M1 muscarinic receptors are necessary for retrieval of remote context fear memory

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HIGHLIGHTS
• Differential role of muscarinic receptors on recent and remote memories retrieval
• Dicyclomine impairs the retrieval of recent and remote contextual fear conditioning.
• Dicyclomine impairs only retrieval of remote tone fear conditioning.
• The M1 muscarinic receptors are not essential for retrieval of inhibitory avoidance.

ABSTRACT
Several studies have investigated the transition of consolidation of recent memory to remote memory in aver-sively motivated tasks, such as contextual fear conditioning (CFC) and inhibitory avoidance (IA). However, the mechanisms that serve the retrieval of remote memories, has not yet been fully understood. Some evidences suggest that the central cholinergic system appears be involved in the modulation of these processes. Therefore, the present study aimed to investigate the effects of a pre-test administration of dicyclomine, a high-affinity M1 muscarinic receptor antagonist, on the retrieval of remote memories in fear conditioning and IA tasks. Male Wistar rats were trained, and after 1 or 28 days, the rats received dicyclomine (16 or 32 mg/kg, intraperitoneally, i.p.) and were tested in CFC, tone fear conditioning (TFC) and IA tasks. At both time intervals, 32 mg/kg dicyclomine induced impairment of CFC. In TFC task only the performance of the rats 28 days after training was impaired. The IA task was not affected in any of the studied intervals. These findings suggest a differential contribution of muscarinic receptors on recent and remote memories retrieval revealing a more generalized role in remote memory.

1. Introduction
The ability to recall and use remotely learned information is critical to survival for humans and animals, for example allowing to avoid dan-gerous places in the future. Thus understanding how remote emotional memories are maintained in the brain may provide important evidence about how memories for unpleasant or noxious experienced events in general are represented. Traumatic memories constitute case in which remote aversive learning impairs the quality of life of individuals. Anxiety disorders such as post-traumatic stress are characterized by retrieval of intrusive memories related to the trauma event that persist for long term after the experience [1]. The difficulty of treatment of post trauma-tic disorders is known [2] possibly because remote memories are more stable than recent memories [3,4]. Therefore, it is important to search the neurobiological faces of remote memories. Although much has been learned about transition of consolidation of recent memory to remote memory, the mechanisms that serve the retrieval of remote memories have not yet been fully understood.

Animal models of fear conditioning are widely used in the investiga-tion of the neurobiological mechanisms of aversive memory and have received considerable attention in the last few decades [5,6]. Generally, in this paradigm a discrete conditioned stimulus (CS, typically a tone) is paired with an aversive unconditioned stimulus (US, typically a footshock) in a given context. After the stimulus pairing, the rat displays a fear response to both the tone and the training context [7,8]. Concerning the neural circuits involved, the amygdala plays a key role in both the contextual fear conditioning (CFC) and tone fear conditioning (TFC) tasks. The hippocampus was seen to exert influence only on CFC [9]. On the other hand, several studies indicated the hippocampus has a time-dependent role and performs a transient function in memory storage [10]. Indeed, Kim & Fanselow [11] showed that electrolytic
lesions of the hippocampus 1 day after training, but not 28 days after, abolished contextual fear memories in rats. After this influential work, other papers have showed that damage to the hippocampus preferentially disrupts recent memories while sparing remote memories using similar times intervals between training and test [12,13]. According to this view, consolidation of recent memories is dependent on activity of the hippocampus whereas the remote memories involve increased activity in a network of cortical sites [10,14]. However, there is also evidence of a permanent role of the hippocampus in contextual memory [15–17].

Inhibitory avoidance (IA) is another behavioral task widely used in the study of the neurobiology of memory associated with emotional and traumatic events [18]. Similar to fear conditioning, the rat is subjected to an aversive experience (footshock) in a given context. However, in this task the rat’s response of entering into dark chamber is punished by a footshock and then the animal avoids reentering that chamber. Moreover, IA is also subjected to interference from lesions in the amygdala [19] and hippocampus [20].

Several studies have shown the involvement of the central cholinergic system in the modulation of memory processes in aversely motivated tasks, including CFC, TFA and IA (for a review see [21]). Experiments employing pharmacological manipulations suggest that the administration of muscarinic cholinergic receptor antagonists impair this kind of learning [22–24]. Studies that used dicyclomine, a M1 muscarinic receptor antagonist, with higher affinity for M1 neuronal receptors [25–27] have reported the critical role of M1 subtype muscarinic receptors in these processes [28–31]. Studies from our lab showed that the pre-training administration of dicyclomine impaired the performance of rats in both CFC and IA tasks, sparing TFC, which suggested that this antagonist exerts its effects on hippocampal-dependent tasks without modifying performance in similar tasks hippocampal-independent [28]. Subsequently, it was indicated that dicyclomine does not modify the consolidation of either CFC or IA, which showed a specific involvement of these receptors in the acquisition of the tasks [29]. In addition, it has been shown that the M1 receptor subtype predominates in the cerebral cortex, hippocampus and amygdala [32,33], areas related with the transition of recent memory to remote memory.

particularly regarding the role of M1 receptors in the retrieval of recent memory, the pre-test administration of intermediate doses of dicyclomine led to impairment in CFC but did not alter TFC or IA, suggesting that retrieval in these tasks was mediated by different neurochemical mechanisms [30]. Nevertheless, the effects of the administration of this antagonist on the retrieval of remote memory in the CFC task, which is putatively independent from hippocampal functional integrity and its effects on TFC and IA tasks cannot be inferred from recent memory studies. Therefore, the aim of the present study was to investigate the effects of dicyclomine, an antagonist with a high affinity for M1 receptors, on remote memory retrieval of CFC, TFC and IA tasks.

2.2. Drug

Dicyclomine hydrochloride (Sigma) was diluted in 0.9% saline solution and injected intraperitoneally (i.p.) at a volume of 1 ml/kg. The doses were 16 and 32 mg/kg. The drug was prepared on the day of testing and maintained in a water bath at 30 °C to avoid salt precipitation. The control group rats only received a 0.9% saline solution.

The doses in this study were based on previous studies, which reported the absence of effects of 2 and 8 mg/kg doses of dicyclomine in the acquisition of CFC and IA tasks. Additionally, 16 and 32 mg/kg doses induced an amnestic effect [28,30].

2.3. Apparatus

The avoidance apparatus was used for both the CFC and IA tasks. This apparatus consisted of 2 acrylic boxes measuring 22 × 21 × 22 cm connected by a guillotine door. The walls of the safe box or chamber were white, thus generating a light environment, whereas black walls in the other chamber generated a dark environment, in which the rats received the shocks. Both chamber tops were composed of clear acrylic. The floor consisted of a metal grid (0.4-cm diameter rods placed 1.2 cm apart) connected to an electric shock generator (AVS – Projetos Especiais), which produced a 0.6 mA electric current that lasted 1 s and caused a footshock. A test chamber was used for the TFC test. This apparatus consisted of a cylindrical white acrylic container that measured 35 cm in diameter × 60 cm high. Each apparatus was maintained in a different room. A buzzer outside of the IA apparatus or the test chamber produced a 60-dB tone, which was used as a CS.

2.4. Behavioral procedures

In early studies we use only one context-shock pairings, which by itself could influence the remote memory retrieval [30]. Then, to compare the effects of the administration of an M1 antagonist (dicyclomine) on the retrieval memory when five context-shock pairings are used, similar parameters were used in retrieval of recent and remote memory tasks.

Each behavioral procedure was performed on different control and experimental groups. Prior to the behavioral procedures, the experimenter individually handled each rat for 2–3 min over 5 days for habituation.

2.4.1. Contextual fear conditioning

The CFC task was performed over 2 days. On the first day (training), each rat was placed directly in the dark chamber of the avoidance apparatus. The access door to the light chamber was closed. After 2 min, the rat received 5 0.6-mA footshocks (US) over 1 s in 30-second-intervals. The rats were removed from the apparatus 1 min after the final shock. The CFC test was performed 1 day after training to assess recent memory. Other groups of animals were tested 28 days after training to assess remote memory. In the test session, the rats were randomly allocated to 3 groups (n = 8–12 per group), which received pharmacological treatment with saline or dicyclomine (16 or 32 mg/kg). Thirty minutes after the injection, each rat was placed into the identical training context, i.e., directly placed into the dark chamber of the avoidance apparatus. The access door to the light chamber remained closed. No footshocks were administered. The freezing time, which was defined as complete body immobility without whisker movement or sniffing activity [7], was recorded for 5 min. The freezing time was scored manually with a chronometer off-line from video-recordings by an experienced observer blinded to the treatment conditions.

2.4.2. Tone fear conditioning

This paradigm was also performed over 2 days. On the first day (training), each rat was placed directly in the dark chamber of the avoidance apparatus. The access door to the light chamber was closed.
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