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Research paper

Treatment with a muscarinic acetylcholine receptor antagonist impairs the acquisition of conditioned reward learning in rats

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HIGHLIGHTS

- The muscarinic antagonist, scopolamine, impairs the acquisition of conditioned reward learning.
- The muscarinic antagonist, scopolamine, does not impair the expression of conditioned reward.
- Muscarinic acetylcholine receptor stimulation is involved in the acquisition of conditioned reward learning.

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ABSTRACT

The neural mechanisms whereby a reward-associated stimulus gains reinforcing properties and comes to function as a conditioned reward (CR) are not understood. We propose that muscarinic acetylcholine (mACh) receptor stimulation is necessary for this type of learning. Here we tested the hypothesis that mACh receptor antagonism, with scopolamine, would attenuate the acquisition by a food-related stimulus of the capacity to function as a CR. Rats were exposed to 5 pre-exposure sessions during which two levers were present, one producing a light and the other a tone when pressed. This was followed by 3 conditioning sessions in which the levers were absent and the rats were presented with 30 light-food pairings delivered randomly. In the test session, the levers were present and presses on both levers were recorded. Different groups of rats received intraperitoneal injections of scopolamine (0, 0.375, 0.75 and 1 mg/kg) either prior to each conditioning session or prior to the test session. All groups showed significantly greater responding on the light lever in the test compared to the pre-exposure sessions, demonstrating the CR effect. In animals treated prior to conditioning the scopolamine groups pressed significantly less on the light lever than the vehicle group. In animals treated prior to the test the increased lever pressing for light was similar for all groups. These data suggest that scopolamine impaired the acquisition of CR but not its expression. The results support the hypothesis that mACh receptor stimulation is important for the acquisition by reward-associated stimuli of the ability to function as CRs.

1. Introduction

Reward-related learning is a vital aspect of an organism's ability to adapt to the environment. Learning about primary rewards (unconditioned stimuli; USs), such as food and water, also leads to learning about reward-related stimuli. A previously neutral stimulus repeatedly paired with a US can become a conditioned stimulus (CS) capable of eliciting behaviors that are similar or related to the US [20]. Furthermore, the CS is also capable of gaining reinforcing properties of its own, thereby, functioning as a conditioned reward (CR) [4,36]. The neural mechanisms whereby CSs gain reinforcing properties and become CRs are not fully understood and, therefore, constitute an aim of the current study.

One possibility is that the neuronal pathways that transmit US signals converge with the neuronal pathways that mediate potential CS signals as inputs to the pathway that mediates the unconditioned response. The dual stimulation by the eventual CS and US of relevant neurons allows the CS to gain the capability of stimulating the same motivational neural circuits as the US and elicit the same motivational behavior, independently of the US [5,8,27,37]. If this is the case, then blocking one of these inputs to the reward-relevant pathway could prevent the acquisition of such learning.

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Previous work suggests that the US signal mediated by food involves, at least, the release of acetylcholine (ACh) in the ventral tegmental area (VTA) [12,23], although ACh in other brain regions may also be important [22]. That ACh activity in the brain serves as a US signal for food is supported by studies showing that the blockade of muscarinic ACh (mACh) receptor stimulation in the VTA reduces eating [23,34] and impairs the acquisition, although not the expression, of food-related learning [35]. If this is so, then the blockade of mACh receptor stimulation should prevent the acquisition of reinforcing properties by a food-paired stimulus or CS. In this event the CS would also not function as a CR. In the present paper, we tested this hypothesis. Specifically, we hypothesized that systemic treatment with scopolamine, a mACh receptor antagonist, would attenuate or prevent the acquisition by a food-related stimulus of the capacity to function as a CR.

2. Methods

2.1. Subjects

Subjects were 64 male, facility bred Long-Evans rats, individually housed in a climate controlled room on a 12 hour light/12 hour dark cycle (lights off at 6 AM). The rats, with initial free feeding weights of approximately 350 g (325–375 g), were maintained at 85% of their free-feeding weights throughout the experiment by daily rationed food portions. Water was freely accessible at all times except during behavioral test sessions.

2.2. Drugs

The mACh receptor antagonist, scopolamine hydrobromide (Sigma–Aldrich, St. Louis, MO), was dissolved in 0.9% saline, at doses of 0, 0.375, 0.75 and 1.5 mg/kg.

2.3. Apparatus

Behavioral testing was conducted in 8 operant conditioning chambers housed in sound- and light-attenuating boxes. Each operant conditioning chamber, measuring 30.5 × 24.0 × 25.0 cm (l × w × h), had two aluminum walls, Plexiglas sidewalls and ceiling. The floor consisted of 0.60-cm diameter stainless steel rods spaced 2.0 cm apart. Each chamber was equipped with two levers, two white stimulus lights, a tone generator, and a food trough, all on the right wall. The food trough, measuring 3.0 × 3.6 × 2.0 cm (l × w × h), was centered between the two levers. Each lever was located 3.0 cm to the right and left of the food trough and 8.0 cm above the floor. Each white stimulus light was positioned 3 cm above each lever.

2.4. Procedure

The conditioned reward paradigm consisted of three phases; pre-exposure, conditioning and test phases. This paradigm tested the acquisition and expression of reward related learning.

2.5. Acquisition

During the pre-exposure phase, rats were placed in the operant conditioning chambers for 40 min sessions held once a day for five consecutive days. Pressing one lever produced a 3-s tone presentation while pressing the other lever resulted in a 3-s light presentation above that lever. The number of presses on each lever was recorded.

The conditioning phase consisted of three 60 min sessions held on separate days with one day between sessions. Thirty minutes prior to being placed in the operant chambers, all rats were treated with an intraperitoneal (IP) injection of one dose of scopolamine (0, 0.375, 0.75 or 1.5 mg/kg). In this phase, both levers were removed from the operant chambers. During each conditioning session, rats were presented with a 3-sec light presentation followed by a food pellet delivery for a total of 30 light stimuli paired with 30 food pellets. After completion of this phase, there was a 2-day rest period.

The test phase consisted of one 40 min session during which both levers were present and pressing one lever produced the tone and the other the light as in the pre-exposure phase. The number of presses on each lever was recorded.

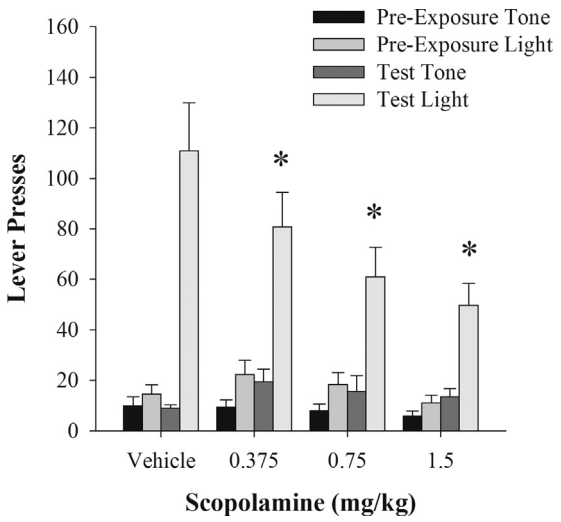

Fig. 1. Mean number of presses on the light and tone levers during the pre-exposure and test phases for rats treated with scopolamine (0, 0.375, 0.75 and 1.5 mg/kg) prior to each conditioning session. Vertical lines represent the standard error of the mean (SEM). * represents a significant difference from vehicle in light lever presses.

2.6. Expression

The conditioned reward paradigm was the same as in acquisition except that IP treatments of scopolamine (0, 0.375, 0.75 and 1.5 mg/kg) were administered prior to the test session and not prior to each conditioning session.

2.7. Data analysis

The average number of responses made on each lever during the pre-exposure phase and the test phase was analyzed for each group. A three-way mixed design analyses of variance (ANOVA) was conducted with the following three factors; phase (pre-exposure and test), scopolamine dose (0, 0.375, 0.75 and 1.5 mg/kg) and lever (light and tone). Significant 3-way interactions were followed by interaction comparisons and tests of simple effects. Significant simple effects were further analyzed using Dunnett's post hoc tests.

3. Results

3.1. Acquisition

In the pre-exposure phase, the numbers of lever presses on the light and tone levers were low with slightly more responding on the light lever. This pattern was similar in all groups (see Fig. 1). In the test phase, the vehicle group showed a large increase in light lever responding with little change in tone lever responding compared to the pre-exposure phase. The scopolamine groups showed no significant changes in responding on the tone lever but significantly large increases in responding on the light lever in the test phase compared to pre-exposure. However, the increases in light lever responding in the scopolamine groups were