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Differential effects of m1 and m2 receptor antagonists in perirhinal cortex on visual recognition memory in monkeys

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ABSTRACT

Microinfusions of the nonselective muscarinic antagonist scopolamine into perirhinal cortex impairs performance on visual recognition tasks, indicating that muscarinic receptors in this region play a pivotal role in recognition memory. To assess the mnemonic effects of selective blockade in perirhinal cortex of muscarinic receptor subtypes, we locally infused either the m1-selective antagonist pirenzepine or the m2-selective antagonist methoctramine in animals performing one-trial visual recognition, and compared these scores with those following infusions of equivalent volumes of saline. Compared to these control infusions, injections of pirenzepine, but not of methoctramine, significantly impaired recognition accuracy. Further, similar doses of scopolamine and pirenzepine yielded similar deficits, suggesting that the deficits obtained earlier with scopolamine were due mainly, if not exclusively, to blockade of m1 receptors. The present findings indicate that m1 and m2 receptors have functionally dissociable roles, and that the formation of new visual memories is critically dependent on the cholinergic activation of m1 receptors located on perirhinal cells.

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1. Introduction

Visual recognition memory depends on activation of cholinergic muscarinic receptors in the perirhinal cortex, as evidenced by the memory impairment that is produced in both rodents and monkeys by intraperirhinal injections of the muscarinic receptor antagonist scopolamine (Tang, Mishkin, & Aigner, 1997; Warburton et al., 2003). Furthermore, this drug-induced impairment is known to result from interference with memory storage as opposed to memory retrieval, since scopolamine is effective when administered shortly before stimulus familiarization but not when it is administered in the period between familiarization and test (Aigner, Walker, & Mishkin, 1991). Although the above findings establish muscarinic receptors as critical players in memory formation, these receptors consist of several different subtypes, and, because scopolamine is a nonselective muscarinic antagonist, it is still unknown which subtypes are the essential ones for the storage of visual memories. The present study aimed to address this issue.

Muscarinic receptors are a non-homogeneous class of G-protein-coupled receptors, composed of five discrete subtypes, m1–m5 (Bonner, 1989; Caulfield, 1993; Wess, 1996). The five subtypes divide naturally into two groups on the basis of their cellular and molecular effects. One group, M1, consists of the m1, m3, and m5 subtypes, which couple to $G_{q/11}$ and thereby produce such changes as activation of phospholipase C, increased MAPK activity, and mobilization of intracellular Ca^{2+}. The other group, M2, consists of the m2 and m4 subtypes, which couple to $G_{i/o}$ and so induce changes such as inhibition of adenylyl cyclase activity and inactivation of Ca^{2+} channels (Eglen, 2006; Ishii & Kurachi, 2006; Lucas-Meunier, Fossier, Baux, & Amar, 2003). Receptor subtypes m1 and m2 are both abundantly expressed in cerebral cortex, but they differ in their laminar distribution as well as in their synaptic location, m1 being expressed mainly on postsynaptic neurons, and m2, mainly on presynaptic terminals (Alcantara et al., 2001; Levey, 1996; Rouse, Marino, Potter, Conn, & Levey, 1999).

Scopolamine does not discriminate between m1 and m2 subtypes as well as some other compounds do. For example, the muscarinic receptor antagonist pirenzepine was found in one *in vitro* assay to have a 57-fold greater affinity for the m1 than for the m2 subtype (Buckley, Bonner, Buckley, & Brann, 1989; Hammer, Berrie, Birdsall, Burgen, & Hulme, 1980), whereas the reverse was the case for the muscarinic receptor antagonist methoctramine, which had an approximately 4.4-fold greater affinity for the m2 than for the m1 receptor subtype (Buckley et al., 1989; Giraldo et al., 1988). Tinsley and colleagues (2011) recently reported that intraperirhinal injections of pirenzepine impaired visual recognition memory in the rat, but there has been no report on the visual memory effects of similarly infusing a selective m2 antagonist. Here, in a study conducted with monkeys, we attempted to

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compare directly m1 and m2 contributions to visual recognition memory by intraperirhinal microinfusions in separate sessions of the m1 blocker pirenzepine and the m2 blocker methoctramine.

2. Materials and methods

2.1. Subjects

The subjects were three naïve male monkeys (*Macaca mulatta*) weighing 4–8 kg at the start of the experiment. They were housed individually or in social pairs in rooms with an automatic lighting schedule (light/dark: 12/12 h). They were fed primate chow (No. 5038, PMI Nutrition International, LLC Brendwood, MO) with a variety of supplements, including fruits and nuts, and they had free access to water. The procedures used in this study were approved by the National Institute of Mental Health Animal Care and Use Committee and conducted in accord with the National Research Council Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

The stimuli were displayed on a 15-in., flat-screen, touch-sensitive monitor (Microtouch, 3M Center, St. Paul, MN) in an unlit, sound-attenuated chamber (Industrial Acoustics Company, Inc., Bronx, NY). The transport chairs in which the monkeys sat for testing allowed them free arm movements. Each correct response was rewarded with a 190-mg food pellet (equal mixture of banana, fruit punch, and grape flavors; Research Diets, New Brunswick, NJ) automatically dispensed into a plastic cup located centrally beneath the monitor.

2.3. Behavioral procedures

The monkeys were habituated for a few sessions to both the transport chair and test apparatus while given free access to pellets in the cup. They were then trained to touch colored pictures on the monitor for pellet rewards. The visual stimuli, 9×9 cm square, appeared against a black background (LabView software; www.ni.com).

The monkeys then started training on the rule for delayed non-matching-to-sample (DNMS) with trial-unique photographs of man-made objects, plants, animals, foods, and nature scenes. For the familiarization phase of each trial, a single sample stimulus appeared centrally on the screen. Touching the sample led to reward and cessation of this stimulus. For the test phase of the trial, presented 10 s later, the sample and a novel stimulus appeared simultaneously, 9 cm apart and equidistant from the center; touching the novel stimulus led to reward and cessation of both stimuli, whereas touching the familiarized sample led only to cessation of the stimuli. There was no correction for errors. Trials were repeated at a constant intertrial interval, ranging between 20 and 30 s depending on the subject, and at the rate of 60 trials per day until the animals met the criterion of 90% correct responses on two consecutive days. Throughout training and subsequent testing on the DNMS rule, pairs of trial-unique stimuli were drawn pseudorandomly from a pool of 8000 stimuli until all were used, after which they were recycled.

Once the animals acquired the DNMS rule, list-length was gradually increased from one sample stimulus to five sample stimuli with 10 s interstimulus intervals (ISIs). In the test phase of each list length, all the sample stimuli were shown again in the same order as before, but now each was paired with a different novel stimulus, with the left–right positions of the sample and novel stimuli changed pseudorandomly. Testing continued at the same rate as before (i.e. 60-trial sessions) until the animals regained the criterion of 90% correct responses.

List lengths and ISIs were then increased either in steps of 5 stimuli or 5 s, until the animal's performance dropped below a stable level of 90% correct responses, at which point the memory demands were reduced one step. This procedure was followed to accommodate individual differences in the animals' recognition memory ability. In the final version of the task, list-lengths across the three monkeys varied from 15 to 25 stimuli presented at ISIs of 15–20 s, resulting in retention intervals between sample and test that ranged from about 4 to 8 min. The monkeys performed 3–5 such lists per session or 75–80 trials per day, 5 days per week. Following training, animals were continued at their final list-length and ISI level until they attained a criterion of 90% correct responses for five consecutive days.

2.4. Surgery

For both headpost and chamber attachment procedures, the animal received glycopyrrolate (0.01 mg/kg, i.m.) and ketamine HCl (10–15 mg/kg, i.m.) prior to intubation, after which the anaesthetic isoflurane (1.0–3.0%, inhalation) was given to effect for the duration of the surgery. Using aseptic techniques, the skin was incised and connective and muscle tissues were retracted in anatomical layers to expose the skull. The titanium headpost was custom-shaped during surgery for optimal fit and held in place by titanium screws (Veterinary Orthopedic Implants; www.vetimplants.com). Using MRI brain scans acquired for the purpose, stereotaxic coordinates were calculated for placement of a rectangular plastic chamber (modified electrode holder) through which the injection-target area could be reached. During surgery, the plastic chamber was individually contoured, placed at the appropriate stereotaxic position, and secured to the skull with dental cement anchored by ceramic screws. Once the cement had hardened fully, the soft tissues were sutured in anatomical layers around the chamber edges, and the chamber was closed with a removable plastic cap.

In another aseptic surgical procedure carried out 2 weeks later, and with the animal anesthetized as before, cranial tissue within the chamber area was removed bilaterally in order to access the target area for microinfusions through a cannula-guide grid inserted in the injection chamber. During each surgical procedure, animals received prophylactic antibiotic treatment (Cefazolin, 25 mg/kg i.m.) and analgesic agents (Ketoprofen, 1 mg/kg i.m. bid). The guide grid was inserted for MRI scanning and for each intraperirhinal injection session, as described below. Before inserting the guide grid and also after removing it following each use, the interior of the plastic chamber and surrounding tissue were thoroughly cleaned with dilute betadine solution followed by sterile saline. Between each use, the chamber was covered with the plastic cap.

2.5. Drug infusions

On completion of surgery, the monkey was given a 2-week recovery period, after which daily DNMS training and testing was resumed. On reattaining the performance criterion, each animal received a second MR scan, this one performed to obtain coordinates for the perirhinal infusions. By filling the injection chamber with a sterile solution of gadolinium diluted in saline (1:1000; Magnevist, Berlex Imaging, Wayne, NJ), the holes of the cannula-guide grid could be visualized and individual MRI-based coordinate maps could be constructed for perirhinal targeting (Saunders, Aigner, & Frank, 1990). The estimated coordinates were confirmed by injection of the gadolinium solution (see Fig. 1). Each animal received a series of bilateral-microinfusion sessions. The compounds tested, pirenzepine and methoctramine (each obtained