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Research report

Olfactory delayed matching to sample performance in mice: Sex differences in the 5XFAD mouse model of Alzheimer's disease

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HIGHLIGHTS

- This is the first successful study of olfactory working memory in mice.
- Mice learned an olfactory delayed matching to sample task with delays up to 30 s.
- The 5XFAD mouse model showed no deficits in olfactory working memory at 6 months.
- Female mice performed better than males on the olfactory working memory task.

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ABSTRACT

While olfactory delayed matching-to-sample tasks have been used to assess working memory in rats, no such tasks have been tested in mice. Olfactory delayed matching-to-sample learning was assessed in male and female 5XFAD mice, a model of Alzheimer's disease, and their wildtype (B6SJL F1) littermates at 6–7 months of age using an operant olfactometer. All 5XFAD and wildtype mice were able to learn the delayed olfactory matching-to-sample task at 2 and 5 s delays. Fewer mice learned with a 10 s delay and only one mouse learned with a 30 s delay. Female mice showed higher levels of performance on the delayed matching-to-sample task than males, indicative of better working memory. These results demonstrate for the first time that mice are able to learn an olfactory delayed matching to sample task.

1. Introduction

Rodents perform remarkably well on olfactory learning tasks. Rats show "learning to learn" when serially presented with olfactory discrimination problems, and can achieve near errorless learning, which was previously thought only to occur in primates [36,37,33–35]. Rats show considerably faster learning on olfactory discrimination tasks than on visual and auditory discrimination tasks [24] and rats are able to perform a matching-to-sample task, with delays of up to 10 s between the sample and comparison odor [22]. The olfactory sensitivity of mice is similar to that of rats and, while mice take longer to complete initial training, spend more time between trials unengaged in the task, and made more errors during acquisition of a task, they were able to reach a level of performance comparable to rats on olfactory discrimination learning tasks, and showed retention of the olfactory memories after 32 days [7]. Mice also rapidly learn a Pavlovian conditioned odor preference task and retain the conditioned odor preference for at least 60 days after testing [32].

As a result of recent advances in genetic engineering techniques, an ever-increasing number of genetically modified mouse models of Alzheimer's disease (AD) have been developed [4,9,16]. Many of these mouse models of AD have learning and memory deficits on visual spatial tasks such as the Morris water maze [39] and the Barnes maze [25], but no studies of olfactory learning and memory have been done on these mice. An advantage of using an olfactometer to study learning and memory in mice is that both olfactory discrimination learning and working memory can be examined. While olfactory matching-to-sample tasks have been used to evaluate working memory in rats [3,22,29], mice have not been tested on olfactory matching-to-sample tasks. Working memory in mice is commonly examined with spontaneous alternation in Y mazes [19,26,27] or cross mazes [17,18]. When placed in either of these mazes mice will spontaneously alternate their entries into the arms, going into the arm which they have entered least recently [21]. The

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problem with these tests is that while they require working memory for the animals to remember the arms last entered, they rely on the concept of innate exploration of novel stimuli, and there are many other factors which could influence performance. If an animal were to simply turn the same direction every time they went to enter another arm they would display perfect alternation. Additionally, both anxiety [5] and spatial memory [21] have been shown to affect spontaneous alternation. Because tests of spontaneous alternation can be confounded in this way, goal directed tasks involving discrete stimulus presentations may better assess working memory, and are thus more valid tests of working memory [13].

The present study is the first to test mice on an olfactory matching-to-sample task. Male and female 5XFAD mice and their wildtype littermates were tested on an olfactory delayed matching-to-sample working memory task at 6–7 months of age. Mice have not previously been evaluated on an olfactory delayed matching-to-sample task, but because both 5XFAD mice [12,18,19] and AD patients [6,14] have deficits in working memory, we hypothesized that the 5XFAD mice would show deficits on the delayed matching-to-sample task. Relative to other transgenic mouse models of AD, the 5XFAD mouse shows an early onset of AD pathology, with Aβ plaques detectable at 2 months of age, as well as high levels of $A\beta_{40}$ and $A\beta_{42}$ in the brain, and low levels of complement factor H, an immune suppressor, decreasing levels of which has been linked to inflammatory neuropathology in AD [2,26].

2. Materials and methods

All animal protocols adhered to the Canadian Council on Animal Care guidelines and were approved by the University Committee on Laboratory Animals (protocol # 11-033).

2.1. Animals

We tested 6–7 month old 5XFAD mice (5 females, 6 males) and their wildtype (B6SJL) littermates (4 females, 9 males). The 5XFAD mouse model of AD has five mutations found in familial AD; three to the APP gene, the Swedish (K670N/M671L), Florida (I716V) and London (V717I) mutations, and two mutations to presenilin 1 (M146L and L286V) [26]. The mice were obtained from an in-house colony bred at Dalhousie University from mice purchased from The Jackson Laboratory (Bar Harbor, ME; strain numbers 006554 and 100012). The mice were weaned at 22 days of age and separated into same sex groups of 2–4 siblings housed in 30 cm × 18 cm × 12 cm polycarbonate cages with wire tops and *ad lib* access to food (Purina Rodent laboratory chow #5001). Genotypes were determined using PCR with DNA from ear punches, and mice testing positive for retinal degeneration ($Pde6b^{rd1}$ gene mutation) were not used. Ten days prior to the start of testing, the mice were individually housed, water deprived and fed with a mash of powdered rodent chow mixed with a measured amount of water. While on water restriction mice were weighed daily and the amount of water given in their mash adjusted to maintain their body weight at 80–85% of free feeding weight. As mice learned to respond in the olfactometer and received increasing amounts of water reward, the level of water restriction was decreased by gradually increasing the amount of water in their mash.

2.2. Olfactometers

Two computer controlled eight-channel liquid diffusion olfactometers (Knosys Olfactometers Inc., Lutz, FL) based on those described by Slotnick and Restrepo [38] were used (Fig. 1). In the olfactometers, filtered air from a compressor was pumped through bottles containing the odor solutions into a final valve, which directed the odor-laden air to an odor sampling port or an exhaust tube. The odor sampling port contained a reinforcement tube delivering water as a reward, and a sensor which detected when the animals were licking the water tube. Odor solutions were made by mixing commercially available odourants with mineral oil. The odors used, cardamom, lavender, dillweed, and patchouli (Aldrich Chemical Company Inc., Milwaukee, WI) were not found to be aversive to the mice in pilot studies, and mice were observed when initially presented with the odors for signs of aversion such as withdrawing their head from the odor sampling port.

2.3. Behavioral procedure

The mice were initially trained on a two odor discrimination and an odor reversal task so that they could learn the procedure for receiving water reward in the olfactometer. The matching-to-sample task started with two days of matching trials. During these trials, mice were presented with a sample odor (A) and then, after a 2 s inter-stimulus delay (ISD), the same odor (A) was presented as a comparison. The mice were rewarded with water for licking the reinforcement tube and there was a 5 s inter-trial interval (ITI). After two days of A–A matching trials, mice were given one day with both matching (A–A) and non-matching (A–B) trials. Non-matching trials were introduced after the mice had been presented with 10 matching trials. During non-matching trials the comparison odor was different than the sample odor and the mice were not rewarded for licking the reinforcement tube. The mice next received two days of B–B matching trials, in which they were rewarded for licking on each trial, followed by one day of mixed B–B matching and B–A non-matching trials, on which they were rewarded for licking only on B–B trials. Mice were trained for one hour or until they completed 100 matching trials. Mice were then presented with all four types of trials, A–A, B–B, A–B, and B–A, with the same odors used during matching to sample training. Trials were divided into blocks of 20, with 5 of each of the 4 types of trials in each block. Mice were considered to have reached criterion, and advanced to the test phase, when they correctly responded to 80% of each of the 4 types of trials in one block.

In the test phase, mice were presented with a new pair of odors (C and D), using the same 2 s ISD and 5 s ITI. After criterion (80% of each of the 4 types of trials) was reached on the 2 s ISD, the ISD was increased to 5 s, and then 10, and 30 s after they reached the 80% criterion at each ISD. The ITIs were 1.1 times the length of the ISD. Prior to advancing from one ISD to the next, the mice were presented with a series of all matching trials with ISDs incrementally increasing from the ISD of the stage previously completed to the next stage. For example, when the ISD was to be increased from 2 to 5 s, mice would first be presented with 2.5, 3, 3.5, 4, and 4.5 s ISDs. This was to ensure the mice would learn to continue to perform the task at the longer delay.

In order to facilitate responding at longer ITIs fifteen of the mice (7 Tg, 8 Wt) were run with a slight variation on this task. This variation provided the mice with small reinforcements during the ISD to encourage them to continue attending to the task during the delay period. Small reinforcements were given every 5 s during the ISD up to 10 s prior to the end of the delay. Additionally, the ITIs were different. Up to 10 s ISD, the ITIs were 6 s, above that ITIs were half the length of the ISD.

The mice were tested for a maximum of 10 blocks of 20 trials per day. The test session was ended before 10 blocks were complete if the mice stopped performing the task. At the 2 s delay mice commonly completed 10 blocks, but as the delays increased, and the amount of time required for the mice to complete 10 blocks increased; mice completed progressively fewer block of trials before they stopped performing the task.