocampus will induce similar effects on both tasks; however, blocking cholinergic function in the striatum may only impair a response learning task.

Therefore, the purpose of the experiments presented here is to compare the effects of intrahippocampal and intrastriatal administration of the muscarinic receptor antagonist scopolamine on both place and response learning in a food-rewarded T-maze. Additionally, we sought to evaluate the environmental conditions under which scopolamine administration in the dorsal hippocampus does or does not impair response learning.

2. Materials and methods

2.1. Animals

Wistar male rats, 3–4 months old, were used as animal models. The animals were bred and raised in the animal facility of the Department of Psychobiology and of the Centro de Desenvolvimento de Modelos Experimentais (CEDEME), both of the Universidade Federal de São Paulo (UNIFESP), and were maintained under controlled temperature (23 ± 2 °C) and 12:12-h light–dark cycle conditions (lights on between 7:00 h and 19:00 h). Food and water were provided *ad libitum* until usage of a food restriction protocol. All procedures followed the local ethical committee of UNIFESP, under number 2000/07, in accordance with international rules for animal use and care.

2.2. Surgery

The animals were anesthetized with ketamine (90 mg/kg, ip) and xylazine (10 mg/kg, ip) and placed in a stereotaxic apparatus. Stainless steel guide cannulae (23 gauge, 8 mm) were implanted bilaterally in the dorsal hippocampus or the dorsal striatum and then fixed with dental cement and micro screws. The following coordinates were used within the dorsal hippocampus: 3.8 mm posterior to bregma, 2.4 mm from the midline and 2.6 mm ventral to the skull surface. Coordinates used for the dorsal striatum were 0.3 mm anterior to bregma, 4.0 mm from the midline and 4.5 mm ventral to the skull surface (Paxinos and Watson, 1997a).

2.3. Apparatus

An elevated plus–maze was used. The maze had four identical arms, with each arm 60 cm long and 10 cm wide, with walls 2 cm high. A removable wooden block was used to block the entry to one of the arms, turning the plus-maze into a T-maze, and another block was used to force the animal to remain in a given segment of the maze, when necessary. Small food wells were positioned at the end of each arm. The maze remained suspended 1 m from the ground and was located in a lighted room that contained a door, windows, and shelves that could serve as extra-maze cues. As the maze walls were low, these extra-maze cues were visible to the animal.

2.4. Handling and maze habituation

After surgery, the animals were individually housed in polypropylene plastic cages and were handled approximately 3 min/day, every day for 7 days before maze habituation. On the first and second days of habituation, the animals were placed individually in the center of the maze without reward. Each animal was allowed to explore the maze for 5 min. On the third, fourth and fifth days, each animal was placed into the arms, with food inside the food well, and confined for 1 min in each arm.

2.5. Food deprivation

On the same day as the first day of the habituation phase, the animals were weighed and submitted to a restricted food regimen. Body weights were gradually reduced and maintained at 85% of their free-feeding weights. During food deprivation, 10 g of feed per day were given to each animal until each animal reached the desired weight. Thereafter, the available food was maintained to keep the animal's body weight constant. From the first day of adaptation to the food deprivation, sucrose balls (50 mg used in the preparation of homeopathic medicines) were placed in the animal's cage, in addition to normal dietary feed for the animal to become accustomed to the type of food that would be used as a training reward.

2.6. Preference test

On the sixth day of habituation, the preferred arm was established in a preference test. The animal was placed at the end of the start-arm and allowed to choose freely between the two other arms of the maze (the arm located in front of the start arm was blocked). A response was recorded when the rat had four feet placed 10 cm inside an arm; no reward was given to the animal in this case.

2.7. Tasks

Separate groups of rats were trained in either a place or a response version of the T-maze. In the place version, the reward was always in the same arm, located in the same position relative to the room cues (e.g., the arm pointing east). In the response version, the animal's performance depended on the use of an egocentric or response strategy based on proprioceptive stimulus (consistently make the same body turn – e.g., turn to the right – at the choice-point for food reward). In all versions, the north and south arms were pseudo randomly selected as start arms for each trial. The east and west arms were considered to be goal arms. When a rat started from the south, the north arm was blocked and vice-versa. The correct (rewarded) arm was the opposite of that chosen by the rat on the preference test. Thus, the animals were rewarded in the arm opposite to their initial preference.

In each trial, the animal was placed at end of the start-arm and was allowed to run through the maze until it entered one of the two arms. Upon entering the correct arm, the rat was allowed to eat the reward. If a rat entered an incorrect arm, the food well at the end of the non-rewarded arm remained empty; the rat remained in either arm for 30 s, and no retracting was allowed. When the rats failed to leave the start arm within 120 s, such trials were considered to be omission errors. After eating the reward or after reaching the 120 s time limit, the rats were submitted to the next trial. The training ended when the rats reached 90 consecutive trials. Training was completed within a single day session. Arm selections and the criterion of 10 consecutive correct choices were recorded as measures of learning. Between each trial, the four arms were cleaned with alcohol (30%) to avoid olfactory cues and to avoid intra-maze cues from the maze. Additionally, the maze was rotated 90° counterclockwise after every five trials or after any three successive correct choices.

2.8. Drugs and microinjections

Scopolamine hydrobromide (Sigma Chemical Co) was dissolved in 0.9% saline (vehicle) and kept frozen until use. The doses used were 15 and 30 μg/0.5 μl. The drug was kept at room temperature on the day of the experiment. The control animals received 0.5 μl of saline per side.

Solutions were injected bilaterally through microinjection needle (30 gauges) that extended 1 mm beyond the tip of the guide cannula. Each microinjection needle was attached to a 10 μl Hamilton micro syringe through polyethylene tubing (PE-10). Infusions were controlled by an infusion pump (Model Bi2000 – Insight Equipment®, Sao Paulo, Brazil), programmed to deliver solution at a constant speed of