Assessing Olfactory Learning and Memory in the 5XFAD Mouse Model of Alzheimer's Disease

by

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Abstract

Using an operant-olfactometer, the long term learning and memory, executive function, olfactory sensitivity, and working memory of the 5XFAD mouse model of Alzheimer's disease was assessed. Six month old male and female 5XFAD and wildtype mice were tested. No deficits were found on an olfactory discrimination task or a reversal learning task. Female and transgenic mice performed better than male and wildtype mice on the higher odour concentrations, but not the lower concentrations, of the sensitivity task, suggesting differences in learning rate or maximum performance on the task, but not olfactory detection. This study demonstrated for the first time that mice are able to learn an olfactory delayed matching to sample task with delays up to 30 seconds long. Female mice showed higher levels of performance on the matching to sample task than male mice, indicative of better working memory.

List of Abbreviations Used

Aβ	Amyloid-β
AD	Alzheimer's disease
AICD	APP intracellular domain
APP	Amyloid precursor protein
BACE1	Beta-site APP cleaving enzyme 1
DNA	Deoxyribonucleic acid
EA	Ethyl acetate
FAD	Familial Alzheimer's disease
ISD	Inter-stimulus delay
ITI	Inter-trial interval
NFT	Neurofibrillary tangle
PCR	Polymerase chain reaction
PS1	Presenilin 1
S+	Positive stimulus
S-	Negative stimulus

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Chapter 1: Introduction

Alzheimer's Disease (AD) is a progressive, neurodegenerative disease characterized by numerous cognitive deficits and by a build up of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFT) within the brain (Epis et al., 2010). As a result of recent advances in genetic engineering techniques, an ever-increasing number of genetically modified mice reported to model AD are being developed, with over 50 such models available (Alexandrov, Pogue, Bhattacharjee, & Lukiw, 2011). One recently developed model of AD in mice is the 5XFAD mouse. The 5XFAD model contains five mutations associated with familial Alzheimer's disease (FAD), three mutations to amyloid precursor protein (APP), and two presenilin 1 (PS1) mutations (Oakley et al., 2006). 5XFAD mice have been shown to develop heavy plaque deposits at an early age.

Before these mice can be used to test possible treatments of AD, the validity of the model must be assessed by examining the symptoms they exhibit. Patients with AD display memory impairments and dementia (Epis et al., 2010). Behavioural tests of working and long-term memory in mice are available, however, the majority of these test rely upon visual tasks, while rodents are primarily olfactory animals (Slotnick, 1993, 1994, 2001). The use of a battery of olfactometer tests, an odour discrimination task, reversal learning, an olfactory sensitivity test, and a delayed matching to sample task, were used to assess long-term memory, executive function, olfactory sensitivity and working memory, respectively.

1.1 Alzheimer's Disease

According to the amyloid hypothesis of AD, aggregation of misfolded Aβ is key to precipitating the pathology. APP can be processed by two pathways, the non-amyloidogenic α-secretase pathway, and the amyloidogenic β-secretase pathway. When processed by the α-secretase pathway, APP is first cleaved by α-secretase into sAPPα and C83. C83 can then be cleaved by γ-secretase, a membrane bound complex which includes PS1 (Duyckaerts, Delatour, & Potier, 2009), into p3 and AICD (APP intracellular domain), which can regulate gene expression. In the amyloidogenic pathway, APP is cleaved by β-secretase, also referred to as beta-site APP cleaving enzyme-1 (BACE1), into sAPPβ and C99; γ-secretase then cleaves C99 into Aβ and AICD (Finder, 2010).

Various isoforms of Aβ can be produced. The common forms of Aβ vary from 39-42 amino acids long with $A\beta_{42}$ the most damaging. $A\beta_{42}$ is more hydrophobic than the shorter peptides due to the additional amino acids on the membrane bound C-terminus, and is thus more likely to precipitate in an aqueous solution (Duyckaerts et al., 2009). The C99 fragment of APP has also been implicated in AD pathology and has been demonstrated to accumulate in mitochondria, resulting in their dysfunction (Devi & Ohno, 2012).

Three types of Aβ deposits can form: diffuse, stellate, and focal (Duyckaerts et al., 2009). Diffuse deposits are large deposits associated with apolipoprotein E and their role in AD pathology has been questioned as diffuse deposits have been found in individuals who show none of the cognitive impairments associated with AD. Stellate deposits are believed to appear in association with astrocytes, but are not well studied and their involvement in AD pathology remains unclear (Duyckaerts et al., 2009). Focal deposits

appear as dense spherical deposits of Aβ and are associated with activated microglia (Arends, Duyckaerts, Rozemuller, Eikelenboom, & Hauw, 2000). These plaques form very rapidly, within a 24 hour period, and the microglia appear shortly after formation of the plaques, suggesting that the microglia are a response to the plaque rather than a cause of them (Meyer-Luehmann et al., 2008).

Tau is involved in the assembly and stabilization of microtubules. When hyperphosphorylated, tau oligomizes into helical filaments, which can aggregate into NFTs which cause a loss of neural function by impairing axonal transport (Finder, 2010). Tau appears to spread through the brain along anatomical connections. This is supported by the case study of a patient who had part of her frontal lobe disconnected from the rest of her brain during surgical removal of a tumour prior to her developing AD. Upon her death it was found that the isolated section of cortex was free of tau in contrast to rest of brain where it was abundant. There were no differences in Aβ accumulation in the isolated cortex compared to the rest of the brain (Duyckaerts, Uchihara, Seilhean, He, & Hauw, 1997).

Neuronal loss and a decrease in synaptic density are associated with Aβ and NTF development and lead to degeneration of the cortex (Lerch et al., 2008). Neurodegeneration is most abundant in cortical layers II and III (Duyckaerts et al., 2009), the amygdala (Vereecken, Vogels, & Nieuwenhuys, 1994), the olfactory bulbs (ter Laak, Renkawek, & van Workum, 1994), and numerous nuclei including the substantia nigra (Uchihara, Kondo, Kosaka, & Tsukagoshi, 1992), the locus coeruleus (Busch, Bohl, & Ohm, 1997), and the raphe nuclei (Aletrino, Vogels, Van Domburg, & Ten Donkelaar, 1992). This degeneration results in a loss of white matter and a decrease in overall brain

volume (Mann, 1991).

Cognitive symptoms in AD include deficits in verbal, contextual, and visuospatial memory, attention, and executive function (Neugroschl & Wang, 2012). Deficits in switching tasks in the Wisconsin card sorting test, indicating high levels of perseveration, are common in AD (Nagahama et al., 2003; Terada et al., 2011), as are working memory impairments (Belleville, Peretz, & Malenfant, 1996; Gagnon & Belleville, 2011). Deficits in odour identification are one of the earliest symptoms to appear in AD (Rahayel, Frasnelli, & Joubert, 2012; Ruan, Zheng, Zhang, Zhu, & Zhu, 2012) and it has been proposed that this could be used as an early test for diagnosing AD (Schofield, Ebrahimi, Jones, Bateman, & Murray, 2012). Thus it is hypothesised that olfactory learning and memory may be disrupted in mouse models of AD.

1.2 The 5XFAD Model of AD

The 5XFAD mouse model of AD was developed by Oakley et al. (2006). This mouse has five mutations found in cases of 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). The mutations to APP are located at the sites where the β and γ secretase enzymes cleave APP. They increased cleavage of APP along the amyloidogenic β secretase pathway resulting in higher levels of Aβ, and specifically bias the pathway toward formation of the $A\beta_{42}$ isoform rather than shorter, and less pathogenic, forms of Aβ.

Relative to other transgenic mouse models of AD, the 5XFAD model shows an early onset of AD pathology, with 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 repressor, decreasing levels of which has been linked to inflammatory neuropathology in AD (Alexandrov et al., 2011).

Impairments in conditioned taste aversion, spontaneous Y maze alteration, and contextual fear conditioning have all been detected in 5XFAD mice. With deficits in conditioned taste aversion detectable at 9 months of age, and deficits of spontaneous Y maze alteration and contextual fear conditioning detected at 6 months of age (Devi & Ohno, 2010a, 2012; Kimura, Devi, & Ohno, 2010). One study found no deficits in conditioned fear to a tone, but did find deficits in 8 month old males to contextual fear (Kaczorowski, Sametsky, Shah, Vassar, & Disterhoft, 2011), while another found no deficits in contextual fear conditioning of 4 month old mice when tested 24 hours after conditioning, but there were deficits when testing was performed 30 days after training. Six month old mice were found to have deficits when tested either 24 hours or 30 days after training (Kimura et al., 2010; Kimura & Ohno, 2009). Spatial memory tests with the Morris water maze also showed impairments in 5 month old male mice (Hongpaisan, Sun, & Alkon, 2011), and spatial working memory, as measured by spontaneous alteration in a cross maze, is impaired in 6 month old mice (Jawhar, Trawicka, Jenneckens, Bayer, & Wirths, 2012). Novel object recognition is also impaired in 6-8 month old males (Joyashiki, Matsuya, & Tohda, 2011).

Interestingly, 5XFAD mice show decreased levels of anxiety in the open field and elevated plus mazes, with no differences in speed or distance traveled in the open field or arm entries in the elevated plus (Jawhar et al., 2012; Joyashiki et al., 2011).

Factors with the potential to confound many of the behavioural assessments include spinal and retinal pathology. The 5XFAD model shows amyloid pathology in the spinal cord as early as 3 months of age, and develops decreased body weight, motor control deficits, as measured by balance beam and string suspension tests, and unusual motor reflexes (Jawhar et al., 2012). They also develop high levels of amyloid deposits in the retina (Alexandrov et al., 2011), which may affect their visual ability and performance on visuo-spatial learning and memory tasks.

1.3 Olfactory Learning and Memory in Rodents

Rodents have been shown to perform remarkably well on olfactory learning tasks. When olfactory stimuli are used, rats are able to show signs of "learning to learn" when serially presented with problems, shown by achievement of near errorless learning, which was previously only thought to occur in primates (Slotnick, 1993, 1994, 2001; Slotnick & Katz, 1974; Slotnick, Kufera, & Silberberg, 1991). Rats trained on a series of two odour discrimination problems made progressively fewer errors during the acquisition of each problem, such that by the end of the series the rats were frequently making only a single error when learning a problem. This suggests that the rats were learning to use a cognitive strategy, likely a win-stay, lose-shift strategy, when presented with a new problem (Slotnick & Katz, 1974). Additionally, increasing the inter-trial interval up to 30 minutes has no effect on odour discrimination learning (Lovelace & Slotnick, 1995), and rats are able to perform a matching to sample task, with delays of up to 10 seconds between the sample and comparison stimuli having no effect on performance (Lu, Slotnick, & Silberberg, 1993).

Rats show much more complex learning in response to olfactory stimuli than to other sensory modalities. Nigrosh, Slotnick, and Nevin (1975) compared the ability of rats to learn on tasks using visual, auditory, and olfactory stimuli. The rats showed considerably faster learning on the olfactory task then on the visual and auditory tasks. When trained with combinations of olfactory and visual cues, then tested on each individual modality, rats were shown to attend to the olfactory cues to a greater extent then to visual cues. Finally, when presented with serial reversals, rats would show near errorless learning if trained on olfactory stimuli, while those trained on visual stimuli or auditory stimuli did not. From these results, it was hypothesized that the rats were using advanced cognitive strategies to complete the tasks, but only in response to olfactory stimuli.

While most of the research on olfactory learning in rodents has been done with rats, mice have also been used. Bodyak and Slotnick (1999) examined the performance of mice on olfactory learning tasks similar to those previously used with rats. They found that mice showed similar olfactory sensitivity to rats, and that while mice took longer to complete initial training, spent 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, and showed retention of the memories after 32 days.

1.3.1 Working Memory Tests

Dudchenko, Talpos, Young, and Baxter (2012) proposed that there are three different ways in which working memory can be assessed in animals: goal maintenance, memory span capacity, and interference control. Goal maintenance tasks require that the

animal have a malleable memory of a stimulus and, after an interval, respond in different manners depending upon the stimulus, in accordance with a learned set of rules for the task. Tests proposed to fall under this category include radial arm maze win-shift paradigms and delayed matching and non-matching to sample or position tasks. Memory span capacity tasks measure the amount of information that can be maintained in the working memory. A test that is claimed to fall under this category is the rodent odour span task (Dudchenko, Wood, & Eichenbaum, 2000) which requires a rodent to remember an increasing number of odours that it has been presented with to obtain a reward. Interference control tasks require that the animal save a representation, such as a stimulus or a location, in their working memory from interference by external stimuli or previous representations. Tests proposed to involve interference control are n-back tasks, in which the animal is presented with a sequence of stimuli and are to respond to a stimulus if it matches the stimulus presented n steps earlier in the sequence, and tasks that require the animals to remember the temporal order of stimuli or positions, such as those used by Jackson-Smith, Kesner, and Chiba (1993).

While the tests proposed by Dudchenko et al. (2012) certainly do involve working memory, there are problems with classifying tests as specifically measuring one of three proposed aspects of working memory as most tests involve more than one of these aspects. For example, during a matching or non matching to sample task, interference by previous trials could affect performance; n-back tasks require the animals to have the memory capacity to remember the last n stimuli in the sequence; and all tasks require some degree of goal maintenance.

Both matching and non-matching to sample tasks using olfactory stimuli have

been used with rats. Lu et al. (1993) used a delayed matching to sample procedure with an olfactometer. The rats were able to reach near errorless performance when presented with a series of different odour sets, and incrementally increasing the delay between the sample and comparison stimuli from 1 sec to 10 sec had no detrimental impact on performance. Other studies have demonstrated that rats can reach high levels of performance on olfactory matching (April, Bruce, & Galizio, 2011; Peña, Pitts, & Galizio, 2006) and non-matching (April et al., 2011) to sample tasks which required the rats to respond by digging in a cup of scented sand to indicate the correct stimuli. However, a possible confound resulting from the apparatus used in these matching and non-matching digging tasks is that the sample stimulus remains in the test chamber when the rats are presented with the comparison stimuli, this would enable the rat to refer back to the sample stimulus rather than having to remember it. Otto and Eichenbaum (1992) used an olfactory test they described as a continuous delayed non-matching to sample task with rats. The task is similar to a 1-back task in that the animals were presented with a sequence of odours and would be rewarded for responding whenever the presented stimulus differed from the preceding stimulus. The difficulty of the task was manipulated in two ways: first by changing the length of the delay between stimulus presentations, using delays of 3, 30, and 60 sec, and secondly, by changing the amount of interference previous stimuli would have by changing the number of odours in the learning sets, using sets of 16, 8, 4, and 2 odours. The task became more difficult for the rats as the length of the delay increased, and as the size of the odour sets decreased. Neither matching or non matching to sample tasks using olfactory stimuli have previously been performed with mice.

To examine the working memory of 5XFAD mice, previous studies have used spontaneous alternation in either Y (Kimura et al., 2010; Oakley et al., 2006; Ohno et al., 2007) or cross mazes (Hillmann et al., 2012; Jawhar et al., 2012). When placed in either of these mazes mice will spontaneously alternate the arm of the maze that they enter, going to the arm which they have entered least recently, due to innate exploration of novel stimuli (Lalonde, 2002). The problem with this test is that while it does require working memory for the animals to remember the arms they were last in, there are many other factors which could confound 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 (Bats et al., 2001), which has been shown to be lower in 5XFAD mice (Jawhar et al., 2012) and spatial memory (Lalonde, 2002) have been shown to affect spontaneous alternation. Due to the effects that these confounding factors can have on spontaneous alternation, it is argued that goal directed tasks involving discrete stimuli presentations better assess the aspects of working memory, as described by Dudchenko et al. (2012), and are thus more valid tests of working memory.

1.3.2 Reversal Learning

Reversal learning tasks involve changing the values of stimuli such that an animal rewarded for responding to stimulus A (A+) but not to stimulus B (B-), is now rewarded for responding to B (B+) and not to A (A-). This requires that the animal display behavioural flexibility and is considered to be an aspect of executive function (Kesner & Churchwell, 2011). Both rats (Nigrosh et al., 1975; Slotnick, Hanford, & Hodos, 2000) and mice (Del'Guidice et al., 2009; Mihalick, Langlois, & Krienke, 2000; Mihalick,

Langlois, Krienke, & Dube, 2000; Phillips, Boman, Österman, Willhite, & Laska, 2011) have been tested on olfactory reversal tasks. These involved the animals first learning to discriminate between an S+/S- odour pair before the values of the odours were switched in the reversal. The rats made fewer errors on the reversal than the initial discrimination task (Nigrosh et al., 1975; Slotnick et al., 2000). In contrast, the mice showed high degrees of perseveration, continuing to respond to the initially learned values of the odours, and thus made a greater number of errors on the reversal tasks than on the initial discrimination tasks (Del'Guidice et al., 2009; Mihalick, Langlois, & Krienke, 2000; Mihalick, Langlois, Krienke, et al., 2000; Phillips et al., 2011).

While reversal learning has not been examined in the 5XFAD model of AD, it has been looked at in other mouse models. Tg2576 mice, which develop high Aβ loads due to an APP mutation, showed impaired reversal in an olfactory task at 6 months of age (Zhuo et al., 2007). Another study found that neither B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax mice expressing increased Aβ due to APP and PS1 mutations, or B6.Cg-MAPT$^{tm1(EGFP)Klt}$Tg(MAPT)8cPdav/J mice expressing hyperphosphorylated tau due to a microtubule-associated protein tau (MAPT) transgene, showed impairments in an olfactory reversal task at ages of 7 to 18 months (Phillips et al., 2011). However, the Phillips et al. (2011) study suffered from low power due to a small sample size of only 3 animals for each of the transgenic strains and the control mice and it is thus difficult to know whether or not there are any deficits.

1.3.3 Olfactory Sensitivity Tests

Olfactory sensitivity has been examined in mice using olfactometers. The ability

of mice to discriminate between increasingly lower concentrations of n-hexanal (Phillips et al., 2011), ethyl acetate (Bodyak & Slotnick, 1999), and octyl aldehyde (Slotnick & Restrepo, 2005) and solvents lacking the odourant has been tested. Mice of the following strains were able to perform at levels above chance on the lowest concentrations tested; C57 mice detected 0.01 ppm n-hexanal (Phillips et al., 2011), CF-1 mice detected 0.00005 % ethyl acetate (Bodyak & Slotnick, 1999), and an unstated strain(s) detected 0.00001 % octyl aldehyde (Slotnick & Restrepo, 2005), with the exception of 18 month old C57 mice tested on the 0.01 ppm n-hexanal, though mice up to 15 months old could discriminate at this low concentration.

1.4 Sex Differences

Sex differences are seldom examined in studies of olfactory learning or transgenic AD mice. However, both Slotnick and Restrepo (2005), using olfactometer tasks, and Schellinck, Arnold, and Rafuse (2004), using an odour discrimination digging task, reported no sex differences on olfactory learning tasks. Mihalick, Langlois, and Krienke (2000) on the other hand, reported better performance by male mice on an olfactory reversal task, but the difference was subtle, and only significant at later stages in a series of reversals.

One paper has shown that female 5XFAD mice show an increase in hippocampal Aβ and related pathological markers of AD in response to stress while males show no such increase (Devi, Alldred, Ginsberg, & Ohno, 2010), but sex differences in 5XFAD have otherwise not been examined, with many studies not even reporting the sex of the mice used in the experiments (Devi & Ohno, 2010a, 2010b, 2012; Kimura et al., 2010;

Kimura & Ohno, 2009; Oakley et al., 2006).

1.5 Rationale for the Present Study

Due to the abundant evidence that rodents display a proficiency for learning olfactory tasks not seen in other sensory modalities, and in light of the motor deficits seen in the 5XFAD mice, I argue that olfactory tasks, which require no visual ability and little motor activity to complete, are the most valid method of testing the cognitive abilities of 5XFAD mice.

This study used a series of tests run on olfactometers to evaluate learning and memory, executive function, olfactory sensitivity, and working memory of 6 month old 5XFAD and wildtype mice. Four tasks were used: an olfactory discrimination task, a reversal task, an odour detection task, and a delayed matching to sample task.

1.6 Hypotheses and Predictions

As 5XFAD mice have been shown to display cognitive deficits in learning and memory at 6 months of age, it is hypothesized that they will show deficits on this battery of olfactory learning and memory tests. Specifically, as long term memory deficits have been found in 5XFAD mice (Kimura et al., 2010; Kimura & Ohno, 2009) it is predicted that transgenic mice will show poorer performance on the olfactory discrimination task than wildtype mice. While executive functioning has not been evaluated in 5XFAD mice, it is predicted that the transgenic mice will show a high degree of perseveration on the reversal task as other mouse models of AD have been shown to be impaired on a reversal task (Zhuo et al., 2007) and AD patients show increased perseveration on the Wisconsin

card sorting test (Nagahama et al., 2003; Terada et al., 2011). Additionally, while male mice have been shown to perform a reversal task better than female mice (Mihalick, Langlois, & Krienke, 2000), this subtle effect was only seen in a serial reversal task and as such no sex differences are predicted to occur on the single reversal used in this study. On the odour detection test it is predicted that there will be no difference in olfactory sensitivity between the transgenic and wildtype mice as the olfactory deficits seen in AD appear to be deficits of odour recognition and naming rather than odour detection threshold (Rahayel et al., 2012). Due to the findings of working memory deficits in both 5XFAD mice (Devi & Ohno, 2012; Jawhar et al., 2012; Kimura et al., 2010) and AD patients (Belleville et al., 1996; Gagnon & Belleville, 2011), transgenic mice are predicted to show deficits on the delayed matching to sample task.

Chapter 2: Methods

2.1 Subjects

Male and female 6 - 6.5 month old 5XFAD mice were used. There were 5 transgenic females, 6 transgenic males, 4 wildtype females, and 9 wildtype males. The mice used were obtained from an in-house colony of mice bred at Dalhousie University from mice purchased from the Jackson Laboratorys (strain numbers 006554 and 100012). Genotypes were determined by PCR using DNA from ear punches. This was done by Dr. Christopher Sinal in the Pharmacology department. The mice were singly housed in 30×18×12cm polycarbonate cages with wire tops and had *ad lib* access to Purina rodent chow. The mice were put on a water restriction schedule beginning 10 days prior to the start of training. While on water restriction mice were weighed daily and given mash, consisting of powdered rodent chow mixed with a measured amount of water to maintain their weight. All protocols were approved the University Committee on Laboratory Animals (protocol # 11-033).

2.2 Apparatus

Two computer controlled eight-channel liquid diffusion olfactometers (Knosys, Fl) were used (Fig 1). These allow for filtered air from a compressor to flow through odour saturation bottles containing the odour solutions, then be directed by the final valve either to an exhaust, or the odour sampling port, which is open to the operant chamber where the animal is placed. The sampling port also contained a reinforcement tube capable of delivering water as a reward, and of detecting when the animals are licking the tube.

Odour solutions were made by mixing commercially available odourants with mineral oil (Table 1). For the sensitivity test ethyl acetate was used, with concentrations that resulted in vapour concentrations of 1, 0.1, 0.01, 0.001, 0.0001, and 0.00001 parts per million (ppm).

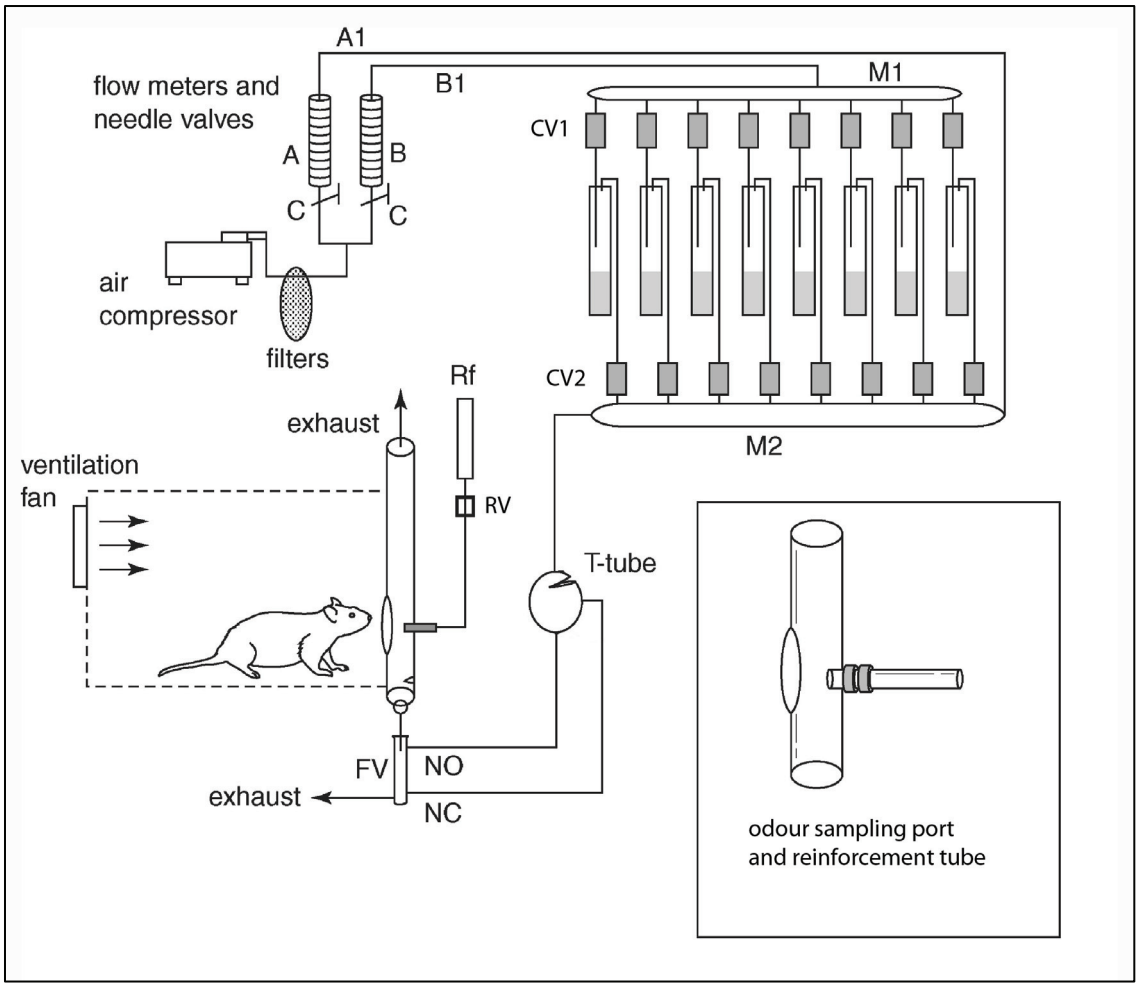


Figure 1: Diagram of the olfactometer. Air from the compressor is first sent through a filter after which it is spilt into two pathways. The first pathway flows through a needle valve (C), which controls the rate of airflow, and a flow meter (A), which measures the airflow. The air then flows through tubing (A1) into a glass manifold (M2) as clean air. The second pathway, which supplies odourized air, flows through a different needle valve and flow meter (B) into a different glass manifold (M1). Pairs of control valves (CV1 and CV2), which are normally closed, control the flow of air along the second pathway from M1, through the odour saturation bottles, and into M2, where the clean and odourized air flows converge. A glass T-tube, with a push-in to create turbulence and mix the airflow from the two pathways, has two outflows controlled by the final valve (FV) which directs airflow either to the odour sampling port via the normally open (NO) port, or to the

exhaust via the normally closed (NC) port. The odour sampling port opens to the animal chamber and contains the reinforcement tube connected to the water storage (Rf). The reinforcement valve (RV) controls the flow of water to the reinforcement tube. Adapted from Slotnick and Restrepo (2005).

Table 1: Odourants used on the various tasks performed in the experiment. All odourants were dissolved in mineral oil.

Task	Odour 1	Odour 2
Initial training	Sage oil	Orange oil
Olfactory discrimination & reversal learning	Eucalyptus oil	Lime oil
Sensitivity	Ethyl acetate	Blank
Matching to sample: training	Cardamom oil	Lavender oil
Matching to sample: test	Dillweed oil	Patchouli oil

2.3 Procedure

The test procedure involved 5 steps, initial training, the olfactory discrimination task, the reversal learning task, the odour sensitivity test, and the matching to sample test (Fig 2).

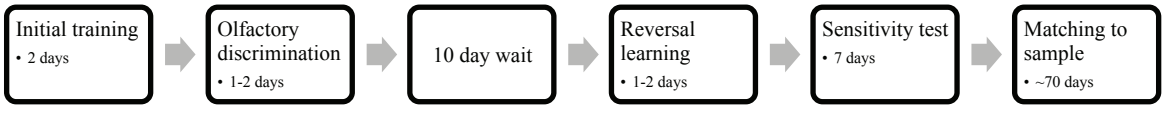


Figure 2: Experimental design of the study showing the tasks the mice were run on and the length of time typically spent on each task.

2.3.1 Initial Training

The mice were initially trained for 20 trials to lick the reinforcement tube to receive a water reward and mice were rewarded for simply licking the reinforcement tube. The inter-trial interval increased from 0.1 sec to 12 seconds over the 20 trials. During the next stage of training an odour was introduced and the mice were required to keep their head in the odour sampling port while the final valve diverted the odour into the port. The amount of time the mice were required to keep their head in the port increased from 0.1 sec to 1.1 sec over 120 trials. This training was completed when the mice performed 20 trials with the final valve on for 1.1 sec. Most mice completed initial training in one day. Mice which did not finish initial training the first day were trained on a second day, starting at the point in the program where they ended the previous day.

2.3.2 Olfactory Discrimination Task

For the olfactory discrimination task, two different odours were used, one designated the positive stimulus (S+) and one designated the negative stimulus (S-). When the mice were presented with the S+ odour, they were rewarded for licking the reinforcement tube, and there was no reward for licking the tube when the S- odour was presented. The first time the mice were run on this task an introduction phase was included at the start of the session which presented the mice with only S+ trials for 30 trials. This go, no-go type task allowed for four possible types of responses, hits, false alarms, correct rejections, and misses. Hits and correct rejections are the two correct

responses and correspond to responding when presented with the S+ and not responding when presented with the S-, respectively. False alarms and misses are errors and correspond to responding to the S- and not responding to the S+, respectively. A mouse was considered to have learned the olfactory discrimination task when it was able to achieve a criterion of 85% correct responses in a block of 20 trials. After reaching criterion on the set of odours used during training, the mice were presented with a new set of odours and tested until they reached criterion. The number of errors made prior to reaching criterion was recorded. Mice were tested on a maximum of 10 blocks of trials per day. Testing was stopped before completing 10 blocks if the mice stopped performing the task or reached criterion.

2.3.3 Reversal Learning

Ten days after the mice had reached criterion with the 2nd odour pair on the olfactory discrimination task they were tested on a reversal task. The same odours were used, except that the values of the odours were reversed, thus the odour that was the S+ became the S-, and the S- odour became the S+. Mice were once again tested until they reached criterion, and the number of errors made prior to reaching criterion was recorded. If mice stopped performing the task prior to reaching criterion, they were removed from the olfactometer and placed in their home cage for approximately 1 hour before being returned to the olfactometer and testing continued. Mice were run for a maximum of 3 sessions per day in the olfactometer until they reached criterion. The mice were given multiple sessions per day during the reversal task because they would often stop performing the task after completing very few trials.

2.3.4 Odour Sensitivity Test

To evaluate the olfactory sensitivity of the mice they were tested on the olfactory discrimination task using decreasing concentrations of ethyl acetate (EA) in mineral oil as the S+, and mineral oil as the S-. The concentrations of EA used were 6.3×10^{-6}, 5.6×10^{-7}, 4.9×10^{-8}, 4.4×10^{-9}, 3.9×10^{-10}, and 3.4×10^{-11}M. These concentrations were used as they result in vapour concentration of EA in the head spaces of the odourant bottles of 1, 0.1, 0.01, 0.001, 0.0001, and 0.00001 ppm respectively. The vapour concentration presented to the mice in the odour sampling port is estimated to be approximately 5% of the concentration in the head space of the odourant bottles (Slotnick & Restrepo, 2005).

The mice were presented first with the highest concentration of EA (1ppm) and given 5 blocks of 20 trials, for a total of 100 trials, at this concentration. The mice were then tested on the remaining concentrations in descending order. They received 100 trials over 5 blocks on all concentrations except for the lowest concentration (0.00001ppm) on which they received 200 trials over 10 blocks. The mice were presented with a single concentration each day and the lowest concentration (0.00001ppm) was presented over 2 days with the mice given 5 blocks of 20 trials each day.

2.3.5 Matching to Sample Task

Mice were first trained on the matching to sample task with two days of A-A matching trials. During these trials, mice are presented with a sample odour (A) then, after a 2 second inter-stimulus delay (ISD), a comparison odour (A). There was a 5 second inter-trial interval (ITI). During matching trials the sample and comparison odours

were the same and the mice are rewarded for licking the reinforcement tube. After the two days of A-A matching trials mice received one day of mixed A-A matching and A-B non-matching trials. Non-matching trials were introduced to the session after the mice had been presented with 10 matching trials. During non-matching trials the comparison odour was different than the sample odour and the mice were not rewarded for licking the reinforcement tube. The mice next received two days of B-B matching trials, followed by one day of mixed B-B matching and B-A non-matching trials. Mice were trained for one hour or until they received 100 matching trials.

Mice were then presented with all four types of trials, A-A, B-B, A-B, and B-A, using the same odours used during matching to sample training. Trials were divided into blocks of 20, with 5 of each of the 4 types of trials. When the mice correctly responded to 80% of each type of trial in one block they were considered to have reached criterion and advanced to the test phase.

Upon reaching criterion, mice were tested with a new pair of odours, with the same 2 sec ISD and 5 sec ITI. After criterion was reached on the 2 sec ISD, the ISD was increased to 5 sec, followed by 10, 30, and 60 sec ISDs upon reaching criterion on each stage. 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 sec, mice would first be presented with 2.5, 3, 3.5, 4, and 4.5 sec ISDs. This was done to ensure the mice would learn to continue to perform the task at the longer delay.

Fifteen of the mice were run with a slight variation on this task. This variation

provided the mice with small reinforcements during the ISD so as to encourage the mouse to continue attending to the task during the delay period. Small reinforcements were given every 5 sec during the ISD up to 10 seconds prior to the end of the delay. Additionally, the ITIs were different. Up to a 10 sec ISD, the ITIs were 6 sec, 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 testing session was ended before 10 blocks if the mice stopped performing the task. At the 2 sec delay mice commonly completed 10 blocks, but as the delays increased, and thus the amount of time required for the mice to complete 10 blocks increased, mice completed progressively fewer blocks before they stopped performing the task.

2.4 Data Analysis

All statistical analysis was performed with R (www.R-project.org). Data from the olfactory discrimination and the reversal learning tests were analyzed by comparing the number of errors made prior to reaching criterion with ANOVAs using genotype and sex as factors. Data from the sensitivity test was analyzed with an ANOVA. The percentage of correct responses in each block of 20 trials was examined with genotype, sex, EA concentration, and block as factors. A χ^2 test was run on the proportions of mice from each condition that were able to achieve at least 80% responses in one block at the lowest odour concentration (0.00001 ppm). The matching to sample task was analyzed with two ANOVAs. The first examined the number of errors until criterion was reached during the training phase (i.e. when initially presented with the four types of trials) and had genotype and sex as factors. The second analyzed the stages after the new odour pair was

introduced. It examined the number of errors until criterion at each stage of the delay and had genotype, sex, and length of delay as factors; at each delay, only data from mice that reached criterion at that delay was included. A Cox proportional hazards regression was run on the test phase of the matching to sample task using failure to reach criterion on delay as the event of interest. First described by Cox (1972), this is a survival analysis that models the relationship between the time it takes for an event to occur and one or more predictor variables (Fox, 2002). As there was a small number of subjects and unequal numbers of subjects in the different conditions, which can violate the assumptions of the ANOVAs, non-parametric ranked data ANOVAs (Conover & Iman, 1981) were run in conjunction with all ANOVAs.

Chapter 3: Results

3.1 Olfactory Discrimination Task

Transgenic mice made fewer errors (M = 13.8, sd = 10.4) than wildtype mice (M = 21.5, sd = 21.2) prior to reaching criterion on the olfactory discrimination task, but this difference was not significant ($F_{1,20} = 1.19$, $p = .29$). Female mice (M = 10.7, sd = 6.2) made fewer errors than males (M = 22.3, sd = 20.3), but this difference was also not significant ($F_{1,20} = 2.16$, $p = .16$). The ranked data ANOVA also showed no significant effects of genotype ($F_{1,20} = 0.42$, $p = .53$) or sex ($F_{1,20} = 1.70$, $p = .21$) (Fig 3).

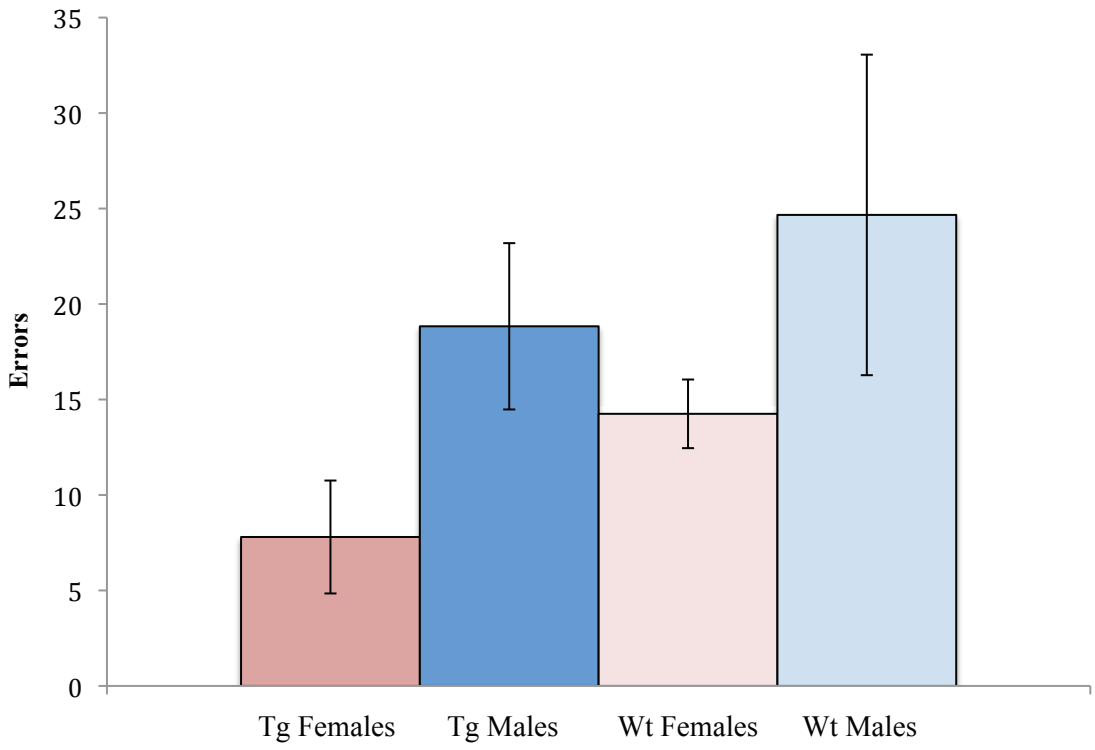


Figure 3: Mean number of errors (± SEM) mice in each group made in the olfactory discrimination task. There were no significant differences between groups.

3.2 Reversal Learning

Transgenic female mice had a higher mean number of errors to reach criterion on the reversal task (M = 104.8, sd = 63.7) than the wildtype females (M = 58.0, sd = 27.4), the difference between the transgenic males (M = 89.3, sd = 26.3) and the wildtype males (M = 79.2, sd = 36.5) was much smaller. But neither the genotype ($F_{1,20} = 2.05$, $p = .17$), nor the sex ($F_{1,20} = 0.03$, $p = .86$), or genotype by sex interaction ($F_{1,20} = 1.14$, $p = .30$) were significant. The ranked data ANOVA also showed no significant effects of genotype ($F_{1,20} = 3.17$, $p = .090$) or sex ($F_{1,20} = 0.07$, $p = .79$) (Fig 4).

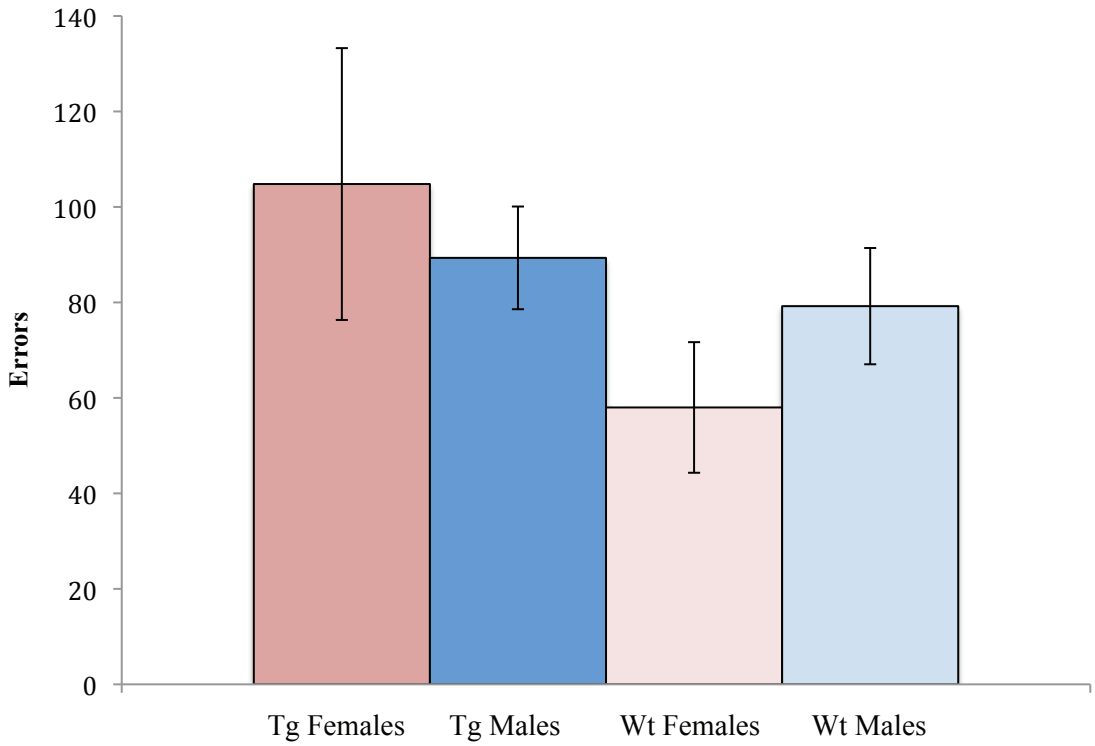


Figure 4: Mean number of errors (± SEM) mice in each group made on the reversal learning task. There were no significant differences between groups.

3.3 Odour Sensitivity Test

There were significant effects of genotype ($F_{1,700} = 9.36$, $p = .0023$), sex ($F_{1,700} = 19.10$, $p = 1.4 \times 10^{-5}$), odour concentration ($F_{5,700} = 65.37$, $p < 2 \times 10^{-16}$), block ($F_{9,700} = 14.69$, $p < 2 \times 10^{-16}$), and a genotype by concentration interaction ($F_{5,700} = 2.27$, $p = .046$) on the percentage of correct responses on the odour sensitivity test. Post hoc analysis was performed by splitting by odour concentration and evaluating each odour concentration with an ANOVA. Transgenic mice preformed better than wildtype mice at odour concentrations of 1 ppm ($F_{1,100} = 6.13$, $p = .015$) and 0.001 ppm ($F_{1,100} = 9.18$, $p = .0031$), while females performed better than males at concentrations of 1 ppm ($F_{1,100} = 5.21$, $p = .025$), 0.1 ppm ($F_{1,100} = 15.46$, $p = .00016$), and 0.01 ppm ($F_{1,100} = 12.84$, $p = .00053$). The block was significant for all but the lowest (0.00001 ppm) odour concentration ($p = .11$) (Fig 5).

The ranked data ANOVA also showed significant effects of genotype ($F_{1,700} = 9.93$, $p = .0017$), sex ($F_{1,700} = 24.46$, $p = 9.5 \times 10^{-7}$), odour concentration ($F_{5,700} = 71.78$, $p < 2 \times 10^{-16}$), block ($F_{9,700} = 17.35$, $p < 2 \times 10^{-16}$), and a genotype by concentration interaction ($F_{5,700} = 2.57$, $p = .026$). Post hoc analysis with ranked data ANOVAs showed that transgenic mice preformed better than wildtype mice at odour concentrations of 1 ppm ($F_{1,100} = 4.35$, $p = .040$), 0.1 ppm ($F_{1,100} = 5.33$, $p = .023$) and 0.001 ppm ($F_{1,100} = 8.94$, $p = .0035$). At 0.01 ppm the effect of genotype was not significant ($F_{1,100} = 0.43$, $p = .51$), but the genotype by sex interaction was ($F_{1,100} = 5.45$, $p = .022$). Splitting the 0.01 ppm concentration by sex revealed that female transgenic mice performed better than female wildtype mice ($F_{1,35} = 4.34$, $p = .045$), but there was no significant difference between transgenic and wildtype males ($F_{1,65} = 1.71$, $p = .20$). Females performed better

than males at concentrations of 1 ppm ($F_{1,100} = 6.18$, $p = .015$), 0.1 ppm ($F_{1,100} = 17.99$, $p = 5.0 \times 10^{-5}$), and 0.01 ppm ($F_{1,100} = 16.41$, $p = .00010$). The block was significant for all but the lowest (0.00001 ppm) odour concentration ($p = .19$).

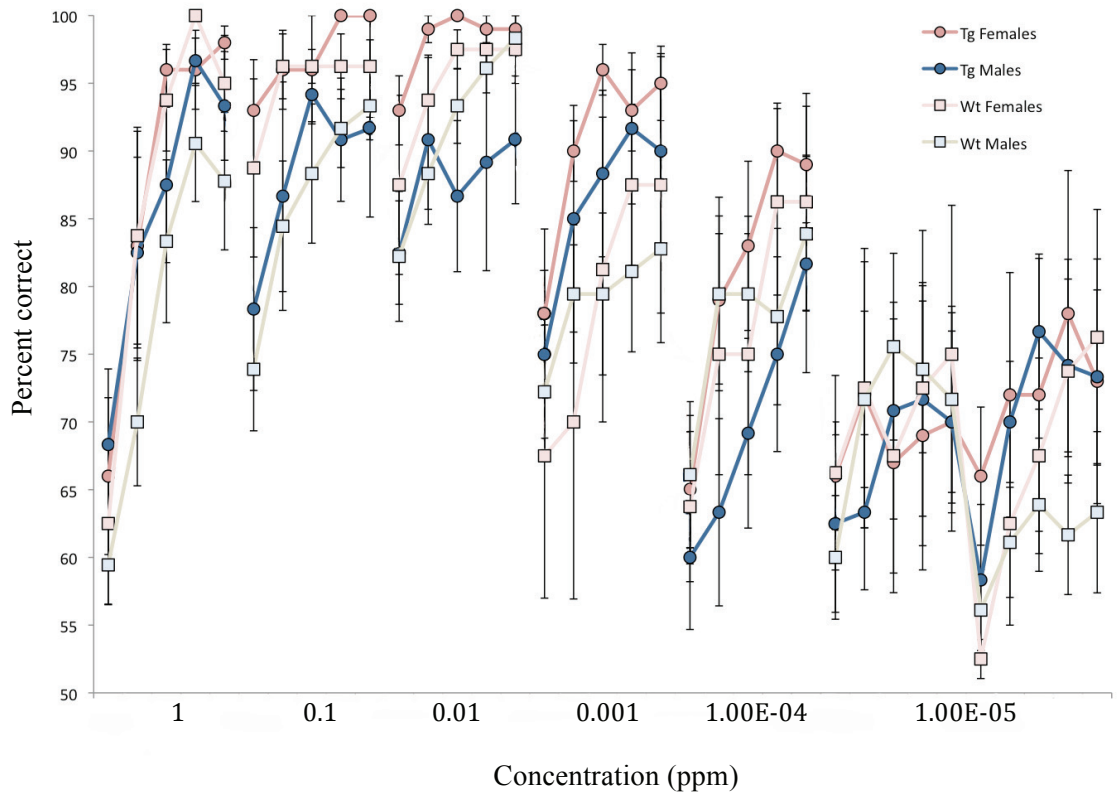


Figure 5: Mean percentage of correct responses (± SEM) in each block of 20 trials on the odour sensitivity task. Transgenic mice performed better than wildtype mice at ethyl acetate vapour concentrations of 1 ppm, 0.1 ppm, 0.001 ppm ($p < .05$). At 0.01 ppm female transgenic mice were better than female wildtype mice ($p = .045$). At vapour concentrations of 1 ppm, 0.1 ppm, and 0.01 ppm female mice performed better than male mice ($p < .05$).

Examining performance of the mice on the lowest odour concentration tested (0.00001 ppm) found that 60% of the transgenic females, 67% of the transgenic males, 75% of the wildtype females and 56% of the wildtype males were able to achieve a

minimum of 80% correct responses in at least one block. A χ^2 test found no significant differences in these proportions ($\chi^2_1 = 1.18$, $p = .28$).

3.4 Matching to Sample Task

One wildtype male mouse died after completing the training for the matching to sample task, data from this mouse is included in the analysis of the training phase, but not of the test phase. During the training phase of the matching to sample task there was an effect of genotype ($F_{1,20} = 12.45$, $p = .0021$) and a genotype by sex interaction ($F_{1,20} = 4.44$, $p = .048$), but not a significant effect of sex ($F_{1,20} = 4.32$, $p = .051$). Post hoc analysis with Tukey HSD tests revealed that wildtype male mice made significantly more errors (M = 372.9 sd = 137.7) ($p < .05$) than transgenic males (M = 171.8, sd = 37.1), wildtype females (M = 194.3, sd = 73.0), and transgenic females (M = 173.8, sd = 82.7). The ranked data ANOVA showed significant effects of genotype ($F_{1,20} = 13.08$, $p = .0017$) and sex ($F_{1,20} = 4.49$, $p = .047$), but no significant interaction ($F_{1,20} = 3.85$, $p = .064$) (Fig 6).

The Cox proportional hazards regression, run on the test phase of the matching to sample task using failure to reach criterion on a delay as the event of interest, found that females preformed better than males ($z = 2.32$, $p = .020$) (Fig 7). An ANOVA was run on the number of errors made at each delay prior to reaching criterion, using only the data at each delay from the mice that were able to meet criterion. Female mice were found to make fewer errors than male mice ($F_{1,43} = 5.02$, $p = .030$). Genotype ($F_{1,43} = 0.30$, $p = .59$) and delay ($F_{3,43} = 0.96$, $p = .42$) caused no significant differences. The ranked data ANOVA also found a significant effect of sex ($F_{1,43} = 6.00$, $p = .019$), but not of genotype

($F_{1,43} = 0.01$, $p = .93$) or delay ($F_{3,43} = 1.40$, $p = .26$) (Fig 8). Individual learning curves were made for all mice that reached criterion at the 2 sec (Fig 9), 5 sec (Fig 10), 10 sec (Fig 11), and 30 sec (Fig 12) delays.

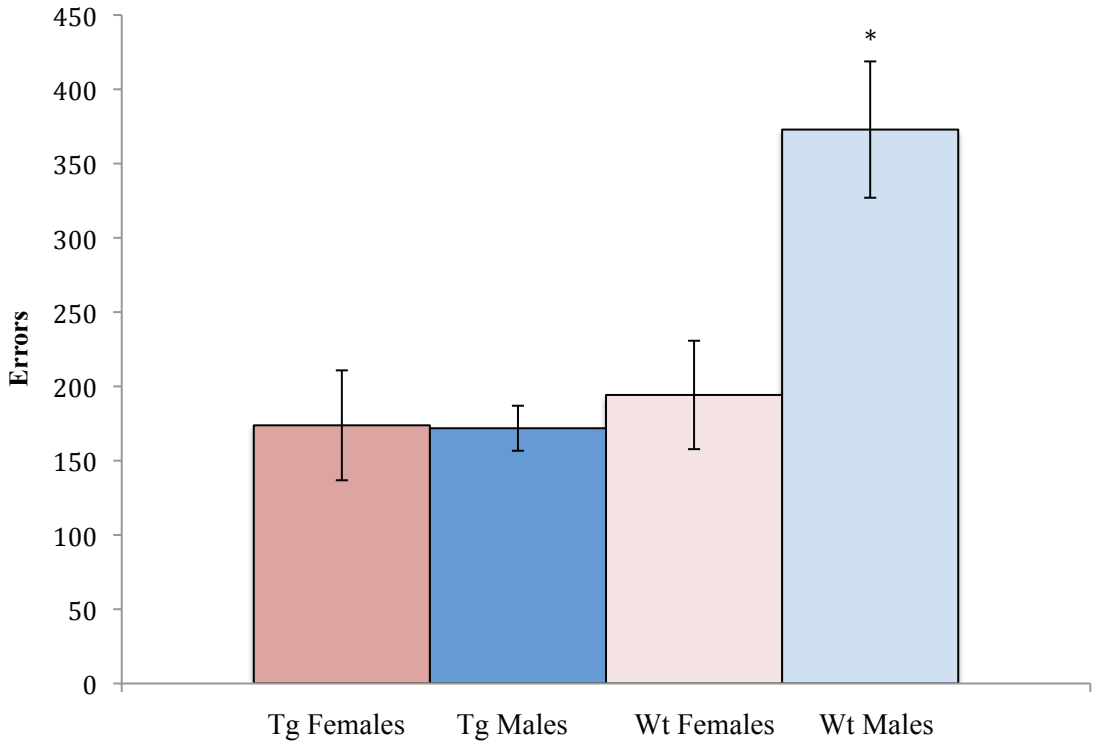


Figure 6: Mean number of errors (± SEM) made by mice of each group on the training phase of the delayed matching to sample task. The wildtype males made more errors that all other groups ($p < .05$).

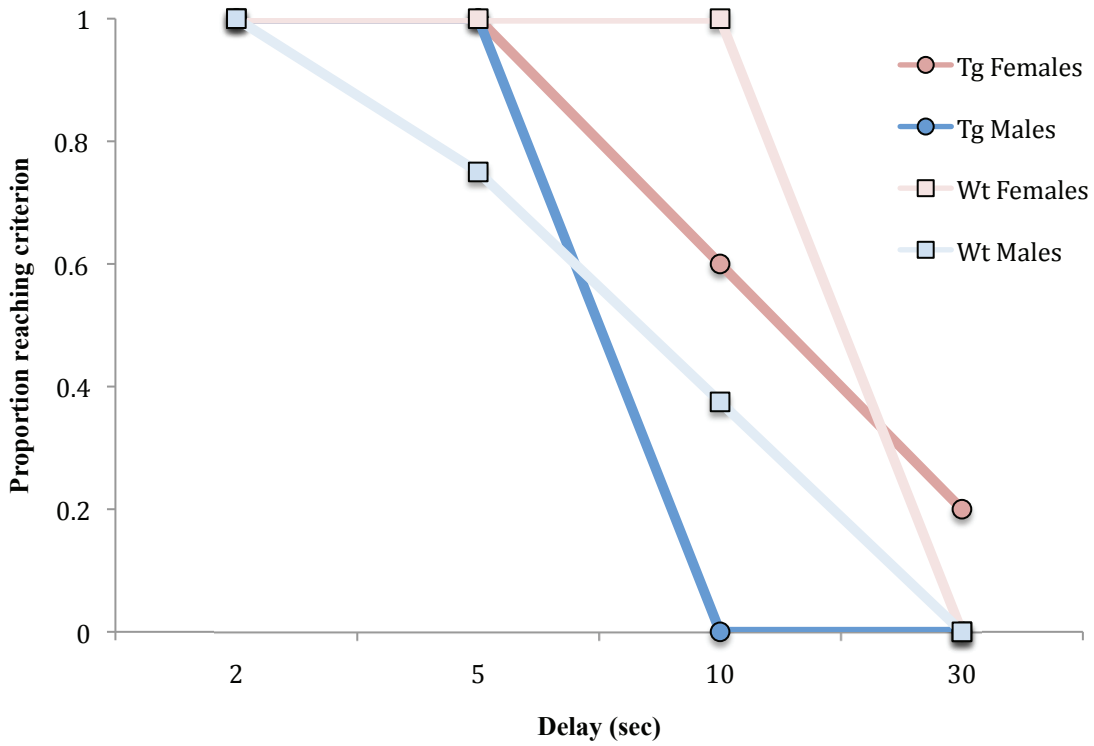


Figure 7: Proportion of mice that were able to reach criterion at each delay in the delayed matching to sample task. Female mice were more likely to reach criterion on a delay than male mice (p = .020).

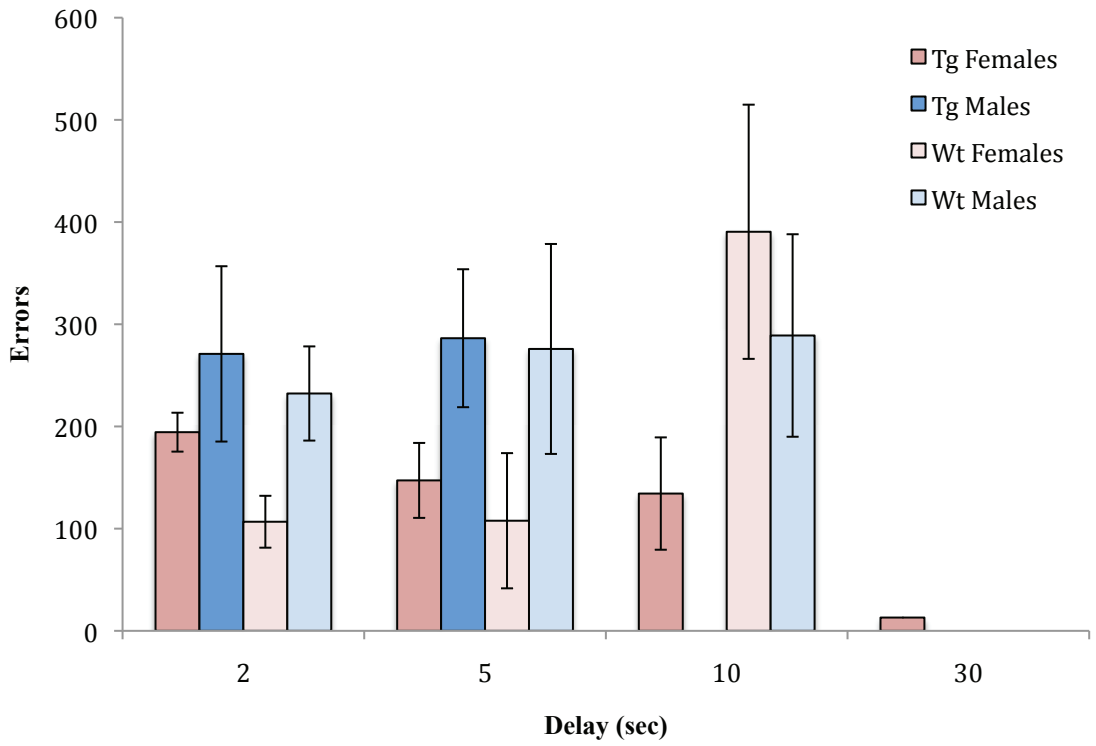


Figure 8: Mean number of errors (± SEM) made at each delay prior to reaching criterion by mice in each group. At each delay only the data from mice which successfully reached criterion are included. Overall, female mice made fewer errors than male mice ($p < .05$).

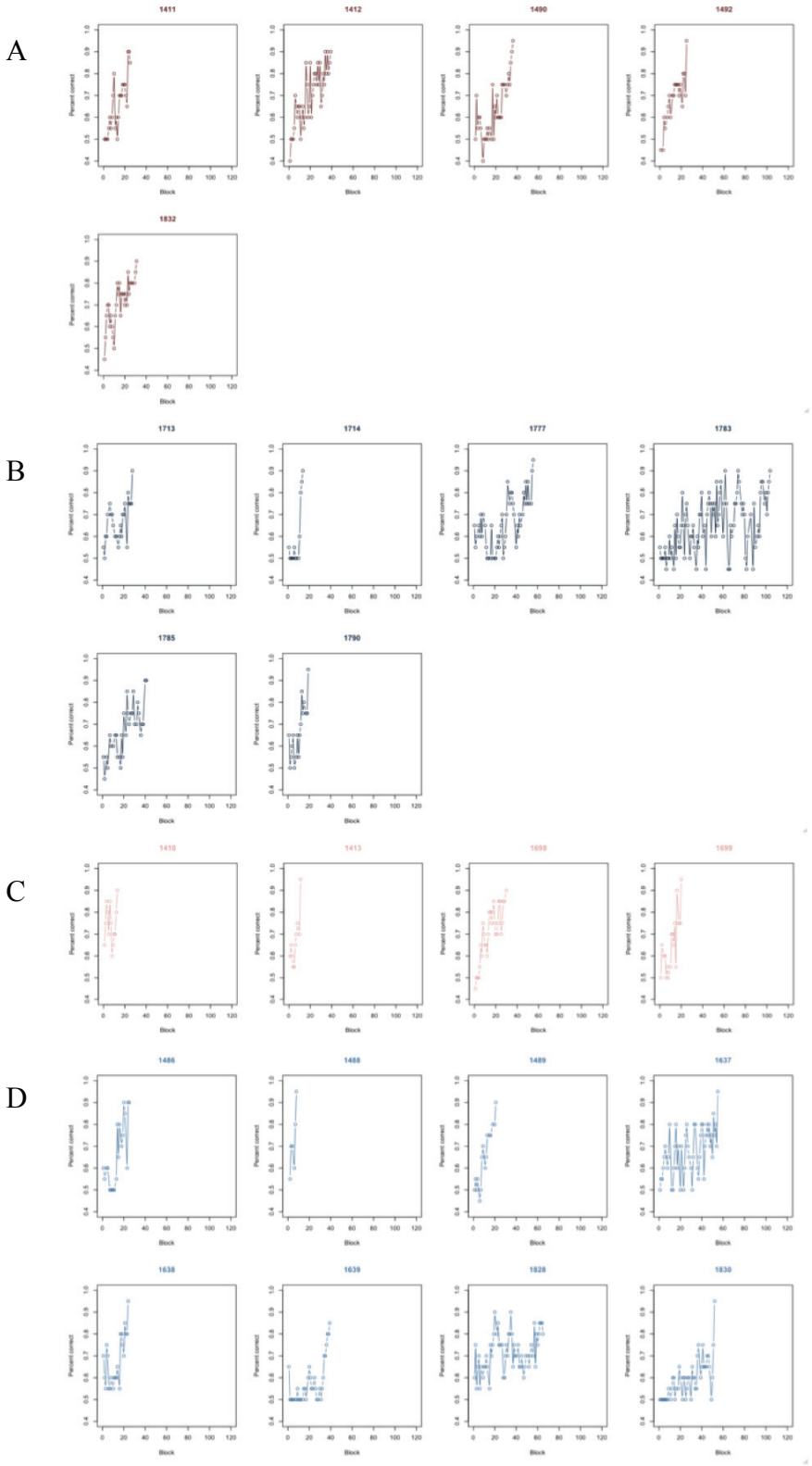


Figure 9: Individual learning curves of the (A) transgenic female, (B) transgenic male, (C) wildtype female, and (D) wildtype male mice which reached criterion on the delayed matching to sample task at the 2 sec delay.

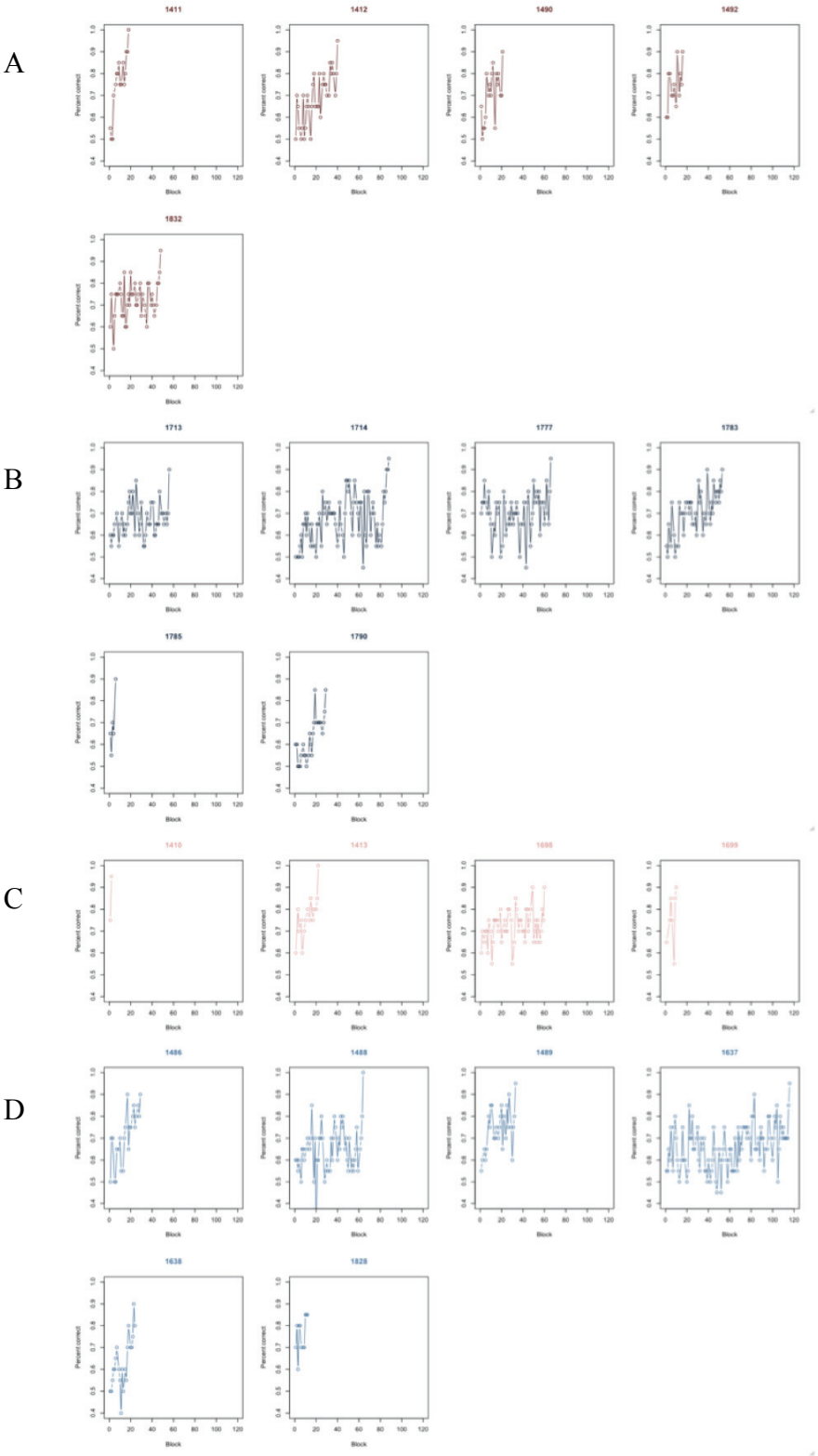
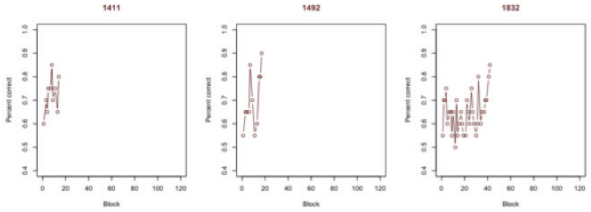
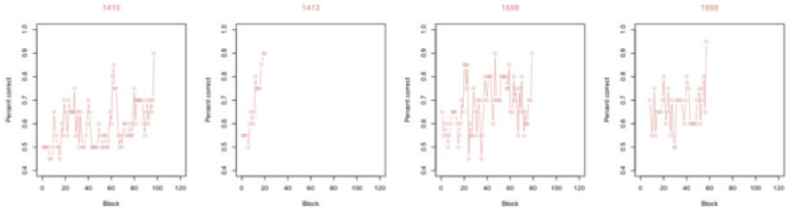


Figure 10: Individual learning curves of the (A) transgenic female, (B) transgenic male, (C) wildtype female, and (D) wildtype male mice which reached criterion on the delayed matching to sample task at the 5 sec delay.

A



C



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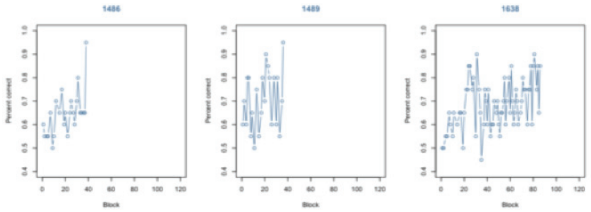

Figure 11: Individual learning curves of the (A) transgenic female, (C) wildtype female, and (D) wildtype male mice which reached criterion on the delayed matching to sample task at the 10 sec delay.

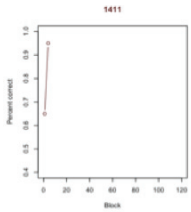

Figure 12: Individual learning curve of the transgenic female mouse which reached criterion on the delayed matching to sample task at the 2 sec delay.

Chapter 4: Discussion

4.1 Olfactory Discrimination

It was hypothesised that the transgenic mice would show impaired performance on the olfactory discrimination task. No such impairment was found and all of the mice were able to learn the olfactory discrimination task without much difficulty. There were no significant effects of genotype or sex on this task, though there were trends towards the female mice making fewer errors on the task than the males, and towards the transgenic mice making fewer errors than the wildtype mice. While it is possible that these trends would become significant if a greater sample size were used, the high level of performance by all of the mice appears to be causing a ceiling effect that would make it difficult to detect any differences on this task. At the start of this task, mice tended to respond to both the S+ and the S- odours, resulting in initial performance of approximately 50% correct. They would then learn not to respond to the S- odour as the approached criterion performance. This suggests that inhibiting their response when presented with the S- is the more difficult aspect of this task for the mice to learn. These results are similar to the findings of Phillips et al. (2011), who found no impairments in a initial odour discrimination task in mouse models of AD, and the rapid learning of the task matches with those of previous mouse studies (Bodyak & Slotnick, 1999; Slotnick & Restrepo, 2005).

4.2 Reversal Learning

The original hypothesis, that the transgenic mice would show increased perseveration on the reversal learning task, was not supported by the data. While the transgenic mice did show high levels of perseveration, it was no different than that of the wildtype mice. In contrast to the olfactory discrimination task, the reversal task was difficult for the mice to complete. All of the mice showed high degrees of perseveration, causing them to continue to respond to the odour that was now the S- as if it was still the S+ despite no longer receiving reinforcement for doing so. Once again there were no significant effects of either genotype or sex, though for the female mice there was a trend towards better performance in the wildtypes compared to the transgenics, a trend which was not seen in the male mice.

Of interest is the pattern of responding that is seen as the mice completed the task. At the start of the reversal they would respond according to the what they had learned in the olfactory discrimination task, causing them to respond to the S- and not to the S+. This resulted in the mice initially performing at near zero percent correct, despite the fact that on such a go no-go type task random performance should be approximately 50% correct, as they are making false alarms and incorrect rejections. This demonstrates that the mice were able to remember the original paradigm they had learned in the olfactory discrimination task 10 days earlier and were perseverating on this. During the next phase of responding the mice would stop responding to the S-, but still not respond to the S+. At this phase they are getting approximately 50% of the trials correct, making correct rejects and misses as they are not responding to either odour. In the third phase they would begin responding to both odours. They were still getting approximately 50% of the

trials correct, only now they were making hits and false alarms. In the final phase they began to stop responding to the S- and their performance improved to above chance and reached criterion.

During the first two phases of responding the mice were not getting reinforcement as they failed to respond to the S+. During these phases, but especially the second phase when they are responding to neither odour, the mice show little motivation to perform the task, waiting for an extended period before initiating the next trial and would often completely stop performing the task, requiring them to be removed to their home cage for a short period. Once they started to respond to the S+ and received reinforcement, they initially responded to every trial before learning to inhibit their response to the S-. This is interesting as they had already learned that they would not get reinforcement when they responded to the S- odour earlier in the task. This suggests that once they started getting reinforcement they treated this as if it was an entirely new task and forgot, or ignored, what they had previously learned about the task. This may also be a reason why the mice made so many more errors on the reversal learning task than on the olfactory discrimination task. On the discrimination task it was learning to inhibit responding to the S- that was the more difficult aspect of the task, and on the reversal task they ended up learning this once, forgetting or ignoring it, then learning it a second time.

The high degree of perseveration on the reversal task by the mice is similar to that seen in other reversal studies with mice (Del'Guidice et al., 2009; Mihalick, Langlois, & Krienke, 2000; Mihalick, Langlois, Krienke, et al., 2000; Phillips et al., 2011), and the lack of reversal deficits in the transgenic mice is in agreement with the findings of Phillips et al. (2011), but not with those of Zhuo et al. (2007). The most likely reason for

the differences in findings is due to Zhuo et al. (2007) using the Tg2576 mouse model of AD while the present study used the 5XFAD model.

4.3 Sensitivity Test

While the hypothesis that there would be no deficits in olfactory sensitivity in the transgenic mice is in agreement with the results of this study, other effects were found. The sensitivity test showed significant effects of genotype and sex, with females performing better than males, and transgenic mice performing better than wildtype mice. There were also significant effects of odour concentration and block. The effect of odour concentration shows that the task became more difficult for the mice as the odour concentration decreased. Related to this, the effect of block for all but the lowest odour concentration showed that when the mice were presented with a lower odour concentration there was a degree of learning to respond to the new concentration, as seen in the learning curves on Fig 5. The lack of such an effect at the lowest concentration is likely due to two factors, the first being that many of the mice were unable to achieve a high level performance at this concentration, and that there was a slight dip in performance at the start of the second day of testing on this concentration.

The effects of genotype and sex do not appear to be related to differences in olfactory sensitivity as they only appear in the higher odour concentrations and there were no significant differences in the proportion of mice from each condition that were able to achieve at least 80% of trials correct in a block at the lowest concentration. While all of the mice were able to achieve high levels of performance at the higher concentrations, the female mice showed exceptionally high performance with the

transgenic females in particular showing near errorless performance on many blocks. It should be noted that these effects are the same as the non-significant trends that are seen in the olfactory discrimination task. On the discrimination task, the mice were stopped when they had reached the criterion of 85% correct, however, in the sensitivity test they were run for 5 blocks which allowed them to reach a higher level of performance. It would seem that these differences are thus related more to either the maximum level of performance that the mice are able to achieve, or the speed at which they are able to reach their maximum level, rather than how well they are able to initially learn the task.

On the lowest odour concentration, two thirds of the mice were able to detect the odour. This indicates that while this concentration is not quite the threshold for detection, it is rather close. It is difficult to compare results between the studies that have examined odour sensitivity in mice due to differences in the odours used and the methods used to express the odour concentrations. The present study used ethyl acetate as the odour, expressed as ppm of the vapour present in the odourant bottle. While Bodyak and Slotnick (1999) also used ethyl acetate, they expressed their concentrations as a percentage of vapour saturation, and while Phillips et al. (2011) also expressed the odour concentrations as ppm of the vapour present in the odourant bottle, they used n-hexanal. Meanwhile, Slotnick and Restrepo (2005) used octyl aldehyde as the odour and expressed the concentration as percent by volume of the liquid odourant solution. Additionally, in all of the above studies mice where still able to detect the lowest odour concentration used.

In contrast to the present finding of improved performance in transgenic mice on the sensitivity task, Phillips et al. (2011) found no differences between the two transgenic

AD strains they tested and their control mice on a sensitivity task. And while it is difficult to compare their sensitivity test to the one used in the present study due to the different odourants used, the fact that the difference found in this study occurred at the higher odour concentrations rather than the lower concentrations should make such a comparison valid.

4.4 Matching to Sample Task

The original hypothesis, that there would be a working memory deficit in the transgenic mice, was not supported. Furthermore, findings of better working memory in female mice was not hypothesised. The mice were able to perform the matching to sample task. While previous studies have shown that rats are able to learn olfactory delayed matching to sample tasks (April et al., 2011; Lu et al., 1993; Otto & Eichenbaum, 1992), this is the first time where it has been demonstrated that mice can also learn an olfactory delayed matching to sample task. While the mice were able to learn the task, it took a long time and was a difficult task for them to do. To run a mouse through all of the tasks in this study took approximately 3 months. Of this time, the olfactory discrimination task, including the initial training, typically required 3 or 4 days, the reversal required 1 or 2 days plus the 10 day waiting period following the discrimination task, and the sensitivity test took 7 days. The remaining approximately 70 days was spent on the matching to sample task. Additionally, mice were making hundreds of errors prior to reaching criterion on both the training and test phases of the task.

Similar to the odour discrimination and reversal learning tests, it was inhibiting their responses that seemed to cause mice the greatest difficulty. The mice would initially

respond to both the matching and non-matching trials before eventually learning not to respond to the non-matching trials. Additionally, the mice seemed largely unable to generalize what they had learned at one delay to subsequent delays. This is shown by the lack of an effect of delay on the number of errors made during the test phase of the matching task, indicating that mice were making approximately the same number of errors prior to reaching criterion at each delay. There were only two instances where a mouse showed any ability generalize what they had learned between delays. A wildtype female mouse, upon reaching criterion on the 2 sec delay and being advanced to the 5 sec delay, made only 6 errors prior to reaching criterion, only to subsequently make 700 errors before reaching criterion on the 10 sec delay. Additionally, a transgenic female upon starting the 30 sec delay was able to reach criterion while making only 13 errors, this was the only mouse to reach criterion on the 30 sec delay.

While the female transgenic and wildtype mice, and the male transgenic mice made a similar amount of errors on the training phase of the matching to sample task, the wildtype males made drastically more errors. This does not appear to be the result of a deficit in working memory in the wildtype male mice, as the test phase of the matching to sample task did not show a similar effect. It would seem that this is the result of the mice having greater difficulty initially learning how to perform the task .

On the test phase of the matching to sample task, a greater proportion of female mice were able to reach criterion at longer delays than male mice, and made fewer errors than the males. This effect of the female mice being able to reach the longer delays suggests that they have improved working memory compared to the male mice. The effect of fewer errors by the females, however, is not necessarily due to differences in

working memory, as at each delay only the data from the mice who were able to reach criterion at that delay were included in the analysis of the number of errors made. Thus even the male mice who were able to perform the task at the same delays as the female still made more errors achieving criterion. This effect of fewer errors by the females than males is similar to the effect seen in the higher odour concentrations of the sensitivity test and the trend seen in the odour discrimination task.

Rats tested on a nearly identical matching to sample task also using an olfactometer were able to learn the task faster and were able to better adjust to increases in the length of the delay (Lu et al., 1993). Working memory tests of 5XFAD mice using spontaneous alternation have found deficits by 6 months of age (Hillmann et al., 2012; Jawhar et al., 2012; Kimura et al., 2010; Oakley et al., 2006; Ohno et al., 2007) in contrast to the lack of deficits found in this study. However, the matching to sample task used here is a very different task than the spontaneous alternation tests.

4.5 Benefits of Tasks Used

There are many benefits of olfactometer based tasks such as the ones employed in the present study. Especially when testing 5XFAD, the possible confounding role of motor deficits are a concern (Jawhar et al., 2012). Tasks using the olfactometer require very little motor control as all the animal is required to do in order to make a response is to lick at a spout. Additionally, the ability of rodents to perform olfactory tasks with greater ease than tasks using other sensory modalities, makes the olfactometer an ideal testing apparatus.

While Phillips et al. (2011) have used olfactometer based tasks to examine various aspects of learning and memory in two strains of AD mice models, there are a number of issues with their study, mostly involving their subjects. They only tested 3 animals each of the two AD strains and the control strain. This small sample size severely limits the power of the study, making it so only large effect would be detected. Additionally, while they used C57 mice as their control mice, neither of the two AD strains they tested are on a purely C57 background. Their Aβ expressing B6.Cg-Tg(APPswe,P- SEN1dE9)85Dbo/J mice are on a C57 by C3H hybrid background, and the hyperphosphylated Tau expressing B6.Cg-MAPT$^{tm1(EGFP)Klt}$Tg(- MAPT)8cPdav/J mice are on a background that is a hybrid of 129, Swiss Webster, C57, and DBA mice. This means that even if a difference were to be detected it is difficult to know whether it was caused by the AD mutations or if it is simply a strain difference.

4.6 Future Research

Some issues with this study are that the sample size is relatively small, and the size of the groups are not balanced. Both of these factors will have an effect on the ability to detect differences. Additionally, the matching to sample task used was very difficult for the mice to learn and perform. The main issue they seemed to have was learning to inhibit responding to the no-go stimulus, and though it occurred to one degree or another on all of the tasks it was much more of a problem on the matching to sample task. A possible way to get around this would be to change the task from a go no-go task to one where after presentation of the sample odour the mice are presented with two comparison odours at the same time and are able to make a choice. Under the current go no-go

paradigm, the mice will only get reinforcement on half of the trials, the go trials, and there is no punishment when responding to the no-go trials. This means that a mouse that simply responds on every trial will receive the same amount of reinforcement as a mouse performing the task with 100% accuracy. Under a choice task, not only would the animals not have to inhibit responding during half of the trials, but they can also receive reinforcement on every trial, and a mistake would result in them not receiving the reinforcement. Another possible way to improve performance would be to us a set of multiple odours on the task rather than just a pair. Otto and Eichenbaum (1992) demonstrated that that using larger sets of odours on an olfactory working memory task reduced the amount of interference previous trials would have on the current trial, and thus improved performance.

Finally, this study began testing the5XFAD mice when they were 6 months old. While some deficits have been detected at this age in the literature, other deficits only manifest at later ages. Additionally, by testing at various ages, the progression of deficits could be tracked.

Chapter 5: Conclusions

The purpose of this study was to use a series of olfactometer based tests to evaluate the long term learning and memory, executive function, olfactory sensitivity, and working memory of 6 month old 5XFAD and wildtype mice. The hypothesised deficits in transgenic mice on the olfactory discrimination task, reversal learning task, and delayed matching to sample tasks were not found. The hypothesis that there would be no differences in olfactory sensitivity between the transgenic and wildtype mice are supported by the results. This study demonstrated for the first time that mice are able to learn an olfactory delayed matching to sample task with delays up to 30 seconds long. It was found that female mice have better working memory than male mice, and show higher levels of performance on the matching to sample task and an odour sensitivity task. Though there was no difference in their ability to detect odours. This effect was also seen on the sensitivity task with transgenic mice showing better performance than wildtype, but no differences on odour detection. The finding of sex differences are of note as they are rarely examined in either the olfactory learning literature or the 5XFAD literature. Finally, further research examining the mice at older ages, and using an easier task, to track the progression of AD related deficits in 5XFAD mice should be done.

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