Report

Current Biology

Working Memory Systems in the Rat

Highlights

- Olfactory memory is resistant to interference from adding a spatial memory load
- Spatial memory is resistant to interference from adding an olfactory memory load
- Olfactory and spatial memory draw on independent working memory systems in the rat
- Independence of working memory systems is evolutionarily quite old

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In Brief

Bratch et al. show that olfactory and spatial memory in rats are resistant to interference from the addition of a memory load in the other domain. These studies suggest that rats process information with multiple, independent working memory systems for olfactory and spatial information.

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Report

Working Memory Systems in the Rat

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SUMMARY

A fundamental feature of memory in humans is the ability to simultaneously work with multiple types of information using independent memory systems. Working memory is conceptualized as two independent memory systems under executive control [1, 2]. Although there is a long history of using the term "working memory" to describe short-term memory in animals, it is not known whether multiple, independent memory systems exist in nonhumans. Here, we used two established short-term memory approaches to test the hypothesis that spatial and olfactory memory operate as independent working memory resources in the rat. In the olfactory memory task, rats chose a novel odor from a gradually incrementing set of old odors [3]. In the spatial memory task, rats searched for a depleting food source at multiple locations [4]. We presented rats with information to hold in memory in one domain (e.g., olfactory) while adding a memory load in the other domain (e.g., spatial). Control conditions equated the retention interval delay without adding a second memory load. In a further experiment, we used proactive interference [5–7] in the spatial domain to compromise spatial memory and evaluated the impact of adding an olfactory memory load. Olfactory and spatial memory are resistant to interference from the addition of a memory load in the other domain. Our data suggest that olfactory and spatial memory draw on independent working memory systems in the rat.

RESULTS AND DISCUSSION

The essential feature of working memory in humans involves the ability to work with information in one domain while maintaining a memory load in another domain, without performance suffering from between-domain interference. Lack of between domain interference in these so-called dual-task paradigms provides evidence for the existence of independent working memory subsystems, which is a fundamental attribute of human cognition. In an everyday example, one often suffers from overloaded memory in a single domain (e.g., too many digits are hard to remember). However, we are able to remember plentiful amounts of information if they come from two domains (e.g., watching a video with images and audio). The theoretical explanation for this remarkable ability in humans is the existence of dedicated working memory systems for two domains. In many experiments, tasks that place information in the visuospatial sketchpad (for manipulating visual images) do not interfere with tasks that tap the phonological loop (for storing speech-based information) [1, 2]. Although there is a long history of using the term "working memory" in animal research [8, 9], working memory has been used in the animal literature in a way that is quite different from the human conceptualization of working memory. In the animal literature, working memory refers to memory for information that changes in status during the completion of a test [10]; thus, working memory in the animal literature is not differentiated from basic short- or long-term memory.

We exploited the well-established proficiency of rats with olfactory and spatial information to test the hypothesis that rats have independent working memory systems for olfactory and spatial information. If rats rely on a single memory resource, then adding information from one domain (e.g., olfactory) to information in the other domain (e.g., spatial) would be expected to produce impaired performance. However, if rats have multiple, independent working memory resources, then the performance of rats would be expected to be resistant to interference when information is added to both domains. To provide a memory load in the olfactory domain, we gave the rats initial training in the odor task (see the Supplemental Experimental Procedures for a description of preliminary training). After the first odor was presented in daily sessions, the rats were presented with pairs of odors; one odor in the pair was novel (not yet presented on that day), whereas the other odor had already been presented earlier in the day. Selection of the novel odor was rewarded with a small piece of food (i.e., selection of an old odor was considered an error). Thus, solving this task requires memory of recently presented odors. To provide a memory load in the spatial domain, we also trained the same rats (on other days) in a spatial task using the eight-arm radial maze (see the Supplemental Experimental Procedures). Each arm was baited with a small piece of food once per day. When the rat visited an arm, it consumed the food; thus, a revisit to a food-depleted location is considered an error. In the study (encoding) phase, the rat chose from four open doors (randomly selected), thereby depleting these arms of food (i.e., closed doors prevented it from entering the remaining four arms). At the end of a retention interval, all eight doors opened, and the rat searched for the last four baited locations (test phase; memory assessment). Thus, solving this task requires memory of spatial locations.

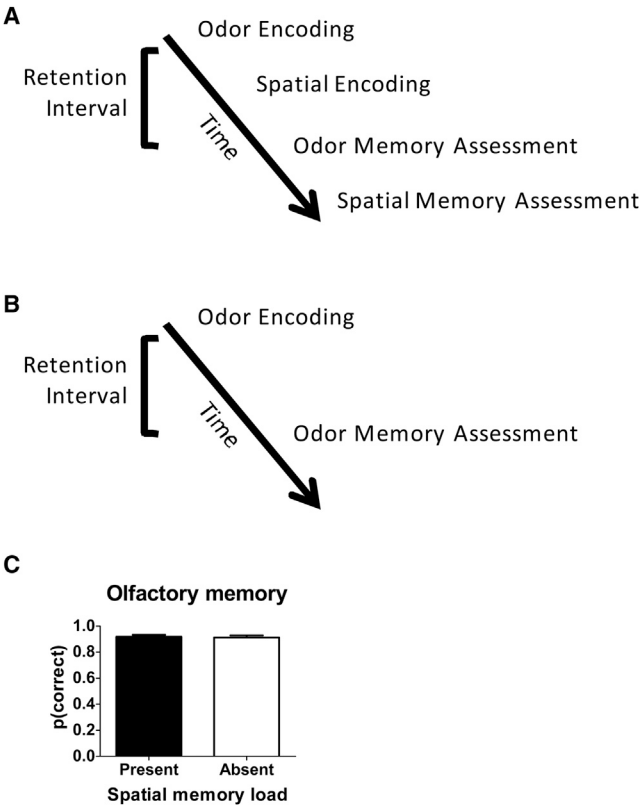

Figure 1. Olfactory Memory Is Resistant to Interference from the Addition of a Spatial Memory Load

(A and B) Schematic of timeline illustrating experimental design. Olfactory memory is assessed in the presence (A) or absence (B) of an added spatial memory load.

(C) Adding a spatial memory load does not impair olfactory memory, as expected with multiple, independent memory systems. Data are shown as mean with 1 SEM.

See also Figure S1 and Table S2.

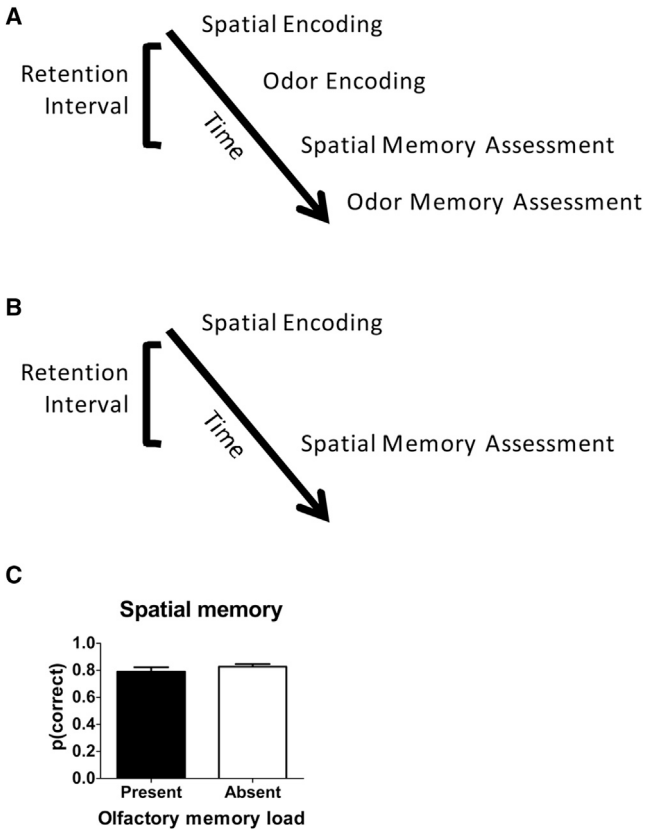

Figure 2. Spatial Memory Is Resistant to Interference from the Addition of an Olfactory Memory Load

(A and B) Schematic of timeline illustrating experimental design. Spatial memory is assessed in the presence (A) or absence (B) of an added olfactory memory load.

(C) Adding an olfactory memory load does not impair spatial memory, as expected with multiple, independent memory systems. Data are shown as mean with 1 SEM.

See also Tables S1 and S2.

To arrange conditions in which a memory load was imposed in both olfactory and spatial memory, we interleaved odor and spatial tasks. In experiment 1 (Figure 1), we began with encoding of olfactory information to evaluate the impact of adding a spatial memory load. When a spatial memory load is present, the sequence of events (Figure 1A) is: olfactory encoding, spatial encoding, olfactory memory assessment, spatial memory assessment. On other days (randomly selected), we used only the olfactory memory task (shown in Figure 1B), without the addition of a spatial memory load. When a spatial memory load is absent, the sequence of events is: olfactory encoding, olfactory memory assessment. Importantly, the delay between olfactory encoding and memory assessment was equated by extending the delay in this condition to match the time taken on other days to complete the spatial encoding. To evaluate the impact of adding a spatial memory load to a pre-existing olfactory memory load, we compared performance on the olfactory memory assessment in the presence and absence of the spatial memory load (Figure 1C). When a spatial memory load was present, olfactory memory was high, similar to the high performance observed when the spatial memory load was absent ($t(10) = 0.73$, $p = 0.48$). Moreover, olfactory memory was above chance when spatial memory was present ($t(10) = 27.4$, $p < 0.001$) and absent ($t(10) = 26.4$, $p < 0.001$). Resistance to interference is consistent with the hypothesis that adding a spatial memory load does not impair olfactory memory, as expected if rats process information with multiple, independent memory systems.

In experiment 2 (Figure 2), we began with the encoding of spatial information to evaluate the impact of adding an olfactory memory load. When an olfactory memory load is present, the sequence of events (Figure 2A) is: spatial encoding, olfactory encoding, spatial memory assessment, olfactory memory assessment. On other days (randomly selected), we used only the spatial memory task (shown in Figure 2B), without the addition of an olfactory memory load. When an olfactory memory load is absent, the sequence of events is: spatial encoding, spatial memory assessment. Importantly, the delay between spatial encoding and memory assessment was equated by extending the delay in this condition to match the time taken on other days to complete the olfactory encoding. To evaluate the impact of adding an olfactory memory load to a pre-existing spatial memory

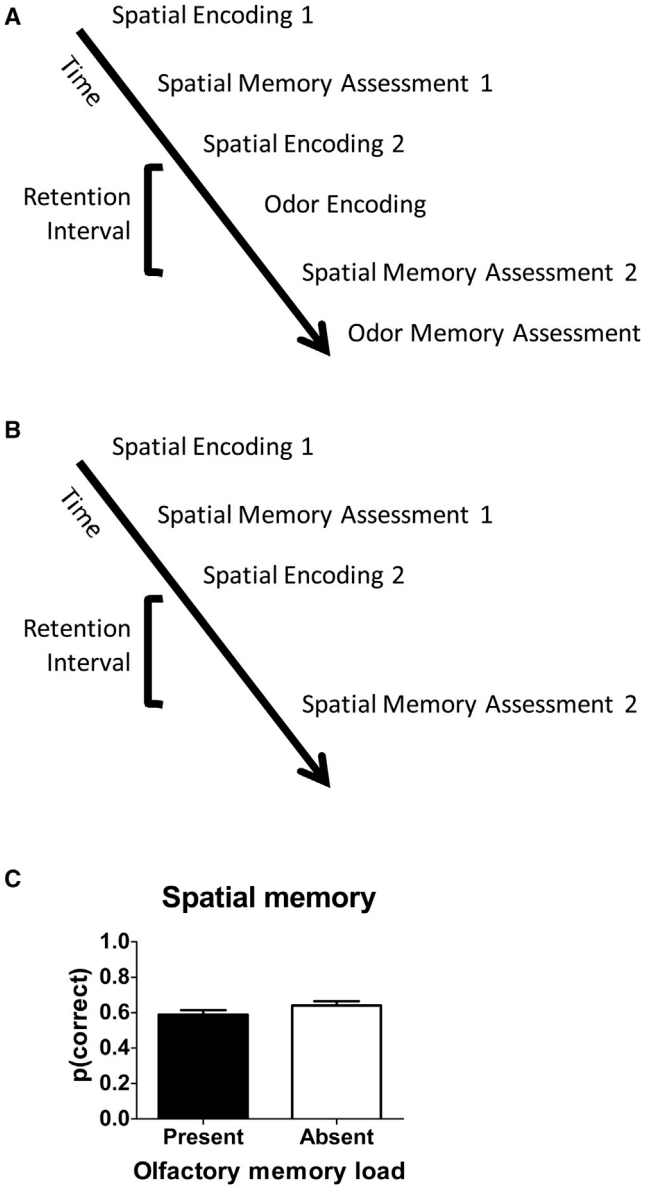

Figure 3. Spatial Memory, when Compromised by the Development of Proactive Interference, Is Resistant to Interference from the Addition of an Olfactory Memory Load

(A and B) Schematic of timeline illustrating experimental design. Spatial memory is assessed in the presence (A) or absence (B) of an added olfactory memory load after the development of proactive interference.

(C) Adding an olfactory memory load does not impair spatial memory even when performance is compromised, as expected with multiple, independent memory systems. Data are shown as mean with 1 SEM.

See also Tables S1 and S2.

load, we compared performance on the spatial memory assessment in the presence and absence of the olfactory memory load (Figure 2C). When a olfactory memory load was present, spatial memory was high, similar to the high performance observed when the olfactory memory load was absent ($t(10) = -1.77$, $p = 0.11$). Moreover, spatial memory was above chance when olfactory memory was present ($t(10) = 11.3$ $p < 0.001$) and absent ($t(10) = 22.3$, $p < 0.001$). Resistance to interference is consistent with the hypothesis that adding a between-domain memory load does not impair spatial memory, as expected if rats process information with multiple, independent memory systems.

Olfactory and spatial memory performance was excellent in experiments 1 and 2. Correcting for different levels of chance (chance is 0.50 and 0.41 for olfactory and spatial memory assessments, respectively), olfactory and spatial memory performance was 0.42 ± 0.02 and 0.40 ± 0.03 above chance, respectively. Experiments 1 and 2 suggest that adding an extra memory load does not impair performance. According to the independent-memory-system hypothesis, resistance to interference is expected to occur not only when performance is high (as in experiments 1 and 2) but also when performance is compromised (i.e., when memory performance is at a relatively low level). Alternatively, perhaps the observed resistance to interference is limited to conditions in which performance is quite high. To test these alternative hypotheses, we characterized the impact of adding a working memory load when one of the domains was compromised. To compromise spatial memory, we used proactive interference [5–7] in the spatial domain and evaluated the impact of adding an olfactory memory load. If resistance to interference from the addition of a memory load is restricted to conditions in which performance is high, susceptibility to interference may be observed when performance is compromised. Alternatively, resistance to interference, despite compromised performance, would validate our conclusion that rats use multiple, independent memory systems.

To generate proactive interference, we conducted two successive spatial memory trials (i.e., spatial encoding trial 1 and spatial memory assessment trial 1, followed by a new trial: spatial encoding trial 2 and spatial memory assessment trial 2). Performance on the second trial is expected to decline relative to performance on the first trial [5–7]. Critically, poor performance on the second trial occurs because the animal remembers information from the first trial [6]. Thus, in experiment 3 (Figure 3), we evaluated the impact of adding an olfactory memory load after the development of proactive interference. When an olfactory memory load is present, the sequence of events (Figure 3A) is: spatial encoding trial 1, spatial memory assessment trial 1, spatial encoding trial 2, olfactory encoding, spatial memory assessment trial 2, olfactory memory assessment. On other days (randomly selected), we used only the spatial memory task (shown in Figure 3B), without the addition of an olfactory memory load. When an olfactory memory load is absent, the sequence of events is as follows: spatial encoding trial 1, spatial memory assessment trial 1, spatial memory encoding trial 2, and spatial memory assessment trial 2. Importantly, the delay between spatial encoding and memory assessment on trial 2 was equated by extending the delay in this condition to match the time taken on other days to complete the olfactory encoding. To evaluate the impact of adding an olfactory memory load to a pre-existing and compromised spatial memory load, we compared performance on the spatial memory assessment in the presence and absence of the olfactory memory load (Figure 3C). When an olfactory memory load was present, spatial memory was modest, similar to the modest performance observed when the olfactory memory load was absent ($t(10) = -1.72$, $p = 0.12$). Critically, performance was reduced on the second spatial memory trial by

0.18 ± 0.03, relative to first trial performance, thereby documenting the development of proactive interference ($t(10) = -6.89$, $p < 0.001$). Despite the reduced level of performance on the second spatial memory trial, spatial memory was still above chance when olfactory memory was either present ($t(10) = 7.00$ $p < 0.001$) or absent ($t(10) = 9.40$, $p < 0.001$), documenting that the rats were successfully attending to the spatial domain, despite their impaired performance. Resistance to interference is consistent with the hypothesis that adding an olfactory memory load does not impair spatial memory, even when performance is compromised, as expected if rats process information with multiple independent memory systems.

The resistance to between-domain interference in olfactory and spatial memory (Figures 1C, 2C, and 3C) is noteworthy because within-domain memory appears to have a limited capacity. Limited capacity is documented by a decline in accuracy as within-domain memory load increases. To provide a memory load in the olfactory domain, without a concurrent spatial task, we assessed olfactory memory using 101 odors (see Figure S1); the olfactory memory load increased as the animal progressed through the session, because an increasing number of odors needed to be remembered as the session progressed. Accuracy declined as a function of olfactory memory load ($F(8,80) = 2.20$, $p < 0.05$; Figure S1). The decline in accuracy as a function of memory load, within a single domain, documents that olfactory memory is capacity limited. Similarly, spatial working memory declines as a function of the number of arms used in radial-maze experiments [11] (see Table S1). The increase in spatial errors as a function of spatial memory load, within a single domain, documents that spatial memory is capacity limited. Although the declines in accuracy are relatively small, they establish the principle that capacity is limited in olfactory and spatial domains. From a comparative perspective, the quantities of olfactory and spatial information that can be maintained in working memory systems in rats are higher than estimates of human working memory [12].

Our findings suggest that independence of working memory systems is evolutionarily quite old. Moreover, our findings support the view that rats may be used to model fundamental aspects of human cognition. Working memory is impaired in aging and Alzheimer's disease [13–16]. The ability to translate successfully from animals to humans will be improved by the development of approaches that include modeling of the specific memory impairments observed in clinical populations (i.e., working memory as validated here, and other aspects of memory, such as episodic memory, source memory, retrieval proactive, and prospective memory, e.g., [17–28]). This approach will advance translational research that may ultimately foster the development of therapeutic approaches to disorders of human memory.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.11.068.

AUTHOR CONTRIBUTIONS

Conceptualization, A.B. and J.D.C.; Formal Analysis, A.B. and J.D.C.; Investigation, A.B., S.K., J.A.C., J.-E. W., N.R.-R., S.D., D.A., A.D., S.C., H.E.C., and M.J.P.; Writing, A.B. and J.D.C.; Visualization, A.B. and J.D.C.; Supervision, A.B., A.R.D., M.J.P., A.E.S., and J.D.C.

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Supplemental Information

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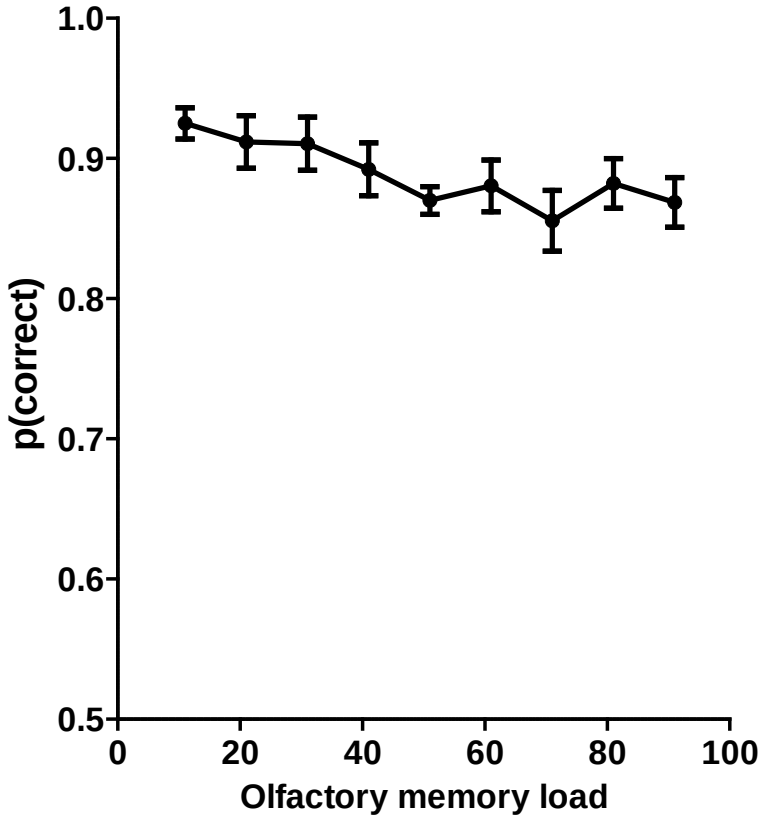

Figure S1. The decline in accuracy as a function of memory load documents that olfactory memory is capacity limited. To equate the influence of retention interval, memory loads were evaluated on trials with retention interval gaps of up to 10 trials; i.e., the gap between the current trial (new odor) and the trial on which the old odor was previously presented as a new odor was held constant at 1-10 trials. Memory loads 11-100 are plotted in blocks of 10. Data are shown as Mean ± SEM. Figure S1 is related to main Figure 1.

Table S1. Number of errors to deplete radial mazes of all food when the number of available arms are 6, 12, 18, and 24.

Number of arms	Number of errors (Mean ± SEM)	Errors expected by chance
6	1.04 ± 0.08	8.76
12	2.65 ± 0.13	25.34
18	3.12 ± 0.12	44.98
24	4.83 ± 0.22	66.44

Data are from Meck and Williams [S1]. The number of errors increased as a function of the memory load imposed by increasing number of arms in the radial maze, suggesting that spatial memory in the radial maze is capacity limited. Data come from independent groups of rats (n=16 per group). Table S1 is related to main Figures 2 and 3.

Table S2. Accuracy (p(correct)) in tasks not reported in main text.

Procedure	Mean ± SEM
unbaited probe	0.97 ± 0.03
experiment 1, spatial memory	0.70 ± 0.03
experiment 2, olfactory memory	0.92 ± 0.01
experiment 3, olfactory memory	0.92 ± 0.02

Table S2 is related to main Figures 1, 2, and 3.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Subjects

Twelve male Sprague-Dawley rats (Harlan, Indianapolis, IN; 81 days old and 289 g, on average, at the start of the experiment)) were housed individually in a colony with light onset at 0615 and offset at 1815. Because one rat did not consistently complete the olfactory task, it was excluded from all experiments. The rats received 45-mg chow and chocolate pellets (F0165 and F0299, respectively; Bio-Serv, Frenchtown, NJ) during spatial and olfactory tasks, respectively, and 15-20 g/day of 5012-Rat-Diet (PMI Nutrition International, St. Louis, MO) after completing each session. Water was available ad lib, except when the rat was tested. All procedures were approved by the Bloomington Institutional Animal Care and Use Committee at Indiana University and followed national guidelines.

Apparatus

The arena used for the presentation of odors was circular with a 94-cm diameter floor and 30-cm high walls made of white acrylic plexiglass. Circular holes (5 cm diameter, 2.5 cm deep) were placed in two concentric circles (12 holes in the outer circle, 6 in the inner circle). Each 59-ml condiment cup could be firmly placed inside a hole with a plastic lid lightly placed on top of the cup.

The radial maze (modified components from Lafayette Instruments) consisted of 8 arms (each ~75 cm length) and a central hub (~33 cm diameter). The arms and hub were surrounded with clear, plexiglass approximately 20 cm high and were open on top.

The ends of each arm contained a 6-cm cup, inlayed in the floor, which could be baited. Guillotine doors, made of clear Plexiglas, separated the hub from each of the arms and could be individually raised using pneumatic cylinders via remote control. Inaccessible pellets were placed at the end of each runway below the food cup.

Chlorhexidine was used to clean the maze after each animal was removed from the maze and to clean the arena after each rat completed a daily session. Pellets were placed outside the maze (i.e., food odors were constant throughout each maze trial). White noise was used to mask outside noise.

Stimuli

Opaque plastic lids were treated with an odorant by placing ~36 lids in a sealed plastic container. Each container was filled with approximately 150 ml of a dry odorant or 90 ml of a wet odorant. A metal grating, which suspended the lids above the odorant, was placed in each container to prevent direct contact between the odorants and lids. A total of 34 odorants were used, with lids treated for at least 2 weeks prior to being presented to rats. The odorants in the plastic containers were replaced every ~2 months. In preliminary training and in Experiments 1-3, odors included: Allspice, anise seed, bay leaf, beet powder, caraway seed, carob powder, celery seed, cherry oil, cinnamon, cloves, coriander, cumin, dill weed, fennel seed, fenugreek seed, garlic powder, ginger, grape oil, marjoram, mustard seed, nutmeg, onion powder, orange oil, Mexican oregano, paprika, peach oil, rosemary leaf, sage leaf, spinach powder, sumac, summer savory, thyme, turmeric, and Mexican vanilla. In Experiment 4, odors included: Allspice, almond oil, amaretto, anise seed, annatto, apple, apricot, asparagus, banana,

bay leaf, beet powder, black pepper, black walnut, blackberry, blue cheese, blueberry, brandy, bubble gum, butter, butterscotch, caraway seed, cardamom, carob powder, celery seed, champagne, cheddar cheese, cherry, chicory root, chocolate, cilantro, cinnamon, cloves, cocoa, coconut, coffee, coriander, cotton candy, crème de menthe, cumin, dill weed, eggnog, fennel seed, fenugreek seed, galangal root, garlic powder, ginger, grape flavor, hazelnut, hickory smoke, honey, horseradish, hot chili oil, Indian curry, Irish cream, juniper berries, lavender, lemon zest, lemongrass, lime, malt vinegar, maple, marjoram, marshmallow, menthol-eucalyptus, Mexican oregano, mushroom, mustard seed, nutmeg, onion powder, orange oil, paprika, peach, peanut butter, pecan, pineapple, pistachio, pumpkin, raspberry, root beer, rosemary leaf, sage leaf, sassafras, sesame, soy sauce, spearmint leaf, spinach powder, strawberry, sumac, summer savory, sweet basil, tangerine, tarragon, thyme, tomato, turmeric, vanilla butternut, Mexican vanilla, wasabi, watermelon, white willow bark, and Worcestershire.

Odor Pre-training

In each pre-training session, a cup (without a lid) was placed in each of the 18 holes of the arena and was baited with 1-2 chocolate pellets. The rat was placed in the center of the arena and was allowed to navigate the arena until all pellets were consumed or 30 minutes elapsed. When a session was completed within 5 minutes, the rat was moved to the next stage of pre-training beginning on the following session. Five sessions were conducted. Sessions were conducted once per day, approximately 5 days per week, for each rat (here and throughout this work, except as noted otherwise).

In the next stage of pre-training, a single cup was placed in a pseudo-randomly selected hole of the arena. The cup was baited with 1 chocolate pellet. On each trial, the rat was placed in the center of the arena and allowed to navigate the arena until he found the baited cup and consumed the pellet or 2 minutes elapsed, after which the rat was removed. This procedure was repeated for 24 trials or until 30 minutes elapsed. At this stage, a cup was used, but without a lid. As soon as a rat successfully completed 24 trials in 30 minute, lid coverage was gradually incremented beginning on the following session: 25%, 50%, 75%, and 100% coverage. As soon as 100% coverage was successfully completed, the rat began Odor Span Training on the following session. Pre-training was conducted for ~7 sessions.

Odor Span Training

Odor Span Training required the rat to select a new odor and avoid old odors. To this end, when a new odor was initially presented in a session, the cup was baited with a chocolate pellet (referred to as an S+ stimulus). After the rat ate the pellet, the next trial involved the presentation of a new odor (baited, S+) and the re-presentation of an old odor which was un-baited and referred to as S-. A correct response was defined as displacement of an S+ lid; an incorrect response was defined as displacement of an S- lid. In this and all subsequent odor procedures, lids were only used once per session to preclude the use of odor cues left by the rats, and the location of cups was randomly determined on each trial for each rat.

On the first trial of a session of Odor Span Training, the first S+ lid with its corresponding baited cup was placed in a pseudo-randomly selected arena location.

The rat was then placed in the center of the arena and was allowed to navigate the arena until the S+ lid was removed or 2 minutes elapsed. The rat was then removed from the arena and the cups and lids were removed from the arena. On each subsequent trial, the set size increased by 1 new odor (in addition to all previously presented odors). Thus each subsequent trial contained a new odor as an S+ and all previously presented odors as S- stimuli. The set size continued to increase until an incorrect response was made, at which point the set size was reset to 1 S+; after the set size was reset, incrementing continued in the same manner until another incorrect response occurred. A correction procedure was used on each trial, meaning that if the rat made an incorrect response, he would be allowed to continue making responses until the correct response was made or until 2 minutes elapsed. If 2 minutes elapsed, the rat was placed in the cage and the trial was scored as incorrect. Given that a session consisted of 25 trials and the arena contained 18 holes, trials that went above 18 odors followed the procedure of randomly selecting which 18 S- stimuli were placed in the arena. Twenty sessions were conducted. We measured the number of consecutive correct choices prior to the first incorrect choice (referred to as span). Span on the first session was less than 1, on average, item and increased to 10.8 ± 1.8 items on the last session. As expected, span increases as a function of sessions ($F(9,99)=132.1$, $p<0.001$).

Two Alternative Forced Choice Task

Next, the rats were introduced to a two-alternative forced choice (2AFC) task, which replaced the Odor Span Task described above. The procedure was the same as

described above in Odor Span Training, with the exception that only a single S- stimulus was randomly selected on each trial to be presented as the S- odor in each trial and the set size of old odors was not reset by an error. The S- stimulus was randomly selected from the list of odors that had previously occurred as S+ in the session. Thus, after the first trial, each subsequent trial presented 2 stimuli: an S+ and an S-. Twenty sessions (each with 25 trials) were conducted.

Unbaited Probes

To test the hypothesis that rats detected the presence of the chocolate pellet under the S+ lids, a series of unbaited probes was conducted. A probe session was conducted for each rat on the last session of initial 2AFC training. One trial was selected for presentation of a probe in each of the following range of trials: 11-15, 16-20, and 21-25 (producing 3 probes per rat). In each probe, a pellet was not placed under the S+ lid (i.e. the cup was initially unbaited before the rat made its choice). Upon a rat's choice of the S+ lid, the experimenter promptly placed a pellet in the cup. One of the rats did not participate in the unbaited probe because it was not reliably completing all trials in a session at this stage of training. Performance in unbaited probes was high (see Table S2).

Radial Maze Pre-training

Pre-training consisted of baiting each of the 8 arms with 3 chow pellets along each arm and 1 chow pellet in the cup. The rat was placed in the hub and, following a brief delay, all 8 guillotine doors were raised. The rat was allowed to explore the maze

until either all pellets were consumed or 30 minutes elapsed. Three pre-training sessions were conducted.

Radial Maze 8-arm Procedure

Each food cup was baited with 1 chow pellet. The rat was then placed in the central hub for a short duration, after which all 8 doors were opened. The rat was allowed to navigate the maze until all 8 pellets were consumed or 15 minutes elapsed. A visit to an arm was recorded if the rat placed all four paws in the arm.

Radial Maze Study-Test Procedure

Study-test training began by baiting each food cup with 1 chow pellet. In the study (encoding) phase, the rat was placed in the hub for a short duration, after which 4 doors (randomly selected on each session for each rat) were opened. The rat was allowed to navigate the maze until all 4 pellets were consumed or 15 minutes elapsed. The rat was then removed and the arms of the maze were cleaned. For the test (assessment) phase, the rat was placed in the hub and all 8 doors were opened after a brief delay; at this stage, food was only available at the 4 arms not visited in the study phase. Visits were scored as described above. The number of baited arms entered in the first four choices of the test phase (expressed as a proportion of four arms) was the dependent measure. If the rats entered arms randomly, the dependent measure expected by chance is 0.41 [S2].

Experiment 1: Olfactory Memory with Spatial Load

Experiment 1 was designed to test the hypothesis that spatial and olfactory working memory systems are independent. This was accomplished by testing performance in the odor task while retaining in memory both an odor and spatial memory load. Two types of sessions were conducted in Experiment 1: experimental sessions and control sessions. In an experimental session, rats first completed 10 trials of the 2AFC task as described above. Next, the rat was placed in the radial maze for a study phase as described above. Next, the rat's memory for the 10 odors was assessed; they performed 10 trials of the 2AFC task in which all S- stimuli were randomly selected with replacement from stimuli used in the first 10 odor trials. Finally, the rat received the test phase in the radial maze as described above. The delay between when a rat was removed from the arena after finishing the first segment of odor trials and when it was placed back in the arena to begin the last segment of odor trials was recorded. These times were used to match retention intervals in the control sessions.

In a control session, the rat first performed 10 trials of the 2AFC task. They were then placed in a cage for a retention interval that matched the rat's previous experimental session. Finally, the rat received 10 trials of the 2AFC task in which all S- stimuli were randomly selected with replacement from the first 10 2AFC trials of the session. One experimental or one control session was conducted on each day; the order of conditions was based on a randomized block design arranged so that 2 experimental and 2 control trials appeared in each block, with the restriction that no

more than 3 identical sessions occurred on consecutive days. Approximately 20 sessions were conducted.

Experiment 2: Spatial Memory with Olfactory Load

Experiment 2 was designed as a complement of Experiment 1 to test the independence of working memory systems by assessing performance in a spatial task while retaining both an odor and spatial memory load. Two types of sessions were conducted in Experiment 2: experimental sessions and control sessions. In an experimental session, the rat first completed the study phase of the radial maze procedure. Next, the rat completed 10 trials of the 2AFC task as described above. The rat's spatial memory was then assessed: the rat was placed back in the radial maze for the test phase as described above. Finally, the rat performed 10 trials of the 2AFC task in which all S- stimuli were randomly selected with replacement from stimuli used in the first 10 odor trials. The delay between when a rat was removed from the radial maze after finishing the study phase and when it was placed back in the radial maze was recorded. These times were used to match retention intervals in the control sessions.

In a control session, the rat first performed the study phase of the radial maze procedure. The rat was placed in a cage for a retention interval that matched the rat's previous experimental session. Finally, the rat was placed back in the radial maze to perform the test phase. Experimental and control session were arranged as described in Experiment 1. Approximately 24 sessions were conducted.

Experiment 3: Spatial Memory with Olfactory Load When Compromised by the Development of Proactive Interference

Experiment 3 was the same as Experiment 2, except that an extra radial maze trial (study and test phases) was conducted on each day. In experimental sessions, the rat completed radial maze study and test sequences for trial 1. Next, a study phase in the same radial maze occurred, but the identity of baited accessible arms was randomly determined (i.e., independent of the accessible baited arms used in the study phase of trial 1). Next, an odor study phase occurred (as described above), followed by the test phase for radial maze trial 2, and finally the odor test phase (described above). The control sessions were identical to experimental sessions, except odor study and odor test phases did not occur. In each control session, the retention interval between study on radial maze trial 2 and test on radial maze trial 2 matched the retention interval from an experimental session.

Experiment 4: 101 odors

Experiment 4 was the same as the Two Alternative Forced Choice Task described above, except each session used 101 trials per session. We test two rats per day, which produced intervals between successive sessions, for each rat, that were approximately 1 week. Four or five sessions were conducted per rat.

SUPPLEMENTAL DATA:

Spatial memory in rats is resistant to interference, primarily when distinct spatial cues are available (e.g., [S3, S4]). Potentially confusable spatial locations are likely to provide a sensitive measure of putative capacity limits in spatial memory. To this end, we examined data published by Meck and Williams [S1], which allowed us to estimate the change in accuracy as a function of increasing number of nearby spatial items. Meck and Williams tested different groups of rats using radial arm mazes with 6, 12, 18, and 24 arms; each arm was baited at the start of a trial, and rats were permitted to search the arms until the last piece of food was found. The number of errors to find all of the food increased as a function of the number of arms in the maze, which is consistent with the hypothesis that spatial memory in the radial maze is capacity limited. To quantify number of errors as a function of number of arms, we measured each data point (from Meck and Williams' Figure 1, using their "CON-RAN" condition). The number of errors as a function of arms are shown in Table S1. The number of errors increased as a function of the number of arms in the maze ($t(2)=7.00$, $p<0.02$). We conducted simulations (25,000 iterations per maze-size condition) to estimate the number of errors that would occur if arms were randomly selected, (see Table S1). The number of errors produced by Meck and Williams' rats cannot be explained by random searching of the maze ($\chi^2(3)=123.2$, $p<0.001$). Note that the number of observed errors is much smaller than the number expected by chance. Moreover, the rate of increase in errors as a function of the number of arms is much more rapid for the predicted than for the observed errors. Limited capacity is documented by a decline in accuracy as within-

domain memory load increases. The increase in the number of errors as the number of spatial locations increased is consistent with the hypothesis that spatial memory in the radial maze is capacity limited.

SUPPLEMENTAL REFERENCES:

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