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Rats answer an unexpected question after incidental encoding

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Summary

A fundamental aspect of episodic memory is that retrieval of information can occur when encoding is incidental and memory assessment is unexpected [1–4]. These features are difficult to model in animals because behavioral training likely gives rise to well-learned expectations about the sequence of events. Thus, the possibility remains that animals may solve an episodic-memory test by using well-learned semantic rules without remembering the episode at memory assessment. Here we show that rats can answer an unexpected question after incidental encoding in a hippocampal-dependent manner, consistent with the use of episodic memory. Rats were initially trained to report about a recent event (food vs. no-food) and separately searched for food where there was no expectation of being asked about the presence of food. To test episodic memory, rats were given the opportunity to incidentally encode the presence or absence of food and were unexpectedly asked to report about the recent event. Temporary inactivation of the CA3 region of the hippocampus with bilateral infusions of lidocaine selectively eliminated the ability of rats to answer the unexpected, but not the expected, question. Our studies suggest that rats remember an earlier episode after incidental encoding based upon hippocampal-dependent episodic memory.

Results and Discussion

Although events are not always known to be important when they occur, people can nonetheless remember details about such events. For example, eyewitness accounts rely on memories for incidental aspects of an earlier episode not known to be important when the episode occurred. Encoding occurs incidentally when apparently unimportant information is stored, but it is not known at the time of encoding that the information may subsequently be useful. By contrast, information is explicitly encoded when it is known that the information is needed later. When information is encoded for use in an upcoming, expected test of retention, it is possible that the explicitly encoded information is used to generate a planned action; according to this view, at the time of the test, the remembered action can occur successfully without remembering the earlier episode. Thus, it is difficult to determine if successful performance is based on a memory of the earlier episode or a planned action generated when information was explicitly encoded [1–4]. Indeed, it is possible that animals may have solved previous tests of episodic memory (e.g., [4–7]) by using well-learned semantic rules without remembering the episode. Formally, learned rules stored in semantic

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Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures.

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memory, a non-episodic memory system devoted to storing generic facts [8], could be used to generate a planned action. By contrast, when information is encoded incidentally, it is not possible to transform information into a specific action plan because the nature of the subsequent memory test is not yet known. Hence, accurate performance observed on an unexpected test after incidental encoding would suggest that this performance is based upon memory of the earlier episode (i.e., retrieval of an episodic memory) [1–4].

We developed a new behavioral technique for evaluating episodic memory in rats that relies on incidental encoding (see Supplemental Experimental Procedures), and thus, is not subject to potential problems of non-episodic memory alternatives. The key insight is that rats must retrieve memory of an earlier episode to “answer” (via its behavior) an unexpected question about information that was encoded incidentally [1–4]. Importantly, at encoding it is not known that the information is subsequently needed (i.e., encoding is incidental) or that it will be requested (i.e., the test is unexpected). We enabled incidental encoding by embedding two different tasks within the same radial maze (Figure 1). In one task, the rats foraged for food at multiple locations (5-arm radial maze task; Figure 1A). In a second task, the rats learned the “reporting” skill (T-maze task; Figure 1B) that would be used later in the unexpected question task. In the T-maze task, rats were rewarded for selecting a left/right turn after being presented with a sample of food or no-food, respectively. Because the animals received extensive training, the T-maze task involves explicit encoding for the purpose of answering an expected question. Thus, presentation of food or no food may generate an action plan to turn left/right. Formally, an action plan based on semantic memory of a rule (e.g., if food → turn left) may be formed, but subsequently, the animal may only remember the response (left turn) without remembering the study episode (food/no-food). Thus, successful performance on the T-maze task does not specifically implicate the use of episodic memory. The 5-arm task provided rats with an opportunity to search for food when there was no expectation of being asked about the earlier availability of food. When foraging, the rat may remember the visited or not-yet-visited locations [9, 10]. Because there is no expectation of being asked about the presence of food, there is no reason for the rat to specifically plan to turn left/right.

To generate incidental encoding, rats began foraging for food and then were unexpectedly confronted with the opportunity to report whether or not they recently encountered food (Figure 2A–B). Thus, a rat that incidentally encoded the availability of food would be able to successfully answer an unexpected question by retrieving a memory of the earlier episode. By contrast, a rat without episodic memory would be unable to answer an unexpected question after incidental encoding; hence, the probability of left and right turns should be equal in the absence of episodic memory.

We, therefore, asked whether rats can answer an unexpected question after incidental encoding. Next, we experimentally manipulated their ability to do so by temporarily inactivating the hippocampus, an anatomical substrate thought to be critical for episodic memory. To our knowledge, our study is the first to document that rats remember an earlier episode that was incidentally encoded and retrieved when unexpectedly requested.

Rats incidentally encode and unexpectedly retrieve an episodic memory

Terminal accuracy in 5-arm and T-maze tasks was 83% and 76%, respectively. To assess the ability of rats to answer an unexpected question, we allowed rats to forage for food in the 5-arm radial maze task, thereby affording the opportunity to incidentally encode either the presence (food probe, Figure 2A) or the absence (no-food probe, Figure 2B) of food. When rats were confronted with the opportunity to report in the T-maze task (via its left/right turn) whether it remembered encountering food in the 5-arm task (Figure 2A–B), they answered

the unexpected question with a level of accuracy similar to that observed in training (Figure 3A).

Temporary inactivation of the CA3 region of the hippocampus selectively impairs the ability to answer an unexpected question

The hippocampus is posited to be a critical processing center for episodic memory in humans [11–13] and episodic-like memory in non-human animals [14–16]. Moreover, the CA3 region is postulated to mediate short-term elements of episodic-memory processing [17–19]. To test the hypothesis that answering an unexpected question requires episodic memory, we asked whether it was similarly hippocampal-dependent (see Supplemental Experimental Procedures). If answering an unexpected question after incidental encoding requires episodic memory, then temporary inactivation of the hippocampus should selectively impair the ability of rats to answer an unexpected question without impacting the ability to answer an expected question. To assess accuracy in answering an unexpected question, we used a no-food probe described above (Figure 2B). To assess accuracy in answering an expected question, we used a control procedure (rotation probe, Figure 2C) that combined elements of the T-maze task while equating other features of the no-food probe. As in the T-maze task (but unlike the no-food probe), the rotation probe presented a no-food sample followed immediately by the opportunity to turn left or right. Thus, this control procedure can be solved by remembering a planned action (following the rationale outlined above for the T-maze task) without remembering the episode. To equate the control procedure with other aspects of the no-food probe, the rotation probe offered a no-food sample, and the sample was presented in the arm opposite to that used in training (i.e., rotated 180° with respect to the usual T-maze sample location); this rotation is equivalent to the average rotation in the no-food probe. Thus, the no-food and rotation probes (Figure 2B–C) varied the episodic-memory demands while equating rotation and absence of food.

Following training, stainless steel guide cannulae were implanted bilaterally above the CA3 region of the hippocampus to enable us to temporarily inactivate this region using infusions of lidocaine. Accuracy was reestablished following surgical recovery, demonstrating that surgery alone did not disrupt performance. We found that temporarily inactivating CA3 of the hippocampus selectively interfered with answering the unexpected question, but did not interfere with answering the expected question. Following local infusion of lidocaine bilaterally into CA3, accuracy in answering the unexpected question was significantly reduced relative to baseline (Figure 3B; $t(14) = -3.34$, $p = 0.002$, 1 tailed; tests for the impact of infusions were one tailed following the a priori prediction that hippocampal inactivation would reduce accuracy), whereas accuracy in answering the expected question was not impaired ($t(14) = -0.56$, $p = 0.29$, 1 tailed). The selective reduction of accuracy on unexpected questions could be specifically attributed to effects of lidocaine infusion because accuracy was not impaired relative to baseline by infusions of vehicle (Figure 3B; no-food probe: $t(14) = -0.55$, $p = 0.29$; rotation probe: $t(14) = -1.01$, $p = 0.16$; 1 tailed). Accuracy in answering an unexpected question was impaired by infusion of lidocaine relative to vehicle infusion ($t(14) = -3.06$, $p = 0.004$; 1 tailed). Moreover, the suppressive effect of lidocaine on memory retrieval was selective for unexpected questions. Accuracy was reduced in the unexpected-relative to expected-question conditions following lidocaine infusion ($t(14) = -1.87$, $p = 0.04$; 1 tailed). Importantly, impairment in answering the unexpected question was selective to inactivation of the hippocampus with lidocaine when an episodic memory needed to be retrieved. These findings cannot be attributed to the order of testing, because the order of probes and infusions was counterbalanced using a Latin Square design. When we restricted our analysis to the very first infusion probe, we observed the same pattern of selective impairment in accuracy; accuracy on the unexpected question with lidocaine was 0.25, which was lower than in the other infusion conditions, which were 0.80, 0.75, and 0.75

($t(15) = 2.01$, $p = 0.03$, 1 tailed). Finally, repeated infusion did not impair accuracy (excluding impaired performance in the unexpected question following lidocaine infusion) as shown by above chance accuracy on the final infusion (0.91 ± 0.09 , mean $\pm$ SEM; $t(10) = 4.50$, $p = 0.001$). Thus, the inactivation data suggest that the hippocampus is necessary to answer the unexpected question under conditions that varied expectations while equating other features. Histological analysis verified that the hippocampus was targeted bilaterally and that the center of the injection sites was localized to CA3 (Figure 3C–D).

Our study demonstrates that rats can answer an unexpected question after incidental encoding of an earlier episode. Inactivation of CA3 eliminated the ability of rats to answer the unexpected question but did not impair performance in answering the expected question. These observations strongly suggest that answering an unexpected question is hippocampal-dependent. We propose that rats needed to retrieve a memory of the recent episode (food/no-food) to accurately answer the unexpected question.

Rats may report the availability of food using either of two strategies. The T-maze task can be solved by a response-mediated strategy in which the rat makes a turning response after sample presentation (e.g., food $\rightarrow$ turn left). An alternative way to solve the task is to use a spatial-mediated strategy in which the rat navigates to a place on the maze after sample presentation (e.g., food $\rightarrow$ left side of maze). In T-maze training, these two strategies led to equivalent performance (i.e., they were confounded). By rotating the sample position in the probes, these two strategies were unconfounded, thereby dissociating response- and spatial-mediated strategies. Indeed, it has previously been shown that response- and spatial-mediated strategies are concurrently available and are mediated by different neural systems involving the hippocampus and striatum, respectively [20–23]; with extended training, rats shift from a hippocampal-dependent spatial strategy to a striatal-dependent response strategy [20–23]. Our data are consistent with the hypothesis that rats used a response-mediated strategy, as expected [20–23]. Performance on probes (excluding impaired performance in the unexpected question following lidocaine infusion) was significantly above chance with respect to a response-mediated strategy (0.77 ± 0.04 , mean $\pm$ SEM; $t(15) = 6.36$, $p < 0.0001$), which is simultaneously below chance performance with respect to a spatial strategy. We hypothesize that a rotation probe does not require episodic memory for two reasons. First, after the study phase, there is nothing unexpected about the test. Second, the study phase is identical to training (despite using a different start location) for a rat that relies on a response-mediated strategy; our rats received 318 (Figure 3A) and at least 504 (Figure 3B) trials in the T-maze task prior to any probe, by which point they are likely to rely on a striatal-response system [20–23]. Thus, the ability to solve the rotation probe was not expected to require an intact hippocampus because well-trained habits have previously been suggested to be striatal-dependent [21, 22].

It is unlikely that rats *expected* “unexpected” questions for three reasons. First, the rats received many 5-arm study phases that were not followed by an assessment of food/no-food. Second, right- and left-turn responses in the 5-arm task were equally likely to be rewarded given the random selection of arm baiting in the study phase of the 5-arm task. Third, hippocampal inactivation eliminated the ability to answer the unexpected but not the expected question. By contrast, the striatum, which is postulated to underlie habit learning, may mediate the ability of rats to answer the expected question [20–23]. Finally, although radial maze tasks use baited locations, eating is incidental to efficient navigation [24]. Moreover, a no-food probe is a particularly novel condition because earlier experience with foraging in 5-arm locations involved encountering food rather than the absence of food.

In conclusion, our results suggest that rats remember an incidentally encoded episode based upon hippocampal-dependent episodic memory. Our earlier work showed that, at the time of

a memory assessment, rats remember a specific earlier event including when in the past the event occurred, what happened, and where it took place [5]. In a previous study, we showed that rats can answer a question when unexpectedly tested in a different context, but a limitation of that work was that the information was not incidentally encoded [4]. Thus, it is possible that rats in the earlier study explicitly encoded information to be used at test, at which point they may have flexibly used that information. This concern does not apply to the present research because encoding was incidental. Moreover, temporary inactivation of the hippocampus, in the present research, allowed us to experimentally manipulate the ability of rats to answer an unexpected question after incidental encoding.

One benefit of studying cognition in animals is that it may provide insight into impairments in cognition observed in people. Cognitive impairments in people are debilitating, and developing insight into the origins of such impairments offers a tool to improve the effectiveness of treatments. Significant obstacles nonetheless impede the development of animal models of disordered cognition with both face and predictive validity. Although there is a long history of studying learning and memory in animals, these types of cognitive processes may not match those observed clinically (e.g., Alzheimer's disease features severe impairments in episodic memory [25–27]). Thus, it is possible that drug-development programs may identify agents effective at the pre-clinical level that subsequently fail when translated to a clinical trial in people. Ultimately, the expansion of the suite of cognitive processes that may be modeled in animals may translate to improved therapies for debilitating memory impairments observed in humans [28].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Rats answer an unexpected question after incidental encoding.
- Answering an unexpected question is hippocampal CA3 dependent.
- Rats remember an incidentally encoded episode based upon hippocampal-dependent episodic memory.

Figure 1. Schematic representation of experimental design of the 5-arm radial maze and T-maze tasks

(A) 5-Arm Task. Each rat was presented with a study (encoding) phase and a test (memory assessment) phase separated by a retention interval (1 trial/day). An example of the accessible arms in the study phase and corresponding test phase is shown. Accessible arms for each session were randomly selected for each rat. Grey shading in the figure highlights arms used in the 5-arm radial maze task. Doors to T-maze (shown in white) were closed. All arms of the actual maze were white. **(B) T-Maze Task.** Each rat was presented with a sample phase and a choice phase separated by an approximately 10-s retention interval. In the sample phase, each rat was either provided with food (6 pellets) or no food (0 pellets). In the choice phase, each rat was rewarded with 6 pellets by interrupting the photobeam after turning left or right. Food and no-food samples led to reward in opposite sides of the T maze (counterbalanced across rats). Six trials were conducted per day with a random sequence of food and no-food samples. Doors to the 5-arm radial maze were closed.

Figure 2. Schematic representation of experimental design of food probe, no-food probe and rotation probe

(A) Food and (B) no-food probes began with a study phase in the 5-arm-radial-maze using arms located 135°, 180° and 225° opposite to the sample arm. In the food probe, rats encountered one pellet at each of the three arms; whereas in the no-food probe, rats visited the three arms but received no food pellets. Next, two choice arms from the T-maze task were opened. (C) The rotation probe was identical to T-maze training (Figure 1B), except the sample was presented in the arm opposite to that used in T-maze training. A–C. All arms of the actual maze were white.

Figure 3. Rats answer an unexpected question after incidental encoding in a hippocampal-dependent manner

(A) Rats answered unexpected questions after incidentally encoding the presence or absence of food. Baseline data come from the first daily T-maze trial in the terminal 5 days before probe testing. (B) Temporary inactivation of CA3 of the hippocampus before memory storage impaired accuracy on the unexpected question relative to baseline but did not interfere with answering the expected question (rotation probe). Accuracy was selectively reduced by lidocaine in the unexpected probe relative to baseline and other probes. Baseline data come from the first daily T-maze trial in the 5 sessions before and 5 sessions after surgery. Each rat was tested once in each probe condition with the order determined by a Latin Square design. Error bars represent 1 SEM. * $p < 0.01$ difference between the unexpected + lidocaine probe and baseline. (C) Representative example of a Nissl-stained section showing bilateral infusion sites centered in the CA3 region of the hippocampus. Scale bar represents 500 μ m. (D) Coronal diagrams show, relative to bregma, locations of bilateral infusions for all rats.