



Measuring olfactory learning and memory in rodents

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ABSTRACT

Age-related cognitive dysfunction is a symptom of many neurodegenerative diseases, and these diseases often involve olfactory deficits. As rodents are the most popular animal models for age-related cognitive dysfunction, it is important to understand their olfactory system and their capacity for olfactory learning and memory in both the main and accessory olfactory systems. After a brief review of learning and memory in the accessory olfactory (primer pheromone) system, we focus on behavioural tasks used to measure odour detection, odour discrimination, working memory, reversal learning, episodic and semantic olfactory based tasks in rodents. We then review our studies using an operant olfactometer to evaluate genotype, sex, and age differences in odour detection ability, olfactory discrimination learning and memory, learning strategies and cognitive flexibility in working memory and reversal learning. The final section reviews our experiments using the Pavlovian conditioned odour preference paradigm to study odour discrimination learning and describes our new one-day conditioning procedure. We discuss the parameters of this one-day test and how it is used to measure genotype, sex and age effects on short-, long- and very long-term memory. The paper thus reviews the methods for studying olfactory learning and memory in rodents and argues that their high level of ability to perform olfactory tasks should be considered as a reliable method for studying cognitive function in rodent models of human neurological disorders.

1. The importance of studying olfactory learning and memory in rodents

1.1. Olfactory deficits in neurodegenerative and neuropsychiatric disorders

Deficits in odour detection and odour identification are observed in age-related neurodegenerative and neuropsychiatric disorders (Alves et al., 2014; Doty, 2017; Yang et al., 2023; Zhang et al., 2023), with olfactory dysfunction being a better predictor of Alzheimer's disease (AD) onset than loss of verbal episodic memory (Devanand et al., 2015). Olfactory deficits also occur in Huntington's disease (HD), Amyotrophic Lateral Sclerosis (ALS), and Parkinson's Disease (PD) (De Cleene et al., 2025). Deficits detected in AD were primarily deficits of odour identification and recognition while deficits in olfactory sensitivity were more apparent in PD (Sun et al., 2025), suggesting that while olfactory deficits may be a symptom common to neurodegenerative diseases, it may be possible to differentiate between the different diseases based on the

nature of the olfactory deficits (De Cleene et al., 2025; Rahayel, et al., 2012). Finally, over 70% of patients suffering from COVID-19 developed some form of olfactory dysfunction (Chang et al., 2024; Doty, 2022). As pointed out by Yang et al. (2023, page 7506), "The olfactory system represents a captivating field of study that intertwines the realms of neuroscience, psychology, and clinical research. By unraveling the intricate relationship between the olfactory system and neurologic conditions, we may uncover the molecular and neural processes that contribute to neuropsychiatric disorders." Since rodents provide the most common animal models for neurological disorders and rodents are olfactory animals (Doty, 1986; Brown, 1985b; Brown & Macdonald, 1985), it is important to examine olfactory function in rodent models of neurological disorders.

1.2. The importance of rodent models in neuroscience research

Rodent models are an essential tool for biomedical research (Dominguez-Oliva, et al., 2023; Nestler & Hyman, 2010; Brown, 2022;

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Borgland, 2026) and transgenic mouse models of human neurological diseases provide a basis for understanding the neurobiological basis of these disorders and for testing the efficacy of vaccines and other treatments for disorders such as COVID-19 (Genzel et al., 2020; Venkataramani & Winkler, 2022). Rodent models are important for studying the neurobiological, cellular and molecular mechanisms underlying neurodevelopmental disorders (Harris, 2021), aging (Mitchell et al., 2015) and neurodegenerative diseases (Dawson et al., 2018; Chen & Zhang, 2022; Zhong et al., 2024). Rodent models increase our understanding of basic brain function (Homberg et al., 2021); brain injury (Ma et al., 2019); neuropsychiatric disorders (Baker et al., 2020) and the efficacy of new drug treatments (Howe et al., 2018; Zhang et al., 2024a). Rodent models are also important for the study of cognitive function throughout the lifespan and in neurological disorders (Alves et al., 2022; Brito et al., 2023; Quillfeldt, 2016; Tanila, 2018), however, most studies of cognitive function in rodents focus on visual-spatial learning and memory (Horner et al., 2013; Vorhees & Williams, 2024) or fear learning (Bergstrom, 2020; Luchkina & Bolshakov, 2019) and do not test aged animals. Thus, it is important to examine the olfactory abilities of rodent models throughout their lifespan (Kondo et al., 2020; Yanai & Endo, 2021).

1.3. Rodents rely heavily on olfaction

Rodents rely on olfactory cues for social and sexual behaviour, food seeking and predator avoidance (Brown, 1979; Brown, 1985b; Brown & Macdonald, 1985; Doty, 1986; Schultz & Tapp, 1973; Takahashi, 2014; Adduci et al., 2021) and are able to detect odours at very low concentrations (Bodyak & Slotnick, 1999; Roddick et al., 2016). Rodents show a remarkable ability to learn complex olfactory based tasks, with both rats and mice showing learning set formation and learning-to-learn (Slotnick & Katz, 1974; Roddick et al., 2023). In discrimination learning tasks, mice and rats show superior performance in discriminating olfactory stimuli over auditory or visual stimuli (Nigrosh et al., 1975; Phillips et al., 2011; Brushfield et al., 2008) (See Fig. 1). There are numerous transgenic rodent models of neurological disorders, including nearly 250 different transgenic mouse models of AD alone (Alzforum, 2026; Zhong et al., 2024) and, combined with the rodents' high olfactory ability, the use of olfactory tasks is an ideal way to study the neurobehavioral effects of these genetic manipulations.

1.4. The rodent olfactory system

The rodent olfactory system is a complex network consisting of the main and accessory olfactory pathways. The traditional view was that the main olfactory system was responsible for detecting and responding to environmental odours, with the accessory olfactory system responsible for responses to pheromones (Scalia & Winans, 1975). However, more recent evidence shows that this strict division of the olfactory system is misleading, with much overlap between the systems, and both playing roles in the detection of odourants and pheromones (Restrepo et al., 2004; de Castro, 2009) (Fig. 2A). Although the mouse and human olfactory receptor genes differ in their evolutionary history (Young et al., 2002), the organization of the olfactory system is phylogenetically conserved, with similarities in odourant receptor proteins and the peripheral and central olfactory pathways (Ache & Young, 2005; Mombaerts, 2006). Furthermore, odour preferences and odour-related behaviors are similar in mice and humans (Laska, 2017; Mandairon et al., 2009). Therefore, mice represent a valuable model for understanding the molecular mechanisms underlying aging- and neurodegeneration-related changes in the olfactory system (Dan et al., 2023).

The main olfactory system begins with the olfactory epithelium in which olfactory receptor neurons express one of the approximately 1500 olfactory receptors encoded in the mouse genome, compared to the approximately 900 found in the human genome (Young et al., 2002).

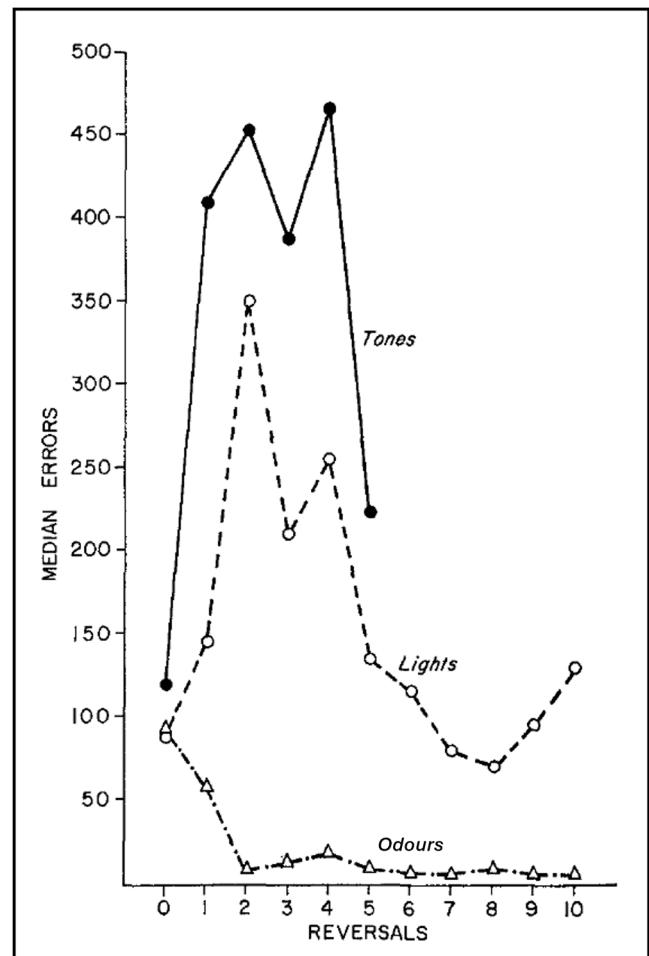


Fig. 1. Rodents excel at olfactory learning and memory. Median number of errors on original learning and successive discrimination reversals for rats trained with tones, lights, or odours. Only rats trained with odours showed positive transfer on the first reversal and acquisition of a reversal set. Other experiments demonstrated that rats preferentially attend to odours when presented in compound with lights or tones; that odours exert more discriminative control than tones in tests using compound stimuli of competing sign; and that after pretraining on the positive stimulus, acquisition of an odour but not a light discrimination occurs with virtually no errors. These results demonstrate the importance of olfactory stimuli in the learning and memory abilities of rodents. From: Nigrosh, B. J., Slotnick, B. M., & Nevin, J. A. (1975). Olfactory discrimination, reversal learning, and stimulus control in rats. Fig. 3. *Journal of Comparative and Physiological Psychology*, 89, 285–94.

The axons of the olfactory receptor neurons project to the olfactory bulb, where neurons expressing the same olfactory receptors converge on the same glomeruli (Mombaerts, 2006). The projections of the olfactory bulb form the lateral olfactory tract, which innervates the olfactory cortex (anterior olfactory nucleus, the olfactory tubercle and piriform cortex), the amygdala, and the entorhinal cortex (de Castro, 2009). There are connections between the structures of the olfactory cortex and the hippocampus, thalamus, and hypothalamus (Ennis et al., 2015). The accessory olfactory system begins with the odour receptors of the vomeronasal organ within the nasal septum which project to the accessory olfactory bulb (AOB) in the dorsal section of the olfactory bulb (Mucignat-Caretta, 2010) and thence to the amygdala, hypothalamus, and the anterior olfactory nucleus (de Castro, 2009; Ennis et al., 2015).

1.5. The neural basis of olfactory learning and memory

Given the complexity of the olfactory system (Fig. 2A) and the

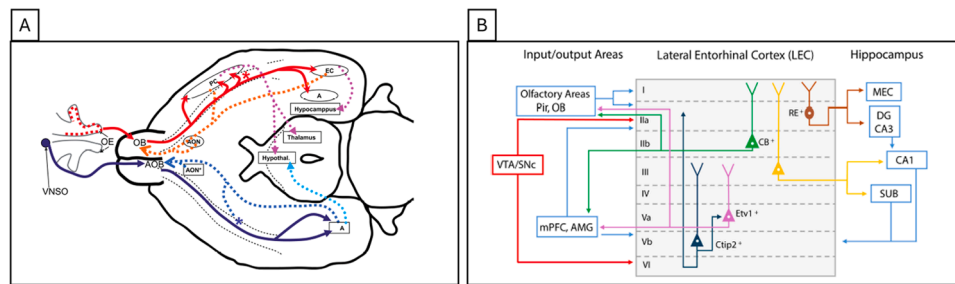


Fig. 2. The rodent olfactory system and olfactory memory pathways.

[2A] An outline of the connections of the main and the accessory olfactory systems in the rodent brain. The axons from the Olfactory Sensory Neurons (red dots within the olfactory epithelium, OE) project to the olfactory bulb (OB), forming the olfactory nerve. The projection neurons from the OB send their axons (the lateral olfactory tract, LOT) to the different structures of the olfactory cortex, including the anterior olfactory nucleus (AON), the olfactory tubercle, the piriform cortex (PC), the amygdaloid complex (A), the entorhinal cortex (EC), and the nucleus of the lateral olfactory tract (nLOT; red asterisk). The olfactory nerve and the LOT are represented with thick red curved arrows. Neurons from the olfactory cortex and the nLOT project back to the OB (orange curved arrows). The main intracortical connections (violet dotted arrows) are towards parts of the neocortex, hippocampus, thalamus and the hypothalamus, as well as to the neocortex and the contralateral olfactory cortex (forming the anterior commissure, not represented).

The axons arising from the vomeronasal organ (VNSO) form the vomeronasal nerve and project to the accessory olfactory bulb (AOB). The projection neurons from this send their axons (as part of the LOT) to the AON and the nLOT (blue asterisk), and to A (thick blue curved arrows), while axons from neurons from these three cortical structures project back to the AOB (light blue dotted arrows). The projections from the accessory olfactory cortex reach the hypothalamus (turquoise dotted arrow) and the nucleus of the lateral olfactory tract.

From: De Castro, F. (2009). Wiring olfaction: the cellular and molecular mechanisms that guide the development of synaptic connections from the nose to the cortex. Fig. 1. *Frontiers in Neuroscience*, 3, 868.

[2B] Circuit dynamics of the olfactory pathway during olfactory learning. A schematic representation of the lateral entorhinal cortex (LEC) layer input/output projections. Inputs from olfactory bulb (OB) and piriform cortex (Pir) reach LEC layer I; Reelin (RE) positive cells project to medial entorhinal cortex (MEC) and dentate gyrus (DG) (shown in dark red); Calbindin (CB) positive pyramidal cells in layer II project to olfactory areas, medial prefrontal cortex (mPFC), and amygdala (AMG) (shown in green); Layer III pyramidal cells mainly project to CA1 and subiculum (SUB) (shown in yellow). Layer Va cells express twenty-six variant transcription factor 1 (Etv1), and project to mPFC and AMG, as well as olfactory areas (shown in purple); Feedback from these areas reach layer Vb. Layer Vb cells express Chicken ovalbumin upstream promoter transcription factor-interacting protein 2 (Ctip2) and project within the LEC (shown in dark blue). LEC layer II and VI receive dopaminergic inputs from midbrain ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (shown in red).

From: Zhang, Y. J., Lee, J. Y. & Igarashi, K. M. (2024). Circuit dynamics of the olfactory pathway during olfactory learning. Fig. 2. *Frontiers in Neural Circuits* 18:1437575. 10.3389/fncir.2024.1437575.

prevalence of olfactory dysfunction in neurological disorders, it is important to know where learning and memory occur in the olfactory system and what neurochemicals facilitate olfactory learning and memory processes (Brennan & Keverne, 1997; Davis, 2004). The olfactory system involves both innate and learned odour processing (Kobayakawa et al., 2007) and the development of the olfactory pathways, like the visual pathways, although innate, is shaped by early olfactory experience during critical periods of development (Mallick et al., 2024; Chen et al., 2023; Sakano, 2020).

1.5.1. Neuroanatomy

Each step in the olfactory pathway has been found to be involved in learning and memory in some way (Fig. 2B). The odourant receptors on the olfactory sensory neurons project to glomeruli in the olfactory bulb (OB) to form "glomerular maps" which are topographically organized so that particular odours activate a specific set of glomeruli (Takeuchi & Sakano, 2014; Weng et al., 2025). The mitral cells and tufted cells of the glomeruli are the first step for the neural processing of olfactory information and relay this information to other brain areas. Thus, the glomeruli in the olfactory bulb contain the first synapses which can be modified during olfactory learning and memory (Cleland, 2014; Tong et al., 2014; Linster et al., 2020; Zhang et al., 2024b). The OB is divided into multiple layers with specific neuron types in each layer and these form a number of complex neural circuits which connect with other cells in the olfactory pathway (Nagayama et al., 2014). Each synapse in the ensuing olfactory pathway also has the capacity for plasticity during the formation of cell assemblies underlying olfactory learning and memory. This includes the anterior olfactory nucleus (AON) (Aqrabawi & Kim, 2020; Brunert et al., 2023), the olfactory tubercle (Murata et al., 2015; Yamaguchi, 2024) and numerous regions of the cerebral cortex (Zhang et al., 2024b), including the piriform cortex (Cohen et al., 2008; Bekkers & Suzuki, 2013), the nucleus of the

lateral olfactory tract (Penker et al., 2024), the lateral entorhinal cortex (Lee et al., 2021; Zhang et al., 2024b), and the orbitofrontal cortex (Wang et al., 2024). Olfactory projections are also sent to the basolateral amygdala (Schoenbaum et al., 2003; Tronel & Sarah, 2002; Buchanan et al., 2003), the dentate gyrus and hippocampus (Kesner, 2018; Restivo, et al., 2006; Woods et al., 2020) which form connections in the olfactory learning and memory pathways (Zhang et al., 2024b).

1.5.2. Neurochemistry

Virtually every neurotransmitter system has been implicated in some aspect of olfactory learning and memory, including dopamine, noradrenaline, glutamate, GABA and acetylcholine (Lothman et al., 2021; Brennan et al., 1998; Zheng et al., 2018; Linster et al., 2020; Lee et al., 2021). Cholinergic signalling in the olfactory bulb modulates olfactory learning and memory, and GABAergic signals innervate all layers of the main olfactory bulb (Torres et al., 2023). In addition, neuropeptides play a prominent role in learning and memory processes (deWied, 1997).

1.6. Learning and memory in the accessory olfactory system

In addition to the main olfactory system, rodents have an accessory olfactory or vomeronasal system used primarily for social (pheromonal) communication (Brown, 1985a; Torres et al., 2023) which has distinct neural pathways, but which also interacts with the main olfactory system (Mucignat-Caretta et al., 2012; Slotnick et al., 2010) (Fig. 2A). The accessory olfactory system involves the vomeronasal organ, the nucleus of the accessory olfactory tract, the medial and cortical amygdala, the bed nucleus of the stria terminalis, the medial preoptic area, and the ventromedial hypothalamus (Brennan & Keverne, 1997; Murata et al., 2024). The connections of the vomeronasal organ and accessory olfactory bulb (AOB) to the neuroendocrine hypothalamus allow pheromones

from male and female mice to regulate the hypothalamic-pituitary-gonadal activity of conspecifics. Like the main olfactory bulb, the glomeruli of the AOB form olfactory maps of conspecific odours, providing information on species, sex and individual identity of social odours (Brown, 1979; 1985a; Hammen et al., 2014). Projections from the main olfactory and vomeronasal systems overlap in the amygdala, allowing the integration of information from the two olfactory systems (Slotnick et al., 2010; Guthman & Vera, 2016; Griffiths & Brennan, 2015) (Fig. 3A).

Pheromones are "social odours" produced by one animal which can have profound effects on the reproductive behaviour of conspecifics (Brown, 1985a; Koyama, 2004). Pheromones act through the accessory olfactory system to modulate the neuroendocrine system of conspecifics, acting as 'external hormones' to regulate the onset of puberty, the female estrous cycle, or block pregnancy by controlling the activation of the Hypothalamic-Pituitary-Gonadal system (Brown, 1985a; Schellinck et al., 1993b; Koyama, 2004; Jouhannau et al., 2013) (Fig. 3B). The ability of pheromones to alter neuroendocrine activity depends on the vomeronasal organ and accessory olfactory system of the mouse learning to recognize and remember the odours of familiar conspecifics and discriminating these from the odours of novel (unfamiliar) conspecifics (Yoles-Frenkel et al., 2022). Although the main function of this paper is to examine methods for conducting behavioural studies of olfactory learning and memory based on the main olfactory pathways, it is important to discuss learning and memory in the accessory olfactory (vomeronasal) system because this is often overlooked in discussions of olfactory learning and memory.

The neurophysiological "primer" effects of pheromones alter the neuroendocrine system and thus affect behaviour only indirectly (Fig. 3C). The neuroendocrine responses to primer pheromones could be

considered 'innate' olfactory responses (Griffiths & Brennan, 2015; Holy, 2018), but they involve the learning and memory of social odours. The accessory olfactory system shows learning and memory for pheromonal odours and relies on neurochemical communication, including the neurotransmitters noradrenaline, GABA, glutamate and acetylcholine and the regulation of the release of hypothalamic-pituitary peptides Luteinizing Hormone Releasing Hormone (LHRH), kisspeptin, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and prolactin (Brown, 1985a; Brennan & Keverne, 1997; Griffiths & Brennan, 2015; Jouhannau et al., 2013). In addition to these neurotransmitters, neural activity in the accessory olfactory (vomeronasal) system is modulated by steroid hormones (Torres et al., 2023; Abaffy et al., 2023) and the neuropeptides oxytocin, vasopressin and Neuropeptide S (Wang et al., 2020; Silkis, 2024; Wacker & Ludwig, 2012; Suyama et al., 2024).

Pheromonal effects occur in both females and males (Brown, 1985a; Koyama, 2016) (Fig. 3) but the most often studied effects are puberty acceleration, modulation of estrous cycles, and pregnancy block in females (Brennan & Keverne, 1997; Koyama, 2004). Each of these phenomena depends on the female mouse being able to discriminate between the odours of familiar and novel males. Exposing juvenile female mice to the odour of a novel male causes them to undergo early puberty (Vandenbergh, 1969) and removal of the vomeronasal organ prevents this effect (Lomas & Keverne, 1982). Mere exposure of juvenile female mice to odours from novel males activates immediate early genes in their accessory olfactory bulb and increases their uterine weights, an effect not shown by exposure to their own odours or peppermint odours (Schellinck et al., 1993b). Estrus can be induced in adult female mice exposed to novel male odours and inhibited by odours from grouped female conspecifics (Wölfl et al., 2023), indicating the ability of their vomeronasal system to distinguish between the odours of males and

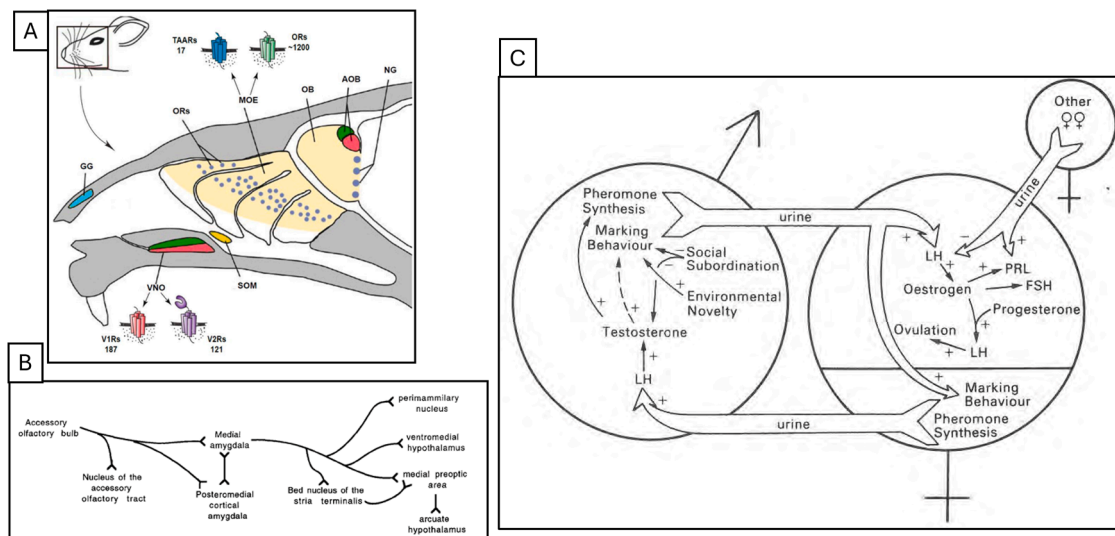


Fig. 3. The accessory olfactory system and the role of learning in primer pheromone effects. [3A] A schematic representation of the mouse olfactory receptor systems. Main olfactory epithelium (MOE), olfactory bulb (OB), accessory olfactory bulb (AOB), Grueneberg ganglion (GG), Vomeronasal organ (VNO), septal organ of Masera (SOM), guanylyl cyclase D (GCD), necklace glomeruli (NG), trace amine associated receptors (TAARs), olfactory receptors (ORs), vomeronasal receptors type1 (V1Rs), vomeronasal receptors type2 (V2Rs).

From: Hussain, A. (2011). The olfactory nervous system of terrestrial and aquatic vertebrates. Fig. 1. *Nature Precedings*, 1–1.

[3B] The subcortical projections of the accessory olfactory bulbs to the medial amygdala and the arcuate nucleus of the hypothalamus.

From: Brennan, P. A., & Keverne, E. B. (1997). Neural mechanisms of mammalian olfactory learning. Fig. 3. *Progress in Neurobiology*, 51(4), 457–481.

[3C] The pheromonal cueing system (primer pheromone effects) in male and female house mice. + indicates stimulation, - indicates inhibition of neuroendocrine activity. The primer effects of rodent social odours regulate the neuroendocrine system controlling puberty and reproductive activity. The male's odour is essential for the proper organization of puberty in females and for the induction of ovulation in adult females. Female odours induce Luteinizing Hormone (LH) secretion and increase pheromone synthesis in males, thus promoting a mutual feedback loop, which functions to stimulate ovulation in the female. Thus, the function of male odours is to induce oestrus and the function of female odours is to induce the male to produce and disseminate his odours, which will then induce oestrus in the female. Activation of male hormone levels may be a female strategy for ensuring the presence of males during her oestrus period, as oestrous females scent mark more than dioestrous females. FSH = Follicle stimulating hormone; PRL = prolactin.

From: Brown, R. E. (1985a). The rodents I: effects of odours on reproductive physiology (primer effects) Figure 8.16. In: *Social odours in mammals*, edited by Richard Brown and David Macdonald. Oxford: Clarendon Press. Pages 245–344.

Table 1
An overview of some tests for studying odour detection, preference, learning and memory in rodents.

Individual odour discrimination by the accessory olfactory system: Primer pheromone effects	
Puberty acceleration/delay	Vandenbergh, 1969
Estrus induction/inhibition	Wöfl et al., 2023
Pregnancy block	Yoles-Frenkel et al., 2022
Unconditioned odour detection tasks	
Nipple recognition in newborn pups	Al Ain et al., 2013
Pup ultrasonic vocalizations	Wagner et al., 2019
Buried food task	Machado et al., 2018
Tasks measuring odour preferences (attraction and aversion).	
Preference tests: attraction and aversion	Brown & Willner, 1983
Odour aversion: defensive reactions	Dell'Omo et al., 1994
Predator odour aversion	Takahashi & Tsuboi, 2017.
Conditioned odour preference and aversion learning.	
Conditioned odour approach/avoidance in pups	Sullivan & Wilson, 1991
Conditioned odour aversion using lithium chloride	Panhuber, 1982
Odour-cued taste avoidance	Slotnick & Coppola, 2015
Pairing odours with footshock	Fletcher, 2012
Tasks measuring olfactory sensitivity	
Simple preference tests; preference scales	Zou et al., 2015
Two odour discrimination in an operant olfactometer	Bodyak & Slotnick, 1999
Tests for simple odour discrimination learning and memory.	
Habituation/dishabituation test	Brown et al., 1987.
Operant olfactometers	Schellinck & Brown, 1995
Olfactory tubing maze	Roman et al., 2002
Pavlovian conditioned odour preference task	Schellinck et al., 2001a
Tests for olfactory working memory.	
Continuous delayed non-matching-to-sample task	Otto & Eichenbaum, 1992
Operant go/no-go delayed matching to sample task	Winters et al., 2000
Olfactory working memory capacity task	Huang et al., 2020
Odour span task	Young et al., 2007
Tests for olfactory reversal learning.	
Reversal learning in an operant olfactometer	Nigrosh et al., 1975
Serial olfactory reversal learning; learning to learn	Slotnick & Katz, 1974
Learning to Learn.	Roddick et al., 2023
Tests for declarative (episodic and semantic) olfactory memory.	
Paired-associates task	Bunsey & Eichenbaum, 1996
Transitive inference task	Dusek & Eichenbaum, 1997
Sequential odour memory task	Fortin et al., 2002
What, where, and when task	Ergorul & Eichenbaum, 2004
Discrimination of elements in a compound odour	
Biconditional olfactory discrimination task	Dreumont-Boudreau et al., 2006
Short-, long- and very long- term olfactory memory	
Pavlovian conditioned odour preference task	Wong & Brown, 2013

females, and novel and familiar males (Brown, 1985a; Lendrem, 1985). Finally, recently mated female mice exposed to the odour of a novel male will have their pregnancy blocked, but this does not occur with re-exposure to the odour of the original stud male (Bruce, 1959; Rosser et al., 1989). Memory is involved in the pregnancy block (the Bruce effect) as the odour of the sire male is learned by the female at the time of mating, enabling her to discriminate the odour of the sire from that of novel males (Rosser & Keverne, 1985; Yoles-Frenkel et al., 2022) and removal of the vomeronasal organ prevents this effect (Bellringer et al., 1980). Noradrenergic projections via the medial forebrain bundle and medial olfactory tract to the main and accessory olfactory bulb are involved in the formation of memory for the odours of sire males (Rosser & Keverne, 1985; Koyama, 2004; Griffiths & Brennan, 2015). Measuring pheromonal learning and memory in the accessory olfactory system thus relies on recording matings, pregnancies and births, investigating hormonal and neurochemical changes, and examining uterine changes (Koyama, 2016; Yang et al., 2026) rather than the type of behavioural analyses discussed in Part 2.

2. An overview of behavioural methods for measuring olfactory learning and memory in rodents

As discussed above, rodents excel in many different olfactory tasks, both unlearned (innate) and learned. Rats and mice can learn simple and complex odour discriminations and the analysis of their olfactory abilities (Bodyak & Slotnick, 1999; Slotnick & Restrepo, 2005) provides

insights into the cognitive processes of these animals which have "a well-developed sense of smell and the neural machinery to think with their noses" (Slotnick, 2001, page 216).

This section is a review and update of previous papers (Schellinck et al., 2008; Schellinck, 2018; Slotnick et al., 2005) covering both unconditioned (ethologically relevant) and conditioning methods for measuring odour detection; olfactory sensitivity; simple odour discrimination learning and memory; reversal learning and learning set formation (learning to learn); working memory; complex olfactory learning; and measuring memory strength (tests for short-, long- and very long-term olfactory memory) as summarized in Table 1.

2.1. Behavioural tasks for measuring odour detection, odour preferences, discrimination, learning and memory

2.1.1. Unconditioned (ethological) odour detection tasks

Odour detection can be demonstrated at an early age in rodents as olfactory cues are essential for newborn pups to find their mother's nipples and suckle (Al Ain et al., 2013; Hongo, 2000). Mice are presented with an odour and a control (water) and if they spend more time sniffing the odour, one infers that it can be detected. It is also possible to use infant rodent ultrasonic vocalizations as a measure of odour attraction and aversion (Wagner et al., 2019). As early as two days of age, mouse pups show a decrease in isolation-induced ultrasonic vocalizations in response to the odour of adult male mice (Lemasson, et al., 2005) and 3-day-old mice show increased ultrasonic vocalizations to

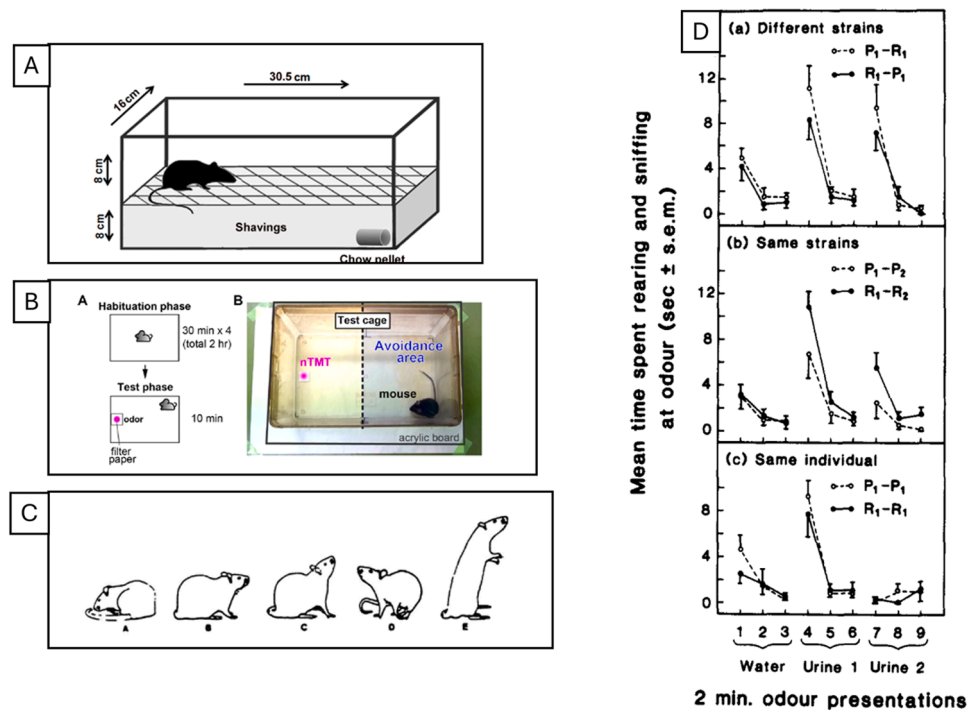


Fig. 4. Unconditioned odour detection and preference tasks.

[4A] The buried food-seeking test. This experimental test measures the rodent's ability to use olfactory cues for foraging. Mice were deprived of food for 24 h but received water ad libitum. The next day, a pellet of regular chow was buried 8 cm beneath the surface of the bedding at one end of a clean test cage. The latency to find the hidden food (the time between when the mouse was placed in the cage and when the mouse uncovers the food pellet) is measured in this test.

From: Machado, C. F., Reis-Silva, T. M., Lyra, C. S., Felicio, L. F., & Malnic, B. (2018). Buried food-seeking test for the assessment of olfactory detection in mice. *Fig. 1. Bio-protocol*, 8(12), e2897.

[4B] Unconditioned odour avoidance test. Mice are habituated to the test arena (A) and then presented with an odour on filter paper at one end of the arena. Both freezing and avoidance times are measured during the 10-min test. 'Freezing time' is the time kept still for more than 3 sec, except for breathing. 'Avoidance time' is the time spent in an area without a filter paper scented with nTMT (an ethologically relevant predator odour compound found in fox feces) when the test cage is divided into two equal areas.

From: Takahashi, H., & Tsuboi, A. (2017). Olfactory avoidance test (Mouse). *Fig. 1. Bio-protocol*, 7(5), e2153.

[4C] The sequence of the orienting response of a rat to an odour presented from above in the habituation-dishabituation task. Before the odour presentation the animal is at rest (A). When the odour is presented, the rat lifts his head and forepart of the body (B, C), moves about (D), and finally rears (E). Only the time spent rearing and sniffing at the odour stimulus was recorded in our studies.

From: Schellinck, H. M., & Brown, R. E. (1995). Methodological questions in the study of the rat's ability to discriminate between the odours of individual conspecifics. *Fig. 1. Advances in the Biosciences*, 93, 427-436.

[4D] Mean time (+/-s.e.m.) rearing and sniffing at odours on each 2-min presentation in Experiments 1a, 1b, and 1c. Water was used as a neutral stimulus odour. Dishabituation of the rearing and sniffing response indicates the ability to discriminate between odours 1 and 2. Abbreviations for stimulus odours: P=PVG and R=PVG.R1 urine samples. In Experiment 1a the urine odours of rats from the two different strains could readily be discriminated, with both subgroups showing the same results. In Experiment 1b, urine odours from two individual rats of the same strain could be discriminated. In Experiment 1c, two urine odours from the same rat could not be discriminated.

From: Brown, R. E., Singh, P. B., & Roser, B. (1987). The Major Histocompatibility Complex and the chemosensory recognition of individuality in rats. *Fig. 1. Physiology & Behavior*, 40, 65-73.

maternal odours (Marchlewska-Koj et al., 1999). A simple task for measuring odour detection in adult rodents is the buried food test (Fig. 4A) which relies on the animal's natural foraging behaviour. Animals are fasted overnight and then introduced to a chamber containing buried pieces of a familiar food that has an odour, such as chocolate (Machado et al., 2018). The number of pieces of food that the animal finds within a given time is scored (Yang & Crawley, 2009).

2.1.2. Tasks measuring odour preferences (attraction and aversion)

Preference tests, presenting two odours at once and measuring the time spent investigating each odour as a measure of preference, can be used to measure the unconditioned (innate) attractiveness or aversiveness of odours to rodents and a series of preference tests can be used to calculate a "preference scale". These preference scales, which rely on the exploratory behaviour of the animals and their motivation to investigate odours, can be used to measure the effects of sexual and social experience, housing conditions and gonadal hormone levels on attraction to

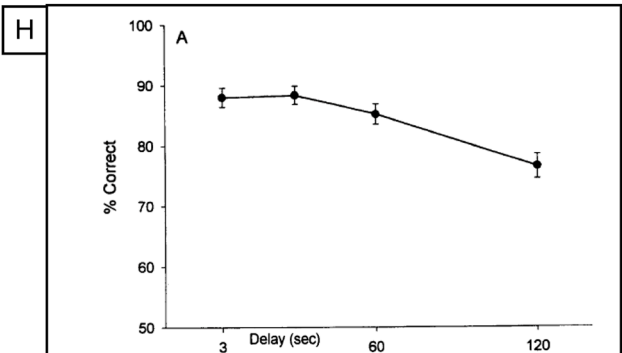
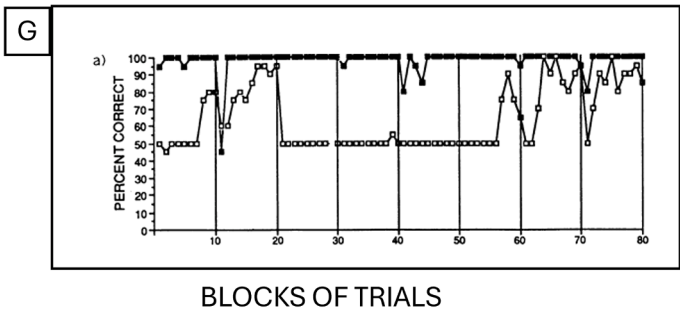
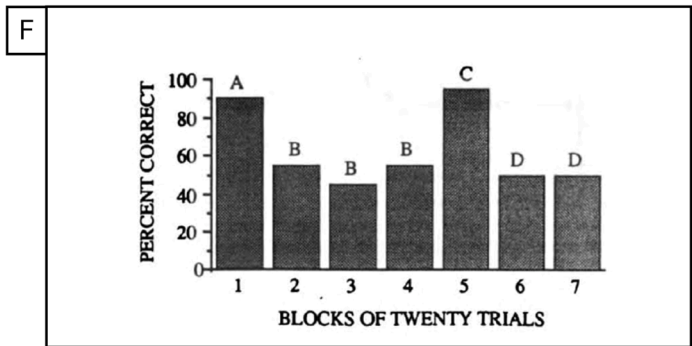
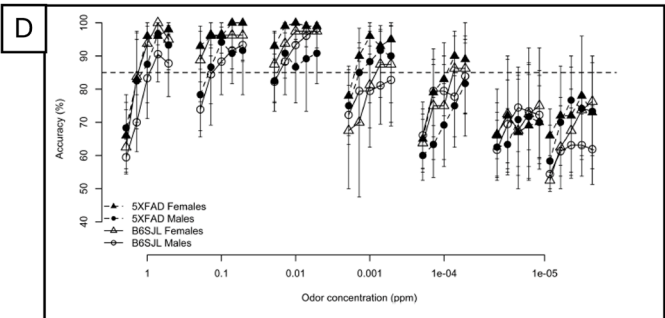
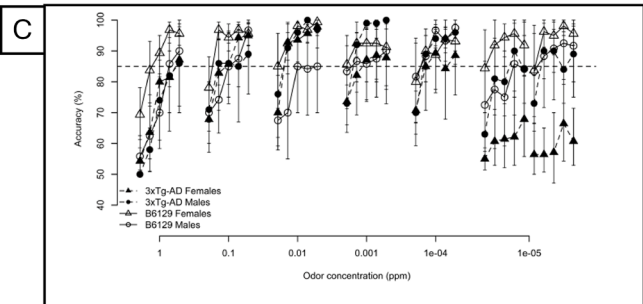
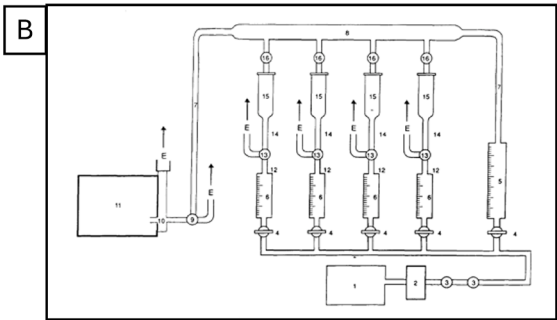
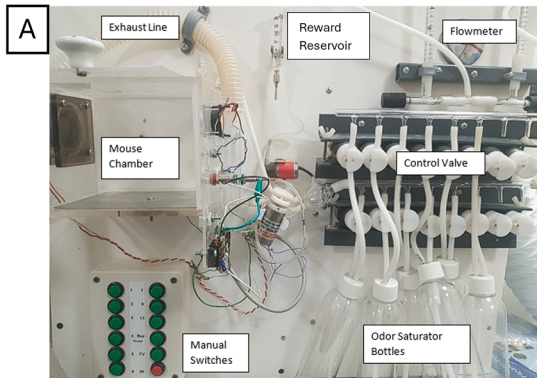
conspecific odours (Brown, 1977; Brown, 1978). Rodents may also show aversions to odours, and a "preference scale" can be constructed using a series of two-choice odour presentations, such that if odour A is investigated more than a neutral odour (water), it is considered attractive, and if it is investigated less than the neutral odour it is considered aversive (Kobayakawa et al., 2007). Using such a preference scale, Brown & Willner (1983) demonstrated that preweaning rat pups show an attraction to their maternal odour and an aversion to peppermint odour. Rodents show a range of unconditioned species-typical responses to the odours of predators, including avoidance, defensive burying, inhibition of activity, reduced feeding and altered neuroendocrine function (Dell'Omo et al., 1994; Apfelbach et al., 2005). A simple task for measuring unconditioned odour avoidance responses in mice (Fig. 4B) was described by Takahashi & Tsuboi (2017).

2.1.3. Conditioned odour preference and aversion learning

Newborn rat pups can be conditioned to approach novel odours

(peppermint) by pairing the odour with gentle stroking using a sable hairbrush and can be conditioned to avoid the same odour by pairing it with foot shock (Sullivan & Wilson, 1991). Adult mice can also be conditioned to avoid odours by pairing them with foot shock; after the odour-shock pairing, mice reduce the time spent with the conditioned odour in a preference test (Fletcher, 2012). Conditioned odour aversion can also be measured using a conditioned taste aversion model in which rats have access to water paired with an odour and are then injected with lithium chloride (LiCl), making them ill. In a memory test one day after LiCl treatment, the rats drank significantly less of the water with the odour paired with LiCl (Panhuber, 1982). A variant of this method is the

taste-mediated odour aversion learning task in which LiCl is injected after rats receive a unique odour-taste combination and later show aversion to the odour (Lopez et al., 2023). Injection of the cancer treatment drug Cisplatin also produces conditioned aversion to odours paired with drug treatment (Yu et al., 2026). Odour-cued taste avoidance is another paradigm used to produce conditioned odour aversion in rodents. In this paradigm, mice associate the odour placed on a drinking tube with a bitter taste of the drinking fluid and avoid drinking from the tube with that odour (Slotnick & Coppola, 2015).



(caption on next page)

Fig. 5. Olfactometer for studying sensitivity to odour concentrations, learning curves, detection of contamination and testing working memory.

[5A] Photograph of the olfactometer and operant chamber. Components are mounted on a PVC board. Air compressor, filters and laptop are not shown here. Photo taken by Wyatt Ortibus

[5B] A schematic representation of the olfactometer showing: (1) GAST compressor pump, (2) activated charcoal meter, (3) fritted glass filters, (4) needle valves, (5) main air flowmeter, (6) stimulus channel flowmeters, (7) 8-mm glass ductwork, (8) glass manifold, (9) final valve, (10) odour-sampling tube, (11) rodent test chamber, (12) Teflon connector, (13) three-way valve, (14) 4-mm glass ductwork, (15) odour stimulus jar, (16) two-way solenoid valve, E = to exhaust.

From: Schellinck, H. M., Brown, R. E., Slotnick, B. M. (1991). Training rats to discriminate between the odours of individual conspecifics. Fig. 1. *Animal Learning & Behavior*, 19(3), 223-233.

[5C and 5D] Testing the sensitivity of mice to odour concentration. Mean accuracy ($\pm$ 95% CI) of the female and male 3xTg-AD mice (C) and 5xFAD mice (D) and their wildtype controls on each concentration of ethyl acetate tested. The mice received 5 blocks of 20 trials on each concentration except for the lowest, on which they received 10 blocks of 20 trials. The dashed lines show the criterion level of 85%.

From: Roddick, K. M., Roberts, A. D., Schellinck, H. M., Brown, R. E. (2016). Sex and genotype differences in odour detection in the 3xTg-AD mice and 5xFAD mice models of Alzheimer's disease at 6 months of age. Fig. 3. *Chemical Senses*, 41(5), 433-440.

[5E] Learning curve from one rat in the discrimination of urine odours from two outbred Long-Evans rats. Different samples were used for the S+ and S- odours during each session of 10 trials. Learning curves within each session can reach 100% correct. By the sixth session, rats attain virtually errorless performance.

From: Schellinck, H. M., Brown, R. E., Slotnick, B. M. (1991). Training rats to discriminate between the odours of individual conspecifics. Fig. 3. *Animal Learning & Behavior*, 19(3), 223-233.

[5F] The percent correct responses in 7 blocks of 20 trials for one subject discriminating between two "clean" stimulus channels after the system was flushed with 70% ethyl alcohol. (A) The ability of the rat to discriminate correctly in 18/20 trials on Block 1 indicates that the system is contaminated. (B) After an odour stimulus valve was replaced the rat found no discriminable cues (random responses; ~50% correct responses) between the two channels (Blocks 2- 4), suggesting that the original valve was contaminated. (C) To confirm this finding, the original valve was replaced (Block 5) and the subject discriminated between the two channels with 95% accuracy. (D) Using the new valve (Blocks 6 and 7) eliminated the discriminable cues.

From: Schellinck, H. M. and Brown, R. E. (1995). Methodological questions in the study of the rat's ability to discriminate between the odours of individual conspecifics. Fig. 3. In: R. Apfelbach, D. Müller-Schwarze, K. Reutter and E. Weiler (eds.) *Chemical Signals in Vertebrates 7*, Pergamon, New York, pp.427-36.

[5G] The learning curves of the fastest and slowest of the five rats in learning to discriminate between the urine odours of two unrelated human males in an operant olfactometer.

From: Schellinck, H. M. and Brown, R. E. (1992). The discrimination of human male urine odours by rats is not influenced by the relatedness of the donors. Fig. 2. In R.L. Doty and D. Müller-Schwarze (eds.) *Chemical Signals in Vertebrates VI*, Plenum Press, New York, 1992, 219-223.

[5H] The mean percent correct ($\pm$ SEM) at each delay from five baseline training sessions during a study of working memory in the olfactometer.

From: Winters, B., Mathieson, W. R., McGregor, I.S., and Brown, R.E. (2000). An automated two-choice test of olfactory working memory in the rat: Effect of scopolamine. Fig. 2. *Psychobiology*, 28 (1), 21-31.

2.1.4. Tasks measuring olfactory sensitivity

Simple preference tests for odour sensitivity involve presenting one odour on a Q-tip or filter paper and a neutral odour (water or vehicle) on a second. The hypothesis is that the odour will be investigated more than the neutral stimulus and by reducing the concentration of the odour, one can calculate the sensitivity of the rodent for odour detection (Zou et al., 2015). Olfactory sensitivity can also be measured using a two-odour discrimination task in an operant olfactometer (Fig. 5A and 5B). The olfactometer developed by Slotnick and Nigrosh (1974) and updated by Slotnick and Restrepo (2005) allows for control of the air flow and temporal control of the odours presented to test animals in operant conditioning paradigms and records how these animals respond to these odour stimuli using a lickometer circuit. The rewarded stimulus is the odour for which sensitivity is being assessed, while the unrewarded stimulus is an odourless substance or clean air. Organic compounds such as ethyl acetate (Roddick et al., 2022; Roddick et al., 2016; Bodyak & Slotnick, 1999), octyl aldehyde (Slotnick & Restrepo, 2005), or n-hexanal (Phillips et al., 2011) are typically used in odour sensitivity tasks. These tasks assess the detection threshold by training the animals to respond to an easily detectable concentration of the odourant, and then decreasing the concentration until the animal is unable to detect it such that performance drops to chance levels (Fig. 5C & 5D).

Slotnick and Schoonover (1984) used a go/no-go method in a dilution olfactometer to measure the sensitivity of rats to amyl acetate odour. Using a criterion of 75% correct, they showed that rats could detect the presence of an odour at about 5×10^{-10} mol/l. Using the same procedure, Bodyak and Slotnick (1999) showed that mice could detect ethyl acetate odour at concentrations of less than 4.1×10^{-10} Molar. Jennings et al. (2022) used an olfactometer to measure the sensitivity of mice to a range of acetate esters and found that C57BL/6J mice could reliably detect acetate esters at concentrations in the parts per billion (ppb) range. They were most sensitive to isobutyl acetate and least sensitive to propyl acetate. The odour thresholds did not differ by sex and were consistent across individuals.

Roddick et al. (2016) used an olfactometer to examine genotype and sex differences in odour detection in two mouse models of Alzheimer's

disease (Fig. 5C and 5D). Using a threshold of 85% correct responses, they found that the 3xTg-AD mice could detect ethyl acetate at 10^{-5} ppm, with males performing better than females. The 5xFAD mice could detect ethyl acetate at 10^{-4} ppm with no sex difference. Odour detection deficits were only present at low concentrations of ethyl acetate, and then only in female 3xTg-AD mice, not in male 3xTg-AD mice. Both male and female 5xFAD mice showed a decline in odour detection at 10^{-5} ppm but did not differ from wildtype controls. Thus, in order to examine olfactory learning and memory deficits in mouse models of age-related neurodegenerative disorders, the sensory and cognitive deficits must be dissociated, as has been done with visual ability in tests of visuo-spatial learning and memory (Wong & Brown, 2012; Wong & Brown, 2013).

2.1.5. Tests for simple odour discrimination learning and memory

Discrimination learning involves presenting rodents with two or more olfactory stimuli and testing their ability to discriminate between them. There are a number of unconditioned preference tests for measuring odour discrimination in rodents, such as the habituation/dishabituation test which involves presenting the animal with one odour, typically on a cotton applicator, and recording the time the animal spends investigating the odour (Fig. 4C). Across subsequent presentations of the same odour the time spent investigating the odour should decrease (habituation). If a novel odour is then presented, the time the animal investigates the new odour should increase (dishabituation). This dishabituation (Fig. 4D) demonstrates that the animal can discriminate between the first and second odours (Brown et al., 1987). However, the habituation-dishabituation task relies on the exploratory behaviour of the animal. They are successful in demonstrating an ability to differentiate two odours, A and B, but suffer from difficulty in interpreting negative results or "non-responses" when the responses to odours A and B do not differ. It is not possible to determine if non-responses are the result of lack of motivation, task difficulty or other unknown variables (Slotnick et al., 2005). For this reason, olfactory discrimination tasks in which learned responses are rewarded are more informative as they measure responses to both rewarded and

unrewarded stimuli and the pattern of responses during learning can be examined (Schellinck & Brown, 1995).

In the **olfactory tubing maze**, for example, mice learn to discriminate odours by moving through the apparatus where they are presented with odours at junctions where they must choose to go left or right (Roman et al., 2002). The tubes for both choices have odourised air pumped into them, with one odour (S+) associated with a water reward, and the other odour (S-) with a buzzer. Balb/c, C57BL/6, CD-1 and 129Sv mice could learn this task, showing increasing accuracy and speed, but DBA/2J mice could not (Restivo et al., 2006).

Odour discrimination ability can best be assessed using operant olfactometers (Bodyak & Slotnick, 1999; Slotnick & Restrepo, 2005). Two odour go/no-go discrimination tasks involve presenting the animal with a rewarded odour (S+) or an unrewarded odour (S-) in an odour sampling port (Fig. 5A & 5B). Animals learn to lick the reward spout in response to the S+ odour and to inhibit licking to the S- odour. Animals are typically tested on such a task until they reach a set criterion such as 85% correct or for a set number of trials, such as 200 trials.

Rats and mice can discriminate between their conspecifics using odours from scent glands and urine (Brown, 1985a) and the operant olfactometer can be used to examine the variables influencing conspecific urine odour discrimination learning and memory. In a two odour discrimination task, rats could learn to discriminate between the urine odours of rats of different MHC genotypes and between the odours of two rats of the same genotype (Fig. 5E), but not between two urine samples from the same rat (Schellinck et al., 1991), urine odours of individual humans (Fig. 5G) (Schellinck & Brown, 1992), the urine odours of Y-chromosome congenic mice (Schellinck et al., 1993a), mice on different diets (Brown et al., 1996), or mice whose gut bacteria had been altered (Schellinck & Brown, 2000). The results suggest that there is both a genetic component and environmental factors that create identifiable individual odours within the urine (Brown et al., 1996). These studies provided unequivocal evidence for the ability of rats to learn about the individual identity of their conspecifics based on their unique body odours (Brown, 1995) as inferred from their responses to primer pheromones discussed above.

2.1.6. Olfactometer methods for rodent odour discrimination

Rodents tested in olfactometers (Fig. 5A & 5B) are first water deprived to increase their motivation to work for a liquid reward such as water or sucrose solution and then trained to discriminate odours in a number of steps (Schellinck et al., 1991; Slotnick & Restrepo, 2005; Slotnick et al., 2005). **Preliminary training (shaping)** consists of training the animals to obtain water reinforcement after breaking the photobeam and sampling the odour for a specific length of time. **Odour/no-Odour discrimination training** involves training the rodents to respond to an odour (S+) and not to respond to no odour (S-) in blocks of 20 trials in daily sessions of 200 trials (Slotnick & Schoonover, 1984). Responding (licking) to the S+ trials and not responding to the S- trials were scored as correct. The S+ trials in which the rat did not lick the drinking tube and S- trials in which it did lick were scored as incorrect. When an animal scored 85% correct or better on a block of 20 trials, it was considered to have met the criterion for acquiring the discrimination. The data for this stage and all successive stages of the experiment were recorded through input to a computer from the digital interface. **Two-odour discrimination training.** After successful completion of the odour-no-odour discrimination, the subjects were required to learn the odour discrimination task to a criterion of 85% correct over 20 odour presentations (10 S+ and 10 S-), with odour A designated as the S+ and odour B as the S-. Most rodents complete shaping and learn to discriminate their first pair of odours to criterion in one or two daily sessions of testing (Fig. 5E & 5G).

Benefits and disadvantages of using olfactometers. The benefits of using olfactometers in olfactory learning and memory research is that the odour presentations can be carefully controlled, and the responses to these odours are not confounded by vision, hearing, or motor deficits,

which occur in many mouse models (Schellinck et al., 2010; Roddick et al., 2014). In addition, animals can be tested in a number of learning and memory paradigms, from simple odour discrimination tasks to working memory and reversal learning tasks, and the learning curves of each individual animal can be tracked. The effects of genotype, age and sex differences on these cognitive processes can also be measured, as can the effects of neural manipulations such as brain lesions (Slotnick & Risser, 1990; Slotnick & Bodyak, 2002).

The disadvantages of using an olfactometer involve maintenance issues and experimenter errors. It is essential to ensure that the odour concentrations within the odourant bottles remain consistent to avoid inconsistencies in odour presentations during olfactory assessments. It is also vital to maintain proper airflow within the valves and tubes of the olfactometer. Due to the sensitive nature of odour detection, the machines must be kept clean in order to eliminate odour contamination as odours can linger on tubes, sample ports or chambers that the test subject will be in contact with, adding unwanted variability to the experiment. Glassware must also be monitored and cleaned to prevent odour contamination, especially the odourant chamber, where the odour and reward solutions are dispensed. It is also important that the reward tube flow-limiters be kept clean as they can become obstructed by very small bits of debris in the reward solution. The blockage of flow-limiters in the reward port necessitated continuous monitoring and manual testing of the reward tubes prior to each testing session to ensure proper reward delivery. The break beam sensors can also malfunction. Malfunctioning break beam sensors (burnt out sensors) are easily identifiable as the olfactometer ceases to operate if these sensors fail, requiring them to be replaced. The most serious olfactometer problem is that odour sample chambers are not fully cleaned between experiments as they could contain unwanted odours from prior experimentation. A test for contamination is to determine if rodents can discriminate between two "clean" odour channels such as two empty odourant bottles in the olfactometer (Fig. 5F). If the bottles are clean, the responses should be random (50% to each "odour"), but if the rodent scores 85% correct with two "clean" bottles, they are contaminated and must be replaced (Schellinck & Brown, 1995).

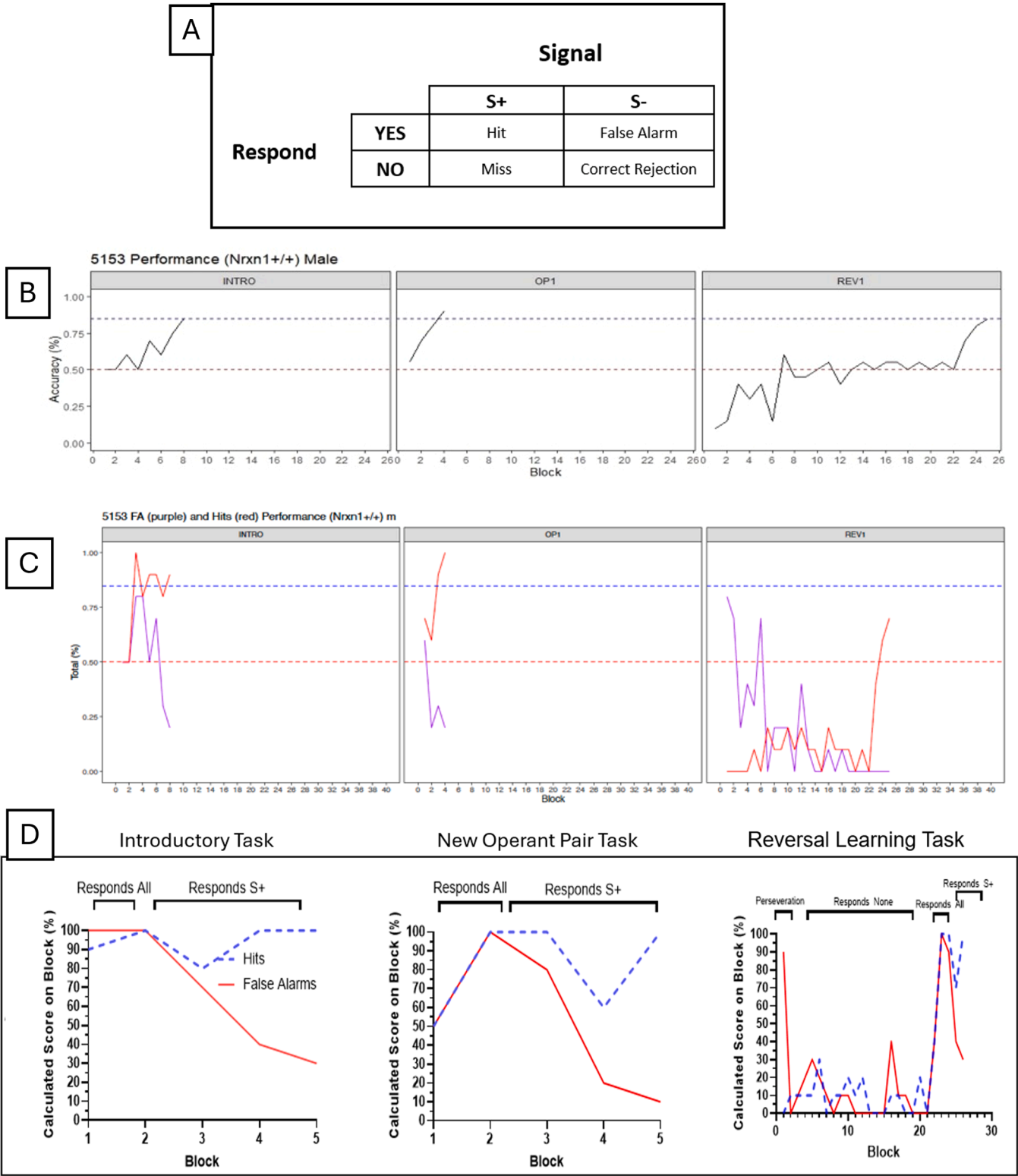
Although the olfactometer experiments require minimal animal handling, as the mouse only needs to be transferred from the home cage to the olfactometer chamber, it is crucial to prevent odour contamination of the testing environment. The experimenters should avoid using strongly scented products, wear gloves, and ensure that environmental odours do not contaminate the designated experimental area (Takahashi & Tsuboi, 2017). The testing environment can potentially influence mouse behaviour due to unexpected odour contaminants, such as odours from other species within the testing facility or airborne contaminants resulting from inadequate air circulation. The sex of the experimenter may also affect mouse behaviour and considerations should be taken to reduce experimenter effects when handling rodents (Georgiou et al., 2022). All of this highlights the importance of conducting pilot tests, to train researchers in the proper methods of animal handling, experimental procedures and diagnosing equipment issues, particularly the buildup of contaminants, to ensure a consistent testing procedure.

It is also possible that some rodents will not learn the shaping procedure. While uncommon, this issue becomes problematic in the olfactometer as the mice cannot progress to the discrimination learning phase of the experiment until they have been shaped to respond to the odours. The most common problems are that some mice have numerous errors (responding to the S- or failing to respond to the S+) or produce short samples (removing their nose from the sample port before the odour stimulus is presented). Typically, these rodents are removed from the study and documented to examine genotype-, sex-, or age-related differences. For example, in a recent experiment, only one out of thirty mice (3.33%) failed to learn the shaping program.

2.1.7. Using signal detection theory to examine response patterns in the olfactometer

Discrimination learning involves two separate learning processes: learning to respond to the S+ odour and learning not to respond to the S- odour. These two learning processes can be dissociated using signal detection theory to examine the details of the operant response patterns (Steckler, 2001; Wixted, 2007). There are four possible response

patterns (Fig. 6A), described as hits (responses to the S+), misses (failures to respond to the S+), false alarms (responses to the S-), and correct rejections (non-responses to the S-). These four measures can help determine individual patterns of learning and memory performance. The simplest measure of learning is percent correct responses (hits plus false alarms) to a criterion of 85% correct over blocks of 20 trials as shown for a single male C57BL/6J mouse in Fig. 6B. These learning



(caption on next page)

Fig. 6. Signal detection & response patterns of a single mouse.

[6A] Using **signal detection methods**, the responses of rodents to the rewarded (S+) and non-rewarded (S-) odours during discrimination and reversal learning in the olfactometer can be scored as Hits, Misses, False Alarms and Correct Rejections.

[6B] **Percent correct** over first (Intro) and second (OP1) odour discrimination learning trials and reversal learning trials to reach a criterion of 85% correct for a single C57BL/6J male mouse (#5153).

[6C] **Data from the same mouse** as in Fig. 6B plotted as hits (red) and false alarms (purple) showing the changing pattern of responses as learning occurs in each phase of the experiment.

All From: [Ortibus, W. \(2025\)](#). Olfactory discrimination, reversal learning and overtraining effects in $Nrxn1^{+/-}$ and $Nrxn1^{+/+}$ mice in an operant olfactometer. <https://hdl.handle.net/10222/85421>.

[6D] **Performance of a single male C57BL/6J mouse** (#5292) to a criterion of 85% accuracy in olfactory discrimination and reversal tasks in the olfactometer. The pattern of hits (dashed blue line) and false alarms (red line) can be used to define the different phases of learning in each phase of the experiment. In the introductory task (A), the mouse responds to both the S+ and the S- odours (Responds All) on the first three blocks of trials and then reduces responding to the S- (Responds S+) as the discrimination is learned. In the new operant pair task (B) the mouse responds to both S+ and S- odours (Responds All) for two blocks of trials and then reduces responding to the S- (Responds S+), as in the Introductory task. In the reversal learning task (C) the S+ odour from the new operant task becomes the S- and vice-versa. The mouse first perseverates in responding to the old S+ (Perseveration) and then stops responding to both odours (Responds None). Finally it begins to respond to both the new S+ and S- (Responds All) and then reduces responding to the S- (Responds S+). The Perseveration and Responds None phases reflect difficulty in cognitive flexibility.

From: [Ortibus, W. \(2025\)](#). Olfactory discrimination, reversal learning and overtraining effects in $Nrxn1^{+/-}$ and $Nrxn1^{+/+}$ mice in an operant olfactometer. See [Lu et al., 2023](#) for information on $Nrxn1^{+/-}$ mice. <https://hdl.handle.net/10222/85421>.

curves show that the second odour discrimination (OP1) was learned faster than the first (Intro) and that reversal learning (Rev1) took much longer than the original discrimination learning, but they do not show the two learning processes, which are depicted as hits and false alarms in Fig. 6C. This shows that in the initial discrimination learning tasks, the mouse first responds to both the S+ and S- odours and then reduces responding to the S- while maintaining responding to the S+; increasing 'hits' and decreasing 'false alarms' ([Ortibus, 2025](#)). The two learning processes can thus be seen to be dissociated.

[Roddick et al. \(2022\)](#) expanded the use of signal detection analysis to study the ability of 12-month-old male and female 5xFAD and WT control mice to detect different concentrations of ethyl acetate in the olfactometer. The 5xFAD mice showed learning deficits compared to the WT mice and males showed better performance than females. The value of using signal detection analysis was that the sensitivity index (d') and bias (c) could be calculated. There were no genotype differences in sensitivity or bias and, while there were no sex differences in sensitivity, the female mice had a more conservative response bias than the male mice. Thus, the signal detection analysis provides a measure of the cognitive strategies used by the mice and allows for the dissociation of cognitive and sensory deficits in mouse models of neurological disorders.

2.1.8. Tests for olfactory reversal learning

Reversal learning can be assessed in a variety of learning and memory tasks ([Mihalick et al., 2000](#)) and serves as a measure of cognitive flexibility ([Izquierdo et al., 2017](#); [Kesner & Churchwell, 2011](#); [Hamilton & Brigman, 2015](#)), providing an important assay for cognitive function in mouse models of human neurodegenerative disorders ([Johnson & Wilbrecht, 2011](#); [Roddick et al., 2023](#)). Olfactory reversal learning tasks require a subject to learn an initial odour discrimination (A+ vs B-) and, once the 'rule' that odour A is rewarded is learned, the rewards are reversed so that the subject must learn that B is now rewarded (B+ vs A-). As pointed out earlier (Fig. 1) rodents learn olfactory discriminations in an olfactometer better than discriminations of lights or tones in an operant chamber and are capable of forming a learning set with serial reversal learning tasks ([Nigrosh, et al., 1975](#); [Slotnick & Katz, 1974](#)). Fig. 6B shows that reversal learning requires many more trials to learn than a simple odour discrimination.

Signal detection analysis (Fig. 6B & 6C) shows that reversal learning in the olfactometer occurred in four stages. In the first stage (**perseveration**) the mice responded to the formerly rewarded odour (A-) rather than the new rewarded odour (B+) and thus scored primarily false alarms and misses. In the second phase (**respond none**), mice quit responding to both odours, as if they were showing "frustrative non-responding". In the third phase, mice responded to both odours (respond

all), and in the fourth phase (**respond S+**) they ceased responding to the S- and continued to respond to the S+. Thus, the first two phases of reversal learning showed perseverance and non-responsiveness, while the third and fourth phases recapitulated the patterns of learning shown in the initial odour discrimination task (Fig. 6D). The signal detection approach allows us to analyze genotype, sex and individual differences in patterns of odour discrimination and reversal learning ([Ortibus, 2025](#)).

2.1.9. Learning to learn

Serial olfactory reversal learning was shown in mice that were trained on four pairs of odour discriminations, each followed by a reversal of that pair ([Caglayan et al., 2021](#)). These mice showed a decrease in errors across both discrimination and reversal pairs, with more errors in reversal learning than in the original odour discriminations. Both rats ([Slotnick & Katz, 1974](#)) and mice ([Roddick et al., 2023](#)) can achieve near errorless performance in serial reversal learning tasks, indicating the ability of learning to learn, or forming olfactory learning sets, a behaviour previously thought to be limited to primates ([Slotnick et al., 1991](#); [Slotnick et al., 2000](#)).

When first reported by [Slotnick & Katz \(1974, page 798\)](#) the idea that rats could perform near errorless learning, acquiring "a learning set for odour stimuli comparable to those achieved by primates in response to visual stimuli" was quite controversial ([Reid & Morris, 1993](#); [Slotnick, 1994](#)). Mice can also learn a serial reversal task, and in a study using 18 serial reversal tasks, male and female 3xTg-AD mice and their WT controls (B6129F2 mice) showed learning to learn as they reduced their errors with each reversal task until they performed at almost error-free levels ([Roddick et al., 2023](#)). In this study, there were more errors in the reversal learning tasks than in the original discriminations, but this difference decreased with training such that by the sixth odour pair there were no more errors during reversal learning than when learning the original discrimination (Fig. 7A), thus, the "learning to learn" occurred at two levels: learning to learn the original discrimination and learning to learn the reversal task. The 3xTg-AD mice performed as well if not better than the WT controls in both of these aspects of learning to learn.

Signal detection analysis of the data from the serial reversal learning study of [Roddick et al. \(2023\)](#) allowed us to identify how the response patterns changed during "Learning to learn". This analysis showed that the number of **perseveration** responses to the former S+ and the number of **responds none** responses were significantly reduced from pair 1 to pair 18 in both the B6129F2/J wildtype control mice and the 3xTg-AD mice. Likewise, as mice progressed through the serial reversal pairs, the time spent in **respond all** decreased and the final phase, **Respond S+** occurred earlier in the series of blocks (Fig. 7A & 7B). The

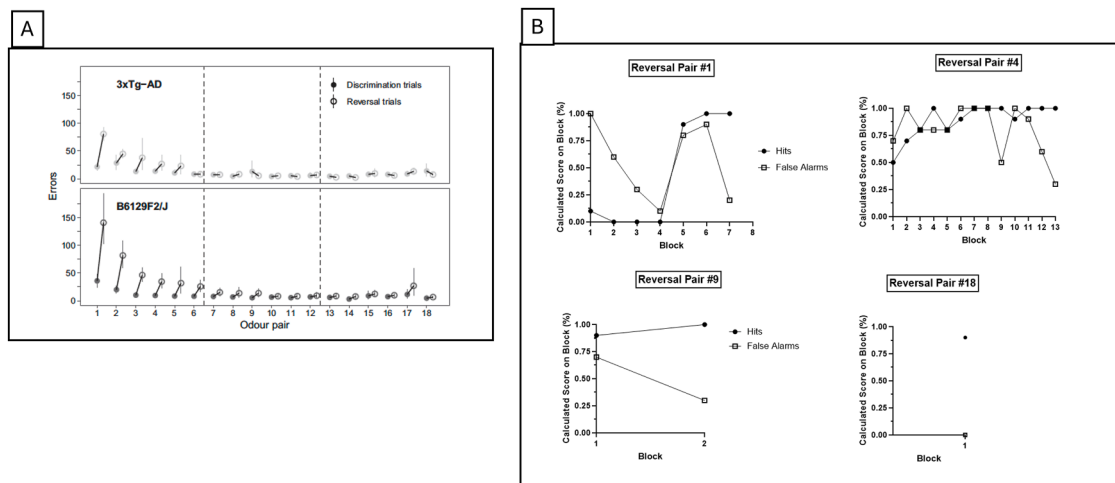


Fig. 7. Serial reversal learning. Learning to learn.

[7A]. Mean number of errors ($\pm 95\%$ CI) made by 3xTg-AD and B6129F2/J mice on the discrimination and reversal trials for each of 18 odour pairs. The difference scores (reversal errors – discrimination errors) are indicated by the lines connecting each odour pair. By the seventh reversal learning pair, the mice are showing virtually errorless learning, demonstrating learning-to-learn.

[7B]. Analysis of the change in performance (Hits vs False alarms) of a single female 3xTg-AD mouse (#2164) in learning a series of two odour discrimination and reversal tasks in the olfactometer to the criterion of 85% correct. Data from pairs #1, #4, #9 and #18 are shown. Reversal pair #1 shows perseveration for three blocks, respond none for one block and then respond all for 2 blocks before Respond S+. By Reversal Pair #4 mice do not perseverate or ignore any presented odours, but rapidly enter the respond all phase followed by responding only to the S+. By Reversal Pair #9 mice perform reversals rapidly, within 1 to 2 blocks of trials. By Pair #18, the reversal shows no perseveration, respond none or respond all; the switch to respond (new) S+ is virtually errorless.

Data from: Roddick KM, Schellinck HM, and Brown RE. (2023). Serial Reversal Learning in an Olfactory Discrimination Task in 3xTg-AD Mice. *Learning and Memory*, 30:310-319, reanalyzed by Ortibus, W. (2025). Olfactory discrimination, reversal learning and overtraining effects in $Nrxn1^{+/-}$ and $Nrxn1^{+/+}$ mice in an operant olfactometer. <https://hdl.handle.net/10222/85421>.

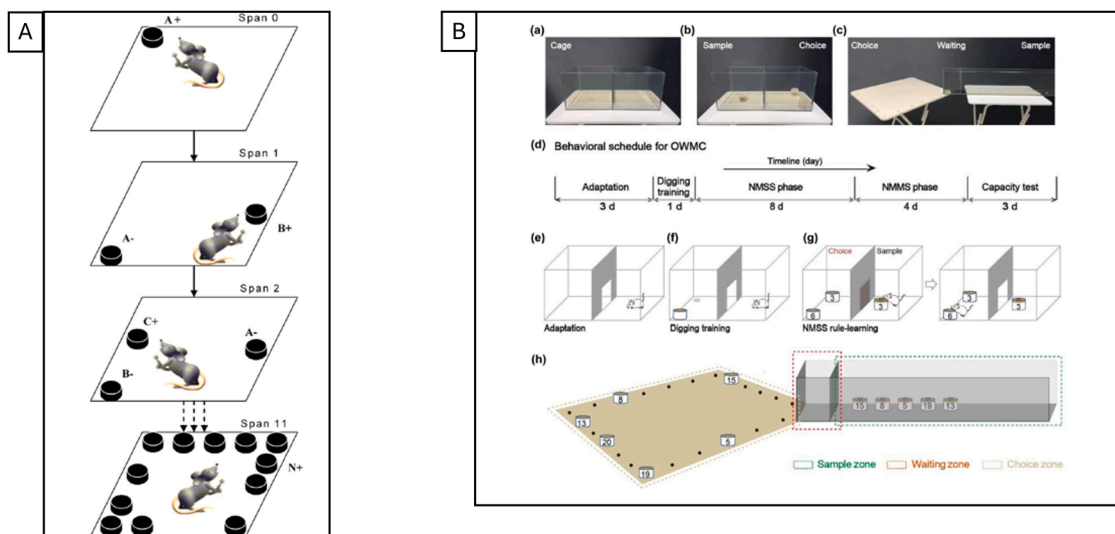


Fig. 8. Tests for working memory.

[8A] Illustration of the mouse odour span task. At span 0, mice are presented with a bowl of odour scented woodchip, containing a buried reward pellet (A+). Upon retrieval and consumption of the reward, the mouse is removed from the platform. For span 1, a novel scented and baited bowl (B+) is introduced whilst the first bowl, now containing no reward (A-) is moved to a random location. When placed back on the platform the mouse is required to remember odour A, but only dig in bowl (B+) with the novel odour. Retrieval of the pellet from B results in mouse removal and initiates span 2 where a third baited novel scented bowl (C+) is placed on the platform and the process repeated. The task continues in this fashion until 12 bowls are presented (span 11) or the animal spends 10 min on the platform. From: Young, J. W., Kerr, L. E., Kelly, J. S., Marston, H. M., Spratt, C., Finlayson, K., & Sharkey, J. (2007). The odour span task: A novel paradigm for assessing working memory in mice. *Fig. 1. Neuropharmacology*, 52, 634–45.

[8B] Training cage and platform for assessing olfactory working memory capacity in mice in five phases. (a) The training cage for context adaptation, digging training, and nonmatching to multiple sample (NMSS) odour rule-learning. (b) Experimental design for the NMSS rule-learning phase. (c) The spliced multipartition platform for NMSS rule-learning and capacity testing. (d) Timeline of the olfactory working memory capacity (OWMC) task. (e–g) The context adaptation, digging training, and NMSS rule-learning phase olfactory working memory capacity of the task. (h) The NMSS learning phase and capacity testing phase of the task.

From: Jiang, L. X., Huang, G. D., Wang, H. L., Zhang, C., & Yu, X. (2022). The protocol for assessing olfactory working memory capacity in mice. *Fig. 1. Brain and Behavior*, 12(8), e2703.

mice reduced their **respond none** responses by the 9th odour pair and began the **respond all** phase as soon as they determined that responding to the former S+ (**perseveration**) did not yield a reward. Our re-analysis indicates that the 3xTg-AD mice had better reversal learning performance than the WT controls and that this was not due to a difference in **perseverative** responses, but to a reduction in **responds none** responses. Thus, as serial reversal learning trials progress, the increase in reversal learning performance, reaching the 85% criterion in fewer and fewer trials, and becoming one trial learning in the latter series, is due to the reduction in number of perseverance, respond none and respond all trials. We are now analyzing this pattern of reversal learning to determine if there are genotype, sex and individual differences in how they show behavioural flexibility and cognitive adaptation to the changing pattern of rewards (Fig. 7B).

2.1.10. Tests for olfactory working memory

Working memory tasks require the animal to store a memory of one or more stimuli and use that memory to determine how to respond at a later timepoint. The three main classifications of working memory tasks used with animals are (1) goal maintenance, (2) memory capacity, and (3) interference control (Dudchenko et al., 2013). Goal maintenance tasks include delayed matching and non-matching to sample tasks, as well as delayed win-shift tasks. In these tasks, the animal is presented with a stimulus and must remember it over a delay period before making a response that depends upon that stimulus. This type of experiment measures short-term recognition memory (working memory) in rodents in a two-choice olfactory discrimination task with increasing retention intervals.

Otto & Eichenbaum (1992) developed an odour-guided continuous delayed non-matching-to-sample task for testing olfactory working memory. Using a go/no-go task in an olfactometer, rats were presented with odour A and then, after a delay of 3, 30 or 60 sec, a second odour was presented, and the rat was rewarded for responding only if the second odour was different from the first odour. Control rats learned this task but rats with orbitofrontal cortex lesions showed impaired performance at the longer delays, showing a reduction in working memory performance.

Memory capacity tests involve the animal retaining a memory of multiple stimuli and using this memory to determine how to respond (Fig. 8A and 8B). For example, in the olfactory working memory capacity task (Huang et al., 2020; Jiang et al., 2022) the animal is presented with a variable number of odour pots before being presented with the same odour pots plus a novel odour pot and must dig in the novel pot to get a reward. This nonmatching to sample task controls the demand on the animals' working memory by varying the number of odours initially presented. The odour span task involves first presenting the animal with an odour pot containing a reward. After the animal has found and consumed the reward it is removed from the testing arena, the now unrewarded odour pot is moved to a random position in the arena and a novel, rewarded, odour pot is added. The animal is then returned to the arena, and should it successfully first dig in the novel odour pot this process of removing the animal, adding a novel, rewarded, odour pot, and randomly positioning the pots is continued until the animal first goes to an incorrect pot. The number of odours added before the animal makes an error and digs in a non-novel odour pot gives a measure of their working memory capacity (Jiang et al., 2024; Young et al., 2007).

In the operant olfactometer both rats (Lu et al., 1993) and mice (Roddick et al., 2014) are able to perform go/no-go delayed matching to sample tasks. Using delays of 3, 30, 60 or 120 seconds between odour presentation and responses, Winters et al. (2000) found a significant delay-related performance decrease in olfactory discrimination ability in rats to a criterion of 80% correct (Fig. 5H). This decrease in olfactory working memory performance was exacerbated by anti-cholinergic treatments using scopolamine, a muscarinic antagonist that produces delay-independent effects in rodent memory paradigms. Roddick et al. (2014) found that the WT controls could learn the matching to sample

task with delays up to 10 sec, but it took many trials for them to reach this criterion. There was no evidence that the 5xFAD mice, a model of AD, showed deficits in working memory, but there was evidence that females of both genotypes performed better than males.

2.1.11. Measuring genotype, sex, age and individual differences in rodents' olfactory learning performance

The olfactometer can be used to examine genotype, sex, age and individual differences in olfactory discrimination and reversal learning as discussed above. In each experiment there has been significant variability of responses within the same genotypes in the odour discrimination and reversal learning curves which can be analyzed using signal detection theory (Roddick et al., 2022). Using the four signal detection measures (Hits, Misses, False Alarms, Correct Rejections) and measures of learning performance (Learning curves, Accuracy, Short samples, Trials per min, etc.) each subject's responses can be recorded to determine their unique pattern of responses in operant discrimination learning and reversal tasks. For example, while some mice learn the reversal task with 100 total errors in 15 blocks, some mice generate four times more errors before achieving 85% accuracy and have a learning curve that stretches over 40 blocks totalling 400 errors. By studying the differences between similar genotype, age and sex cohorts in our experiments we can determine if there are different patterns of responses in our operant learning experiments and how the individuality of each rodent's memory performance is demonstrated (Fig. 9).

When comparing the performance of old (Mean = 581 days) and young (Mean = 92 days) male and female *Nrxn1*^{+/+} and transgenic *Nrxn1*^{+/-} mice, we found that ~35% of the old WT (C57BL/6J) and ~67% of the old *Nrxn1*^{+/-} mice failed to complete all phases of the study (Shaping, Introductory Odour Pair, New Operant Pair, Reversal) while ~20% of the young mice of both genotypes failed to complete the study. However, those old mice which completed all phases of the experiment did not differ in performance (errors to criterion) from young mice (Delaney-Smith et al., 2026; Ortibus, 2025). We continue to analyze the interactions of sex, age and genotype differences in olfactory reversal learning in the olfactometer.

There were also considerable individual differences in working memory performance in mouse models of neurological disorders as these mice often have age-related motor and visual deficits which affect their performance in visuo-spatial tests of cognitive function, but do not seem to affect olfactory learning and memory (Wong & Brown, 2007; Brown & Wong, 2007; O'Leary et al., 2018).

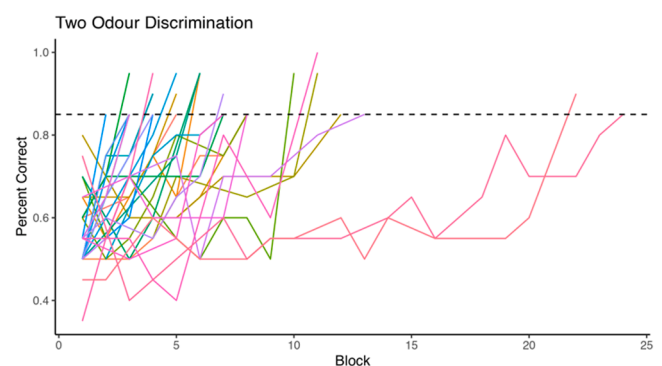


Fig. 9. Individual differences in learning curves [number of blocks of trials to reach a criterion of 85% correct] in a two-odour discrimination in the olfactometer by male and female 3xTg-AD mice and B6129F2/J wild-type controls between 5 and 24 m of age. Some mice reach criterion in under 5 blocks of trials, others require more up to 10 blocks, and a few require more than 20 blocks.

Data from: Roddick, Schellinck, Brown (2023). Serial reversal learning in an olfactory discrimination task in 3xTg-AD mice. *Learning & Memory*, 30 (12), 310–319.

2.1.12. Tests for declarative (episodic and semantic) olfactory memory

Olfactory episodic and semantic memory systems are closely allied and Eichenbaum (2001, page 202) proposed that, for rodents, olfactory semantic memory is based on a series of episodic memories. According to this view, "semantic memory is accrued from linking multiple experiences that share some of the same information". Eichenbaum (1998, page 659) argued that the hippocampal declarative memory system plays a role in converting individual olfactory episodic memories into an integrated olfactory semantic memory system through the organization and expression of odour memories. Based on the concept that declarative memory involves "the capacity to compare and contrast items in memory, and to make inferences from and generalizations about memories, the role of the hippocampus is to encode relations among

items in memory and to support the organization of memories within a structured knowledge network." A fundamental property of such memory networks is the capacity to make inferences about items that have never been experienced together directly but are indirectly related within the network structure. Such 'flexibility' of memory expression is taken as a fundamental aspect of declarative memory performance (Eichenbaum, 1998 p. 660).

Eichenbaum (2010, page 482) delineated four features of episodic memory which lead to semantic memory. First, **episodic** recollection of previous experiences is distinguished from familiarity; second, episodic memories for events involve the **context** (when and where) the event occurred; third, episodic memory involves the **temporal organization** of the events in the episodic experience; and, fourth, "specific episodic

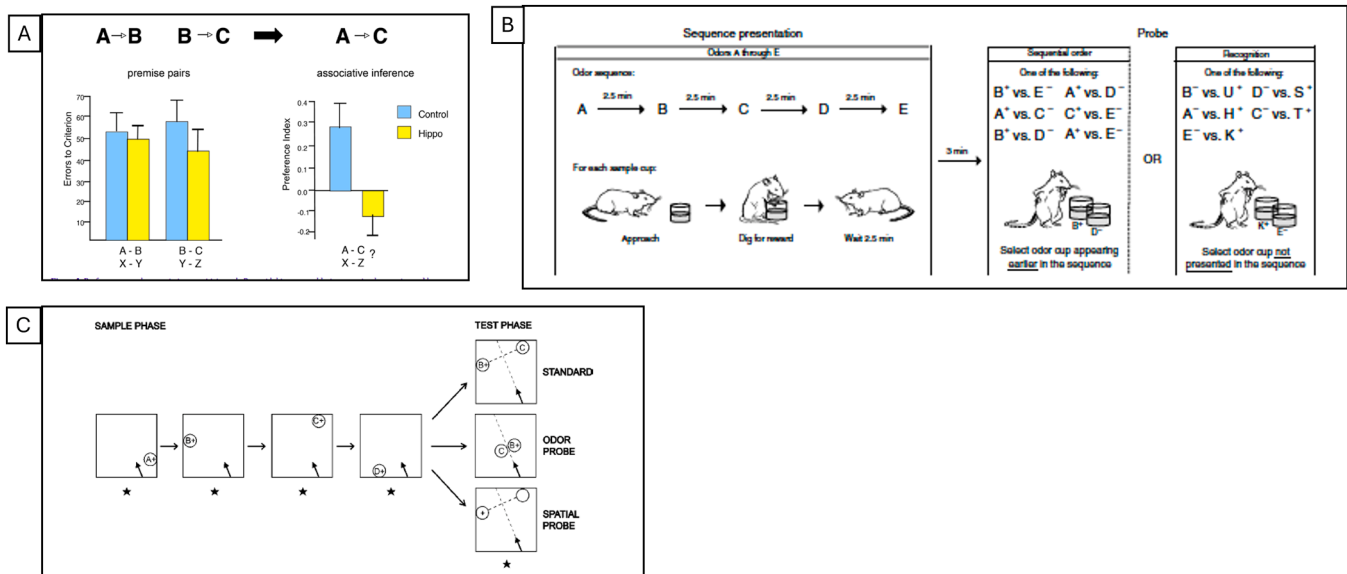


Fig. 10. Tests for declarative (episodic & semantic) olfactory memory.

[10A] Transitive inference task. Performance on the associative transitivity task. Rats with hippocampal lesions acquire the original odour discrimination problems (episodic memory) as readily as intact rats. However, the intact rats demonstrate transitive inference by a preference in the appropriate indirectly related stimulus, but the rats with hippocampal lesions do not show transitivity, indicating they have not represented the indirect (semantic) relations. (Hippo = hippocampal lesion).

From: Eichenbaum, H. (2007). Comparative cognition, hippocampal function, and recollection. Fig. 5. *Comparative Cognition & Behavior Reviews*, 2(1), 47–66.

[10B] Sequential order and odour recognition task. Food-deprived rats were trained to dig for pieces of cereal rewards buried in cups of unscented sand. During sequence presentation of odours A-E, odour cups were presented singly in the home cage and the rat was required to dig in the sand to obtain the reward. For the training trial with each odour in both the sequential order and recognition tests, five odours were randomly selected and these were presented singly with cereal pieces buried at the bottom of the cup. A variable (average 2.5-min) inter-stimulus interval was interposed between stimulus presentations, and a 3-min delay was interposed before the probe test. The inter-trial interval was 30–60 min.

During the sequential order probe test, the rat had to choose between two odours that had appeared in non-adjacent positions in the series (semantic memory). The correct choice was the odour that was presented earlier in the series. A choice response was scored when the animal touched the sand in one of the stimulus cups with its paw.

During the recognition probe test, the rat had to discriminate between a familiar odour that appeared in the series (A to E) and a novel odour which was not in the earlier series. The reward for a correct choice of the novel odour was one whole piece of cereal. Animals received 6 trials per day. +, reinforced odor; -, nonreinforced odor.

From: Fortin, N. J., Agster, K. L., & Eichenbaum, H. B. (2002). Critical role of the hippocampus in memory for sequences of events. Fig. 1. *Nature Neuroscience*, 5, 458–62.

[10C] In the What-Where-When Task, each trial was composed of a sample phase followed by a test phase. In the sample phase, the rat was presented with four odours in a series (A+ → B+ → C+ → D+), each at a different location on a platform. The first rewarded odour (A+) was placed in a location along one wall of the platform, and the rat was placed at the starting point (Shown by an arrow) and was allowed to sample the odour and dig in the sand to retrieve a buried cereal reward. After consumption of the reward, the rat was returned to its home cage for ~7 sec. during which the first odour was removed, and a second rewarded odour (B+) was placed in a different location on the platform. The rat was then re-placed at the starting point and allowed to sample the second stimulus. After the rat ate the reward, it was again returned to its cage for ~7 sec. and this cycle was repeated with four different rewarded odours placed at four different locations on different sides of the platform (A+ → B+ → C+ → D+).

The test phase had three different tasks. In the **standard Probe** test, the rat was placed at the starting point and allowed to choose between odours B and C which were presented in their sample locations and the odour presented earlier was rewarded (B+). The choice response was scored as the first cup in which the rat began to dig, indicated by touching the sand with its paw. In the **Odour Probe** test, two of the sampled odours were presented in adjacent locations in the middle of the platform and the odour that was presented earlier in the sample series was rewarded (B+). In the **Spatial Probe** test, cups filled with unscented sand were presented at two of the sampled locations and the location presented earlier in the sample series was rewarded (+). The * indicates the location of the experimenter.

From: Ergorul, C., & Eichenbaum, H. (2004). The Hippocampus and Memory for "What," "Where," and "When." Fig. 1. *Learning & Memory*, 11, 397–405.

memories are integrated with other memories that contain substantial common information, a capacity that bridges to semantic memory, a network of knowledge gained from integration of many episodic memories". Eichenbaum (2010, page 483) summarized his views on the integration of episodic and semantic memory by saying that "in the view presented here, semantic memory is not independent of episodic memory; rather, semantic memories are a natural product of the networking of memories for events and episodes." With respect to olfactory memories, Eichenbaum (2010) postulated that simple episodic memories are encoded in the perirhinal and/or entorhinal cortex, whereas the context, temporal organization, and integration of episodic memories into semantic memories requires the para-hippocampal and hippocampal areas of the brain.

In order to investigate the difference between episodic (non-hippocampal) and semantic (hippocampal) aspects of olfactory memory, Bunsey and Eichenbaum (1996) developed a paired-associates task in which rats were trained to dig in a sand solution containing a sample odour A or X for a cereal reward and were then presented with a choice between two different odour pots: B and Y. Both intact and hippocampal lesioned rats could learn the paired-associates task, thus the hippocampus was not required for the episodic paired-associates memory. Rats were then tested in a "Transitivity experiment" in which they were trained to associate odours A and B, and then odours B and C, and then, when presented with A as a sample odour, they had to choose between odours C and X. Intact rats were able to perform this transitivity task, selecting odour C, but rats with hippocampal damage could not. The ability of rats to perform such transitive inference tasks was taken to demonstrate olfactory semantic memory (See Eichenbaum, 2010; Eichenbaum, 2016). Likewise control rats trained that sample odour B predicted reward in odour C but not X on the paired associate trial could also perform the "Symmetry test", that odour C predicted reward in odour B, but rats with hippocampal damage could not learn this task.

Dusek & Eichenbaum (1997) used tests of transitive inference to examine the ability of rats to learn a relational representation among a set of odours (Fig. 10A). Rats were trained to dig in a pot containing odour A (CS+) for a cereal reward, and a second pot containing odour B (CS-) for no reward. They were then trained on new pairs of odours, B+ vs C-, C+ vs D-, D+ vs E-, and given non-rewarded probe trials to determine if they could discriminate between two odours which had not been paired together. Rats with hippocampal damage did not differ from intact control rats in simple discriminations (B vs C; C vs D), but control rats could learn the correct discrimination in the probe tests for transitivity involving pairs B+ vs D- and A+ vs E-, but rats with hippocampal damage could not, indicating the importance of the hippocampus for learning and remembering relationships among olfactory stimuli which had not previously been paired. Fortin et al. (2002) developed a test of sequential odour memory in which rats were presented with a series of five odours in odour pots and then, in a two-choice probe trial, they had to choose the odour that was presented first (Fig. 10B). Thus, in the sequence ABCDE, if the probe trial had odours A vs C, A would be correct as it was presented before C. Control rats could choose the correct odour, but rats with hippocampal lesions were impaired. Other versions of the sequential odour memory task have also been developed for the study of olfactory episodic memory in rats (Panoz-Brown et al., 2018; Sheridan et al., 2025).

In order to examine the role of the hippocampus in contextual olfactory memory, Ergorut & Eichenbaum (2004) developed tests for the combination of spatial and olfactory memory in a "what, where, and when" paradigm (Fig. 10C). Rats were trained with a sequence of four rewarded odours (A, B, C, D), each in a different quadrant of an open field. They were then given odour probe trials to test their memory for the earlier presented odour (A+ before C-) and spatial probe trials to test their memory for the location of the rewarded odour (matching an odour with a spatial location). Control rats could learn the spatial-odour pairings but rats with hippocampal lesions were impaired, indicating the role of the hippocampus in integrating olfactory and spatial

information in memory. These complex odour discrimination experiments identify the importance of the connections between the hippocampus and the olfactory and prefrontal areas of the cortex in complex olfactory memory tasks (Eichenbaum, 2017). This series of complex olfactory learning and memory tasks from Eichenbaum's lab showed that the hippocampus was not essential for simple odour discriminations, but was necessary to form representations of relationships among olfactory memories, thus combining spatial (where), and olfactory (what) cues to recall when an event occurred in episodic memory (Eichenbaum, 1998).

In attempting to understand the characteristics of these complex olfactory episodic and semantic memory processes, Eichenbaum (2010; 2016; 2018) used the concept of Hebbian cell assemblies and phase sequences. In the examples above, a simple episodic olfactory memory could be represented as a single cell assembly and these episodic memories could then be linked with contextual and temporal associations into phase sequences (Eichenbaum, 2010, page 218). Eichenbaum (2018, page 92) argued that Hebbian cell assemblies formed the basic "units of information processing" in perception and cognition and that their coupling into phase sequences could be used to explain "how anatomically separated but interconnected [neural] networks interact within functional systems" such as found in olfactory semantic memories.

2.2. The Pavlovian conditioned odour preference task for testing short-, long-, and very long-term olfactory memory

2.2.1. The original Pavlovian conditioned odour preference task

Schellinck et al. (2001a; 2001b) developed a simple Pavlovian conditioned odour preference task (akin to autoshaping) to evaluate learning and memory in mice that relies on their natural foraging behaviour. During training, mice were presented with one odour pot containing sawdust with an odour paired with sugar (CS+), and a second odour pot containing another odour (CS-) without sugar, in separate cages (Fig. 11A & 11B). In the original procedure, CD1 mice received eight 10-minute training trials per day for four days: four trials with the CS+ odour and four with the CS- odour. Over the four days, mice completed sixteen CS+ and sixteen CS- trials, followed by a 24-hr odour preference memory test on day 5 in a three-chamber apparatus containing a CS+ odour pot at one end and a CS- odour pot at the other, but without sugar in either one (Fig. 11C). Learning and memory were determined by measuring the amount of time spent digging in the CS+ odour pot (searching for the sugar reward) relative to the time digging in the CS- odour pot (Fig. 12A). This task has been used to examine a number of aspects of olfactory learning and memory as discussed below.

2.2.2. The problem of motivation

Although mice that have not been food-restricted can learn to discriminate between rewarded and non-rewarded odours (Moss et al., 2025), mice that are food-restricted express a conditioned odour preference (digging in the CS+ odour pot) more readily and to a greater extent than mice that are not food-restricted in memory tests (Forestell et al., 2001). Food-restriction (Makowiecki et al., 2012) and sugar reward (Dalm et al., 2009), however, increase the motivation for learning and yield more reliable results in the memory tests.

2.2.3. Measuring memory strength using the Pavlovian conditioned odour preference task

Memory strength is a product of memory encoding (learning) and storage (consolidation) as measured by ability to recall (remember) the memory over time. However, "memory" itself cannot be measured, and we rely on some measure of performance which reflects memory strength, and as noted by Cahill et al. (2001), "We ignore the learning/performance distinction at our peril". While measuring memory is difficult, measuring "memory strength" is even more difficult (Brady et al., 2023; Wixted, 2004). Although our attempts to determine memory strength appear quite primitive, we have used the classical methods

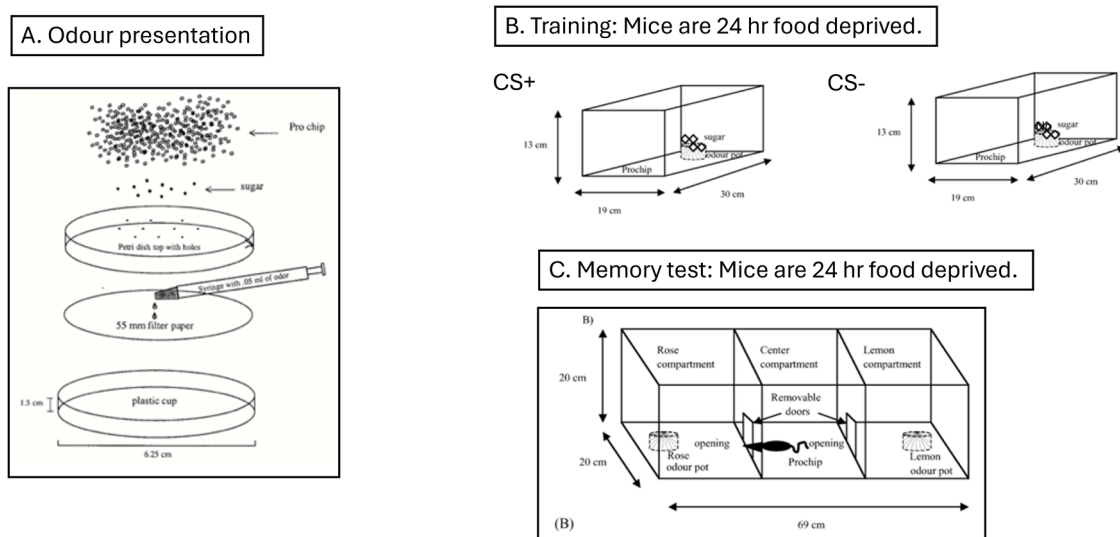


Fig. 11. Pavlovian conditioned odour preference task.

[11A] **Odour presentation.** The CS+ odour pot had a .05 ml drop of liquid odour on filter paper covered by a perforated plastic lid covered with bedding and containing 8 pieces of sugar. The CS- odour pot was identical but had no sugar pieces.

[11B] **Training takes place in cages** identical to the home cages with each odour presented in a different room. The CS+ odour pot has 8 pieces of sugar; the CS- odour pot has no sugar.

[11C] **Memory tests** are conducted in a 3-chamber box with one odour pot at each end. Neither the CS+ nor CS- odour pot contains sugar.

[A] is From: Schellinck, H. M., Forestell, C. A., LoLordo, V. M. (2001a). A Simple and Reliable Test of Olfactory Learning and Memory in Mice. Fig. 1. *Chemical Senses*, 26, 663–72. [B] and [C] are unpublished figures.

(used by weightlifters to assess muscle strength) of using different numbers of training trials and different durations of memory assessment.

Number of training trials. As noted above, the "original procedure" was to give eight training trials (4 CS+ and 4 CS-) per day for four days and then test for memory on day five. Mice could also learn the odour discrimination with six training trials per day (3 CS+ and 3CS-) instead of eight, and they could show memory for this discrimination for up to 60 days. However, there was a significant decrease in time spent digging in the CS+ odour pot on the second day of discrimination tests, an indication of extinction of the conditioned odour preference. Schellinck et al. (2001a; 2001b) found that male DBA/2Ncr/BR mice given two (one CS+ and one CS-) or four (2 CS+ and 2 CS-) training trials per day successfully formed a conditioned odour preference as shown in the 24-hr memory test (Fig. 12 C & 12D). However, mice that received fewer training trials showed increased digging in the CS- odour pot in the memory test, indicating that additional training strengthens the association between the CS+ odour and reward, as well as between the CS- odour and no reward, thereby increasing discrimination.

Duration of memory. Using the original four-day training procedure, we have found short-, long- and very long-term memory in the Pavlovian conditioned odour preference test. Schellinck et al. (2001a) showed olfactory memory at 24-hr, 14, 30 and 60 days following discrimination training (Fig. 12E). Likewise, Wong & Brown (2013) found that DBA/2J mice could remember the odour discrimination for 3, 6 and 9 months, even when given other, possibly interfering, odour discrimination tests 3 and 6 months after the initial odour discrimination training. Likewise, 5xFAD mice and wild-type control (C57BL/6J x SJL/J) mice which were trained at three months of age and given a memory test 90 days later, showed no decay of memory over the 3-month retention period (O'Leary et al., 2020). Thus the "original" conditioned odour preference task can produce short-, long- and very long-term memories (Brown et al., 2009).

2.2.4. The biconditional olfactory discrimination task

Drumont-Boudreau et al. (2006) used the conditioned odour preference task to evaluate the ability of mice to learn about the separate elements of a compound odour. Mice were trained to dig for sugar

reward in odour pots with compounds of two odours (eg., AC+, or BD+) versus non-rewarded odour pots with compounds of two other odours (eg., AD-, BC-) during training. During the subsequent 24h probe trial memory test, the mice were able to identify the rewarded versus the non-rewarded odours in each compound odour stimulus (AD+ vs AC-, BC+ vs BD-, etc.) by spending more time digging in the odour pot which contained the rewarded odour pair, thus indicating that they were capable of olfactory configural learning when solving a biconditional discrimination problem.

2.2.5. Genotype, sex and age effects

Genotype. Using the original parameters described above, Schellinck et al. (2001a) showed that the Pavlovian conditioned odour preference task effectively assesses olfactory memory in male CD1, C57BL/6Ncr/BR and DBA/2Ncr/BR mice, all of which exhibited a significant preference for digging in the CS+ odour 24 hr after the final training trial (Fig. 12B). Although Gheusi et al. (2000) reported that Neural Cell Adhesion Molecule (NCAM) knockout mice did not show odour discrimination in a habituation-dishabituation test; Schellinck et al. (2004) found that these NCAM knockout mice were able to learn an olfactory discrimination in the conditioned odour preference task, as indicated by increased time spent digging in the CS+ odour pot compared to the CS- odour pot in the 24h memory test. Brown & Wong (2007) showed that mice of 13 different strains which differed in visual ability showed olfactory discrimination learning in the Pavlovian conditioned odour preference task (Fig. 13). In this study, the mice with visual impairments had poor performance in visuo-spatial learning and memory in the Morris Water Maze, but enhanced performance on the conditioned odour preference task, suggesting that the mice which had visual impairments, compensated with an enhanced olfactory discrimination ability.

Sex. Sex differences in olfactory learning and memory in the Pavlovian conditioned odour preference task are rare. Schellinck et al. (2001b) found no sex difference in CD-1 mice in the 24-hr memory test; males and females both learned the task and did not differ in time spent digging in the CS+ or CS- odour pots. Brown & Wong (2007) found no significant sex differences in the 13 strains of mice they tested; male

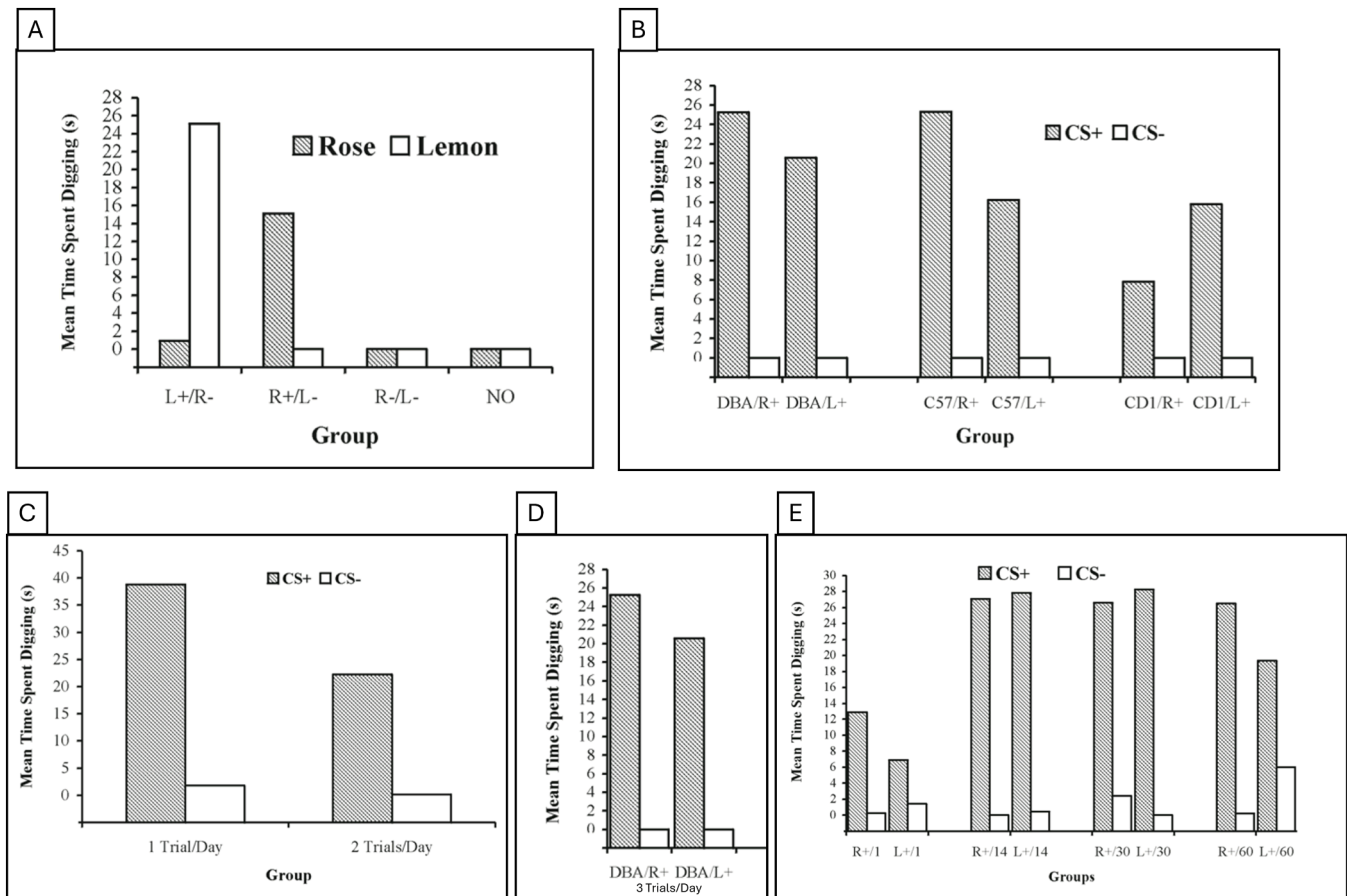


Fig. 12. Number of training trials and duration of memory.

[12A] Mean time spent digging in rewarded and non-rewarded odour pots during the 24 hr memory test by male CD-1 mice trained with lemon as the CS+ (L+/R-); rose as the CS+ (R+/L-), neither odour rewarded (R-/L-), or no odours or sugar reward during training (NO). The results show that significantly more digging was performed in the rewarded odour pots.

[12B] Mean time spent digging in the CS+ and CS- odour pots in the 24 hr memory test by male mice of three different genotypes (DBA, C57, and CD1). All mice learned to associate the odour with the reward.

[12C and D] Mean time spent digging in the CS+ and CS- odour pots in the 24 hr memory test by male DBA mice trained with one, two or three trials/day with each odour. All learned to associate the odour with the reward.

[12E] Mean time spent digging in the CS+ and CS- odour pots by male CD1 mice trained for 3 trials/day with each odour for 4 days in memory tests given 1, 14, 30 or 60 days after training. These results show that the 4 day training procedure produced short-, long- and very long-term memory.

Data from: Schellinck, H.M., Forestell, C.A., LoLordo, V.M. (2001a). A simple and reliable test of olfactory learning and memory in mice. *Chemical Senses*, 26, 663–72.

and female mice learned the task equally well. Gunn et al. (2011) used the "original" training procedure of Schellinck et al. (2001a), to assess olfactory learning and memory in male and female Coloboma mutant C3Sn.Cg-Cm/J (Cm) mice, a proposed model of attention-deficit hyperactivity disorder (ADHD). While both male and female C3H/HeSnJ (C3H) wild-type control mice showed a significant conditioned preference for the CS+ odour in the 24-hr memory test, there was a sex difference in the coloboma mice. Female Coloboma mice learned the discrimination, digging significantly more in the CS+ odour pot in the memory test, but the male Coloboma mice failed to dig more in the CS+ pot than the CS- pot, suggesting that male Coloboma mice may exhibit impairments in olfactory learning and memory in addition to the motor coordination and hyperactivity deficits observed in this mouse model. O'Leary et al. (2020) found no sex differences in olfactory memory in 5xFAD mouse models of AD or wild-type control (C57BL/6J x SJL/J) mice.

Age. Male and female C57BL/6J and DBA/2J mice were able to learn the Pavlovian conditioned odour preference task, at 6, 12, 18 and 24 months of age, and their performance was not affected by the age-related decline in visual ability of the DBA/2J mice (Wong & Brown, 2007). Wong & Brown (2013) also reported that visual ability did not

significantly predict performance in the Pavlovian conditioned odour preference task at any age, although mice with poorer vision tended to spend more time digging in the CS+ odour at 12 months of age. Likewise, O'Leary et al. (2020) found no impairment in the 24-hr olfactory memory test in male and female 5xFAD and wild-type control (C57BL/6J x SJL/J) mice when tested from 3 to 15 months of age, despite the 5xFAD mice displaying physiological symptoms of Alzheimer's disease, as seen through A β plaque deposition in the olfactory pathways in the brain.

Overall, these findings validate the **Pavlovian conditioned odour preference** task as a reliable paradigm for studying olfactory learning and memory in mice. The task proved effective across both outbred and inbred strains, and required little training time, making it a reliable and practical tool for studying associative learning.

2.2.6. The problem of individual differences

In the original studies of Schellinck et al. (2001a; 2001b) and in other studies where data from individual mice are shown (eg. Forestell et al., 2001), there were considerable individual differences in performance of mice within genotypes in the 24-hr memory test. While most mice showed more digging in the CS+ than the CS- during memory tests, the

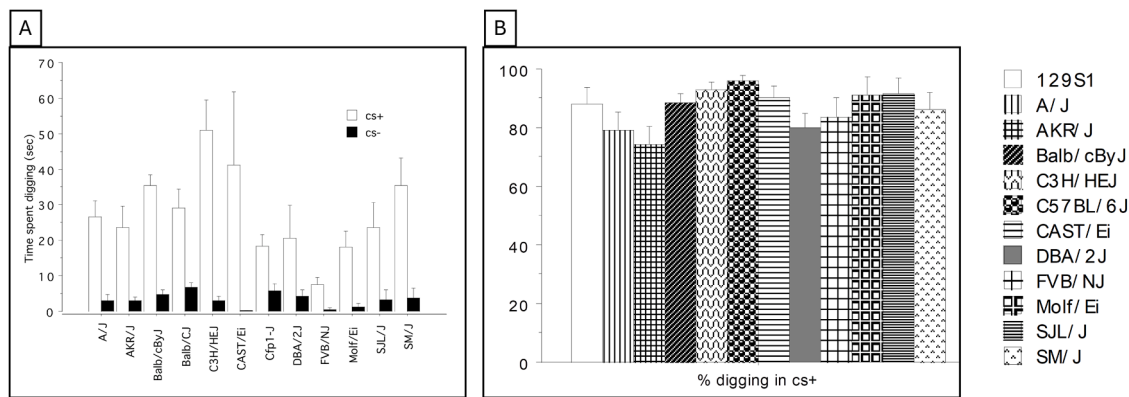


Fig. 13. Strain differences in conditioned odour preference.

All inbred strains tested in a 4-day conditioning paradigm learned to discriminate CS+ and CS- odours and remembered this discrimination for 24hr.

[13A] There were strain differences in time spent digging ($p < 0.001$) in the 24-hr memory test, however all mice dug significantly more in the CS+ odour pot than in the CS- odour pot ($p < 0.0001$).

[13B] The CS+ preference scores ($CS+ / ((CS+) + (CS-))$) for data shown in [A] show that all mice showed a preference for digging in the CS+ odour pot in the 24-hr memory test. The preference test allows all mice to be evaluated on the same scale. [data for 129S1 and C57BL/6J mice are shown in B but not A].

Data redrawn from: Brown, R. E. & Wong, A. A. (2007). The influence of visual ability on learning and memory performance in 13 strains of mice. *Learning & Memory*, 14(3), 134–44.

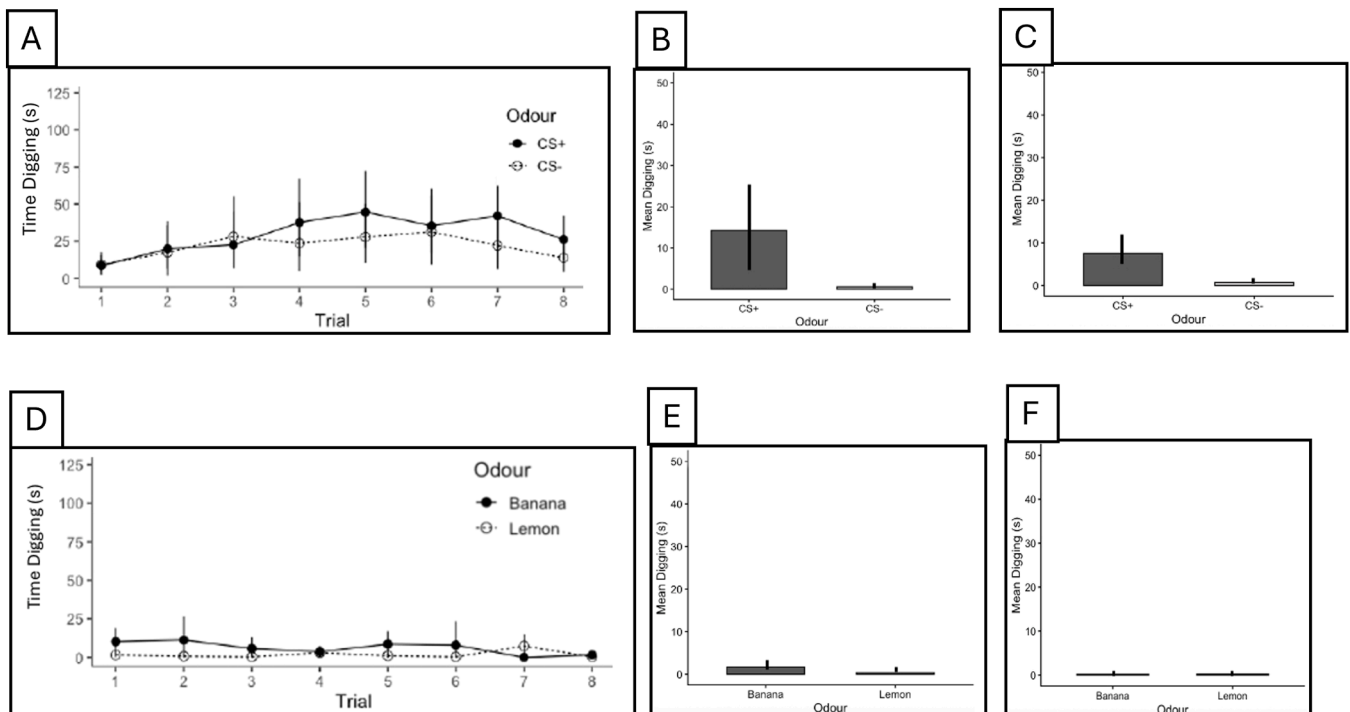


Fig. 14. Mice trained with a CS+ and a CS- odour pot learned to discriminate the two odours, but mice trained with two CS- odour pots did not.

[14A] Mean ($\pm$ 95% CI) time (sec) spent by B6SJL mice digging in the CS+ (rewarded) and CS- (non-rewarded) odour pots during each of the eight training trials. Mean CS+ preference score (time digging in CS+ / total time digging) during training trials was 0.61.

[14B] The mean ($\pm$ 95% CI) time (sec) spent digging in the CS+ odour pot in the 24-hr memory test was significantly greater ($p < 0.01$) than the time digging in the CS- odour pot for mice trained with a CS+ reward. Mean preference score in the 24-hr memory test was 0.88.

[14C] The mean ($\pm$ 95% CI) time (sec) spent digging in the CS+ odour pot in the 7-day memory test was significantly greater ($p < 0.05$) than the time digging in the CS- odour pot for mice trained with a CS+ reward. Mean preference score in the 7-day memory test was 0.85.

[14D] Mean ($\pm$ 95% CI) time (sec) spent by B6SJL mice digging in each of the two non-rewarded (CS-) odour pots (Banana and lemon odours) during each of the eight training trials.

[14E] The mean ($\pm$ 95% CI) time (sec) spent digging in the banana CS- odour pot in the 24-hr memory test did not differ from the time digging in the lemon CS- odour pot for mice trained with no CS+ reward.

[14F] The mean ($\pm$ 95% CI) time (sec) spent digging in the banana CS- odour pot in the 7-day memory test did not differ from the time digging in the lemon CS- odour pot for mice trained with no CS+ reward.

Data from Oliver Schnare (2022). Measuring memory strength in mice in an olfactory discrimination task. Unpublished thesis, Dalhousie University, April 2022.

time spent digging in the CS+ ranged from 5 to 70 sec; some mice spent more time digging in the CS- than the CS+ odour pot; and some mice did not dig in either odour pot. The source of this individual variability is a question for future investigations.

2.2.7. Development of a one-day training paradigm

When studying the neurobiology of learning and memory, it has become popular to use a one-day training paradigm, so that there is a specific, short time period in which learning can occur (Zamorina, et al., 2025). The Y-maze spontaneous alternation test (Kraeuter et al., 2019); the box maze escape task (Darvas et al., 2020); the one-trial object recognition task (Ennaceur, 2010; Antunes & Biala, 2012) and, most frequently, the cued and/or contextual fear conditioning task (Bevins & Ayres, 1995; Wehner & Radcliffe, 2004; Josselyn, 2010) have all been used to examine the neurobiological changes underlying learning, consolidation and recall of information. Based on the procedure of Schellinck et al. (2001a; 2001b) that showed that odour preferences could be conditioned with either eight, six, four, or two, training trials per odour per day (distributed practice) we developed a one-day Pavlovian conditioned odour preference task (massed practice) that could be used for neurobiological studies of olfactory learning and memory (Brown et al., 2023; 2024).

While the majority of our studies for the 1-day training procedure were done with C57BL/6J mice, we also tested male and female B6SJL, 5xFAD, *Nrxn1*^{+/−}, *Nrxn1*^{DS5−}, and *Nrxn1*^{DS5/DS5} mice from 3 to 20 months of age and the data from these genotypes will be presented in another paper (Brown et al., 2026, in progress). The results presented here are from C57BL/6J and B6SJL mice.

Motivation. Mice trained in the one-day conditioned odour preference task with a sugar reward associated with the CS+ odour and no sugar reward with the CS- odour learned the discrimination (showed significantly more digging in the CS+ odour in the 24-hr and 7-day memory tests) but mice given no sugar reward (two non-rewarded CS- odours) showed no preference for digging in either CS- odour (Fig. 14). Thus, the reward is necessary to motivate the mice to learn in the one-day conditioned odour preference procedure, as it is in the 4-day training procedure (see Fig. 12A).

Learning curves. In their analysis of the learning curves during training in the "original" 4-day training Pavlovian conditioned odour preference procedure, Forestell et al. (2001) found that male CD-1 mice showed a reduced latency to dig and an increased time spent digging in the CS+ odour pots over the 14 training trials over 4 days (distributed practice). However, in the one-day training procedure (massed practice), it is difficult to discern such learning curves. During the training

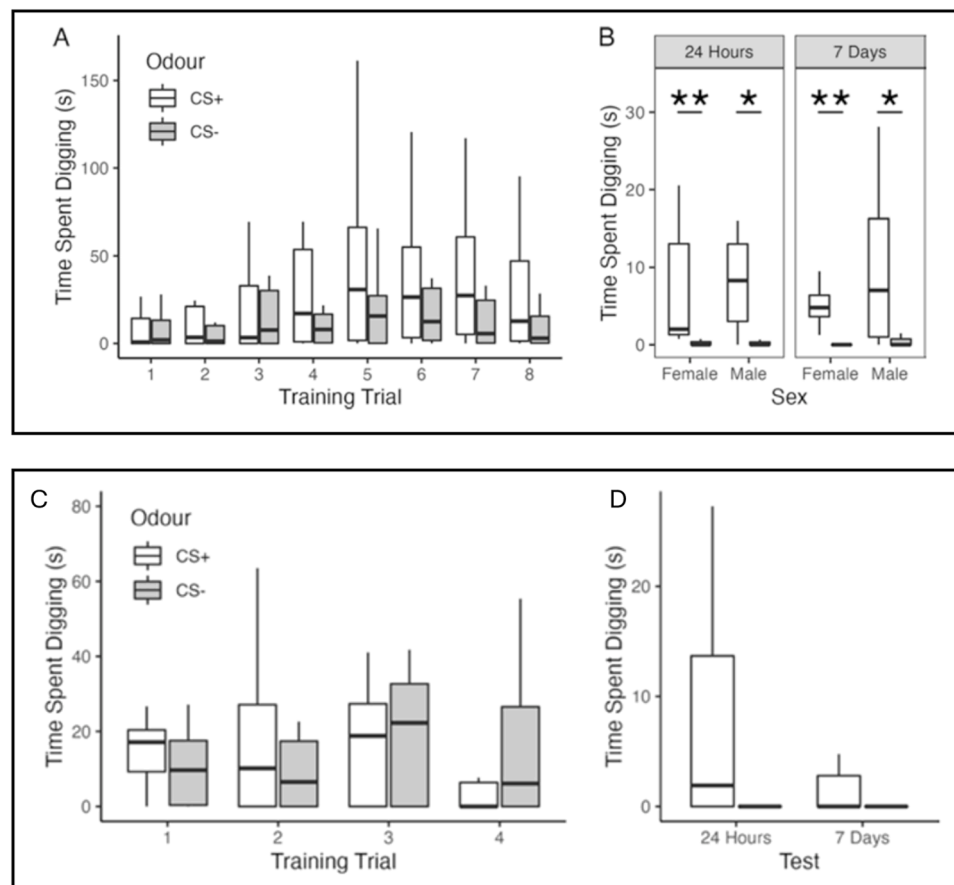


Fig. 15. Mice show 24-hr and 7-day memory with 8 CS+ and 8 CS- trials, but not 4 CS+ and 4 CS- trials in one day of training.

[15 A & B] Box and whisker plots displaying the median time (in seconds) spent digging by B6SJL mice in the CS+ and CS- odour pots in (A) the eight acquisition trials and (B) the 24-h and 7-day memory tests. The boxes represent the inter-quartile range (IQR) with the bars in the middle showing the medians, the boxes showing the 25th and 75th percentiles and the whiskers extending to the furthest points within 1.5 inter-quartile ranges. During acquisition the difference in time digging in the CS+ and CS- odour pots did not differ significantly for sex or days. In the 24-hr and 7-day memory tests there was significantly more digging in the CS+ than the CS- odour pot for both male and female mice. ** = $p < 0.01$; * = $p < 0.05$.

[15 C & D] Box and whisker plots displaying the median time (in seconds) spent digging by B6SJL mice in the CS+ and CS- odour pots in (C) the four acquisition trials and (D) the 24-h and 7-day memory tests. The difference in time digging in the CS+ and CS- odour pots did not differ significantly during the acquisition trials nor during the 24-hr or 7-day memory tests.

From: Brown RE, Schnare OK, Habib E, & Roddick K. (2023). Development of a one-day test of olfactory learning and memory in mice. In: Benoit Schaal, Diane Rekow, Matthieu Keller & Fabrice Damon (Editors) *Chemical Signals in Vertebrates 15*, pages 39-53. Cham, Switzerland, Springer Nature Switzerland.

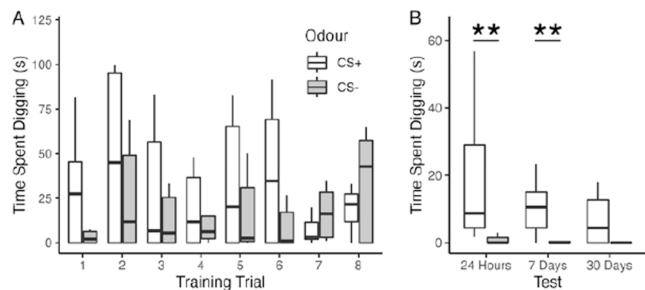


Fig. 16. Short-, long- and very-long-term memory in mice.

[16 A] The median time (in seconds) spent digging by C57BL/6J mice in the CS+ and CS- odour pots in the eight acquisition trials did not differ significantly.

[16 B] In the 24-hr and 7-day memory tests there was significantly more digging in the CS+ than the CS- odour pot, but on the 30 day memory test, the time spent digging in the CS+ and CS- odour pots did not quite reach significance ($p = 0.0625$). ** = $p < 0.01$; * = $p < 0.05$.

From: Brown RE, Schnare OK, Habib, E, & Roddick K. (2023). Development of a one-day test of olfactory learning and memory in mice. In: Benoit Schaal, Diane Rekow, Matthieu Keller & Fabrice Damon (Editors) *Chemical Signals in Vertebrates 15*, pages 39-53. Cham, Switzerland, Springer Nature Switzerland.

trials the mice dug in both the CS+ and CS- odour pots and, while some mice showed a clear preference for digging in the CS+ odour pot, other mice showed no significant difference in time spent digging in the CS+ and CS- odour pots during the training trials (see training trial data in Figs. 13–16). Thus, the only way to determine if the odour discrimination was learned is to rely on the data from the memory tasks which

indicated that the mice did learn and consolidate the conditioned odour discrimination (Brown et al., 2023).

Measuring memory strength in the one-day Pavlovian conditioned odour preference paradigm. We used three methods to examine memory strength in the one-day conditioned odour preference training paradigm: number of training trials, memory duration, and extinction and spontaneous recovery tasks.

Number of training trials. Male and female B6SJL mice given 8 CS+ and 8 CS- training trials in the one-day training procedure showed significant memory for the CS+ odour in both the 24-hr and 7-day memory tests (Fig. 15), but B6SJL mice given four CS+ and four CS- training trials in a single day did not spend significantly more time digging in the CS+ odour than in the CS- odour pot during the 24-hour and 7-day memory tests (Brown et al., 2023). This indicates that the number of training trials is important in forming the conditioned odour preference: 8 trials is sufficient to produce a memory for 7 days, but 4 trials is not.

Duration of memory. Male and female C57BL/6J mice were given eight training trials with each of the rewarded (CS+) and non-rewarded (CS-) odours during the one-day training session (Brown et al., 2023). During the training trials there was no evidence of a learning curve, but these mice showed significantly more digging in the CS+ odour pot than the CS- odour pot during 24-hr and 7-day memory tests, demonstrating retention of the conditioned odour preference (Fig. 16). There were no significant sex differences during either the 24-hr or 7-day memory tests, indicating comparable performance between sexes in memory retention. Although these mice dug more in the CS+ than the CS- odour pot during the 30-day memory test, the difference was not significant. Thus, the one-day training procedure appears to produce a significant memory for the CS+ odour that lasts up to 7 days but not 30 days.

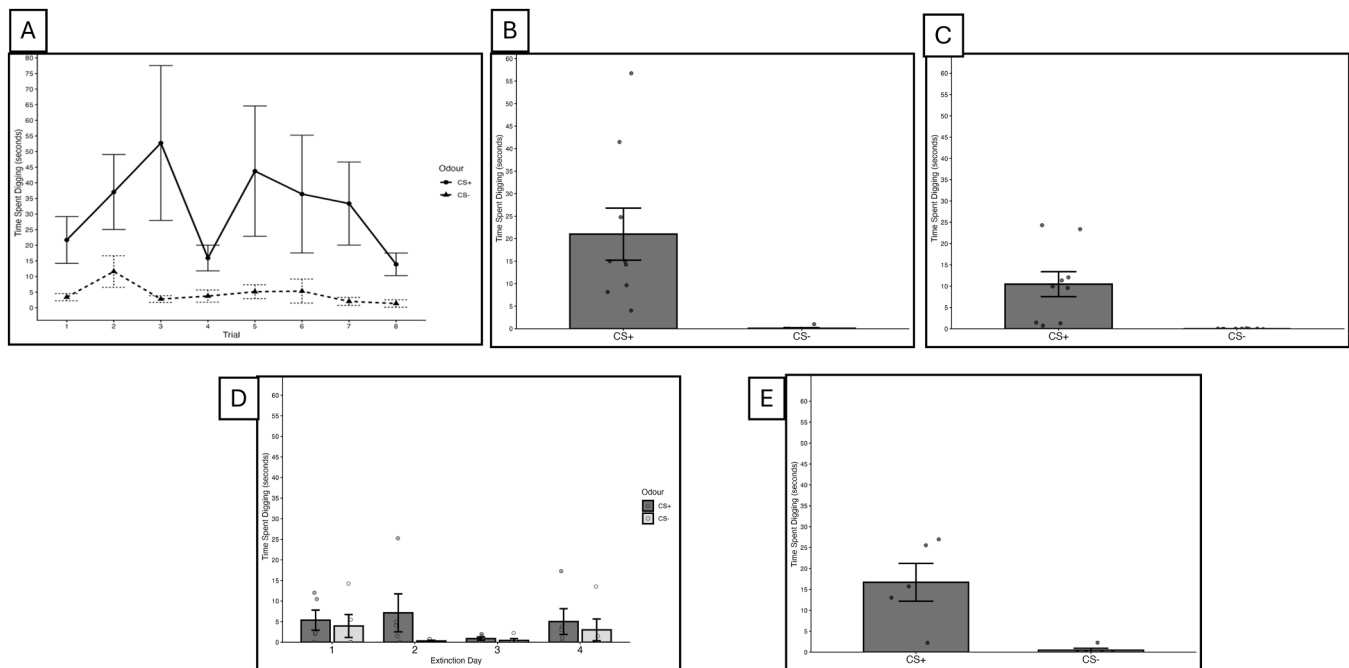


Fig. 17. Extinction and spontaneous recovery in the one-day training procedure.

[17 A] Mean (± sem) time (sec) spent digging by male and female C57BL/6J mice in the CS+ and CS- odour pots during each of the 8 CS+ and 8 CS- trials in the one-day training procedure. These mice showed significantly more time digging in the CS+ than the CS- odour pot during training ($p = 0.004$). Mean discrimination ratio ((CS+ - CS-) / (CS+ + CS-)) during training trials was 0.66.

[17 B] In the 24-hr ($p = 0.004$; Mean discrimination ratio = 0.99) and [17C] the 7-day memory test ($p = 0.004$; mean discrimination ratio = 1.00), mice dug significantly more in the CS+ odour pot than in the CS- odour pot.

[17 D] Mean (± sem) time (sec) spent digging in the CS+ and CS- odour pots during each of the four days of extinction trials after the 7-day memory test.

[17 E] In the spontaneous recovery test given 7 days after the last extinction day, the mice dug significantly more in the CS+ odour pot than in the CS- odour pot ($p = 0.025$; mean discrimination ratio = 0.94).

Data from: Habib (2020). The Optimization of the Training Parameters for the Conditioned Odour Preference Task. Unpublished manuscript, Dalhousie University, 11 December 2020.

Extinction and spontaneous recovery tasks. Male and female C57BL/6J mice trained with 8 CS+ and 8 CS- odour trials (Fig. 17A) were given a 24-hr memory test and a 7-day memory test (Fig. 17B and 17C), followed by five days of extinction (non-rewarded) trials (including the 7-day memory probe test) (Habib, 2020). During the extinction trials, mice showed low levels of digging behaviour overall, and no significant differences were observed across extinction sessions in the time spent digging in the CS+ and CS- odour pots (Fig. 17D). Seven days following the last extinction trial, the mice were assessed in a one-day spontaneous recovery test to determine if digging behaviour in the CS+ was restored. During this spontaneous recovery test, mice showed the majority of digging behaviour in the CS+ odour pot with minimum digging in the CS- odour pot (Fig. 17E). Thus, after two memory probe tests and four more days of extinction trials, mice retained the ability to selectively dig in the CS+ odour pot in the spontaneous recovery test. While the time spent digging during spontaneous recovery was relatively low, mice consistently dug in the CS+ odour and exhibited an odour discrimination. These findings show that the one-day training procedure produces a resilient odour discrimination memory.

Comparison of memory performance in the 4-day and 1-day training procedure. These findings indicated that C57BL6/J and B6SJL mice given eight CS+ and eight CS- training trials in a single session were able to form a short-term (24-hr) and a long-term (7-day), but not a very-long-term (30-day) conditioned odour preference. How do the results of the one-day training procedure (8CS+ and 8CS- trials) compare with those of the original 4-day training procedure? There are three ways to measure the conditioned odour preference: the raw scores (in seconds) for time digging in each odour pot; the difference scores (in seconds) between the time digging in the CS+ and CS- odour pots ((CS+) - (CS-)); and the preference (proportion) score for digging in the CS+ odour pot $(CS+)/((CS+) + (CS-))$. In the past we have used the preference score as a measure of memory as it is independent of the magnitude of the raw data (See Schellinck et al., 2001a; 2001b; Brown & Wong, 2007; O'Leary & Brown, 2020). Table 2 shows that each of these measures for the 24-hr memory test from two experiments in which a 4-day training procedure was used (Schellinck et al., 2001a) and two experiments in which a 1-day training procedure was used (Brown et al., 2023) were quite similar. Table 3 shows that the memory test results from the 24-hr, 7-day, 14-day, 30-day, and 60-day memory tests from the 4-day and 1-day training procedure, where available were also quite comparable. We have previously analyzed the replicability of our behavioural studies (Brown, 2024) and the data shown in Tables 2 and 3 extends this analysis; indicating that the memory results of studies using 4-day and 1-day training procedures done more than 20 years apart give remarkably similar results.

Table 2

Summary table comparing time spent digging (sec.) in the CS+ and CS- odour pots, difference scores ((CS+) - (CS-)) and preference scores (proportion of time digging in the CS+ odour pot) in the 24-hr memory test for mice that completed two experiments using the 4-day training procedure or the 1-day procedure.

	24-Hour Memory Test			
	4-Day Procedure (Schellinck et al., 2001a)		1-Day Procedure (Brown et al., 2023)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
CS+ Digging	19.73	9.87	14.28	18.74
CS- Digging	0.43	0.85	0.55	0.99
Difference Scores	19.30	9.02	13.73	17.75
Preference Scores	0.98	0.85	0.91	0.89

Note. Data from mice included in the 4-day procedure are from Tables 1 & 2 in Schellinck et al. (2001a) and data from mice included in the 1-day procedure are from Figs. 2 & 3 in Brown et al. (2023).

Table 3

Summary table comparing preference scores (proportion of time digging in the CS+ odour pot) in the 24-hr, 7-day, 14-day, 30-day, and/or 60-day memory tests for mice that completed the 4-day procedure or the 1-day procedure.

	Preference Scores	
	4-Day Procedure (Schellinck et al., 2001a)	1-Day Procedure (Brown et al., 2023)
24-Hour Test	0.85	0.89
7-Day Test	N/A	0.93
14-Day Test	0.99	N/A
30-Day Test	0.87	0.80
60-Day Test	0.91	N/A

Note. Data for mice included in the 4-day procedure are from Table 2 in Schellinck et al. (2001a) and data from mice included in the 1-day procedure are from Fig. 3 in Brown et al. (2023).

2.3. Summary and discussion

In this paper we have shown that olfactory deficits occur in many neurodegenerative and neuropsychiatric disorders and thus it is important to study olfaction in animal models of these disorders. Rodents rely on olfaction for social and sexual behaviour, food detection and predator avoidance. Rodents have a dual olfactory system with the main olfactory pathways connecting the olfactory receptors to the olfactory bulb, anterior olfactory nucleus, piriform cortex, amygdala and hippocampus. The accessory olfactory system connects the vomeronasal organs to the accessory olfactory bulb, the medial preoptic area, hypothalamus and amygdala, and is involved in the regulation of the neuroendocrine system by primer pheromones. The main and accessory olfactory systems interact at the amygdala and both systems are involved in olfactory learning and memory. Examination of learning and memory in the accessory olfactory ("primer pheromone") system involves studying neuroendocrine and reproductive system changes in response to familiar and novel social odours, while the study of learning and memory in the main olfactory system involves behavioural methods.

Rodents show a remarkable ability to learn olfactory based tasks, including odour detection, odour discrimination, working memory, reversal learning, and complex tasks such as sequential odour and transitive inference tests (Table 1). A full range of behavioural tasks used in studying olfactory learning and memory is described. Together, these highlight the importance of considering the role of olfactory learning and memory in the life of rodents, and point to the value of using rodent olfactory learning and memory processes for modelling human disorders of cognitive function.

The operant olfactometer provides a number of advantages for the study of olfactory sensitivity, odour discrimination and reversal learning, but it requires careful attention to maintenance and cleaning and extensive training of experimenters. The ability to measure sensitivity to different odours and record learning curves to odour discriminations provides a replicable quantitative measure of olfactory sensitivity and learning ability in rodent models and in human subjects. The ability to use signal detection theory to analyze response patterns in discrimination and reversal learning enables one to determine exactly where the deficits in olfactory decision-making lie: in sensitivity to odours, shaping responses, initial odour discrimination ability or in the complex pattern of responses in reversal learning. The use of olfactometers in combination with fMRI will aid in the early detection of neuropsychiatric and neurodegenerative disorders in humans and in mouse models.

The Pavlovian conditioned odour preference task developed by Schellinck et al. (2001a; 2001b) is a reliable easy-to-use test for measuring short, long and very-long term olfactory memories. Since training in the "original" procedure for this test takes four days, we developed a one-day training paradigm and showed that this also produced short- and long-term odour discrimination memory. The one-day

training procedure results in a shorter window during which learning and memory formation occur, making it a more appealing task for the molecular and cellular study of learning and memory.

The concept of multiple, interacting memory systems can be used to explain the neural pathways underlying the odour memory cell assemblies. Each cell assembly may involve one memory system (White et al., 2015) and then the phase sequence connects a set of cell assemblies such that odour information can be combined with other information for complex cognitive processes (as discussed by Sherman et al., 2024). By using rodent models for olfactory function, information may be gained about the neurobiological processes in human cognitive dysfunction in neurodegenerative and neuropsychiatric disorders (Bhatia-Dey & Heinbockel, 2021; Chen & Kostka, 2024; Dan et al., 2021; Fatuzzo, et al., 2023; Yang, et al., 2023).

Deficits in olfactory learning and memory have been shown in Tg2576, APP/PS1, 3xTg-AD and 5xFAD mouse models of AD (Roddick, et al., 2016; Zhang et al., 2022) mouse models of aging (Tzeng et al., 2021), Parkinson's disease (Johnson et al., 2020; Yuan et al., 2023) and other neurodegenerative disorders (Fatuzzo et al., 2023) as well as neurodevelopmental disorders (Lyons-Warren et al., 2021). The use of olfactometers allows for the characterization of brain structures related to olfaction and cognitive deficits in these mouse models as well as humans by identifying brain activation from the application of olfactory stimuli (Good & Sullivan, 2015; Sezille et al., 2013). Using bold functional MRI to investigate the response in the human brain to odour stimuli it is possible to examine the different brain metabolic patterns between patients with psychotic disorders to determine deficits in olfactory cue identification (Gates et al., 2001; Sommer et al., 2012). Based on the results of the complex odour learning experiments discussed above for episodic and declarative memory and for cognitive flexibility, and using the signal detection analysis, it should be possible to dissociate olfactory sensitivity and cognitive functions that involve the hippocampus, orbitofrontal cortex, piriform cortex, and cerebellum (Sommer et al., 2012; Eichenbaum, 2017). In addition, using the olfactometer should aid in determining the roles of neurotransmitters such as dopamine in olfactory learning and memory (Yuan et al., 2023) and in detecting the early onset of neurodegenerative diseases (Zhou et al., 2025). Thus, the study of olfactory learning in memory is an important aspect of the use of rodent models of human neurodevelopmental, neuropsychiatric and neurodegenerative disorders.

Ethics declaration

This study was conducted in accordance with the following guidelines for animal welfare and/or reporting: Canadian Council on Animal Care (CCAC). This study was approved by the University Committee on Laboratory Animals (UCLA), Dalhousie University.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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