Olfactory recognition memory in mice depends on task parameters

Alison Buseck*, Katherine McPherson*, Christiane Linster [$]

Computational Physiology Lab
Dept. Of Neurobiology and Behavior
Cornell University

*Contributed equally to the work
[$] to whom all correspondence should be addressed at CL243@cornell.edu

Abstract

We use a simple two trial odor recognition paradigm to test memory duration, span and specificity in adult mice. Our paradigm allows mice to encode and/or recall multiple odors in one trial and necessitates no training or food/water deprivation. We show that this paradigm can be used for encoding and/or testing of multiple odors in single trials, leading to shorter behavioral testing. Using this simple paradigm we show that mice can remember a single odor for up to 10 but no more than 15 minutes and two odors for up to 5 minutes. Mice could not remember three odors at any delays tested here. We also show that specificity for the encoded odor decreases as delay increases. Our results are important for setting baseline levels of testing for experiments in which memory parameters are expected to be modulated.

Acknowledgments. The authors thank Matthew Einhorn and technical assistance and Jocasta Lewis, William Hu, Yexuan Zhu, Eric Chen, Colette Gazonas and Naseem Dabiran for experimental assistance. This work was supported by CRCNS: NSF 1724221 to CL.

Introduction

Odor object recognition is a standard behavioral paradigm used to test olfactory memory in rodents (Petrulis et al., 2000, Petrulis et al., 2004, Bevins and Besheer, 2006, Manella et al., 2013, Manella et al., 2016, Oettl et al., 2016, Oettl and Kelsch, 2018), with a focus on social recognition in many cases (Oettl et al. 2018). An odor object recognition task most commonly consists of 2 trials: an encoding trial in which the animal is allowed to investigate an odor, and a recognition trial, in which the animal is presented with the encoded and a novel odor after a specific delay (Hackett et al., 2015). If the animal investigates the encoded odor source less vigorously during the recognition trial than during the encoding trial, and/or investigates the novel odor more vigorously than the encoded odor during the recognition trial, then this is taken as a sign that the animal remembers the encoded odor and can differentiate it from the novel odors (Bevins and Besheer, 2006). Odor recognition and the very similar habituation tasks, in which a series of encoding trials are used followed by one or more recognition trials, have been successfully used to probe mechanisms of social recognition and odor learning and contributions of various brain areas to these processes (Ferreira et al., 1999, Terrazas et al., 1999, Johnston and Peng, 2000, Petrulis et al., 2000, Petrulis et al., 2004, Petrulis et al., 2005, Bevins and Besheer, 2006, McNamara et al., 2008, Wilson and Linster, 2008, Petrulis, 2009). The similar non-olfactory object recognition task has been used to test many types of questions including noradrenergic activation of the amygdala (Roozendaal et al., 2009) and the effects of stress on memory (Cazakoff et al., 2010).

Odor recognition tasks have been implemented using a large array of paradigms and methods, ranging from presenting odors in the homecage to specialized automated platforms that measure investigation time (Youngentob et al., 2006, Mandairon et al., 2009, Manella et al., 2013). We present a new variation of the paradigm in which multiple odors can be presented during a single encoding or recognition trial, eliminating the need for multiple trials when more than one odor is being tested. We use this paradigm to test memory duration, the number of odors that can be remembered, and discrimination

between encoded and novel odors. We here show that when encoding non-social odors, mice can recall a single odor for at least 10, but not 15 minutes, and two odors for at least 5 but not 10 minutes. The ability to discriminate perceptually similar odors from an encoded odor decreases with time after encoding. The data presented here are important in that they allow investigators to choose task parameters for specific questions which will increase the like hood of positive results. For example, if a manipulation is expected to increase memory duration or memory span, choosing the appropriate delay parameters based on our results will enhance the like hood of positive results. Similarly, if a manipulation is expected to increase odor discrimination our data allows to decide on the best delay and odors to choose for an experiment. Thus, our findings, while not conceptually novel, will be important when setting baseline parameters for this task in the investigation of factors influencing learning and memory in mice.

Materials and methods

Male C57BL/6J mice, aged approximately 2 months at the beginning the of the experiment were used, 17 for Experiment 1 and 12 for Experiments 2 and 3. Mice were group housed - between 3 and 5 per cage, kept on a reversed 12hr/12hr light cycle and had free access to food and water throughout the experiment. Trials were run during the dark part of the cycle. On three consecutive days, mice were familiarized with the testing apparatus for five-minutes per day. All tasks were performed according to the standard procedures of, and approved by, the Cornell University Institutional Animal Care and Use Committee.

Experimental Setup

Experiments were run in an house made apparatus designed to use more than one odor stimulus in an odor recognition task (similar to that described in Hackett et al. (2015) for rats). Mice were run in a 60x60 cm red plastic chamber outfitted with two (Experiment 1) or six (Experiments 2&3) identical circular slots (4 cm diameter) for in house made holders (4 cm diameter and 2 cm high) for Eppendorf tubes. These were spaced at equal distances in a circle around the box in which odors were presented. Before a given trial, 60 µL of odorant was pipetted into an 0.6 mL Eppendorf tube which was then placed

into a base. The bases and apparatus were wiped down with 70% ethyl alcohol between trials. During each trial mice were allowed to freely explore the apparatus and their movements were recorded by an overhead camera suspended approximately 50 cm above the mice in such a manner as to view the whole field.

Behavioral paradigm

For each trial the odors were placed in the bases and arranged in the box according to a pre-determined, randomized order. The mouse was then placed in the box for a 2 minute (Experiment 1) or 5 minute (Experiments 2&3) encoding trial after which it was removed for the pre-determined delay. During the delay, the odors were replaced with new odors, the mice were placed in a separate clear cage (Experiment 1) or in their homecage (Experiments 2&3) and the apparatus was wiped down with 70% ethyl alcohol. Fresh tubes and bases were used to ensure no cross-contamination of odors. After the allotted delay, the mouse was placed back in the box in the presence of new odor tubes for the 2 minute (Experiment 1) or 5 minute (Experiment 2) recognition trial. At the end of the recognition trial, the mouse was placed back in its cage and the box and bases were cleaned with 70% ethyl alcohol and allowed to dry. Mice were tested once a day on a given condition. In each case, the order of conditions was pseudo-randomized using a in house program to create a different order for each.

In this paradigm, the time spent investigating the odors in each trial is the dependent variable. Memory for the encoded odor is indicated when mice investigate the encoded odor significantly less during the recognition trial than during the encoding trial. If the novel odor is perceptually very dissimilar from the encoded odor, memory for the encoded odor is also indicated by significantly longer investigation of the novel odor than the encoded odor during the recognition trial. Discrimination between the encoded odor and the novel odor is assessed by significantly longer investigation of the novel odor during the recognition trial. In the case mice cannot discriminate novel and encoded odor, they will investigate both equally.

Odors

A total of 12 odors (Table 1) were used for Experiments 1&2. Odors were randomly allocated to each delay/mouse combination for Experiment 1 and to each delay/number of odors/mouse combination for Experiment 2. An odor set consisting of an encoded odor (C), and a series of 4 related odors with decreasing degrees of similarity to the encoded odor (C+1, C+2, C+3, C+4; (Cleland et al., 2002, Mandairon et al., 2006, Cleland et al., 2009)) were used for Experiment 3 (Table 1). All odors were diluted to obtain approximate vapor partial pressures of 1 Pa (Cleland et al., 2002).

Experiment 1: Memory duration. During the encoding trial, one odor vial with the encoded odor (E) and one vial containing only the carrier (mineral oil, MO) were presented to the mice (Figure 1A). After a variable delay of 2, 5, 10 or 15 minutes, mice underwent a recognition trial during which they were presented with the encoded odor (E) and a novel odor (N). Odor pairs were randomly chosen from odors in Table 1 and the order of delays was pseudo-randomized among mice. On each day and only once a day a mouse was run on a different delay with a different odor pair. This experiment was run twice, with 7 mice run at the 5, 10 and 15 minute delays the first round, and 10 mice run at the 2, 5, 10 and 15 minute delays the second round and the data combined for analysis. Memory for the encoded odor at each delay was assessed by statistically comparing the response to the encoded odor during the first trial (E1) and the second trial (E2) and the response to the novel odor (N) and encoded odor (E2) during the recognition trial.

Experiment 2: Memory span. During the encoding trial, mice were presented with 1, 2 or 3 odorants and 5, 4 or 3 vials of MO such that all six odor slots were filled (Figure 2A). After a variable delay of 2, 5 or 10 minutes mice were presented with the same encoded odorants plus one novel odor and the remaining slots were filled with vials of MO. Each day and only once a day a mouse was tested with a different combination of delay and number of familiar odors, with odors randomly chosen (Table 1) and order of delay and number of encoded odors varied and counterbalanced. Memory for the encoded odors was assessed by statistically comparing the response to the encoded odors during the first and second trial

as well as by comparing the investigation of the encoded odors to the novel odor in trial 2. In case of multiple encoded odors the average investigation time to these odors was used for analysis.

Experiment 3**:** Memory specificity. During the encoding trial, mice were presented with one encoded odor C, and five mineral oil vials such as to fill all the available odor slots (Figure 3A). After a variable delay of 2, 5, or 10 minutes, mice were presented with the encoded odor C, four novel odors differing by 1, 2, 3 or 4 carbons from C (C+1, C+2, C+3, C+4; Table 2) and one vial of MO. The order of delay mice were tested at was varied and counterbalanced among mice. Memory for the encoded odor C was assessed by comparing the response to C during trials 1 and 2. Discrimination between the encoded odor C and the novel odors was assessed by comparing the responses to odor C and the novel odors during the recognition trial: if C is remembered and a novel odor is investigated significantly more than C, it is assumed that mice discriminate the novel odor from C.

Data analysis. The time mice spent investigating each odor vial was determined offline. Each video was watched and scored by an investigator blinded to the identity of the odors using an in-house software. Mice were considered to investigate an odor vial when they were actively sniffing at a distance closer than 1 cm from the odor vial (Cleland et al., 2002, Hackett et al., 2015). The time spent sniffing was used as the dependent variable for statistical analysis, with odor as within subjects factor and delay and number of encoded odorants as between conditions variable. For trials involving multiple encoded odors, time spent investigating these odors for each trial was averaged for comparison to novel odor investigation. Outlier trials more than 2 standard deviations from the mean were excluded from the analysis. For each experiment a repeated measures analysis was followed by pairwise comparisons to test in which conditions mice investigated the encoded odor less during trial 2 than trial 1 and the novel odor more than the encoded odor during trial 2. To directly compare memory and discrimination across delay conditions, the memory index was calculated from the responses to the encoded odor during trials 1&2 $((R_{trial1}-R_{trial2})/(R_{trial1}+R_{trial2})$ and the discrimination index was calculated from the responses to the novel and encoded odors in trial 2 $((R_{novel}-R_{encoded})/(R_{novel}+R_{encoded}))$, These indices vary between 1 (good

memory or discrimination) and -1 (bad memory or discrimination) and were correlated with the delay time using Pearson's R.

Results

We tested how long mice remember a single odor without reinforcement (Experiment 1), how many odors can be remembered using this paradigm (Experiment 2) and how specific the memory is for the encoded odor (Experiment 3).

Experiment 1. Olfactory recognition memory duration

Mice were first allowed to investigate an encoded odor (E1) and MO for 2 minutes. After a variable delay (2, 5, 10 or 15 minutes) they were allowed to investigate the same encoded odor (E2) and a novel odor (N) for 2 minutes (Figure 1A).

Mice investigated the encoded odor significantly less during the recognition trial after a 2, 5 or 10 but not 15 minute delay (Figure 1B). Similarly, they investigated the novel odor (N) significantly more than the encoded odor (E2) during the recognition trial after 2, 5 and 10 minutes (Figure 1B). Repeated measures analysis using investigation times as the dependent variable, delay as between conditions factor and odor (E1, E2 and N) as within subjects factor showed a significant effect of odor ($F(2, 44) = 32.431$; $p < 0.001$) and a significant interaction between odor and delay ($F(6, 88) = 2.368$; $p = 0.036$ with Wilk's Lambda) showing that mice investigated odors differentially depending on delay between encoding and recall. Posthoc pairwise comparisons (Wilks' Lambda) showed a significant decay in investigation time between the encoded odor during encoding and recognition at the three shorter delays tested (2 minutes: $p = 0.012$; 5 minutes: $p = 0.010$; 10 minutes: $p = 0.038$) but not the longest delay (15 minutes : $p = 0.669$). Comparison between the investigation times in response to the encoded and novel odor during the recognition trial showed a significant difference after the 2 minute ($p = 0.001$), 5 minute ($p=0.001$) and 10 minute ($p = 0.003$), but not the 15 minute delay ($p=0.139$).

These results show that mice can remember an odor for at least 10 but not 15 minutes after a single 2 minute encoding trial in this paradigm (Figure 1B). Both the memory index measuring the degree

of memory for the encoded odor and the discrimination index were strongly inversely correlated with delay (R_{memory} = -0.337; $p < 0.01$ and $R_{discrimination}$ = -0.369; $p < 0.01$; Figure 1C) showing that memory for the encoded odor decreases as delay between encoding and recognition increases.

Experiment 2: Number of encoded odors that can be recalled

Mice were first allowed to investigate one, two or three encoded odors (averaged to E1) and MO for 5 minutes. After a variable delay (2, 5 or 10 minutes) they were allowed to investigate the same encoded odors (averaged to E2) and a novel odor (N) for 5 minutes (Figure 2A). The 15 minute delay was omitted here because Experiment 1 showed that in our paradigm one encoded odor cannot be recalled after a 15 minute delay.

Mice remembered 1 encoded odor for 2, 5 and 10 minutes, 2 encoded odors for 2 and 5 minutes and did not remember 3 encoded odors for any delay tested (Figure 2B). Repeated measures analysis with investigation time as dependent variable, delay (2, 5 or 10 minutes) and number of encoded odors (1, 2 or 3) as between condition factors and odor (E1, E2 or N) as within subjects factor showed a significant effect of odor ($F(2, 84) = 14.387$; $p < 0.001$), and a significant interaction between odor and number of encoded odors ($F(4, 168) = 4.586$; $p = 0.002$ using Wilk's Lambda). Significant differences between the investigation of the encoded odors during encoding and recognition trials were found at the 2 minute delay with 1 and 2 encoded odors ($p = 0.015$ and $p = 0.036$), at the 5 minute delay with 1 and 2 odors ($p = 0.02$ and $p = 0.023$) and at the 10 minute delay with 1 encoded odor only ($p = 0.04$). Results were similar when responses to the encoded and novel odors during recognition were compared (ITI=2 minutes, 1 odor : $p = 0.05$; 2 odors : $p = 0.045$; 3 odors $p = 0.962$; ITI=5 minutes, 1 odor $p = 0.012$l 2 odors $p = 0.028$, 3 odors $p = 0.608$; ITI=10 minutes , 1 odor $p = 0.001$, 2 odors $p = 0.634$ and 3 odors $p = 0.762$). These results show that mice can remember one odor for up to 10 minutes (as shown in Experiment 1), and two odors for up to 5 but not 10 minutes and cannot remember 3 odors at the shortest delay tested here (2 minutes). For a given delay, memory and discrimination indices decrease with number of odors encoded,

as could be expected, with a significant negative correlation between the number of encoded odors and memory index at the 2 minute delay ($R=-0.238$; $p < 0.05$).

Experiment 3. Specificity of odor memory

Mice were first allowed to investigate an encoded odor in the presence of 5 blank odor sources. After a variable delay (2, 5 or 10 minutes), mice were allowed to investigate the encoded odor in the presence of 4 novel odors and one blank odor source (Figure 3A). The novel odors were chosen to present a range of perceptual similarities with the encoded odor by increasing differences in carbon chain length (C+1, C+2, C+3, C+4; Linster and Hasselmo, 1999, Cleland et al., 2002). Each trial lasted 5 minutes and the order of delays was varied among mice and counterbalanced. Each mouse was tested once on an experimental day.

Mice remembered the encoded odor at all delays tested, as evidenced by lower investigation of the encoded odor C during recognition as compared to encoding. Discrimination of a novel test odor in that case is evidenced by mice investigating a novel odor significantly more then the encoded odor during the recognition trial. Mice showed decreased ability to discriminate the novel odors from the encoded odor as delay increased (Figure 3B), with all novel odors discriminated after a 2 minute delay, the two least similar odors (C+3, C+4) discriminated after a 5 minute delay and only the least similar odor (C+4) discriminated after 10 minutes. Repeated measures analysis with investigation time as dependent variable, delay as between conditions factor and odor (C_{trial1}, C_{trial2}, C+1, C+2, C+3, C+4) as within subjects factor showed a strong effect of odor ($F(5, 21) = 5.536$; $p < 0.001$) but no interaction with delay ($F(10, 44) = 1.542$; $p = 0.163$). Pairwise comparisons showed that mice remembered the encoded odor at 2, 5 and 10 minute delays (comparison between investigation of odor C during encoding and recognition; $p = 0.037$, $p = 0.014$ and $p = 0.030$ with Wilk's Lambda), as expected from Experiments 1 and 2. Mice discriminated the encoded odor from novel odors less well as delay increased, with all odors differentiated from the encoded odor after 2 minutes (C+1: $p = 0.029$, C+2: $p = 0.032$, C+3: $p = 0.044$ and C+4: $p = 0.019$), the two least similar after 5 minutes (C+3: $p = 0.018$ and C+4: $p = 0.037$) and only the most different

differentiated after 10 minutes (C+4: $p = 0.045$). In other words, as the memory for the encoded odor fades, its specificity also fades; this is put into evidence by a significant correlation between memory index and discrimination indices ($R=0.287$; $p < 0.01$; Figure 3C). Discrimination indices also decrease with delay ($R=-0.208$; $p < 0.05$; Figure 3D) as expected.

Discussion

We present a behavioral paradigm to test mouse odor memory in two short trials. Our paradigm allows for the testing of multiple odors per trial and hence avoids testing over multiple days and or trials in the way previous paradigms did (Linster and Hasselmo, 1999, Cleland et al., 2002, Cleland et al., 2009, Escanilla et al., 2010, Freedman et al., 2013). We show that after a single encoding trial, mice can remember one odor for more than 10 but no more than 15 minutes (Figure 1), and two odors up to 5 but not 10 minutes (Figure 2). In our paradigm, mice were not able to remember more than two encoded odors after any delay. Specificity of the memory for the encoded odor decreased over time and was correlated with the degree of memory for the encoded odor (Figure 3). Our results are in agreement with data from previous experiments using a multiple encoding trial paradigm (habituation paradigm) and show similar effects of delay time on memory duration and memory specificity (McNamara et al., 2008, Dillon et al., 2013, Freedman et al., 2013). In previous experiments we showed that when using 4 encoding trials separated by 5 minutes, mice could remember an odor up to 30 but not 60 minutes (McNamara et al., 2008, Dillon et al., 2013, Freedman et al., 2013). Reducing estrogen levels in the olfactory bulb decreased this duration to under 30 minutes (Dillon et al., 2013). As in the present results, memory specificity was reduced when the delay between the last encoding trial and the recognition trial was increased (Freedman et al., 2013). We have proposed the same paradigm for rats, showing that rats can remember a single odor for up to 50 minutes, and up to 3 odors at shorter delays (Hackett et al., 2015). Differences in memory between mice and rats could be attributed to numerous physiological differences, life span and ecology. In this paradigm both memory duration and specificity could be

manipulated by external factors such as noradrenaline release, social isolation or food deprivation (Manella et al., 2013, Manella et al., 2016).

Odor object recognition is a commonly used behavioral paradigm in social interaction research in mice, rats and hamsters, in fact this paradigm was pioneered by Petrulis and Johnson (1997) to study scent marking in hamsters. We here show that this paradigm is a fast, easy to implement tool for olfactory research in general and systematically investigated the parameters of this paradigm, Knowing these parameters is important when designing experiments, for example, if we expected a manipulation to increase memory duration, we would want to test at a time point that allows for this observation (15 minutes), but if we expected a manipulation to reduce memory duration we would test at 10 minutes for example. Most importantly, the present paradigm does not require training nor food deprivation, can be conducted in a simple non automated arena and allows researchers to test multiple odors in a single trial making it a useful methods for further exploration of neural mechanisms governing olfactory memory.

Bevins RA, Besheer J (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc 1:1306-1311.

Cazakoff BN, Johnson KJ, Howland JG (2010) Converging effects of acute stress on spatial and recognition memory in rodents: a review of recent behavioural and pharmacological findings. Prog Neuropsychopharmacol Biol Psychiatry 34:733-741.

Chaudhury D, Manella L, Arellanos A, Escanilla O, Cleland TA, Linster C (2010) Olfactory bulb habituation to odor stimuli. Behav Neurosci 124:490-499.

Cleland TA, Morse A, Yue EL, Linster C (2002) Behavioral models of odor similarity. Behav Neurosci 116:222-231.

Cleland TA, Narla VA, Boudadi K (2009) Multiple learning parameters differentially regulate olfactory generalization. Behav Neurosci 123:26-35.

Dillon TS, Fox LC, Han C, Linster C (2013) 17beta-estradiol enhances memory duration in the main olfactory bulb in CD-1 mice. Behav Neurosci 127:923-931.

Escanilla O, Arrellanos A, Karnow A, Ennis M, Linster C (2010) Noradrenergic modulation of behavioral odor detection and discrimination thresholds in the olfactory bulb. Eur J Neurosci 32:458-468.

Escanilla O, Yuhas C, Marzan D, Linster C (2009) Dopaminergic modulation of olfactory bulb processing affects odor discrimination learning in rats. Behav Neurosci 123:828-833.

Ferreira G, Gervais R, Durkin TP, Levy F (1999) Postacquisition scopolamine treatments reveal the time course for the formation of lamb odor recognition memory in parturient ewes. Behav Neurosci 113:136-142.

Freedman KG, Radhakrishna S, Escanilla O, Linster C (2013) Duration and specificity of olfactory nonassociative memory. Chem Senses 38:369-375.

Hackett C, Choi C, O'Brien B, Shin P, Linster C (2015) Odor Memory and Discrimination Covary as a Function of Delay between Encoding and Recall in Rats. Chem Senses 40:315-323.

Johnston RE, Peng M (2000) The vomeronasal organ is involved in discrimination of individual odors by males but not by females in golden hamsters. Physiol Behav 70:537-549.

Linster C, Hasselmo ME (1999) Behavioral responses to aliphatic aldehydes can be predicted from known electrophysiological responses of mitral cells in the olfactory bulb. Physiol Behav 66:497-502.

Mandairon N, Ferretti CJ, Stack CM, Rubin DB, Cleland TA, Linster C (2006) Cholinergic modulation in the olfactory bulb influences spontaneous olfactory discrimination in adult rats. Eur J Neurosci 24:3234-3244.

Mandairon N, Peace S, Karnow A, Kim J, Ennis M, Linster C (2008) Noradrenergic modulation in the olfactory bulb influences spontaneous and reward-motivated discrimination, but not the formation of habituation memory. Eur J Neurosci 27:1210-1219.

Mandairon N, Sultan S, Rey N, Kermen F, Moreno M, Busto G, Farget V, Messaoudi B, Thevenet M, Didier A (2009) A computer-assisted odorized hole-board for testing olfactory perception in mice. J Neurosci Methods 180:296-303.

Manella L, Woldeyohannes L, McMahon D, Linster C (2016) Effects of experimentally necessary changes in husbandry on olfactory memory: Chronic food restriction and social isolation. Physiol Behav 155:38-45.

Manella LC, Alperin S, Linster C (2013) Stressors impair odor recognition memory via an olfactory bulb-dependent noradrenergic mechanism. Front Integr Neurosci 7:97.

McNamara AM, Magidson PD, Linster C, Wilson DA, Cleland TA (2008) Distinct neural mechanisms mediate olfactory memory formation at different timescales. Learn Mem 15:117-125.

Oettl LL, Kelsch W (2018) Oxytocin and Olfaction. Curr Top Behav Neurosci 35:55-75.

Oettl LL, Ravi N, Schneider M, Scheller MF, Schneider P, Mitre M, da Silva Gouveia M, Froemke RC, Chao MV, Young WS, Meyer-Lindenberg A, Grinevich V, Shusterman R, Kelsch W (2016) Oxytocin Enhances Social Recognition by Modulating Cortical Control of Early Olfactory Processing. Neuron 90:609-621.

Petrulis A (2009) Neural mechanisms of individual and sexual recognition in Syrian hamsters (Mesocricetus auratus). Behav Brain Res 200:260-267.

Petrulis A, Alvarez P, Eichenbaum H (2005) Neural correlates of social odor recognition and the representation of individual distinctive social odors within entorhinal cortex and ventral subiculum. Neuroscience 130:259-274.

Petrulis A, Peng M, Johnston RE (2000) The role of the hippocampal system in social odor discrimination and scent-marking in female golden hamsters (Mesocricetus auratus). Behav Neurosci 114:184-195.

Petrulis A, Weidner M, Johnston RE (2004) Recognition of competitors by male golden hamsters. Physiol Behav 81:629-638.

Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. Nat Rev Neurosci 10:423-433.

Terrazas A, Ferreira G, Levy F, Nowak R, Serafin N, Orgeur P, Soto R, Poindron P (1999) Do ewes recognize their lambs within the first day postpartum without the help of olfactory cues? Behav Processes 47:19-29.

Wilson DA, Linster C (2008) Neurobiology of a simple memory. J Neurophysiol 100:2-7.

Youngentob SL, Johnson BA, Leon M, Sheehe PR, Kent PF (2006) Predicting odorant quality perceptions from multidimensional scaling of olfactory bulb glomerular activity patterns. Behav Neurosci 120:1337-1345.

Figure 1. Memory duration. **A**. Schematic depiction of experimental paradigm. During the encoding trial, mice we put in the arena for 2 minutes and allowed to investigate an odorized and a vial with mineral oil only (blank). After a delay mice were re-introduced in the arena with two odorized vials, the encoded odor or a novel odor for two minutes. **B**. The graph shows average investigation times of the encoded odor (E1) during the encoding trial as well as average investigation times of the encoded odor (E2) and a novel odor (Novel) during the recognition trial as a function of delay between encoding and recognition. $^{\$}$ denotes a significant decrease between investigation of E1 and E2 and * denotes a significant increase in investigation between the N and E2 during recognition. **C**. The graphs show individual memory (Ci) and discrimination indices (Cii) as a function of delay time between encoding and l trials. Dotted lines are regression lines for each index.

Figure 2. Memory span. **A**. Schematic depiction of the experimental paradigm for memory span. During the encoding trial, mice are presented with 1, 2 or 3 encoded odors in the presence of 5, 4 or 3 blank vials (to keep total number of vials constant). After a delay, mice are put back into the arena with the same encoded odors plus a novel odor and enough blanks to have 6 total vials. **B**. The graphs show average investigation times in response to the encoded odors (average of all E1 investigation times) during encoding as well as average investigation of encoded odors (average of all E2 investigation times) and the novel odor (N) during the recognition trial as a function of delay (B_i: 1 encoded odor, B_{ii}: 2 encoded odors, E_{iii}: 3 encoded odors). $^{\$}$ denote a significant decrease between E1 and E2 and * denote significantly higher investigation of the novel odor as compared to encoded odors during the recognition trial.

Figure 3 Odor memory specificity. **A**. Schematic depiction of experimental paradigm. Mice are put into the arena in the presence of one encoded odor and 5 blank vials for 2 minutes. After a delay, mice are reintroduced to the arena in the presence of the encoded odor, 4 novel odors and a blank vial. **B**. The graph shows average investigation times in response to the encoded odor (C during the encoding trial and in response to the encoded odor (C and 4 novel odors (C+1-C+4) during the recognition trial. $ depicts a significant decrease between the response to C during encoding and recognition trials and * depicts novel odors with significantly higher investigation times than the encoded odor during recognition. **C**. The graph shows individual discrimination indices as a function of memory indices (all data points for all delays). Note that these two vary together; the dotted line shows the regression line. **D**. The graph shows individual discrimination indices as a function of delay between encoding and recognition. The dotted line shows the regression line.