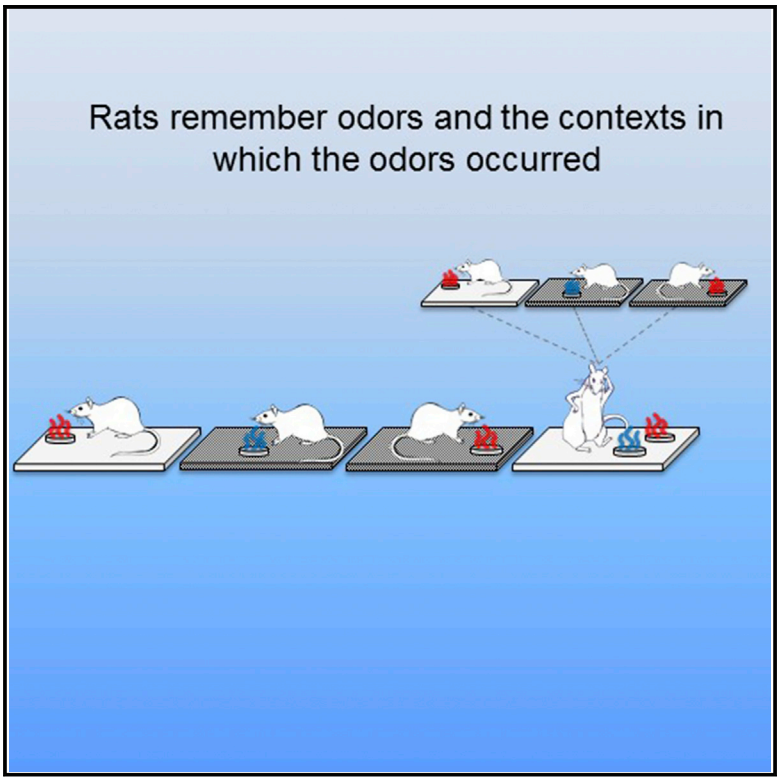
Report

Current Biology

Rats Remember Items in Context Using Episodic Memory

Graphical Abstract



Authors

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In Brief

Panoz-Brown et al. show that rats remember many unique events and the contexts in which they occurred using episodic memory. These studies suggest that rats remember at least 32 items in context, episodic memory can withstand at least 15 unpredictable transitions between contexts, and item-in-context memory persists for at least 45 min.

Highlights

- Rats remember many unique events using episodic memory
- Rats remembered items and the contexts in which they occurred using episodic memory
- The ability to represent numerous episodic memories is evolutionarily quite old
- Rats may be used to model fundamental aspects of human memory

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Current Biology
Report

Rats Remember Items in Context Using Episodic Memory

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SUMMARY

Vivid episodic memories in people have been characterized as the replay of unique events in sequential order [1–3]. Animal models of episodic memory have successfully documented episodic memory of a single event (e.g., [4–8]). However, a fundamental feature of episodic memory in people is that it involves multiple events, and notably, episodic memory impairments in human diseases are not limited to a single event. Critically, it is not known whether animals remember many unique events using episodic memory. Here, we show that rats remember many unique events and the contexts in which the events occurred using episodic memory. We used an olfactory memory assessment in which new (but not old) odors were rewarded using 32 items. Rats were presented with 16 odors in one context and the same odors in a second context. To attain high accuracy, the rats needed to remember item in context because each odor was rewarded as a new item in each context. The demands on item-in-context memory were varied by assessing memory with 2, 3, 5, or 15 unpredictable transitions between contexts, and item-in-context memory survived a 45 min retention interval challenge. When the memory of item in context was put in conflict with non-episodic familiarity cues, rats relied on item in context using episodic memory. Our findings suggest that rats remember multiple unique events and the contexts in which these events occurred using episodic memory and support the view that rats may be used to model fundamental aspects of human cognition.

RESULTS AND DISCUSSION

A fundamental attribute in human cognition is the ability to remember multiple unique events using episodic memory [1, 9, 10]. In an everyday example, imagine you are watching an episode of your favorite television show when you see actors that look vaguely familiar make cameo appearances. You know that you have seen the actors before in another show or movie, but you cannot remember where. This common scenario illustrates two types of memory situations: one involves episodic-based remembering of items in context, in this case, the cameo actors and the movies or series you originally saw them in, whereas the other involves a vague sense of familiarity of the actor but without memory of the episode or context in which they occurred. Episodic memory involves remembering an event and the contextual details of the episode, whereas familiarity is the somewhat vague judgment that an item is known [3, 11–14], as highlighted in the example above.

Importantly, disorders of episodic memory (such as Alzheimer's disease) are often not limited to the loss of a single event, but rather present with the widespread impairment of multiple episodic memories, which makes the disorder debilitating [15–18]. Increasingly, animal models of human memory are developed to gain insight and understanding of basic biological mechanisms of memory and to validate therapeutic approaches to treat memory diseases that are observed in humans [19, 20]. Prior work on animal models of episodic memory has been successful at documenting that animals are capable of remembering a single event [4–8]. Although it is possible for single events to contain multiple features, it is currently unknown whether animals can remember many episodic memories. Animal models of episodic memory have used a variety of animals, such as scrub jays [4], rats [5–8], and non-human primates [21]. How to define episodic memory in animal models has been a subject of debate [22, 23], but a major view about episodic memory is that it involves memory of an event and the contextual details of the episode (i.e., item in context information) [13]. Our approach focuses on the objective content of episodic memory [19, 24–27] rather than subjective experiences that are thought to accompany episodic memory in people, while carefully eliminating non-episodic memory alternatives. In the current study, we asked rats to identify specific items and the contexts in which the items were encountered for multiple unique events (i.e., item-in-context memory).

We exploited the well-established proficiency of rats with olfactory information [28, 29]. Rats were individually tested in two distinctive circular arenas with "food holes" covered by scented opaque lids (see the Supplemental Experimental Procedures). In our approach, rats were trained to pick the "new" odor and avoid the "old" odor when presented with pairs of odors across multiple contexts. Specifically, one odor was always "new" (S+) to the current context, whereas the other was "old" (S−; i.e., it had already been presented within that context earlier in the day). A new odor item was presented to individual rats using

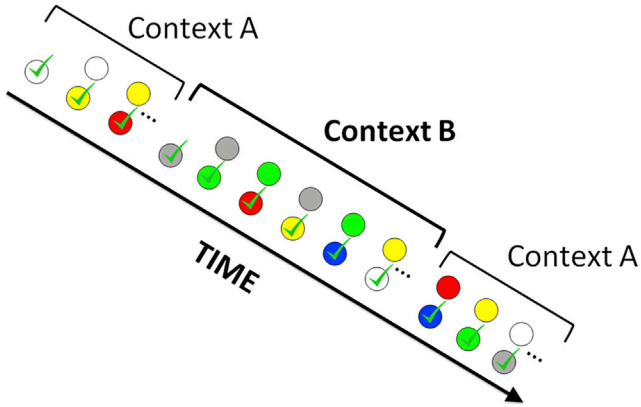

Figure 1. Schematic of Odor Sequence Presented across Multiple Contexts

Schematic illustrating the sequence of new odors (depicted as colors) in experiment 1 with two context transitions. The "new" item (i.e., odor) in each trial is rewarded (S+, denoted by "√"), whereas the "old" item is not rewarded (S−). In the first segment, the rats were presented with half of the items in context A. Next, the rats were presented with all of the odors in context B, including those already presented in context A. Finally, in context A, the rats were presented with the remaining half of the items that had not yet been presented in context A. Six of 16 S+ items in each context are shown. See also Figure S1 and Table S1.

a scented lid placed on top of a small cup, together with an old odor placed on a different cup (at randomly selected locations in the arenas). We defined a choice as displacement of a lid. Selection of a new item was rewarded with food, whereas selection of the old item was not. In preliminary training, rats were presented with multiple odors across two contexts in rapid succession (see Figure S1 and Supplemental Experimental Procedures); to make the contexts distinctive, we used two circular arenas that differed in diameter, black-and-white patterns on the floor, number of holes on the floor, and access to visual cues outside the arena. Initially, we presented the rats with a set of 16 odors in the first context (context A). Next, in the second context (context B), the same 16 odors were presented as "new," despite the earlier presentation of these odors in the other context. Training accuracy was high (Table S1). However, because the odors are presented in each context sequentially, high accuracy could be attained by selecting the least familiar item (i.e., by using a semantic rule [30], namely "avoid familiar items"), without using episodic memory of items in context.

To investigate memory of item in context, we rapidly interleaved presentations of new odors across contexts by presenting an additional transition (context A→context B→context A; Figure 1; experiment 1). Initially, rats were presented with half of the items (i.e., eight odors) in the first context, followed by all 16 items in a second context (i.e., context B presentation included items that had and had not been presented in context A). Finally, the rats returned to the first context, where they received the remaining half of the odors (i.e., the eight items that had not yet appeared in the initial context). Each day, 16 odors were randomly selected from a pool of 40 odors, and odors were presented in random order. Figure 2 illustrates how small changes in the order of items provide insights into the type of memory that may support selection of the "new" item. In Figure 2A, the animal could find the "new" item by selecting the least familiar odor, without using episodic memory. To illustrate how familiarity cues can promote selection of the "new" item, consider a snapshot of four items presented across two contexts (Figure 2A). For a particular pair of odors (e.g., banana and basil, depicted as yellow and green in Figure 2A), one item (banana), but not the other (basil), occurs in the first context. Next, both items occur in the second context (notably basil followed by banana). Finally, upon return to the first context, a choice between banana and basil is offered; basil is rewarded because it is "new" to context A (i.e., the item-in-context correct choice). Because banana was rewarded after basil in the second context in Figure 2A, when the choice occurs, banana would be more familiar than basil. Thus, an animal that used a semantic rule ("avoid familiar items") would successfully choose the basil in the final context (i.e., the correct item-in-context choice in Figure 2A) based on judgments of relative familiarity. Figure 2A illustrates that, in some odor sequences, a correct choice of the "new" item could be based on familiarity or remembering item in context. Although accuracy in selecting the new item is high (Table S1) when both familiarity and item-in-context cues are available, these data do not isolate item-in-context memory while eliminating the use of familiarity cues. Importantly, a small change in the sequence of odors unconfounds these two alternatives, as described next.

To dissociate episodic memory from familiarity judgments in the last segment, we identified sequences of odors that put familiarity cues and item-in-context memory in conflict. For a particular pair of odors (e.g., strawberry and blueberry, depicted as red and blue in Figure 2B), we presented one item (strawberry), but not the other (blueberry), in the first context. Next, both items were presented in the second context (notably strawberry followed by blueberry). Finally, the memory assessment occurred upon return to the first context. In the memory assessment, the rats were confronted with a choice between strawberry and blueberry. The correct choice, based on item in context, is blueberry because it has not yet been presented in the first context; indeed, blueberry is rewarded when chosen in this test, and our measure of accuracy is the proportion of choices of the rewarded item. Importantly, prior to the memory assessment, blueberry was presented more recently than strawberry (see Figure 2B). Consequently, in the memory assessment, strawberry would be less familiar relative to blueberry. Thus, an animal that relied on judgments of relative familiarity would choose the strawberry in the memory assessment (i.e., following the semantic rule "avoid familiar items"). By our measure of accuracy, such a choice would result in accuracy below chance in the memory assessment shown in Figure 2B. On the other hand, an animal that relied on item-in-context memory would choose blueberry in the memory assessment, which would in turn result in above chance accuracy. Notably, this memory assessment dissociates item-in-context memory (above chance) from judgments of relative familiarity (below chance). Because the arrangement shown in Figure 2B dissociates item-in-context memory and judgments of relative familiarity, we used the memory assessment shown in Figure 2B for each experiment (data shown in Figure 3) and excluded the arrangement shown in Figure 2A.

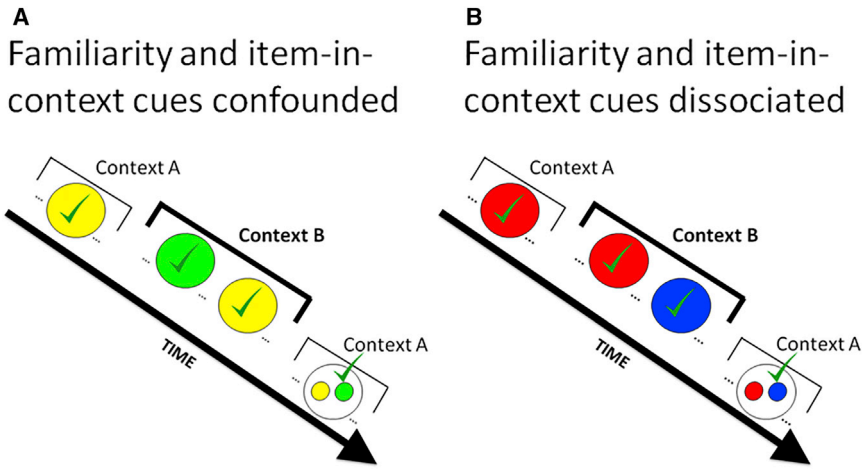

Figure 2. Dissociating Episodic Item-in-Context Memory from Familiarity Cues

(A) Familiarity and item-in-context memories are confounded. Banana and basil odors are depicted here as yellow and green, respectively. Initially, we presented banana in context A, and we presented both basil and banana in context B. Notably, basil was not presented in context A, and importantly, basil occurred before banana in context B. Finally, the memory test occurred in context A. In the memory test, the rats were presented with a choice between banana and basil in context A. Critically, both familiarity and item-in-context memories would lead an animal to choose basil, the correct choice in the memory test. A small change in the order of items in context B unconfounds these two alternatives, as shown in (B).

(B) Familiarity and item-in-context memories are dissociated. Strawberry and blueberry odors are depicted here as red and blue, respectively. Initially, we presented strawberry in context A, and we presented both strawberry and blueberry in context B. Notably, blueberry was not presented in context A, and strawberry occurred before blueberry in context B. Finally, the memory assessment occurred in context A. In the memory assessment, the rats were presented with a choice between strawberry and blueberry. The correct choice, based on item in context, is blueberry because it has not yet been presented in context A. Blueberry is rewarded when chosen in this test, and our measure of accuracy is the proportion of choices of the rewarded item. Importantly, prior to the memory assessment, blueberry was presented more recently than strawberry. Consequently, in the memory assessment, strawberry would be less familiar relative to blueberry. Thus, an animal that relied on judgments of relative familiarity would choose the strawberry in the memory assessment. By our measure of accuracy, this choice results in accuracy below chance. By contrast, an animal that relied on item-in-context memory would choose blueberry in the memory assessment, which results in above-chance accuracy. Notably, this memory assessment dissociates item-in-context memory (above chance) from judgments of relative familiarity (below chance).

(A and B) The presence of additional odors (not shown) is identified by "…" in the schematic. The schematic focuses on S+ items (denoted by "√") by omitting comparison S− items prior to the memory assessment. Trials depicted in (A) and (B) were randomly intermingled throughout daily testing.

To test whether the rats were relying on item-in-context episodic memory or non-episodic judgments of familiarity, we examined the rats' accuracy (Figure 3A) in the memory assessment (Figure 2B). Notably, the most compelling evidence for our dissociation of memory lies within the rats' *initial* memory-assessment performance when these conditions are novel (i.e., before receiving feedback from reward in the novel condition). Consequently, the critical data for evaluating item-in-context memory come from the initial trials (labeled "initial" in Figure 3A). If rats were relying on item-in-context episodic memory, performance in the initial memory assessment would be above chance, whereas using familiarity cues would produce below-chance performance. When the identity of items in context was put in conflict with familiarity cues, initial performance was above chance ($t(11) = 7.71$; $p < 0.001$; Figure 3A; experiment 1). Similar to the initial performance, high accuracy was observed in subsequent memory assessments (labeled "terminal" in Figure 3A). High accuracy provides compelling evidence that rats relied on episodic item-in-context memory rather than judgments of familiarity.

Our data suggest that, after performing in the second context, the rats remembered the items presented in the first context. To establish that rats can also remember items from the second context, we added an additional context transition. We divided the day into four segments by using three context transitions (context A→context B→context A→context B). In sequence, we presented the rat with half of the items in each context during the first two segments. For the third and fourth segments, the rat returned to the first and second context, respectively, and was presented with the remaining half of the items in each. Therefore, in order to attain high accuracy in the fourth-segment memory assessment, the rat must be able to identify which items had and had not previously appeared in each context. Further, this

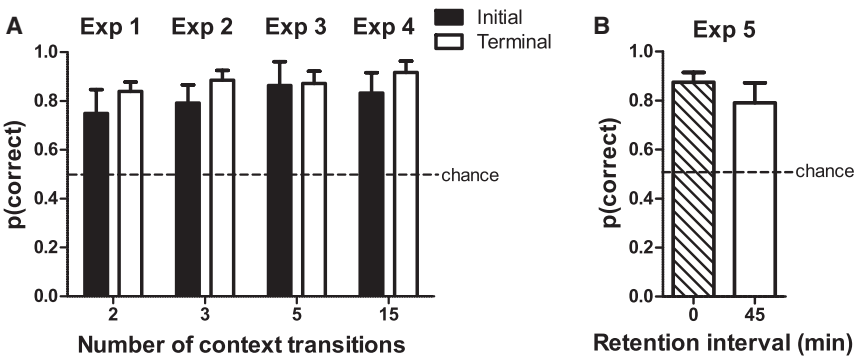

Figure 3. Rats Remember Items in Context Using Episodic Memory

(A) Item-in-context memory is shown by above-chance accuracy following 2, 3, 5, and 15 context transitions. Initial performance comes from the first two memory assessments in each experiment; subsequent memory assessment performance is labeled terminal. See also Table S1.

(B) Item-in-context memory survives a long retention-interval challenge.

(A and B) Data are shown as mean +1 SEM.

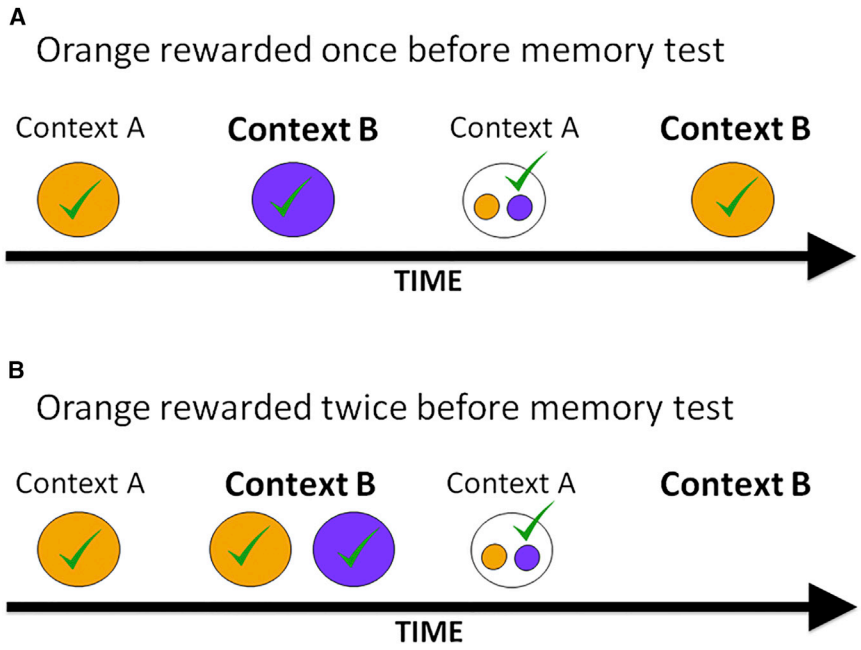

Figure 4. Schematic of Odor Sequences to Compare the Impact of Rewarding an Item Once or Twice

An item (orange) is rewarded once (A) or twice (B) prior to a common memory test (choice between orange and purple in context A). The other item (purple) is rewarded once in context B. The second presentation of orange occurs after the memory test in (A). The trials depicted in (A) and (B) come from experiment 2 (contexts A→B→A→B).

approach allowed us to recreate novel conditions, which again allowed us to examine performance, prior to the opportunity for new learning. When the identity of item in context was put in conflict with familiarity cues in the novel fourth segment, initial performance in the fourth segment was above chance ($t(11) = 10.65$; $p < 0.001$; Figure 3A; experiment 2). Similarly, high accuracy was observed in subsequent memory assessments (terminal performance). These data suggest that the rats remember items in both contexts.

We recreated novel conditions again by adding two new transitions. Days were divided into six segments (context A→context B→context A→context B→context A→context B), which produced novel transitions in the last two segments. When we recreated novel conditions that included not one but two new context transitions, initial performance was above chance ($t(10) = 8.86$; $p < 0.001$; Figure 3A; experiment 3), and high accuracy was also observed in subsequent memory assessments (terminal performance).

Next, we challenged the rats' memory further by randomly arranging memory assessments that were maximally unpredictable from trial to trial (i.e., by randomly determining whether a context transition would occur after each trial). The novelty of our conditions was enhanced because context transitions were unpredictable item to item and day to day. Under these conditions, it is not possible to use previous transitions to predict the next transition. On average, 15 unpredictable context transitions occurred each day. When item in context was put in conflict with familiarity cues using unpredictable transitions, initial memory-assessment performance was above chance ($t(8) = 10.0$; $p < 0.001$; Figure 3A; experiment 4), and high accuracy was also observed in subsequent memory assessments. These data suggest that the rats relied on item-in-context memory when the change in contexts was unpredictable.

Episodic memory is posited to be a part of long-term memory [31]. To test the hypothesis that rats were relying on long-term episodic memory in our approach, we asked whether the rats' performance could survive a 45 min retention-interval challenge inserted between memory encoding and memory assessment. We used three context transitions (as in experiment 2) and inserted the 45 min delay between the third and fourth segments; the data for the 0 min delay come from experiment 2. In memory assessments after 0 and 45 min retention intervals, performance was above chance ($t(11) = 9.4$, $p < 0.001$ and $t(8) = 3.6$, $p < 0.01$, respectively; Figure 3B; experiment 5) with no significant decline in performance as a function of delay ($t(8) = -1.3$; $p = 0.24$). These data suggest that the rats were able to remember item in context following a long retention interval challenge and are consistent with the hypothesis that rats relied on long-term episodic memory.

It is noteworthy that, in our memory assessment (Figure 2B), the familiarity choice was rewarded twice (red in Figure 2B) and the item-in-context choice was rewarded once (blue in Figure 2B). This observation raises an important concern, namely that the rats may have chosen the correct item in our memory assessment (blue in Figure 2B) because the incorrect item had been rewarded twice, whereas the correct item was only rewarded once. Alternatively, the number of rewarded presentations may not impact accuracy. To address this issue, we compared performance on other occasions, when the item was rewarded in one or two contexts (see Figure 4 and Supplemental Experimental Procedures). In this analysis, choice between two items (depicted as orange and purple in Figure 4) was compared after a single rewarded presentation of the incorrect item (orange, Figure 4A) versus two rewarded presentations of the incorrect item (Figure 4B). The addition of a rewarded presentation did not significantly impact the accuracy in selecting the correct item ($t(12) = 1.45$; $p = 0.17$). Because the absence of evidence (from the traditional null hypothesis significance test) is not necessarily evidence for the absence of a reward-frequency effect, we used a Bayesian statistical approach. Bayesian statistics can be used to "prove the null hypothesis" [32, 33], which in this case corresponds to the hypothesis that equivalent performance occurs when the number of rewarded presentations is varied. The JZS Bayes factor is 4.0; that is, the null hypothesis is four times more likely than the hypothesis that reward frequency impacts performance. A Bayes factor of this size is described as *substantial* evidence that the null hypothesis is correct [32]. Thus, these data provide substantial

evidence that our memory-assessment performance (Figure 3) is not influenced by the number of rewarded presentations.

Four lines of evidence suggest that rats remember multiple items in context using episodic memory. First, rats remember at least 32 items in context. Second, episodic-memory performance can sustain at least 15 transitions between contexts. Third, item-in-context memory survives a long retention-interval challenge. And, fourth, we conducted four transfers to novel, unpredictable context transitions, which document that our data cannot be explained by learning rules that govern predictable changes in context (Figure 3A; initial versus terminal, $F(1,8) = 2.21$, $p = 0.18$; number of context transitions, $F(3,24) = 1.77$, $p = 0.18$; and interaction, $F(3,24) = 0.28$, $p = 0.84$). Moreover, all of our data come from memory assessments that dissociate item-in-context memory from non-episodic judgments of familiarity. Thus, the high level of accuracy (84% across experiments 1–4) provides dramatic evidence for episodic memory that rules out non-episodic contributions to performance. Moreover, our data cannot be explained by the ability to detect the presence of pellets under S+ lids because we conducted unbaited probes and observed high levels of accuracy (see the Supplemental Experimental Procedures).

Our data suggest that rats remember the context in which odors were presented. When the memory of items in context was put in conflict with familiarity cues, rats relied on item-in-context memory rather than familiarity. We conclude that rats remember multiple unique events and the contexts in which these events occurred using episodic memory. Our findings enhance the translational potential for utilizing animal models of episodic memory to both explore the biological mechanisms of memory and memory disorders and to validate therapeutic approaches for disorders of episodic memory. Moreover, our findings suggest that the ability to represent numerous episodic memories is quite old in the evolutionary timescale. More broadly, our work supports the view that rats may be used to model fundamental aspects of human memory.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.08.023.

AUTHOR CONTRIBUTIONS

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

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REFERENCES

1. Tulving, E. (2002). Episodic memory: from mind to brain. Annu. Rev. Psychol. *53*, 1–25.
2. Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. Nat. Rev. Neurosci. *1*, 41–50.
3. Eichenbaum, H., Yonelinas, A.P., and Ranganath, C. (2007). The medial temporal lobe and recognition memory. Annu. Rev. Neurosci. *30*, 123–152.
4. Clayton, N.S., and Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. Nature *395*, 272–274.
5. Ergorul, C., and Eichenbaum, H. (2004). The hippocampus and memory for "what," "where," and "when". Learn. Mem. *11*, 397–405.
6. Eacott, M.J., Easton, A., and Zinkivskay, A. (2005). Recollection in an episodic-like memory task in the rat. Learn. Mem. *12*, 221–223.
7. Roberts, W.A., Feeney, M.C., Macpherson, K., Petter, M., McMillan, N., and Musolino, E. (2008). Episodic-like memory in rats: is it based on when or how long ago? Science *320*, 113–115.
8. Zhou, W., and Crystal, J.D. (2009). Evidence for remembering when events occurred in a rodent model of episodic memory. Proc. Natl. Acad. Sci. USA *106*, 9525–9529.
9. Tulving, E. (2001). Episodic memory and common sense: how far apart? Philos. Trans. R. Soc. Lond. B Biol. Sci. *356*, 1505–1515.
10. Tulving, E., and Markowitsch, H.J. (1998). Episodic and declarative memory: role of the hippocampus. Hippocampus *8*, 198–204.
11. Yonelinas, A.P., and Levy, B.J. (2002). Dissociating familiarity from recollection in human recognition memory: different rates of forgetting over short retention intervals. Psychon. Bull. Rev. *9*, 575–582.
12. Yonelinas, A.P. (2001). Components of episodic memory: the contribution of recollection and familiarity. Philos. Trans. R. Soc. Lond. B Biol. Sci. *356*, 1363–1374.
13. Eichenbaum, H. (2007). Comparative cognition, hippocampal function, and recollection. Comp. Cogn. Behav. Rev. *2*, 47–66.
14. Eichenbaum, H., Sauvage, M., Fortin, N., Komorowski, R., and Lipton, P. (2012). Towards a functional organization of episodic memory in the medial temporal lobe. Neurosci. Biobehav. Rev. *36*, 1597–1608.
15. Bäckman, L., Andersson, J.L., Nyberg, L., Winblad, B., Nordberg, A., and Almkvist, O. (1999). Brain regions associated with episodic retrieval in normal aging and Alzheimer's disease. Neurology *52*, 1861–1870.
16. Fodero-Tavoletti, M.T., Rowe, C.C., McLean, C.A., Leone, L., Li, Q.X., Masters, C.L., Cappai, R., and Villemagne, V.L. (2009). Characterization of PiB binding to white matter in Alzheimer disease and other dementias. J. Nucl. Med. *50*, 198–204.
17. Leube, D.T., Weis, S., Freymann, K., Erb, M., Jessen, F., Heun, R., Grodd, W., and Kircher, T.T. (2008). Neural correlates of verbal episodic memory in patients with MCI and Alzheimer's disease–a VBM study. Int. J. Geriatr. Psychiatry *23*, 1114–1118.
18. Schwindt, G.C., and Black, S.E. (2009). Functional imaging studies of episodic memory in Alzheimer's disease: a quantitative meta-analysis. Neuroimage *45*, 181–190.
19. Crystal, J.D. (2012). Animal models of human cognition. In Oxford Handbook of Comparative Evolutionary Psychology, J. Vonk, and T.K. Shackelford, eds. (Oxford University Press), pp. 261–270.
20. Crystal, J.D., and Glanzman, D.L. (2013). A biological perspective on memory. Curr. Biol. *23*, R728–R731.
21. Martin-Ordas, G., Berntsen, D., and Call, J. (2013). Memory for distant past events in chimpanzees and orangutans. Curr. Biol. *23*, 1438–1441.
22. Suddendorf, T., and Corballis, M.C. (1997). Mental time travel and the evolution of the human mind. Genet. Soc. Gen. Psychol. Monogr. *123*, 133–167.
23. Corballis, M.C. (2013). Mental time travel: a case for evolutionary continuity. Trends Cogn. Sci. *17*, 5–6.

24. Crystal, J.D. (2009). Elements of episodic-like memory in animal models. Behav. Processes *80*, 269–277.

25. Crystal, J.D. (2010). Episodic-like memory in animals. Behav. Brain Res. *215*, 235–243.

26. Crystal, J.D. (2013). Remembering the past and planning for the future in rats. Behav. Processes *93*, 39–49.

27. Crystal, J.D. (2016). Animal models of source memory. J. Exp. Anal. Behav. *105*, 56–67.

28. April, L.B., Bruce, K., and Galizio, M. (2013). The magic number 70 (plus or minus 20): variables determining performance in the rodent odor span task. Learn. Motiv. *44*, 143–158.

29. Bratch, A., Kann, S., Cain, J.A., Wu, J.E., Rivera-Reyes, N., Dalecki, S., Arman, D., Dunn, A., Cooper, S., Corbin, H.E., Doyle, A.R., Pizzo, M.J., Smith, A.E., and Crystal, J.D. (2016). Working memory systems in the rat. Curr. Biol. *26*, 351–355.

30. Zentall, T.R., Clement, T.S., Bhatt, R.S., and Allen, J. (2001). Episodic-like memory in pigeons. Psychon. Bull. Rev. *8*, 685–690.

31. Tulving, E. (1983). Elements of Episodic Memory (Oxford University Press).

32. Gallistel, C.R. (2009). The importance of proving the null. Psychol. Rev. *116*, 439–453.

33. Rouder, J.N., Speckman, P.L., Sun, D., Morey, R.D., and Iverson, G. (2009). Bayesian t tests for accepting and rejecting the null hypothesis. Psychon. Bull. Rev. *16*, 225–237.

Current Biology, Volume *26*

Supplemental Information

Rats Remember Items in Context Using Episodic Memory

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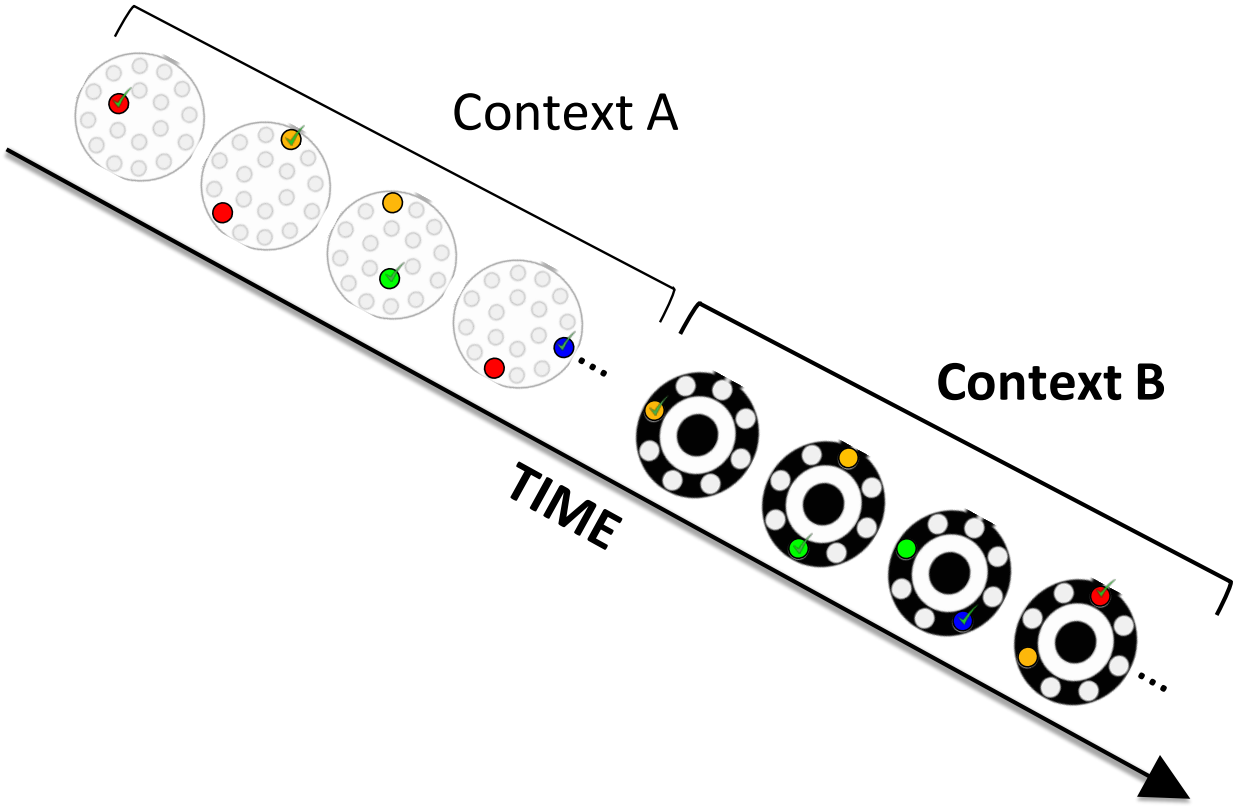

Figure S1. Preliminary training procedure. Schematic illustrates a subset of the olfactory new-old procedure presented across two contexts in rapid succession; colors represent odors. In Context A, after the first trial, the rat is presented with pairs of odors. In each pair, one odor is always new to the context (within the current session) and the other is always old (i.e., has already been rewarded in that context during the current session). Only responses to new items are rewarded (depicted by "√"). This process continues until all odors (i.e. items) have been rewarded in Context A. Next, the same odors were presented again in Context B (in a randomly selected order) and treated as "new" despite their earlier presentation in Context A. The first 4 of 16 trials are shown in the schematic. Figure S1 is related to main Figure 1.

Table S1. Overall accuracy p(S+ response | S+ vs. S- choice).

Number of context transitions	Overall Accuracy (Mean ± SEM)
1	0.91 ± 0.01
2	0.92 ± 0.01
3	0.88 ± 0.01
5	0.92 ± 0.02
15	0.90 ± 0.02

Overall accuracy is the mean accuracy of all trials conducted in each session, including memory assessments. Table S1 is related to main Figures 1 and 3.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Subjects

Twelve male Sprague-Dawley rats (Harlan, Indianapolis, IN; 80 days old, on average 297g at the start of the experiment) were housed individually and maintained on a 12-h light/dark cycle with light onset at 0715 and offset at 1915. The rats received 45-mg chocolate pellets (F0299; Bio-Serv, Frenchtown, NJ) during experimental sessions followed by 15 g/day of 5012-Rat-Diet (PMI Nutrition International, St. Louis, MO). Water was available ad libitum, except during testing sessions. All procedures followed national guidelines and were approved by the Bloomington institutional animal care and use committee at Indiana University. Scheduling conflicts, long interruptions in testing, and deterioration in baseline performance lead to: Two rats were not assessed in the unbaited probes. One rat was not assessed in Experiments 3-5. Two rats were not assessed in Experiments 4 and 5.

Apparatus

Two open-field arenas were used for odor presentation and served as the distinctive contexts. Arena A was circular in shape with a 94-cm diameter white floor and enclosed with a 30-cm high wall constructed from white acrylic plexiglass. Eighteen circular holes (5 cm diameter, 2.5 cm deep) were arranged in two concentric circles with 6 and 12 holes in the inner and outer rings, respectively. Arena B was circular in shape with a 46-cm diameter floor and a transparent 30-cm high wall. The floor of Arena B contained an array of 3 concentric circles that alternated in color. The innermost circle was black, the middle circle was white, and the outer circle was black. The inside of Arena B consisted of eight equidistant circular holes (5 cm diameter, 2.5 cm deep) positioned along the walls. Each condiment cup (59-ml) was firmly snapped in place inside a hole so that it lay flush with the floor and was covered with a plastic lid loosely placed on top. White noise was used throughout to mask outside noise. After each animal completed its daily session, both arenas were cleaned with 2% chlorhexidine solution.

Stimuli

Opaque plastic lids were used to present odors. Lids were odorized through a process of storing approximately 40 lids in sealed plastic containers, each filled with approximately 150 ml of a spice odorant or 90 ml of an oil odorant. To prevent direct contact in the containers, a metal grating separated the lids and the odorant. Lids were odorized for at least 2 weeks before being presented to the rats. In order to maintain scent consistency, odorants were refreshed approximately every 2 months. Odors included: Almond oil, amaretto oil, banana, asparagus, blueberry oil, brandy oil, butterscotch oil, caraway seed, celery seed, chicory root, cinnamon, coffee oil, cumin, dill weed, garlic powder, hickory smoke, honey oil, horseradish, Irish cream oil, lavender, lemon zest, maple, menthol-eucalyptus, Mexican oregano, mustard seed, onion powder, orange oil, pecan oil, pineapple oil, root beer oil, rosemary leaf, sage leaf, sesame oil, spinach powder, strawberry oil, summer savory, thyme, tomato, Mexican vanilla, and watermelon oil.

General Methods

A session occurred once per day, approximately 5 days per week. During testing sessions, a rat was removed from its home cage and placed in a holding cage, where it remained during inter-trial intervals. Holding cages were identical to the cages used in vivarium housing, except that bedding, food, and water were not available. In each session, a number of variables were randomly selected, including: the first context, the identity of the odors, and the location of the odors inside the arenas.

Odor Pre-training

In each session of phase one pre-training, a cup was placed in all locations of the first testing context and baited with a single chocolate pellet. The rat was then removed from the holding cage and placed in the arena where it remained until the chocolate pellets were located and consumed, or until 15 min elapsed, whichever occurred first. Immediately following pre-training in the first context, this same procedure was repeated in the other context. Once the rat could complete these sessions within 5 min, it was advanced to the next phase of pre-training.

The second phase of pre-training consisted of mock trial sessions. In these sessions, the rat was presented with a total of 30 mock trials, with 15 in each context. Each trial consisted of a single cup being placed in a randomly determined location and baited with a single chocolate pellet. The rat was removed from the holding cage and placed in the first arena where it remained until the rat located the baited cup and consumed the chocolate pellet, or approximately 2 min elapsed, whichever came first, at which point the rat was removed from the arena and placed in the holding cage. This procedure continued in the initial context for 15 trials, or until 15 min elapsed. The same mock trial procedure was repeated in the second context. Phase two pre-training sessions continued until the rat

completed all 30 mock trials in 30 min or less. Once the rat met this criterion, phase three pre-training began in the following session.

Phase three pre-training was the same as phase two pre-training, with the addition of unscented plastic lids for lid response shaping. Initially, in each trial one baited cup was placed in a random location in the arena with a lid positioned adjacent to the cup. In subsequent trials, the lid position was gradually adjusted so that the coverage was incremented until it reached 100%. As soon as the rat completed 30 trials at 100% coverage, Odor Span Training began in the following session.

Odor Span Training

During Odor Span Training (OST) sessions, 11 odors were randomly selected from a pool of 40 odors. Twenty-two trials made up each session, with 11 in each context. The first trial in each context consisted of one baited cup that was completely covered with an odorized lid. Next, the rat was placed in the arena and allowed to navigate until it located the odor, displaced the lid, and consumed the chocolate pellet, or 2 min elapsed. Once a response was made, or 2 min elapsed, the rat was removed from the arena and returned to the holding cage. The second trial in each context consisted of two odors in the arena, a new odor (referred to as a baited S+), and an unbaited re-presentation of the old odor that had already been presented within the current context in the current session (referred to as the S-). Thus, a correct response was defined as the first lid displacement directed toward an S+. An incorrect response was defined as a first response (lid displacement) to an S-. If the rat made an incorrect response, a correction procedure was implemented wherein the rat was allowed to continue to navigate the arena until a response to the S+ was made; selection of the S+ after selection of an S- was not included in calculations of accuracy. Although odors could appear as stimuli multiple times in each context per session, new lids were used in every trial to prevent the rat from using its own scent marking. For instance, in trial 2, the scented lid from the first trial (previously presented S+, now S-) was replaced with a new lid of the same odor, and placed in the arena along with the new S+. If the rat did not make a response within 2 min, the trail was scored as an error and repeated, but only with the S+ odor (i.e., without the corresponding S- odor(s)) and a new lid. If the rat failed to make a response during the repeat trial, OST trials were resumed. If the rat made two consecutive correct responses, the subsequent third trial continued to increment in this fashion such that it consisted of one new S+ placed in the arena along with the two previous S- odors. Trials continued to increment in this manner until an incorrect response was made or until 8 lids occupied the arena (one S+, seven S-). If the rat made an incorrect response, the number of stimuli in the arena was reset to one (only the S+) in the subsequent trial; this also reset the collection of presented S- odors. Incrementing continued with each subsequent correct response until another incorrect response occurred, a maximum of 8 stimuli occupied the arena, or all 11 odors had been presented as an S+ and rewarded in the each context. Given that each session consisted of 11 trials in each context and Arena B only contained eight cup locations, in trials that followed 8 consecutive correct responses in either context, we randomly selected the seven S- comparison stimuli from within the available odors. Immediately after all 11 odors had been rewarded in the first context, the same procedure was implemented in the second context, with the odors in a new randomly selected order. For instance, in a session with Context A and Context B as the testing order, immediately after all 11 items were presented in Context A, the same 11 odors were then presented as new (i.e., baited) in Context B, because each of the 11 items were new to that specific context. Fifteen sessions of OST were conducted.

Preliminary Training

Once the rat completed OST, two-alternative forced choice preliminary training began. This phase consisted of 32 trials per session, with 16 items in each context. The preliminary training procedure was similar to the OST, except that in preliminary training, every trial after the first trial consisted of two stimuli, one new S+ (odor that was new to the present context in the current session), and one old S- (odor previously rewarded in present context within the session; see Figure S1). The two-choice procedure continued in this manner until all 16 items had been rewarded in the first context. Immediately after all of the odors had been presented in the initial context, the same procedure was repeated with the same odors in the second context using a randomly selected order of odor presentation (Figure S1). Approximately 7 sessions were conducted. Terminal accuracy was 0.93 ± 0.01 (mean ± SEM) in the first context and 0.89 ± 0.01 in the second context.

Data Analysis: Experiments 1-5

Memory assessments that dissociated item-in-context memory from judgments of relative familiarity (Figure 2) were based on odor arrangements that met the following criteria: First, the S+ item in the memory assessment had previously been rewarded in the other context. Second, the S- item in the memory assessment had also been previously rewarded in the other context. Third, the S- item had not yet been presented as an S- earlier in

the current segment. Fourth, both the S+ and S- in the memory assessment had been rewarded in the other context, but the S- occurred before the S+ (see Figure 2B). Because the order of odors was randomly assigned for each segment, we searched all odors to identify items that met the above criteria.

To test the hypothesis that the number of rewarded presentations of an item impacts memory performance, we identified trials with odor arrangements that varied the number of times the S+ item was previously rewarded (see Figure 4). We compared accuracy in choosing a to-be-rewarded item after (1) that item was presented earlier in the day (i.e., it was rewarded in the other context), or (2) that item was absent (i.e., it had not yet been rewarded in the other context). We searched all odors in sessions with four segments (Experiment 2) to identify items that met the above criteria. Because the absence of evidence (from the traditional null hypothesis significance test) is not necessarily evidence for the absence of a reward-frequency effect, we used a Bayesian statistical approach. Bayesian statistics can be used to "prove the null hypothesis" [S1, S2], which in this case corresponds to the hypothesis that equivalent performance occurs when the number of rewarded presentations is varied. The prior hypothesis used the effect size derived from Figure 3A.

Experiment 1: Item-in-context Memory with Two Transitions

In preliminary training, correct selection of the "new" odor in the second context may be accomplished using memory for item in context, but it is necessary to rule out the use of non-episodic familiarity cues. Experiment 1 was designed to dissociate item-in-context episodic memory and non-episodic familiarity judgments. This was accomplished by subdividing the session into three segments (Context A→Context B→Context A). Experiment 1 was similar to preliminary training, except the rat only completed half of the trials in the first context before moving to the second context. In the second context, the rat completed all 16 trials. Thus, within those 16 trials, half of the items were new to the context, but half had also previously been presented in the first context. Note that in the second context, half of the odors were presented as new to both the session and the context (i.e., they had not yet been presented in the first context). Finally, once the rat completed the trials in the second context, it was returned to the first context where it finished the second half of the trials (i.e., the remaining 8 odors that had not yet been presented in the first context). Approximately 9 sessions were conducted.

Unbaited Probes:

In order to test for possible odor detection of the chocolate pellets in the baited cups, a series of unbaited probes were conducted. Unbaited trials were conducted during the last session of Experiment 1. For each rat, four probe trials were conducted in random order, with the constraints that at least one probe trial occurred in each context and within the following range of trials: 2-8 in the first context, 2-8 and 9-16 in the second context, and 9-16 in the first context. In probe trials, the new S+ was placed in the arena unbaited, along with the old comparison stimulus. Next, the experimenter manually delivered the chocolate pellet to the cup immediately following the rat's selection of the S+ lid. In unbaited probe trials, the proportion of S+ selection was 0.92 ± 0.05 (mean ± SEM), documenting high accuracy in the absence of food odors.

Experiment 2: Item-in-context Memory with Three Transitions

Experiment 2 was designed to test whether the rats remember items from both contexts. Experiment 2 sessions were conducted using the same procedure as Experiment 1, except that a third context transition occurred. In Experiment 1, it is possible for the rats to obtain high accuracy in the last segment (i.e., second half of the first context) by remembering events from the first context without remembering items in the second context. In Experiment 2, we used three context transitions to present odors across four segments (Context A→Context B→Context A→Context B). In this design, the rat was presented with half of the items from each context in each segment. Thus, the order of events was as follows: The rat completed the first half of the items in Context A, followed by the first half of items in Context B. Next, the rat was returned to the first context where the remaining half of the items were presented. Finally, the rat was returned back to the second context to complete the last segment with the remaining half of the items. Data analysis comes from the fourth segment. High accuracy would require memory of items that had been presented earlier in both contexts. Approximately 9 sessions were conducted.

Experiment 3: Item-in-context Memory with Five Transitions

Experiment 3 presented the rat with five context transitions (Context A→Context B→Context A→Context B→Context A→Context B). In each context, six items were presented in the first and second segments, followed by five items in each subsequent segment. Data analysis examined memory-assessment performance in the fifth and sixth segments. Approximately 8 sessions were conducted.

Experiment 4: Item-in-context Memory with Unpredictable Transitions

Experiment 4 was similar to the approach described above, except context transitions occurred at random. In other words, the probability of a transition in context (0.5) was equal from trial to trial for the entire session. The number of transitions per session in Experiment 4 ranged from eight to twenty-one. Approximately 8 sessions were conducted.

Experiment 5: Item-in-context with a 45-min Retention Interval Challenge

Experiment 5 was designed to test whether item-in-context memory could withstand a 45-min retention interval challenge. In Experiment 5, sessions were the same as those described in Experiment 2, except a 45-min retention interval occurred between the third and fourth segments. The rat was returned to its home cage outside of the testing room during the retention interval. Once the 45-min delay had elapsed, the rat was returned to the testing room to complete the final segment. Experiment 5 was conducted after Experiment 3. Approximately 5 sessions were conducted. The data from the 0-min delay come from Experiment 2.

SUPPLEMENTAL REFERENCES:

S1. Gallistel, C.R. (2009). The importance of proving the null. Psychol Rev 116, 439-453.

S2. Rouder, J.N., Speckman, P.L., Sun, D., Morey, R.D., and Iverson, G. (2009). Bayesian t tests for accepting and rejecting the null hypothesis. Psychon Bull Rev 16, 225-237.