EVALUATING KNOWLEDGE OF WHAT, WHEN, AND WHERE IN RATS: IMPLICATIONS FOR EPISODIC-LIKE MEMORY

by

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(Under the Direction of Jonathon D. Crystal)

ABSTRACT

Episodic memory was originally defined by Tulving (1972) as the recall of a personal, past event, and where and when the event occurred. A series of experiments using an 8-arm radial maze were designed to identify whether rats could discriminate what, when, and where. In each experiment, four forced locations were chosen to provide food (randomly chosen each trial; study phase). In Experiments 1, 2, and 3, three of these locations provided regular pellets, and one was randomly selected to provide a unique food (chocolate pellets). In Experiment 4, two locations provided regular pellets, and two randomly chosen locations each provided 3 pellets of a different unique food type (e.g., grape or raspberry pellets). The rats were then removed from the radial maze. After a short or long retention interval, the rats were returned to the maze, all 8 locations were available, and the locations that were not available in the study phase provided regular pellets (test phase). After the long retention interval, the unique location(s) also provided food. The rats made more revisits to the unique locations after the long retention interval than after the short retention interval. In Experiment 1, chocolate was paired with lithium chloride (LiCl) outside of regular testing. In subsequent trials using long retention intervals, the rats avoided the location that provided the devalued chocolate. This discrimination of what (food

type), when (long vs. short retention interval), and where (location of the randomly chosen arm for each trial) could have been based on (a) time of day, (b) failure to encode the location of the unique food, (c) an inherent strategy to revisit the chocolate location more after a long than after a short retention interval, (d) switching from a win-stay to a win-shift strategy after the long retention interval, and/or (e) failure to specifically encode the content of the unique location. These possible alternative explanations were tested by controlling for time of day, administering the LiCl during the retention interval (i.e., after encoding its location in the study phase), comparing revisits to the unique location(s) after the retention intervals during initial and terminal blocks of training, and using two unique flavors and selectively devaluing one unique food type while leaving the other unaffected. These alternative explanations were rejected in subsequent experiments. The data support the hypothesis that rats have detailed knowledge of what, when, and where, and provide evidence of episodic-like memory.

INDEX WORDS: What-when-where; Episodic memory; Episodic-like memory; Time of day

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CHAPTER 1

INTRODUCTION

Semantic memory and episodic memory are both forms of declarative memory, or memory that can be brought into conscious awareness. Separate, distinct semantic and episodic memory systems were originally proposed by Tulving (1972). Semantic memory is memory for general facts about the world, such as knowing that the earth is round. Episodic memory is a personal, unique, past-oriented memory system; for example, memories from one's own twelfth birthday party.

Tulving's (1972) original definition of episodic memory included spatial and temporal relations among events (i.e., what happened, where did it happen, and when did it occur). Although what, when, and where remain important elements to the definition of episodic memory, more recent additions focus on the subjective experiences that may accompany episodic memory recall (Tulving, 1983; Tulving & Markowitsch, 1998). These additions usually include three features: chronesthesia, autonoetic consciousness, and knowledge of a "self". Chronesthesia refers to the mental ability to travel in time, to relive a past event or to imagine a future event; autonoetic consciousness refers to the subjective re-enactment of a personal event; and knowledge of self refers to knowledge of one's particular mental states, including one's beliefs, desires, and sensations. These three features are believed to be required for mental time travel, or the ability to subjectively re-experience an event. These features are difficult, if not impossible, to test in non-human, non-verbal animals.

There are other potential challenges when testing episodic memory in animals. For example, some authors have agreed that non-human animals are bound to the present by their current motivational state and cannot anticipate future needs (referred to as the Bischof-Kohler hypothesis; Bischof, 1978; Bischof-Kohler, 1985; Suddendorf & Corballis, 1997). However, recent work with squirrel monkeys has shown they can anticipate future consequences of their choices (McKenzie, Cherman, Bird, Naqshbandi, & Roberts, 2004). The monkeys learned to switch their preferences from a larger amount of food to a smaller amount of food in anticipation of future pilfering or replenishing.

An important aspect of episodic memory is a concept of time. Roberts (2002) argued that representation of time of day, interval timing, and the decay of a memory trace do not require a concept of time as a continuous dimension with past, present, and future. Circadian rhythms and interval timing do not represent personal experiences backward into the past or forward into the future. Memory of the order of events, which has been documented in nonhuman animals (Devine et al., 1979; MacDonald, 1993; Parker, 1984; Shimp & Moffitt, 1974; Shimp, 1976; Weisman & DiFranco, 1981), can be based on discrimination of memory strength instead of a sense of time (Roberts, 2002).

Another difficult aspect to overcome when testing episodic memory in animals is that the strategy for solving a problem should not be rule-based (Zentall, Clement, Bhatt, & Allen, 2001). A task such as delayed match-to-sample that could be solved using the rule of relative familiarity (i.e., “choose the familiar stimulus”) would not be considered episodic memory.

Clayton, Bussey, Emery, and Dickinson (2003) argued that when testing episodic memory in animals, researchers should not attempt to focus on the subjective states involving knowledge of self and mental time travel, and should instead use behavioral criteria. These

behavioral criteria include content, structure, and flexibility. The content is knowledge of what, when, and where an event occurred (following Tulving's (1972) original definition). The structure refers to an integrated representation of what, when, and where. Memory should also show flexible use of information. Because these behavioral criteria do not include the subjective states of the animal, Clayton and colleagues referred to episodic memory in animals as "episodic-like".

Clayton and colleagues have conducted numerous studies with food-storing birds that demonstrate knowledge of what, when, and where (Clayton et al., 2003; Clayton, Bussey, & Dickinson, 2003; Clayton & Dickinson, 1998, 1999a, 1999b; Clayton, Griffiths, & Dickinson, 2000; Clayton, Griffiths, Emery, & Dickinson, 2001; Clayton, Yu, & Dickinson, 2001, 2003). Wild scrub jays naturally cache food over wide geographical areas, and have to remember those locations for days, weeks, and months in order to recover them. In Clayton and Dickinson's (1998) initial demonstration, captive scrub jays were given the opportunity to learn that a preferred food (wax worms) decayed after a long period of time, but peanuts (a less preferred food) did not decay. The birds first cached one of the two types of foods in either side of a caching tray (i.e., an ice cube tray filled with sand), and after a retention interval (RI) were allowed to cache the second food type in the other side of the tray. During a cache recovery period, the animals learned to recover either the wax worms or peanuts contingent upon the amount of time that had passed since caching (i.e., the status of the worms, palatable or decayed, depended on the amount of time since caching). If the worms had been cached recently (4 hrs. ago) and were still fresh, the birds recovered worms; if a long period of time (124 hrs.) had elapsed since the worms had been cached, and the worms were no longer palatable, the birds

recovered the peanuts. Therefore, the animals learned to discriminate what (food type), where (cache location), and when (retention interval).

To ensure the birds were not searching the peanut caches on the 124-hr period due to rapid forgetting of worm vs. peanut caches, another group of birds experienced a replenishment of fresh worms after 124 hours. Therefore, worms were always fresh during the recovery phase, and the birds never had the opportunity to learn that worms degrade over time. All the birds in this group directed their first inspection of the caching tray to the side of the tray that contained worms, after both 4 and 124 hours. This is evidence that the birds that had experience with decayed worms did not show a peanut-side preference due to differential forgetting of worm caches.

Roberts (2002) argued that the scrub jays may have learned that a weak memory trace for mealworm locations predicted decayed worms and that a strong memory trace predicted palatable worms; therefore, the birds may have based their recovery strategy on the rule, “If memory for worms is strong, recover worms; if memory for worms is weak, recover peanuts”. If the task can be solved using rule-based learning such as relative familiarity, this would not be an example of episodic-like memory. If the task can be solved based on the simple learning of rules, then there is no evidence that each episode is uniquely remembered.

In a related study, Clayton and Dickinson (1999a) allowed the birds to cache either peanuts or dog kibble. Next, the birds were satiated to either the peanuts or kibble (i.e., one food type was devalued) during the RI between caching and recovery. After both the long and short RIs, the birds directed their first searches to the tray that contained the non-devalued food. These data suggest that the birds inspected the trays based upon their ability to recall the contents of the caches and the current incentive value of the food. However, if the birds were using this kind of

rule during recovery, after the peanuts or dog kibble were devalued using satiation, the birds would not have preferentially searched for the non-devalued food type. Therefore, the satiation manipulation rules out relative familiarity.

Clayton and colleagues' studies with food-storing birds fulfill the behavioral criteria for episodic-like memory in animals: integrated knowledge of what, when, and where, and the flexibility to update their memory systems. However, because scrub jays are food-storing birds, they may have an adaptive specialization for remembering the temporal-spatial locations for food caches. A mammalian model of episodic memory is needed in order to better understand the neural, molecular, and behavioral mechanisms underlying disorders and diseases that exhibit a loss of episodic memory, such as Alzheimer's disease.

Clayton and colleagues' strategy for evaluating episodic-like memory in scrub jays was modified in a study with rats. A radial maze was used to evaluate discrimination of what, when, and where. Experiment 1 evaluated the feasibility of this approach using 1 and 4 hour retention intervals (RIs). In the study phase, the rats were given four forced choices, one of which always contained chocolate (randomly chosen for each trial). After a RI of 1 hour (short retention interval; SRI) or 4 hours (long retention interval; LRI), the rats were returned to the radial maze for a test phase, in which all 8 arms were accessible. After a SRI, only the four locations not available in the study phase provided food, and after the LRI, the four locations plus the chocolate location provided food (i.e., the chocolate location replenished). The rats showed knowledge of what (food type), when (SRI vs. LRI), and where (randomly chosen arm on the radial maze) by revisiting the chocolate location more after the LRI than after the SRI. Chocolate was then paired with lithium chloride (Batson, Best, Phillips, Patel, & Gilleland, 1986; Melcer &

Timberlake, 1985) outside of regular testing, which resulted in an acquired taste aversion to the chocolate food.

The rats reduced revisits to the chocolate location when tested in the LRI after the LiCl manipulation, which ruled out the possibility that the rats were basing their revisit strategy on relative familiarity. However, it was possible the rats were discriminating the "when" component based on time of day, or that the rats simply failed to encode the location of the chocolate in the study phase. If the rats based their revisit strategy on morning vs. afternoon using a circadian oscillator, this would not be a representation of the "when" component. If the rats failed to encode the location of the chocolate in the study phase because chocolate was devalued outside of regular testing, the rats would not be using an integrated representation of what, when, and where in the study phase.

Therefore, Experiment 2 controlled for time of day by using 1 (SRI) and 25 hour (LRI) RIs. The rats were unable to base their revisit strategy on morning vs. afternoon, because the test phase always occurred at the same time of day. In this study, the rats experienced chocolate paired with LiCl during the 25-hr RI, or after encoding the chocolate location in the study phase. The rats continued to display knowledge of what, when, and where. Therefore, the rats were not solving the "when" component using time of day, and they flexibly updated their memory system during the long retention interval, after encoding the location of the chocolate.

Experiment 3 showed that rats would transfer their revisit strategy after the LRI to grape (i.e., a new flavor), which suggests that their revisit strategy was not specific to the chocolate, but rather was based on a location that provided a unique food.

Experiment 4 evaluated the selectivity of episodic-like memory by using two unique foods, and devaluing one food type using satiation or LiCl, while not manipulating the value of

the second food type (i.e., the representation of the second food type was not devalued). In Experiments 1 and 2, there was the possibility that, with regards to the chocolate location, the rats were switching from a win-stay strategy to a win-shift strategy after the LiCl manipulation. However, if the rats specifically encode the content and location of both unique food types, they would selectively reduce revisits to the devalued unique location while maintaining a high number of revisits to the non-devalued unique location.

CHAPTER 2

LITERATURE REVIEW

Gallistel (1990) proposed that when an event occurs, animals record the time and place at which the event occurred, together with the features of the event. According to this proposal, the content of memory is a record of what occurred, when it occurred, and where it occurred. Memories of what, when, and where an event occurred may be recalled later and may form the basis for predicting future events (e.g., classical conditioning) according to Gallistel. Information about the temporal-spatial features of a specific event is one aspect of episodic memory.

Declarative (explicit) memory is made up of knowledge of facts (semantic memory) and memory of events (episodic memory). Semantic memory is a permanent memory store of general world knowledge. This includes the meaning of words, facts, knowledge about holidays, objects, famous people, etc. Memories of these facts do not represent memories of personally experienced events. In contrast, episodic memory centers on remembering personally experienced events. Memories of these events include components of what (content), where (spatial information), and when (the time or date the event occurred).

Tulving (1972) originally distinguished between episodic memory (the encoding and retrieval from memory of unique, personal past experiences) and semantic memory. This early distinction focused on the difference between personally experienced events and knowledge of general facts about the world. In particular, Tulving (1972) proposed that “Episodic memory

receives and stores information about temporally dated phases or events, and temporal-spatial relations among these events" (p. 385).

Although knowledge of what, when, and where remains an important component of episodic memory (Nyberg, et al., 1996), it is not the only feature of episodic memory. In particular, Tulving and colleagues (e.g., Tulving, 1983; Tulving & Markowitsch 1998) have focused on three additional features of episodic memory: the ability to recognize subjective time, autonoetic consciousness, and knowledge of a "self", all of which are deemed necessary for mental time travel (i.e., the ability to subjectively re-experience an event). According to this proposal, these features distinguish between recalling a personal past experience and remembering an impersonal fact. However, these attributes are difficult, if not impossible, to test in non-verbal animals (Hampton & Schwartz, 2004; W.A. Roberts, 2002; Suddendorf & Busby, 2003; Suddendorf & Corballis, 1997).

In humans, episodic memory is the first kind of memory to degenerate with age, and the last to be acquired as a child (Tulving, 2002). Patients with neurodegenerative diseases such as Alzheimer's show a loss of episodic memory. The hippocampus plays an important role in the acquisition of new memories; it incorporates temporal information from the frontal lobes, thus providing a basis for "when" (Burgess, Maguire, & O'Keefe, 2002). The hippocampus has been implicated in spatial memory tasks, and may provide a basis for "where" (O'Keefe & Nadel, 1978). The right hippocampus processes spatial locations, while the left hippocampus is involved in episodic memory (Burgess, et al., 2002; Maguire, 2001). The left and right prefrontal cortices are implicated in encoding and retrieval of episodic memory, respectively (Tulving, 2002).

Development of a rodent model for episodic memory could lead to a better understanding of the neural, molecular, and behavioral mechanisms of episodic memory (Griffiths & Clayton,

2001). The availability of a rodent model could also permit the screening of putative pharmacotherapies for human memory disorders such as Alzheimer's (Clayton & Griffiths, 2002).

As stated previously, aspects of the new definition of episodic memory (Tulving, 1983; Tulving & Markowitsch, 1998) are difficult or impossible to test in non-verbal, nonhuman animals. There are also other criticisms and problems to consider when testing episodic memory in animals.

For example, Suddendorf and Corballis (1997) refute the idea that animals possess episodic memory on the basis that there is no solid evidence for the preconditions of human episodic memory (i.e., meta-representation and sense of self). They believe future planning in chimpanzees, such as tool creation and carrying stones to a location that has no stones for cracking nuts, is based on present motivational states instead of true anticipation of the future. The authors support the Bischof-Kohler hypothesis (Bischof, 1978, 1985; Bischof-Kohler, 1985), which suggests that non-human animals, specifically apes, cannot anticipate future needs and are bound to the present, which is defined by their current motivational state. However, recent studies with squirrel monkeys (McKenzie, Cherman, Bird, Naqshbandi, & Roberts, 2004) have suggested that squirrel monkeys may be able to anticipate events at least 15 minutes into the future.

Suddendorf and Corballis (1997) agree with Tulving's (1983, 1984, 1985) hypothesis that episodic memory evolved after a primitive form of semantic memory, which then allowed this primitive semantic memory system to develop more fully. The authors base this argument on the evidence that young children and non-human animals possess a semantic memory system but do not seem to have an episodic memory system. They state the preconditions to mental time travel

may have evolved as a result of the pressures of a complex social structure. The emergence of “multiple monitoring” in primates and later Homo sapiens may be correlated both with neurological evolution and the development of language. Language and mental time travel both make use of more general attributional, dissociative, and generative abilities, and language is the ideal method for recalling episodic memories.

An important aspect of episodic memory is a concept of time. Roberts (2002) argues that a representation of time of day, short-interval timing, and a decaying memory trace do not require a concept of time as a continuous dimension with past, present, and future. Circadian rhythms and interval timing do not represent personal experiences backward into the past or forward into the future.

If the location of two or more events in time can be remembered, their order can be inferred, and the ability to remember order of events in time is critical to episodic memory.

There is evidence that animals can remember the order of events (Shimp & Moffitt, 1974; Shimp, 1976, Weisman & DiFranco, 1981; MacDonald, 1993; Devine et al., 1979; Parker, 1984), but Roberts argues that the animals could have performed this discrimination on the basis of memory strength.

Zentall, Clement, Bhatt, and Allen (2001) have emphasized another feature of episodic-like memory. In particular, it should be possible to answer an unexpected question based on episodic memory. Zentall and colleagues argued that the discrimination of what-when-where could be based on the acquisition of a set of explicitly trained if-then rules; the rules could be applied when a food item is initially encountered. To avoid the concern, Zentall and colleagues trained pigeons to respond differently after having pecked or not pecked (i.e., nonverbally answering the question "Did you just peck or refrain from pecking?"). In a separate

discrimination, the pigeons were placed in a situation in which they pecked one stimulus but not another, without requiring them to do so. Next, the pigeons were given the opportunity to report whether they had pecked or not. The data suggest that the pigeons could remember a specific detail about their past experience, and were not using rule-based learning to solve the task.

Clayton and colleagues (Clayton, Bussey, & Dickinson, 2003; Clayton, Griffiths, & Dickinson, 2000; Clayton, Griffiths, Emery, & Dickinson, 2001) have argued that behavioral studies of episodic memory should focus on knowledge of what, when, and where as criteria for episodic-like memory in animals.

Studies with food-storing birds (Clayton & Dickinson, 1998, 1999a, 1999b; Clayton, Yu, & Dickinson, 2001, 2003) have shown that scrub jays can remember what type of food they cached, where they cached it, and when they cached it; there is evidence that food-storing birds have an adaptive specialization of memory (e.g., Pravosudov & Clayton, 2002; Shettleworth, 1998). If characterizing episodic memory in animals is based on the information encoded rather than the subjective experience that accompanies the memory, then there is evidence that scrub jays possess episodic-like memory.

Scrub jays were given the opportunity to cache different food types and recover them after a retention interval (RI; Clayton & Dickinson, 1998). Birds in the degrade group were trained to expect that wax worms, a preferred food, decayed after a delay of 124 hr, whereas peanuts, a less preferred food, did not. The birds were then allowed to cache either peanuts or wax worms on one side of a visuospatially distinct tray. After a retention interval of 120 hr, the birds were then allowed to cache the second food type in the opposite side of the tray. Four hours later, the birds were allowed to recover food from one side of the tray. The birds searched for the food type that was preferred only if it was still fresh (i.e., birds recovered more worms when they

cached peanuts before worms, but more peanuts were recovered when the birds had cached worms before peanuts).

To ensure that the different search strategies after different delays were not due to different levels of forgetting of worm and peanut caches (i.e., relative familiarity), a replenish group was created, in which the birds never had the opportunity to learn that the wax worms decay after long periods of time. Unlike the birds in the degrade group, the birds in the replenish group inspected the worm side first in all delay conditions.

In the wild, the longer caches are left without being recovered, the more likely they are to be stolen by another animal. Therefore, a pilfer group was trained to expect that wax worms had been removed from the tray after 124 hr. The pilfer group directed more of their first inspections to the peanut side of the tray if worms were cached long enough to be pilfered.

These data suggest scrub jays can remember what type of food they cached, where in the tray they cached, and when they cached it. The same conclusion was reached in a similar study that used three food types that became unavailable (degraded or pilfered) after three RIs (Clayton, Yu, & Dickinson, 2001).

Clayton, Yu, and Dickinson (2003) provided evidence that information about the status of a stored food type can be updated after the food has been cached. Clayton and colleagues gave scrub jays the opportunity to store crickets and peanuts and recover them after RIs of 2, 3, and 5 days. For one group of birds, the crickets degraded after 2-3 days but not after 5 days; for another group, the stored food was always fresh at recovery. As in previous studies, this experience resulted in a preference for peanuts after the longest delay for birds that had experience with the decayed crickets. Next, the ability to flexibly update memory about the stored food was assessed by introducing new information during the RI. The birds cached

peanuts and crickets in three trays across successive days and recovered their caches from the first two trays after a 3-day RI. For one group, the crickets remained fresh and palatable; for the second group, the crickets decayed. The birds that had found fresh crickets in the first two trays continued to search for crickets in the third tray. However, the birds that had found decayed crickets in the first two trays searched preferentially for peanuts in the third tray. These data suggest that the birds integrated information about the cached food with new information presented during the RI.

Clayton and Dickinson (1999a) provided additional evidence that scrub jays could remember the types of food that they cached. The motivation to consume a particular food type was manipulated between the time of caching and recovery by prefeeding the birds with one of two food types. The birds cached peanuts and dog kibble, and they were then prefed one of the foods after a retention interval (4 or 172 hr). After both RIs, the birds directed their first searches to the tray that contained the food they had not been prefed. These data suggest that the birds inspected the trays based upon their ability to recall the contents of the caches and the current incentive value of the food.

To determine if the birds could remember which type of food had been recovered, they were allowed to cache 3 peanuts on one side of each tray, and 3 dog-food kibbles on the other side of each tray. After a 3-hr RI, the birds were given two recovery phases in which they were allowed to recover kibble from one tray and peanuts from the other tray. The birds were then prefed one of the food items and then presented with both caching trays. The birds directed their searches to the tray that still contained the preferred (i.e., non-prefed) food. Therefore, the birds were able to encode information about the type of food they cached, update their memory of

whether or not the cache site contained food, and integrate the information about the content of the cache with the incentive value of the food at recovery.

Another aspect of episodic memory involves encoding of unique events. For food-caching animals, retrieval of the food cache depends on a single, brief encoding event. Clayton and colleagues placed distinctive Lego structures at each caching tray to provide unique encoding events (Clayton & Dickinson, 1998, 1999a, 1999b; Clayton, Yu, & Dickinson, 2001, 2003). In a study with nonfood-storing animals, rats were provided with trial-unique objects in each arm of the radial maze (Babb & Crystal, 2003). However, the rats navigated with respect to a representation of spatial locations and did not follow trial-unique cues.

Babb and Crystal (2005) documented that the rats could discriminate what, when, and where in the 8-arm radial maze. Daily testing was divided into forced-choice and free-choice phases, respectively, and separated by either a short (0.5-hr) or long (4-hr) retention interval (RI). In the forced-choice phase, one randomly selected arm was always baited with a chocolate pellet, a preferred food type. The rats were trained to discriminate between a short- and long-RI. After the short retention interval (SRI), the four locations not seen in the forced-choice phase provided food. On days with a long retention interval (LRI), the free-choice phase was identical to the SRI condition, except that the chocolate arm replenished. On any given day, either SRI or LRI (but not both) was tested. Rats revisited the chocolate location more after the LRI than after the SRI, suggesting that they remembered where in the radial maze the chocolate was placed that day, and when, or how long ago, they had seen it (i.e., what, where and when).

Chocolate was then paired with lithium chloride (LiCl) in an attempt to change a rat that revisited the chocolate location after the LRI to a rat that did not revisit the chocolate location after the LRI. This manipulation was conducted to ensure that the rats were not using relative

familiarity to guide their behavior. For example, the rats could have solved the task using a conditional rule: do not revisit if familiarity with chocolate is high, and revisit if familiarity with chocolate is low. The rats revisited chocolate less in the LRI after chocolate was paired with LiCl than in the LRI before chocolate was paired with LiCl. The conditional rule predicted revisits to chocolate at a high rate, rather than the observed low rate. Therefore, the authors proposed that knowledge of what, when, and where as the interpretation for selective revisits to chocolate before LiCl and the decline in visits to chocolate after LiCl.

In the previous study, the rats could have been discriminating the "when" component using time of day (Hampton & Schwartz, 2005). Two types of timing mechanisms may be proposed to discriminate between the short and long retention intervals. The rats may have used an interval timing mechanism (Gibbon, 1991) to discriminate 0.5 and 4 hr. Alternatively, the rats may have used a circadian oscillator (Mistlberger, 1994) to discriminate time of day (e.g., morning vs. afternoon).

The observation of what-when-where information contrasts with other recent reports with rats. W. A. Roberts and S. Roberts (2002) assessed the ability of rats to remember the order in which they entered arms on a radial maze. The rats were unable to discriminate the first arm they encountered from the other arms. Bird, W. A. Roberts, Abroms, Kit and Crupi (2003) induced rats to carry food items (cheese and pretzels) from the center of a radial maze to boxes at the end of each arm; the rats were removed from the maze arm before the food was fully consumed. The rats hid and retrieved cheese preferentially over pretzels. However, the rats were insensitive to pilfering of the food. When Bird and colleagues degraded cheese at one delay interval but not the other, the rats did not selectively avoid locations with the degraded food. Taken together, the experiments by W. A. Roberts and colleagues provide evidence for

knowledge of what type of food they hid and where they hid it, but no evidence that rats remembered when they hid it.

In contrast, Eichenbaum and colleagues (e.g., Ergorul & Eichenbaum, 2004; Fortin, Agster, & Eichenbaum, 2002) have documented that rats quickly learn the sequence of randomly ordered odors. After being presented with a sequential order, the rats learned to select an odor that had not been presented in the sequence. The rats were then divided into hippocampal lesion or sham groups. The rats with the hippocampal lesions performed as well as the control rats on an odor recognition task, but were impaired on tests of sequential order. These data are consistent with the hypothesis that the hippocampus is involved in memory for sequences of events. Eichenbaum and Fortin (2003) argued that memory for the sequential order of unique events provides a model for episodic memory.

Studies with nonhuman primates have not yielded much evidence for memory of what, when, and where. Tinklepaugh (1932) studied where (spatial location) and what (food type) memories using single-trial learning with juvenile chimpanzees. He hid food in one of two containers and made the chimps wait long intervals before recovering them. In some instances Tinklepaugh replaced the food in the container with a more or less desirable food, and noted that the chimpanzees seemed surprised when they discovered the new food in the container. However, these results are difficult to interpret because the chimpanzees demonstrated the same behavior when they made an error and did not receive food.

Menzel (1973) demonstrated single-trial “where” learning with chimpanzees. He hid 18 pieces of food in an open enclosure while one of 4 chimpanzees watched. After a 2-min RI, all 4 chimpanzees were released into the enclosure, and the chimpanzee that had observed the food

being hidden found the majority of the food, and in a second experiment the chimp recovered the more desirable food first, suggesting it remembered “what” information as well as “where”.

Menzel (1999) used the Clayton-Dickinson model of episodic memory with a chimpanzee that had received extensive language training and could communicate about many different objects using a lexical keyboard. An experimenter again hid foods and different objects outside the enclosure and in view of the chimpanzee. The chimpanzee was then moved inside and the caretakers noted the spontaneous communications the chimpanzee made with them. Even after 16 hours, the chimp could indicate both memory for the food type (what) and could point in the general location it was hidden (where). However, the chimp was not required to discriminate between any intervals, so this study contained no “when” component.

Savage-Rumbaugh (1999) found that a language-trained bonobo could remember “who” memories, “what” memories, and “where memories”. MacDonald and Agnes (1999) used single-trial spatial-memory tests to document that orangutans could remember “where” information.

An adult gorilla recalled “what” and “who” information using picture cards after both short and long RI (Schwartz, Colon, & Sanchez, 2002). This study demonstrates gorillas may be capable of exhibiting episodic-like memory, but like the previous primate studies, the what-when-where components have not been simultaneously demonstrated.

Recently, Hampton, Hampstead, and Murray (2005) showed that rhesus monkeys could not learn to search for preferential food based on both retention interval and spatial location.

It is possible that non-human primates have episodic-like memory, although none of the current studies show evidence of retrieval of multiple components, and do not fully meet the what-when-where criteria.

Knowledge of what, when, and where is a necessary condition for the establishment of episodic memory. However, it is not a sufficient condition. For example, in the what-when-where study with rats (Babb & Crystal, 2005), there is no information about the subjective state of the animal when the animal makes a decision to revisit the chocolate location. There is no method to know if the animal is engaged in mental time travel or if it is subjectively re-experiencing the previous event of finding chocolate. Therefore, it is possible that a three-way conditional discrimination of what, when, and where occurs in the absence of any accompanying subjective states. Clayton, Bussey, Emery, and Dickinson (2003) have argued that reconstructive generative processes and meta-representations at retrieval should not be considered necessary criteria for episodic memory.

Clayton, Bussey, and Dickinson (2003) have agued that behavioral criteria for episodic-like memory include content, structure, and flexibility. According to this proposal, the content is a recollection of what occurred, where it occurred, and when it occurred based on a specific past experience. The structure is an integrated what-where-when representation. Flexibility proposes that episodic memory is embedded within a declarative-memory framework that involves flexible use of information.

Babb and Crystal's (2005; *in press*) studies meet Clayton, Bussey, and Dickinson's (2003) content criterion. Clayton, Yu, and Dickinson (2001) have demonstrated that scrub jays can distinguish between multiple episodes in which they cached the same food type in different locations; these data suggest that what-when-where information is integrated. Clayton, Yu, and Dickinson (2003) have demonstrated that when scrub jays are presented with new information about the perishability of worms after having stored the worms, the birds switched their preferences for worms and peanuts accordingly; these data suggest flexibility in updating

information after the time of encoding. Additional research with rats will be needed to assess the integrated and flexibility criteria. One approach toward addressing these questions is to satiate the rats to a specific food type during the RI (Colwill & Rescorla, 1985; Balleine & Dickinson, 1998).

Roberts (2002) offered a rule-based criticism of Clayton and colleagues' demonstration of episodic-like memory. In particular, Roberts argued that the scrub jays might have learned that a relatively weak memory trace for mealworm locations predicts decayed worms and that a relatively strong memory trace predicts palatable worms. A conditional rule can be constructed to describe Babb and Crystal's rat studies, applying Roberts's explanation. In particular, the memory trace for a chocolate location would be relatively strong after a short RI and relatively weak after a long RI. Therefore, the rat could adopt the rule, "if the memory of the chocolate location is weak, revisit that location." However, the use of this rule does not predict the data observed after LiCl treatment. Testing after the LiCl treatment used a long RI. Therefore, application of the rule outlined above would predict revisits at a high rate, rather than the observed low rate. LiCl treatment may change the memory representation of chocolate (e.g., Trapold & Overmier, 1972). However, if LiCl treatment affects the strength of the memory trace, it is not clear whether the memory trace is strengthened or weakened.

As mentioned previously, episodic memory is the first kind of memory to degenerate with age (Tulving, 2002). Patients with neurodegenerative diseases such as Alzheimer's disease show a loss of episodic memory. Development of a rodent model for episodic memory could lead to a better understanding of the neural, molecular, and behavioral mechanisms of episodic memory (Griffiths & Clayton, 2001). The availability of a rodent model could also permit the

screening of putative pharmacotherapies for human memory disorders such as Alzheimer's (Clayton & Griffiths, 2002).

CHAPTER 3

DISCRIMINATION OF WHAT, WHEN, AND WHERE: IMPLICATIONS FOR EPISODIC-LIKE MEMORY IN RATS[1]

[1] Babb, S.J., & Crystal, J.D. (2005). *Learning and Motivation, 36,* 177-189.
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ABSTRACT

We investigated discrimination of what, when, and where in rats (n=6) on the radial maze, and controlled for time of day. Phase 1 consisted of four choices, one of which contained chocolate. In Phase 2, all eight arms were available. After a short (1 hour) retention interval (SRI), the four arms not available in Phase 1 provided food. After a long (25-h) retention interval, the four arms, plus the chocolate arm, provided food. The rats visited chocolate more after the LRI than after the SRI. Chocolate was then paired with lithium chloride after Phase 1, during the LRI. Following the taste-aversion manipulation, the rats visited chocolate after the LRI less often than before LiCl. These data demonstrate knowledge of what, when, and where that cannot be based on time of day. The data also suggest flexibility to update memory based on information acquired in a new situation.

EXPERIMENT 1

Gallistel (1990) proposed that when an event occurs, animals record the time and place at which the event occurred, together with the features of the event. According to this proposal, the content of memory is a record of what occurred, when it occurred, and where it occurred. Memories of what, when, and where an event occurred may be recalled later and may form the basis for predicting future events (e.g., classical conditioning) according to Gallistel. Information about the temporal-spatial features of a specific event is one aspect of episodic memory.

Tulving (1972) distinguished between episodic memory (the encoding and retrieval from memory of unique, personal past experiences) and other types of memory. This early distinction focused on the difference between personally experienced events and knowledge of general facts about the world. In particular, Tulving (1972) proposed that "Episodic memory receives and stores information about temporally dated phases or events, and temporal-spatial relations among these events" (p. 385).

Although knowledge of what, when, and where remains an important component of episodic memory (Nyberg, et al., 1996), it is not the only feature of episodic memory. In particular, Tulving and colleagues (e.g., Tulving, 1983; Tulving & Markowitsch 1998) have focused on three additional features of episodic memory: the ability to recognize subjective time, autonoetic consciousness, and knowledge of a "self", all of which are deemed necessary for mental time travel (i.e., the ability to subjectively re-experience an event). According to this proposal, these features distinguish between recalling a personal past experience and remembering an impersonal fact. However, these attributes are difficult, if not impossible, to test

in non-verbal animals (Hampton & Schwartz, 2004; W.A. Roberts, 2002; Suddendorf & Busby, 2003; Suddendorf & Corballis, 1997). Consequently, Clayton and colleagues (Clayton, Bussey, & Dickinson, 2003; Clayton, Griffiths, & Dickinson, 2000; Clayton, Griffiths, Emery, & Dickinson, 2001) have argued that behavioral studies of episodic memory should focus on knowledge of what, when, and where as criteria for episodic-like memory in animals.

Studies with food-storing birds (Clayton & Dickinson, 1998, 1999a, 1999b; Clayton, Yu, & Dickinson, 2001, 2003) have shown that scrub jays can remember what type of food they cached, where they cached it, and when they cached it; there is evidence that food-storing birds have an adaptive specialization of memory (e.g., Pravosudov & Clayton, 2002; Shettleworth, 1998). If characterizing episodic memory in animals is based on the information encoded rather than the subjective experience that accompanies the memory, then there is evidence that scrub jays possess episodic-like memory.

Scrub jays were given the opportunity to cache different food types and recover them after a retention interval (RI; Clayton & Dickinson, 1998). Birds in the degrade group were trained to expect that wax worms, a preferred food, decayed after a delay of 124 hr, whereas peanuts, a less preferred food, did not. The birds were then allowed to cache either peanuts or wax worms on one side of a visuospatially distinct tray. After a retention interval of 120 hr, the birds were then allowed to cache the second food type in the opposite side of the tray. Four hours later, the birds were allowed to recover food from one side of the tray. The birds searched for the food type that was preferred only if it was still fresh (i.e., birds recovered more worms when they cached peanuts before worms, but more peanuts were recovered when the birds had cached worms before peanuts).

To ensure that the different search strategies after different delays were not due to different levels of forgetting of worm and peanut caches (i.e., relative familiarity), a replenish group was created, in which the birds never had the opportunity to learn that the wax worms decay after long periods of time. Unlike the birds in the degrade group, the birds in the replenish group inspected the worm side first in all delay conditions.

In the wild, the longer caches are left without being recovered, the more likely they are to be stolen by another animal. Therefore, a pilfer group was trained to expect that wax worms had been removed from the tray after 124 hr. The pilfer group directed more of their first inspections to the peanut side of the tray if worms were cached long enough to be pilfered.

These data suggest scrub jays can remember what type of food they cached, where in the tray they cached, and when they cached it. The same conclusion was reached in a similar study that used three food types that became unavailable (degraded or pilfered) after three RIs (Clayton, Yu, & Dickinson, 2001).

Clayton, Yu, and Dickinson (2003) provided evidence that information about the status of a stored food type can be updated after the food has been cached. Clayton and colleagues gave scrub jays the opportunity to store crickets and peanuts and recover them after RIs of 2, 3, and 5 days. For one group of birds, the crickets degraded after 2-3 days but not after 5 days; for another group, the stored food was always fresh at recovery. As in previous studies, this experience resulted in a preference for peanuts after the longest delay for birds that had experience with the decayed crickets. Next, the ability to flexibly update memory about the stored food was assessed by introducing new information during the RI. The birds cached peanuts and crickets in three trays across successive days and recovered their caches from the first two trays after a 3-day RI. For one group, the crickets remained fresh and palatable; for the

second group, the crickets decayed. The birds that had found fresh crickets in the first two trays continued to search for crickets in the third tray. However, the birds that had found decayed crickets in the first two trays searched preferentially for peanuts in the third tray. These data suggest that the birds integrated information about the cached food with new information presented during the RI.

Clayton and Dickinson (1999a) provided additional evidence that scrub jays could remember the types of food that they cached. The motivation to consume a particular food type was manipulated between the time of caching and recovery by prefeeding the birds with one of two food types. The birds cached peanuts and dog kibble, and they were then prefed one of the foods after a retention interval (4 or 172 hr). After both RIs, the birds directed their first searches to the tray that contained the food they had not been prefed. These data suggest that the birds inspected the trays based upon their ability to recall the contents of the caches and the current incentive value of the food.

To determine if the birds could remember which type of food had been recovered, they were allowed to cache 3 peanuts on one side of each tray, and 3 dog-food kibbles on the other side of each tray. After a 3-hr RI, the birds were given two recovery phases in which they were allowed to recover kibble from one tray and peanuts from the other tray. The birds were then prefed one of the food items and then presented with both caching trays. The birds directed their searches to the tray that still contained the preferred (i.e., non-prefed) food. Therefore, the birds were able to encode information about the type of food they cached, update their memory of whether or not the cache site contained food, and integrate the information about the content of the cache with the incentive value of the food at recovery.

The present study was designed based on Clayton and colleagues' approach to episodic-like memory. The purpose of the present experiment was to document knowledge of what, when, and where using rats as subjects, without relying on food caching. Rats were trained to find a food pellet at each of eight arms in a radial maze (Olton & Samuelson, 1976); one of the arms, randomly selected, was baited with a chocolate pellet, a preferred food type. Daily testing was divided into forced-choice (which always included chocolate) and free-choice phases, separated by a retention interval. The rats were trained to discriminate between a short and long retention interval. After the long retention interval, the chocolate replenished (i.e., revisiting the chocolate location resulted in a pellet). After the short retention interval, the chocolate bait did not replenish. After the animals had learned to selectively revisit the chocolate location following a long retention interval, chocolate was paired with lithium chloride (LiCl; Batson, Best, Phillips, Patel, & Gilleland, 1986; Melcer & Timberlake, 1985). The goal was to change a rat that revisited the chocolate location into a rat that did not revisit the chocolate location. By not revisiting the chocolate location after LiCl treatment, the rats would demonstrate knowledge of what, when, and where.

METHOD

Subjects

Five male Long Evans rats (Harlan, Madison, WI) were individually housed in a colony with light onset at 0700 and offset at 1900. The rats were given unlimited access to 5001 Rodent Diet (Lab Diet, Brentwood, MO) for one week, and then given 20 g for one day and 15 g per day on subsequent days. Water was available at all times, except during brief testing periods. The rats

were approximately 6 months old at the beginning of the experiment with an average weight of 326 g. The rats had served (69 days) in a previous, related study (Babb & Crystal, 2003). In addition, the rats received pilot testing to expose the rats to the contingency that chocolate replenished on multiple visits. The animals were given several two-alternative forced choices between chocolate, new, and old locations. Because the rats continued to make only a single revisit to the chocolate location, the procedure described below required only a single revisit to the chocolate location.

Materials

Testing was conducted in an eight-arm radial maze. The central hub (white polypropylene octagonal base [28.6 cm in diameter, 11.4 cm sides], metal walls [33.3 cm high], and a clear polycarbonate lid, MED Associates, ENV-538) was equipped with eight computer-controlled guillotine doors (ENV-540). The arms (76.2 cm long, 8.9 cm wide with 17.5-cm high clear polycarbonate walls and topped with polycarbonate) radiated from the center hub with equal spacing between each arm. A food trough (ENV-200R1M) was placed at the end of each arm. A photobeam (ENV-254, approximately 1 cm inside each food trough, 1 cm from the trough bottom) detected head entries. A 45-mg pellet dispenser (ENV-203) was placed behind each food trough. Additional photobeams were located in each arm at 3.8 and 5.1 cm from the guillotine doors.

The maze was positioned on stools 81.3 cm above the floor. White noise (67 db from a speaker located in the ceiling above the hub) masked outside noise. A 500-MHz computer in an adjacent room, running MED-PC for Windows (Version 1.15), controlled experimental events (guillotine doors and food) and recorded the data (photobeam breaks) with 10-msec resolution. A video camera in the ceiling above the center of the maze was used to observe the rats.

Procedure

Training consisted of two shifts per day for each rat. The rats were individually placed in the maze beginning at 0900 for Phase 1 (forced-choice). Four doors (randomly chosen for each rat each day) were then opened, with the restriction that one of the arms dispensed chocolate pellets (F0299, Bio-Serv, Frenchtown, NJ); all other arms dispensed regular pellets (PJA/I-0045, Research Diets Inc., New Brunswick, NJ). A pellet was delivered to each of the accessible food troughs contingent on the first head entry into the photobeam located in each of the troughs. The animals were later returned for Phase 2 (free-choice) in which all 8 doors were open; food was available at each of the arms not previously accessible in the forced-choice phase. The interval between the forced- and the free-choice phases served as a discriminative cue. On days with a short retention interval (SRI), the interval between Phases 1 and 2 was 0.5 hr, and the only arms that provided food were the four arms not available in Phase 1. On days with a long retention interval (LRI), the interval between Phases 1 and 2 was 4 hr; the free-choice phase was identical to the SRI condition, except that the chocolate arm replenished (i.e., the chocolate was available during the free-choice phase at the location that provided chocolate during the forced-choice phase). The rat was required to visit each arm to collect the remaining pieces of food after a retention interval; the free-choice phase ended after the 4 or 5 pellets were collected in SRI or LRI, respectively. On any given day, either SRI or LRI (but not both) was tested.

The rats received alternating blocks of SRI followed by blocks of LRI sessions. Each block differed only in retention interval and consisted of 7-12 days of training. A total of 17 and 19 SRI and LRI sessions were conducted, respectively. The animals were then given 42 days of mixed SRI and LRI conditions. The order of SRI and LRI was randomized across days. We analyzed the last 17 days of mixed training.

Next, the rats received pairings of chocolate and LiCl. The animals were fed 50 g of chocolate pellets for 30 minutes, and 10 minutes after removing the food they were injected with an isotonic solution of LiCl in distilled water (0.75 mol/L, 0.6-ml/100 g of body weight ip). The animals were given LiCl treatments as described above for three days, with at least one day of regular food and no LiCl in between each LiCl-treatment day. Testing resumed after the third day of regular food following the third LiCl treatment. Testing after LiCl treatments was conducted for three days. The animals were tested with the LRI condition described above; however, the free-choice phase ended when the animals had visited four locations, and the chocolate dispenser was disabled.

For comparison, a pilot study with experimentally naïve Long Evans rats ($\underline{n} = 6$) documented that animals consumed more chocolate pellets than regular pellets. The rats were given two bowls, each containing 50 chocolate or 50 regular pellets, respectively. The bowls were removed and the pellets were counted after five minutes or after one bowl was depleted. Each rat ate all available chocolate pellets. The percentage of chocolate pellets consumed was 97.4% ± .01 (mean ± SEM). A preference test was given after the LiCl treatment (described above) to evaluate the effectiveness of the taste-aversion manipulation. The percentage of chocolate pellets consumed was 2.5% ± .02 (mean ± SEM); each rat ate all available regular pellets.

Throughout the experiment, the arms of the maze were cleaned with Nolvasan (Fort Dodge Animal Health, Fort Dodge, Iowa) each day between forced- and free-choice phases. A plastic bag with holes and filled with chocolate and regular pellets was taped on the stool at the end of each arm beside the filled pellet dispensers (i.e., food odors were constant throughout all parts of the experiment).

Throughout this article, the criterion for statistical significance is .05.

RESULTS

The proportion correct in the first four choices after the retention interval is presented in Table 1. There were no significant differences in these proportions, $F(4,16) = 1.41$. During block testing with SRI, the proportion of visits to the chocolate location in the first four choices was .25. During block testing with LRI, the proportion of visits to the chocolate location in the first four choices was .54.

Figure 1.1 (top panel) plots the proportion of first four choices that included chocolate during mixed SRI and LRI testing. The rats visited the chocolate site more often in LRI than in SRI conditions, $t(4) = 2.90$.

The probability of revisiting chocolate in the first four choices from LRI in testing after the lithium chloride is shown in Figure 1.1 (bottom panel). After LiCl, the rats revisited chocolate in LRI less often than they did prior to LiCl in LRI, $t(4) = 3.07$.

The probability of revisiting chocolate was not statistically different in SRI before LiCl treatment and LRI after LiCl treatment, $t(4) < 1$. The proportion correct in the first four choices after the retention interval was lower after LiCl compared to pre-LiCl performance; however, this difference was not significant, $t(4) = 1.37$ (see Table 1).

The rats ate the chocolate when they encountered it in the forced-choice phase (i.e., prior to the retention interval). Therefore, encountering a chocolate pellet was not sufficient to reject eating it. Nevertheless, when tested after the retention interval, the rats avoided the location

Table 1

Mean (and SEM) of proportion correct in the first four choices after the retention interval

	Retention Interval	
	Short	Long
Block	0.89(0.02)	0.86 (0.03)
Mixed	0.91 (0.03)	0.91(0.02)
Lithium chloride		0.78 (0.07)

Note. After a short RI, a visit to the four arms that were not available in Phase 1 was defined as correct. After a long RI, a visit to the four remaining arms plus the arm containing chocolate was defined as correct.

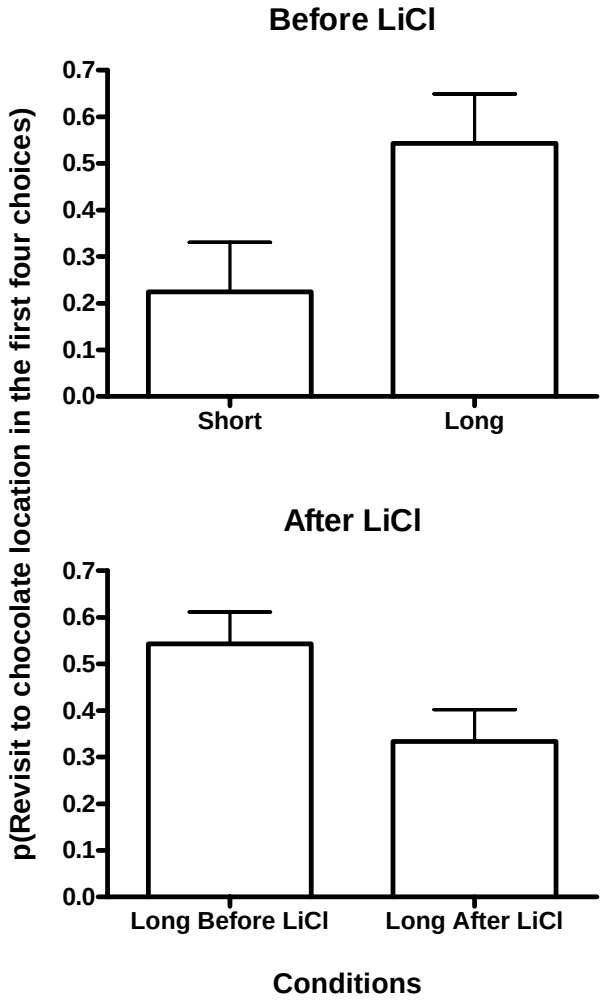

Figure 1.1

Mean proportion of chocolate revisits in the first four choices of Phase 2 in mixed testing is plotted as a function of experimental conditions. Top and bottom panels display data from testing without and with LiCl treatment, respectively (see text for details). Error bars represent 1 SEM.

known to contain chocolate, reversing their initial preference to choose the chocolate location after a long RI.

It is unlikely that the different proportions of visits to the chocolate location in SRI and LRI conditions are due to more forgetting of forced-choice locations after LRI than SRI. The proportion correct did not decline reliably across the retention intervals (see Table 1), $t(4) < 1$. However, the comparison of SRI and LRI in Table 1 includes a different number of correct locations per trial (4 and 5, respectively); a visit to the chocolate location counted as a correct choice after a long retention interval but as an incorrect choice after a short retention interval. These differences complicate the comparison of the proportions in Table 1. Therefore, we examined the proportion correct excluding the chocolate location in mixed SRI (0.96 ± 0.01; mean ± SEM) and LRI (0.90 ± 0.02) conditions. The proportion correct at non-chocolate locations did not decline reliably across the retention intervals, $t(4) = 2.08$. Therefore, the higher rate of revisits to the chocolate location after the long retention interval cannot be due to forgetting which location contained chocolate.

DISCUSSION

Rats were required to visit four randomly-chosen locations, one of which was randomly selected to provide chocolate. The animals were later returned to the maze after either a short or long retention interval, with all 8 locations available. After the short retention interval, only the four locations not available in the first phase provided food; after the long retention interval, the four locations not available in the first phase, plus the chocolate location, provided food. The rats made more visits to the chocolate location after the long than after the short retention interval.

Next, the animals received a taste-aversion treatment, in which chocolate was paired with lithium chloride. The animals were subsequently tested using the long retention interval (i.e., a condition in which the rats previously revisited the chocolate at a high rate). The rats made fewer revisits to the chocolate location after the lithium chloride treatment than in previous testing. The animals could not have reduced the rate of revisits to the chocolate location without knowledge of what, when, and where.

One potential alternative explanation is that the animals were using relative familiarity to guide their behavior after the different retention intervals. The rats could have solved the task by using the conditional rule: do not revisit if familiarity with chocolate is high (i.e., after a short delay), and revisit if familiarity is low (i.e., after a long delay). However, the conditional rule cannot explain performance after lithium chloride. Post-LiCl testing was conducted with a long RI. Therefore, the conditional rule predicts revisits to chocolate at a high rate, rather than the observed low rate. Because chocolate revisits declined after LiCl treatment, in conditions that controlled the level of relative familiarity, knowledge of what, when, and where is a single parsimonious interpretation for the selective revisits to chocolate before LiCl (Figure 1.1, top panel) and the decline in visits to chocolate after LiCl (Figure 1.1, bottom panel).

An aspect of episodic memory involves encoding of unique events. In the present experiment the rats demonstrated memory of the daily location of the chocolate. For food-caching animals, retrieval of the food cache depends on a single, brief encoding event. Clayton and colleagues placed distinctive Lego structures at each caching tray to provide unique encoding events (Clayton & Dickinson, 1998, 1999a, 1999b; Clayton, Yu, & Dickinson, 2001, 2003). We approached this issue in a previous study by providing rats with trial-unique objects in

each arm of the radial maze. However, we found that rats navigated with respect to a representation of spatial locations and did not follow trial-unique cues (Babb & Crystal, 2003).

The observation of what-when-where information contrasts with other recent reports with rats. W. A. Roberts and S. Roberts (2002) assessed the ability of rats to remember the order in which they entered arms on a radial maze. The rats were unable to discriminate the first arm they encountered from the other arms. Bird, W. A. Roberts, Abroms, Kit and Crupi (2003) induced rats to carry food items (cheese and pretzels) from the center of a radial maze to boxes at the end of each arm; the rats were removed from the maze arm before the food was fully consumed. The rats hid and retrieved cheese preferentially over pretzels. However, the rats were insensitive to pilfering of the food. When Bird and colleagues degraded cheese at one delay interval but not the other, the rats did not selectively avoid locations with the degraded food. Taken together, the experiments by W. A. Roberts and colleagues provide evidence for knowledge of what type of food they hid and where they hid it, but no evidence that rats remembered when they hid it.

In contrast, Eichenbaum and colleagues (e.g., Ergorul & Eichenbaum, 2004; Fortin, Agster, & Eichenbaum, 2002) have documented that rats quickly learn the sequence of randomly ordered odors. After being presented with a sequential order, the rats learned to select an odor that had not been presented in the sequence. The rats were then divided into hippocampal lesion or sham groups. The rats with the hippocampal lesions performed as well as the control rats on an odor recognition task, but were impaired on tests of sequential order. These data are consistent with the hypothesis that the hippocampus is involved in memory for sequences of events. Eichenbaum and Fortin (2003) argued that memory for the sequential order of unique events provides a model for episodic memory.

The present experiment documents knowledge of what, when, and where. However, it does not identify the mechanisms responsible for these types of knowledge. In the paragraphs that follow, we outline several potential mechanisms that may subserve knowledge of what, when, and where.

Two types of timing mechanisms may be proposed to discriminate between the short and long retention intervals. The rats may have used an interval timing mechanism (Gibbon, 1991) to discriminate 0.5 and 4 hr. Alternatively, the rats may have used a circadian oscillator (Mistlberger, 1994) to discriminate time of day (e.g., morning vs. afternoon).

The rats restricted their revisits to conditions in which chocolate replenished, and the rate of revisits to chocolate was reduced after LiCl treatment. This suggests that the content of "what" consisted of knowledge of chocolate. However, the mechanism remains to be identified. For example, the animals may have been using a response-outcome association to avoid revisiting the chocolate location after LiCl treatment (Colwill & Rescorla, 1985). According to this view, the animals did not revisit the chocolate location after the long retention interval because the outcome was undesirable.

The location of the chocolate arm varied randomly from day to day. The content of "where" is the location of the chocolate arm. However, the mechanism of spatial navigation remains to be identified. For example, the rats may have navigated with respect to a spatial representation of the global geometric framework (i.e., a cognitive map), dead reckoning (i.e., path integration) or landmarks (Gallistel, 1990).

The present experiment documents the use of what-when-where information. In the paragraphs that follow, we outline the implications for episodic-like memory.

Knowledge of what, when, and where is a necessary condition for the establishment of episodic memory. However, it is not a sufficient condition. For example, in the present study, there is no information about the subjective state of the animal when the animal makes a decision to revisit the chocolate location. We have no method to know if the animal is engaged in mental time travel or if it is subjectively re-experiencing the previous event of finding chocolate. Therefore, it is possible that a three-way conditional discrimination of what, when, and where occurs in the absence of any accompanying subjective states. Clayton, Bussey, Emery, and Dickinson (2003) have argued that reconstructive generative processes and meta-representations at retrieval should not be considered necessary criteria for episodic memory.

Clayton, Bussey, and Dickinson (2003) have argued that behavioral criteria for episodic-like memory include content, structure, and flexibility. According to this proposal, the content is a recollection of what occurred, where it occurred, and when it occurred based on a specific past experience. The structure is an integrated what-where-when representation. Flexibility proposes that episodic memory is embedded within a declarative-memory framework which involves flexible use of information.

We view the present demonstration of what-when-where in rats as meeting Clayton, Bussey, and Dickinson's (2003) content criterion. In contrast, Clayton, Yu, and Dickinson (2001) have demonstrated that scrub jays can distinguish between multiple episodes in which they cached the same food type in different locations; these data suggest that what-when-where information is integrated. Clayton, Yu, and Dickinson (2003) have demonstrated that when scrub jays are presented with new information about the perishability of worms after having stored the worms, the birds switched their preferences for worms and peanuts accordingly; these data suggest flexibility in updating information after the time of encoding. Additional research

with rats will be needed to assess the integrated and flexibility criteria. One approach toward addressing these questions is to satiate the rats to a specific food type during the RI (Colwill & Rescorla, 1985; Balleine & Dickinson, 1998). In pilot studies we were unable to identify parameters to satiate rats to chocolate.

Zentall, Clement, Bhatt, and Allen (2001) have emphasized another feature of episodic-like memory. In particular, it should be possible to answer an unexpected question based on episodic memory. Zentall and colleagues argued that what-when-where information could be based on the acquisition of a set of explicitly trained if-then rules; the rules could be applied when a food item is initially encountered. To avoid the concern, Zentall and colleagues trained pigeons to respond differently after having pecked or not pecked (i.e., nonverbally answering the question "Did you just peck or refrain from pecking?"). In a separate discrimination, the pigeons were placed in a situation in which they pecked one stimulus but not another, without requiring them to do so. Next, the pigeons were given the opportunity to report whether they had pecked or not. The data suggest that the pigeons could remember a specific detail about their past experience. The current study, like Clayton and Dickinson's (1998, 1999b) initial demonstrations of what-when-where information, could be solved by rule-based learning at the time a food item is encountered.

W.A. Roberts (2002) offered a related rule-based criticism of Clayton and colleagues' demonstration of episodic-like memory. In particular, Roberts argued that the scrub jays may have learned that a relatively weak memory trace for meal worm locations predicts decayed worms and that a relatively strong memory trace predicts palatable worms. A conditional rule can be constructed to describe our training data, applying Roberts's explanation. In particular, the memory trace for a chocolate location would be relatively strong after a short RI and

relatively weak after a long RI. Therefore, the rat could adopt the rule, "if the memory of the chocolate location is weak, revisit that location." However, the use of this rule does not predict the data observed after LiCl treatment. Testing after the LiCl treatment used a long RI. Therefore, application of the rule outlined above would predict revisits at a high rate, rather than the observed low rate. LiCl treatment may change the memory representation of chocolate (e.g.,Trapold & Overmier, 1972). However, if LiCl treatment affects the strength of the memory trace, it is not clear whether the memory trace is strengthened or weakened.

Episodic memory is the first kind of memory to degenerate with age, and the last to be acquired as a child (Tulving, 2002). Patients with neurodegenerative diseases such as Alzheimer's show a loss of episodic memory. The hippocampus plays an important role in the acquisition of new memories; it incorporates temporal information from the frontal lobes, thus providing a basis for "when" (Burgess, Maguire, & O'Keefe, 2002). The hippocampus has been implicated in spatial memory tasks, and may provide a basis for "where" (O'Keefe & Nadel, 1978). The right hippocampus processes spatial locations, while the left hippocampus is involved in episodic memory (Burgess, et al., 2002; Maguire, 2001). The left and right prefrontal cortices are implicated in encoding and retrieval of episodic memory, respectively (Tulving, 2002).

Development of a rodent model for episodic memory could lead to a better understanding of the neural, molecular, and behavioral mechanisms of episodic memory (Griffiths & Clayton, 2001). The availability of a rodent model could also permit the screening of putative pharmacotherapies for human memory disorders such as Alzheimer's (Clayton & Griffiths, 2002).

The present study demonstrates that rats possess what, when, and where components of episodic memory. This study adds to the growing literature which suggests that animals may

possess episodic-like memory (Clayton & Dickinson, 1998; Clayton & Dickinson, 1999a, 1999b; Clayton, Yu, & Dickinson, 2001, 2003). Although it may be impossible to demonstrate autonoetic consciousness in animals, relying on Tulving's (1972) definition of episodic memory may represent a critical strategy for testing episodic-like memory in rats.

CHAPTER 4

DISCRIMINATION OF WHAT, WHEN, AND WHERE IN RATS IS NOT BASED ON TIME OF DAY[2]

[2] Babb, S.J., & Crystal, J.D. (*in press*). *Learning and Behavior*. Reprinted here with permission of publisher.

ABSTRACT

Rats (n=6) visited four baited locations (randomly chosen on each trial; study phase), one of which was randomly selected to provide chocolate. After short (1 hr; SRI) or long (25 hr; LRI) retention intervals, all 8 locations were available and the four locations not available in the study phase provided food (test phase); the chocolate location also provided food after LRI. More visits to the chocolate location occurred after LRI than after SRI. Next, chocolate was paired with lithium chloride (LiCl) during the LRI (i.e., after encoding the chocolate location). Fewer revisits to the chocolate location occurred after LiCl than in previous testing with the LRI. The rats demonstrated complete transfer when grape replaced chocolate after LiCl-chocolate pairing. The discrimination of what, when, and where could not be based on adopting different revisit strategies at different times of testing.

EXPERIMENT 2

Tulving (1972) proposed a distinction between episodic memory and semantic memory. Episodic memory refers to the encoding and retrieval of personal, unique past experiences. In contrast, semantic memory is knowledge of general facts, such as knowing that the earth is round. Tulving's (1972) classic definition of episodic memory states that "Episodic memory receives and stores information about temporally dated episodes or events, and temporal-spatial relations among these events" (p. 385). According to this definition, episodic recall involves retrieval of information about three aspects of an event or episode: what occurred, when did it transpire, and where did it take place (what-when-where, *WWW*).

The definition of episodic memory has been refined to include experiences such as an awareness that the event happened in the past, or chronesthesia, a feeling of re-experiencing the past event, or autonoesis, and a realization that the memories belong to oneself, or knowledge of self (e.g., Tulving, 1983, 2005; Tulving & Markowitsch, 1998). Unfortunately, these terms are difficult, if not impossible, to test in non-verbal animals (Griffiths, Dickinson, & Clayton, 1999; Roberts, 2002; Suddendorf & Busby, 2003; Suddendorf & Corballis, 1997). Because focusing on WWW only captures a subset of the current definition of episodic memory, the study of WWW in non-human animals is referred to as "episodic-like" (Clayton & Dickinson, 1998).

Clayton and colleagues (Clayton, Bussey, & Dickinson, 2003) have argued that tests of episodic memory in animals should focus on the behavioral aspects of episodic memory, instead of the phenomenological experiences that may accompany these memories. They proposed three criteria: content, structure, and flexibility. According to Clayton and colleagues, knowledge of what, when, and where is evidence for episodic-like memory if the structure is an integrated

representation that can be used flexibly (Clayton, Bussey, & Dickinson, 2003; Clayton, Bussey, Emery, & Dickinson, 2003; de Kort, Dickinson, & Clayton, 2005). Clayton, Bussey, and Dickinson (2003) argued that an integrated representation of what-when-and-where means that retrieval of any one feature of an episode automatically retrieves the other features. This integrated structure permits the discrimination of multiple events that share some, but not all, common features. The memory must also be flexibly updated and generalized over situations.

Some birds cache food, and recover it days, weeks, or months later. The recovery of stored food is guided by memory for the spatial location of the cache site and also memory for the content of the caches, and these memories are encoded during a single brief caching episode (e.g., Clayton & Dickinson, 1998; Shettleworth, 1998). Clayton and Dickinson (1998) provided the first evidence of discrimination of WWW in food-storing scrub jays. They provided jays with the opportunity to cache peanuts or wax worms. The birds either stored peanuts followed by worms or, on other trials, worms followed by peanuts. The opportunity to retrieve the cached foods occurred after either a short or long RI. For some birds, the worms were decayed after the long RI, and for other birds they were replenished with fresh worms (peanuts did not decay). The birds preferred the worm rather than peanut cache sites when the worms were fresh, but reversed this preference when the worms were decayed. These data suggest that the jays are sensitive to what (food type), when (time of caching and recovery), and where (location in the tray). Since this initial demonstration, Clayton and colleagues have established a growing body of research to indicate that scrub jays have a detailed representation of what, when, and where food was cached. For example, jays (1) remember the specific food types that they cache and recover (Clayton & Dickinson, 1999a, 1999b), (2) remember the relative time and location of caches (Clayton & Dickinson, 1999b), (3) form integrated memories for the location and time of

caching of particular foods (Clayton, Yu, & Dickinson, 2001), and (4) flexibly update information about caching episodes with new information acquired during the RI (Clayton, Yu, & Dickinson, 2003). Changing the expected value of the to-be-recovered food item by degrading it (i.e., decreased value; Clayton & Dickinson, 1998, 1999b; Clayton, Yu, & Dickinson, 2001, 2003), ripening it (i.e., increased value; de Kort et al., 2005), and satiation of that food type (i.e., decreased value; Clayton & Dickinson, 1999a, 1999b) have been used to demonstrate discrimination of WWW. Clayton and colleagues have argued that the relative familiarity of the tray cues from the caching episode cannot explain the discrimination of WWW.

We recently used Clayton's behavioral approach to test rats' ability to discriminate WWW in an 8-arm radial maze (Babb & Crystal, 2005). Daily testing was divided into forced-choice and free-choice phases, which were separated by either a short (0.5 hr) or long (4 hr) retention interval (RI). In the forced-choice phase, the rats were required to visit four baited locations (randomly chosen on each trial for each rat), one of which was randomly selected to provide chocolate. The rats were trained to discriminate between short and long RIs. After the short retention interval (SRI), the four locations not seen in the forced-choice phase provided regular food. On days with a long retention interval (LRI), the free-choice phase was identical to the SRI condition, except that the chocolate arm replenished (i.e., provided an additional chocolate pellet). On any given day, either SRI or LRI (but not both) was tested. We found that rats revisited the chocolate location more after the LRI than after the SRI. Chocolate was then paired with lithium chloride (LiCl) in an attempt to reduce the likelihood of a revisit to the chocolate location after the LRI; the rats were given three separate pairings of chocolate and LiCl outside of regular testing (i.e., in the intertrial interval) which eliminated the preference for chocolate. The animals were subsequently tested using the long RI (i.e., a condition in which the

rats previously revisited the chocolate location at a high rate). The rats made fewer revisits to the chocolate location after the LiCl treatment than in previous testing with the long RI. The animals could not have reduced the rate of revisits to the chocolate location without discriminating WWW. Because chocolate revisits declined after LiCl, in conditions that controlled the level of relative familiarity of chocolate, discrimination of WWW is a single parsimonious interpretation for the selective revisits to chocolate before LiCl and the decline in visits to chocolate after LiCl.

Hampton, Hampstead, and Murray (2005) argued that our rats could have solved the discrimination of WWW by using time of day. Our rats were always tested in the forced-choice phase in the morning, and the free-choice phase occurred in the morning or afternoon after the SRI or LRI, respectively. Therefore, the rats may have used a representation of time of day from a circadian oscillator (e.g., Mistlberger, 1994) to discriminate between the short- and long-retention intervals. Consequently, the rats could have adopted different revisiting strategies in morning and afternoon tests. Hampton et al. (2005) argued that this strategy is not sufficient to demonstrate "when" memory in the sense required for WWW memory. In contrast, Hampton et al. argued that a demonstration of "when" memory involves requiring the animal "to know how *long ago* they learned the locations of food, rather than what time of day is associated with the availability of a certain food" (p. 256), as has been demonstrated by Clayton and colleagues. Moreover, Hampton et al. point out that encoding a rule about the availability of chocolate at different times of day is analogous to semantic, rather than episodic, memory.

Hampton et al. (2005) also emphasized the importance of documenting that the discrimination of WWW is acquired through experience with the contingencies of the experiment. The influence of experience may be evaluated by comparing the preference for revisits at the beginning and end of training. Presumably, there would not be a preference for

revisits to the chocolate location in the initial block of training. However, by the end of training, we would expect to document a reliable preference; these two predictions may also be evaluated by testing the interaction of training and RI.

In the present study, we controlled for time of day by using retention intervals of 1 hour (SRI) and 25 hours (LRI). For example, a rat that was tested in the forced-choice phase beginning at approximately 1200 would be returned to the maze for the free-choice phase at approximately 1300 the same day (SRI) or 1300 the next day (LRI). Therefore, the rats always received the free-choice phase at the same time of day. In a separate experiment, we examined forgetting functions of spatial memory and found that rats' accuracy in the radial maze was still high (62%) after 25 hours (Babb & Crystal, *in preparation*). The intertrial interval (ITI) was at least 48 hours in order to reduce the similarity of RI and ITI. After the animals had learned to selectively revisit the chocolate location, chocolate was paired with LiCl. However, in the present study the taste-aversion manipulation was inserted into the RI; the rats were given a forced-choice phase, and during the 25-hr RI, they were fed chocolate and injected with LiCl before completing the free-choice phase. Because the animals encountered chocolate prior to LiCl treatment, avoiding chocolate after LiCl treatment cannot be explained by failure to encode the location of chocolate. A reduction in visits to the chocolate location after encoding would document some degree of flexibility to update memory based on newly acquired information (i.e., that chocolate is bad).

METHOD

Subjects

Six male Long Evans rats (Harlan, Madison, WI) were individually housed in a colony with light onset at 0700 and offset at 1900. The rats were given unlimited access to 5001 Rodent Diet (Lab Diet, Brentwood, MO) for one week, and then given 20 g for one day and 15 g per day on subsequent days. Water was available at all times, except during brief testing periods. The rats were approximately 77 days old at the beginning of the experiment with an average weight of 239 g.

Materials

Testing was conducted in an eight-arm radial maze. The central hub (white polypropylene octagonal base [28.6 cm in diameter, 11.4 cm sides], metal walls [33.3 cm high], and a clear polycarbonate lid, MED Associates, ENV-538) was equipped with eight computer-controlled guillotine doors (ENV-540). The arms (76.2 cm long, 8.9 cm wide with 17.5-cm high clear polycarbonate walls and topped with polycarbonate) radiated from the hub with equal spacing between each arm. A food trough (ENV-200R1M) was placed at the end of each arm. A photobeam (ENV-254, approximately 1 cm inside each food trough, 1 cm from the trough bottom) detected head entries. A 45-mg pellet dispenser (ENV-203) was placed behind each food trough. Additional photobeams were located in each arm at 3.8 and 5.1 cm from the guillotine doors.

The maze was positioned on stools 81.3 cm above the floor. White noise (67 db from a speaker located in the ceiling above the hub) minimized outside noise. A 500-MHz computer in an adjacent room, running MED-PC for Windows (Version 1.15), controlled experimental events

(guillotine doors and food) and recorded the data (photobeam breaks) with 10-msec resolution. A video camera in the ceiling above the center of the maze was used to observe the rats.

Procedure

Pretraining consisted of three days in which three pellets (PJA/I-0045, Research Diets Inc., New Brunswick, NJ) were placed along each arm, and one pellet was placed in each food trough to provide the animals with the experience of obtaining food in the maze. Each day one randomly chosen arm contained chocolate pellets (F0299, Bio-Serv, Frenchtown, NJ). The rats were individually placed on the maze for an average of approximately 10.0, 10.0, and 7.7 minutes on days 1, 2, and 3, respectively.

During initial training, the rats were individually placed in the central hub beginning at 1200; all 8 doors were then opened. The session ended when the animals had depleted all arms or 10 minutes had elapsed. Training was conducted once per day for 10 trials. Each trial was separated by at least a 48-hr ITI.

For the remainder of the study, testing consisted of two shifts per day for each rat. The rats were individually placed in the maze beginning at 1200 for Phase 1 (forced-choice). Four doors (randomly chosen for each rat each day) were then opened, with the restriction that one of the arms dispensed chocolate pellets (*chocolate arm*); all other arms dispensed regular pellets. A pellet was delivered to each of the accessible food troughs contingent on the first head entry recorded by the photobeam located in each of the troughs. The animals were later returned for Phase 2 (free-choice) in which all 8 doors were open. The interval between the forced- and the free-choice phases served as a discriminative cue. On days with a short retention interval (SRI), the interval between Phases 1 and 2 was 1 hr, and the only arms that provided food were the four arms not available in Phase 1; regular pellets were dispensed at these Phase 2 locations. On days

with a long retention interval (LRI), the interval between Phases 1 and 2 was 25 hr; the free-choice phase after a LRI was identical to the SRI condition, except that the location that had provided chocolate in Phase 1 also provided a chocolate pellet in Phase 2 (i.e., the chocolate arm replenished). The rats were required to visit each arm to collect the remaining pieces of food after a retention interval; the free-choice phase ended after the 4 or 5 pellets were collected in SRI or LRI, respectively. On any given day, either SRI or LRI (but not both) were tested. Each trial was separated by at least a 48-hr ITI.

The rats received alternating blocks of SRI followed by blocks of LRI sessions. Each block differed only in retention interval and consisted of 10-15 days of training. A total of 44 and 43 SRI and LRI training sessions were conducted, respectively. The animals were then given 16 days of mixed SRI and LRI conditions. The order of SRI and LRI was randomized across sessions in the mixed condition.

Next, the rats received pairings of chocolate and LiCl. Ten minutes after all rats completed a Phase 1 shift (i.e., during the retention interval), the animals were given access to 50 g of chocolate pellets for 30 minutes, and 10 minutes after removing the food they were injected with an isotonic solution of LiCl in distilled water (0.75 mol/L, 0.6-ml/100 g of body weight ip). The animals were returned to the maze 25 hours after Phase 1 for a LRI Phase 2 shift; the Phase 2 shift was identical to previous free-choices after LRI, except the shift ended after the fourth regular pellet was dispensed or 20 min elapsed. Two trials with LiCl treatment were conducted.

Throughout the experiment, the arms of the maze were cleaned with Nolvasan (Fort Dodge Animal Health, Fort Dodge, Iowa) each day between forced- and free-choice phases. A plastic bag with holes and filled with chocolate and regular pellets was taped on the stool at the

end of each arm beside the filled pellet dispensers (i.e., food odors were constant throughout all parts of the experiment).

Throughout this article, the criterion for statistical significance is .05.

RESULTS AND DISCUSSION

During block testing with SRI, the proportion of visits to the chocolate location in the first four choices was .22. During block testing with LRI, the proportion of visits to the chocolate location in the first four choices was .51. The proportion correct after the retention interval is shown in Table 2; the data shown in Table 2 is based on an analysis that was restricted to the first four choices among the seven non-chocolate arms.

Figure 2.1 (top panel) plots the proportion of first-four choices that included chocolate during mixed SRI and LRI testing. The rats visited the chocolate site more often in LRI than in SRI conditions ($t(5) = 5.37$, $p < .01$). Because the data shown in Figure 2.1 (top panel) and Table 2 were derived from independent segments of the data (i.e., chocolate and non-chocolate arm entries, respectively), we used these data to compare error rates in the mixed condition. The probability of a revisit to a non-chocolate arm after a short retention interval (i.e., a baseline error rate) was .05 ± .02 (mean ± SEM). Although this baseline is lower than the probability of a revisit to the chocolate location after a short retention interval, this difference was not statistically significant ($t(5) = 2.24$, $p > .05$). Therefore, after short retention intervals, the rats revisited the chocolate locations at a level comparable with the revisit rate for non-chocolate locations. In contrast, after long retention intervals, the rats revisited the chocolate location at a higher level than would be expected based on revisits to non-chocolate arms. In particular, the

Table 2

Mean (and SEM) of proportion correct in the first four choices excluding the chocolate location after the retention interval in Experiments 2 and 3.

	Retention Interval	
Trial Type	Short	Long
Experiment 2		
Block	.87 (.02)	.68 (.01)
Mixed	.95 (.02)	.65 (.03)
Lithium Chloride		.75 (.06)
Experiment 3		
Initial	.90 (.06)	.75 (.08)
Terminal	.94 (.04)	.70 (.03)

Note. The analysis of the first four choices was restricted to the seven non-chocolate arms.

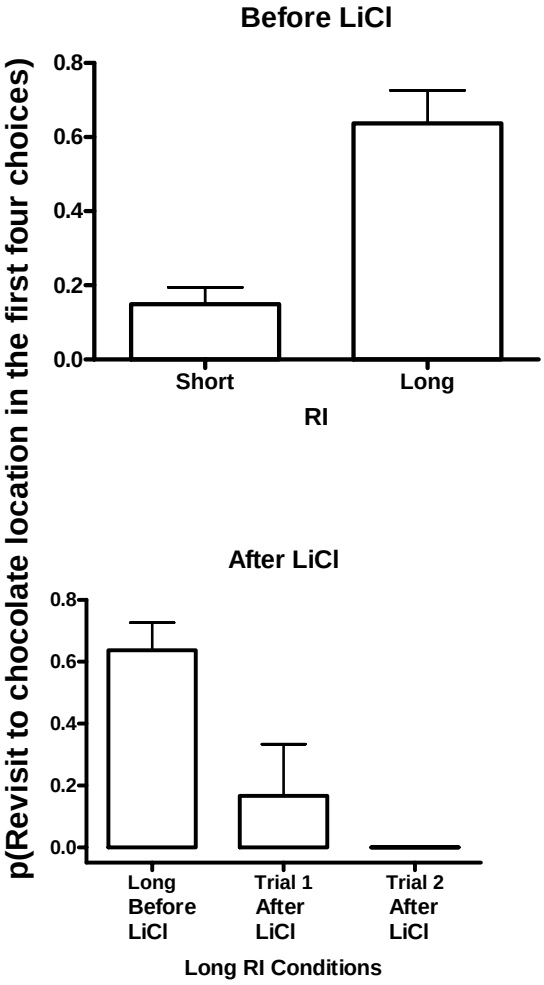

Figure 2.1

Mean proportion of chocolate revisits in the first four choices of Phase 2 in mixed testing is plotted as a function of retention intervals in Experiment 2. Top and bottom panels display data from testing without and with LiCl treatment, respectively (see text for details). Error bars represent SEM.

probability of a revisit to a non-chocolate arm after a long retention interval (i.e., baseline error rate) was .35 ± .03 (mean ± SEM). The probability of a revisit to the chocolate location after a long retention interval was significantly higher than this baseline error rate ($t(5) = 3.01$, $p < .05$).

In summary, revisits to the chocolate location occurred a rate higher than expected from the baseline error rate after long, but not short, retention intervals.

Performance from LRI in testing after the LiCl treatment is shown in Figure 2.1 (bottom panel) together with the expected probability of revisiting chocolate (i.e., LRI before LiCl treatment). The rate of revisits varied significantly across long RI conditions ($F(2, 10) = 6.74$, $p < .05$). The revisit rate was significantly lower on the first ($F(1, 10) = 6.84$, $p < .05$) and second ($F(1, 10) = 12.55$, $p < .01$) trials after LiCl relative to pre-LiCl testing. There was no statistical difference between SRI before LiCl treatment (Figure 2, top panel) and LRI after LiCl treatment (Figure 2, bottom panel), $t(5) < 1$.

Because the post-LiCl testing in Phase 2 terminated after the fourth regular pellet was dispensed, each rat did not encounter chocolate. After LiCl treatment, chocolate could be encountered on three occasions: first trial Phase 2, second trial Phase 1, and second trial Phase 2. In the first trial Phase 2, 4 of the rats encountered chocolate, and 3 of them ate it. In the second trial Phase 1, 6 rats encountered chocolate, and all 6 rats ate it. In the second trial Phase 2, 2 rats encountered chocolate, and 0 ate it. Therefore, encountering a chocolate pellet was not sufficient to reject eating it. Nevertheless, when tested after the retention interval, the rats avoided the location known to contain chocolate, reversing their initial preference to choose the chocolate location after a LRI.

Figure 2.2 documents that the discrimination of WWW was acquired through experience. The initial and terminal blocks consist of the first and last 5 trials, respectively, from SRI and LRI block training. There was a significant interaction between training and RI ($F(1, 5) = 6.92$, $p < .05$), reflecting a difference between SRI and LRI in terminal ($t(5) = 4.00$, $p < .05$) but not initial ($t(5) < 1$) training.

A preference test was given immediately after the final LiCl-trial to evaluate the effectiveness of the taste-aversion manipulation using two bowls, each containing 50 chocolate or 50 regular pellets. The percentage of chocolate pellets consumed was 0.4% ± 0.4 (mean ± SEM); each rat ate 100% of the regular pellets. By contrast, Babb and Crystal (2005) reported that rats not treated with LiCl consumed 97% of chocolate pellets when given the same two-bowl test.

In summary, the rats made more visits to the chocolate location after the long than after the short retention interval. Next, chocolate was paired with lithium chloride during the LRI. The rats made fewer revisits to the chocolate location after the lithium chloride treatment than in previous testing. The animals could not have reduced the rate of revisits to the chocolate location without knowledge of what, when, and where.

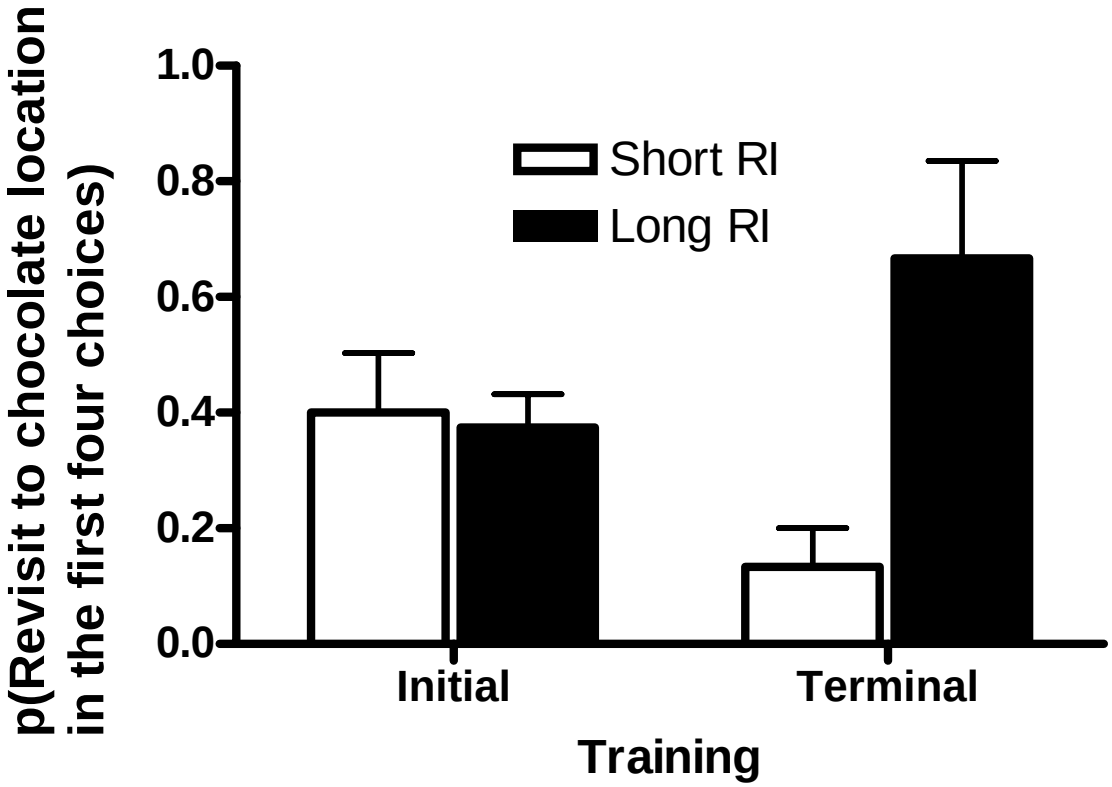

Figure 2.2

The discrimination of WWW was acquired through experience. The initial and terminal blocks consist of the first and last 5 trials, respectively, from SRI and LRI block training in Experiment 2. Error bars represent SEM.

EXPERIMENT 3

Experiment 3 investigated whether the rats would exhibit transfer to a new distinctive food type (grape pellets) after chocolate was paired with LiCl. The purpose of the transfer test was to evaluate the hypothesis that what the rats learned in Experiment 2 was specific to the chocolate food. Alternatively, the rats may have learned that the location with a *distinctive* food type replenished. If the rats had learned that the distinctive food type replenished, then we would expect that the revisit strategy would transfer to a new distinctive food type (grape pellets) without additional training. In contrast, if the revisit strategy was limited to the stimulus with which the rats had experience, then we would expect that the revisit strategy would not transfer to a new distinctive flavor.

METHOD

Subjects

The subjects were the rats used in Experiment 2, except that one animal was not tested because of illness.

Materials

The apparatus was the same as described in Experiment 2.

Procedure

After completion of post-LiCl trials with chocolate, the rats received 22 days without testing, and the rats then received 12 trials of mixed SRI and LRI testing. The procedure was the same as the mixed condition in Experiment 2, except that grape was used as the distinctive pellet

(i.e., grape replaced chocolate). The first and second trials of Experiment 2 were LRI and SRI, respectively.

RESULTS AND DISCUSSION

The proportion correct after the retention interval is shown in Table 2. Figure 3.1 shows that the rats exhibited complete transfer to grape distinctive pellets after the pairing of chocolate and LiCl. In the first two trials (LRI and SRI, respectively), revisits to the grape location were more frequent after the LRI than SRI ($t(4) = 4.00$, $p < .05$; Figure 3.1, top panel). During the remaining 10 trials, revisits to the grape location were more frequent after the LRI than after the SRI ($t(4) = 9.80$, $p < .01$; Figure 3.1, bottom panel). The magnitude of the difference between SRI and LRI in Experiment 2 (Figure 2.1, top panel) was not significantly different from the magnitude of the difference between SRI and LRI in Experiment 2 (Figure 3.1: top panel, $t(4) = 2.41$; bottom panel, $t(4) < 1$; p's $> .05$). The revisit strategy during training transferred to a new distinctive food, suggesting that what the rats learned in Experiment 2 was not specific to the chocolate food.

Because the probability of a revisit to the grape location after a long retention interval was higher in the initial test compared to terminal performance ($t(4) = -9.37$, $p < .01$), this apparent decline may reflect the growth of generalized flavor aversion over time; initial and terminal revisit probabilities after short retention intervals in Figure 3.1 did not differ ($t(4) = -0.65$, $p > .05$). However, it is noteworthy that the decline in the probability of a revisit from initial to terminal tests after long retention intervals is primarily due to a higher than expected probability on the initial long retention interval. In particular, the probability of a revisit to grape

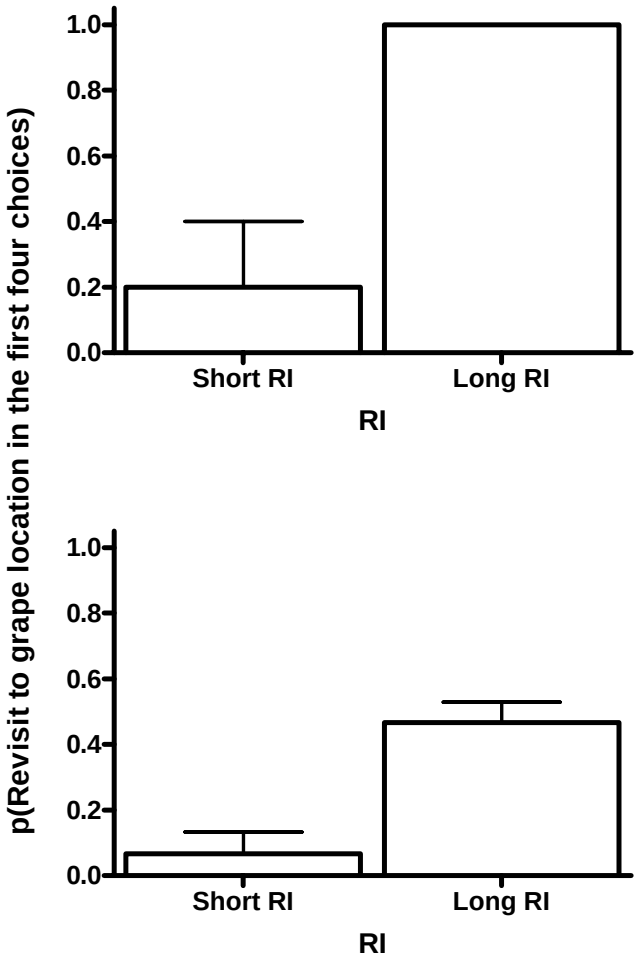

Figure 3.1

Mean proportion of revisits to the unique location (grape) during Phase 2 of the transfer test in Experiment 3. Top and bottom panels display data from the initial and terminal trials, respectively. Error bars represent SEM.

in the initial LRI (Figure 3.1, top panel) was higher than the probability of a revisit to chocolate in Experiment 2 (Figure 2.1, top panel, long retention interval; $t(4) = 3.25$, $p < .05$); note that this higher than expected probability is based on a single trial in which each rat revisited the grape location in the first four choices. By contrast, the probability of a revisit to grape in the terminal long retention intervals (Figure 3.1, bottom panel) was not reliably different from the probability of a revisit to chocolate in Experiment 2 (Figure 2.1, top panel, long retention interval; $t(4) = -1.61$, $p > .05$). In summary, the probability of revisiting the grape location after LRIs in Experiment 3 was at least as high as the probability of revisiting the chocolate location after LRIs in Experiment 2 (i.e., complete transfer was observed in both initial and terminal trials).

GENERAL DISCUSSION

In Experiment 2, rats were required to visit four baited, randomly chosen locations, one of which was randomly selected to provide chocolate, during the study phase. The animals were later returned to the maze after either a short or long retention interval, with all 8 locations available. After the SRI, the four locations not available in the study phase provided food; after the LRI, the four locations not available in the study phase, plus the chocolate location, provided food. The rats made more visits to the chocolate location after the LRI than after the SRI. Next, the animals received a taste-aversion treatment during the long retention interval, in which chocolate was paired with LiCl. The animals were subsequently tested at the end of the long retention interval (i.e., a condition in which the rats previously revisited the chocolate at a high rate). The rats made fewer revisits to the chocolate location after the LiCl treatment than in

previous testing. The animals could not have reduced the rate of revisits to the chocolate location without knowledge of WWW.

In Experiment 3, grape replaced chocolate pellets after chocolate was paired with LiCl. The rats exhibited complete transfer to grape distinctive pellets in both the first grape trials and in the subsequent mixed training with short and long RIs. The transfer data suggest that the revisit strategy learned in Experiment 2 was not specific to the chocolate food. Instead, the rats learned that the location with a distinctive food type replenished.

These results are consistent with the findings from our previous study (Babb & Crystal, 2005). However, in the previous study, there was the possibility that the rats solved the discrimination of WWW problem by using time of day. The rats in our earlier experiment were tested in the free-choice phase in either the morning or afternoon; therefore, they may have used a circadian representation of time of day to discriminate between the SRI and LRI. Because the current study used 1- and 25-hr RIs (i.e., test phases were conducted at the same time of day), time of day could not have served as a discriminative cue. Consequently, the discrimination of WWW in the current study satisfies Hampton et al.'s (2005) definition of "when" memory.

In Babb and Crystal (2005), chocolate was paired with LiCl during the intertrial interval. By contrast, in the present study, chocolate was paired with LiCl during the long retention interval. Consequently, the observed reduction in revisits to the chocolate location (Figure 2.1) cannot be explained by a failure to encode the chocolate location after the chocolate was devalued. The revisiting strategy was acquired through experience with the replenishment contingency (Figure 2.2). Therefore, if the rats were responding to the RI as in prior training, they would have continued to revisit the chocolate location at a high rate. Consequently, the

decline in revisits to chocolate documents some degree of flexibility to update memory based on new information (i.e., chocolate is bad) acquired during the RI.

Roberts (2002) offered a rule-based criticism of Clayton and colleagues' demonstration of episodic-like memory that can be constructed to describe our training data. According to Roberts' criticism, the memory trace for a chocolate location would be relatively strong after a short RI and relatively weak after a long RI. Therefore, the rat could adopt the rule, "if the memory of the chocolate location is weak, revisit that location." However, the use of this rule does not predict the data observed after LiCl treatment. Testing after the LiCl treatment used a long RI. Therefore, application of the rule outlined above would predict revisits at a high rate, rather than the observed low rate. Nevertheless, if the rats learned the expectancy "if the memory of chocolate is weak, expect more chocolate", then the rats would not revisit the chocolate location because chocolate is repellant after LiCl. Zentall (2005; Zentall, Clement, Bhatt, & Allen, 2001) has also emphasized the need to document that episodic-like memory cannot be solved using rule-based learning.

Because there is no information about the subjective state of the animal when the animal makes a decision to revisit the chocolate location, it is possible that a three-way conditional discrimination of what, when, and where occurs in the absence of any accompanying subjective states. One interpretation of the discrimination of WWW is that the rats retrospectively re-experience an event from memory to assess when it occurred in the past. However, the discrimination of WWW could also be based on an interval timing mechanism. According to the interval-timing hypothesis, discrimination of WWW is based on the discrimination of elapsing intervals with respect to a resetting stimulus (i.e., a pacemaker-accumulator mechanism; Church, Meck, & Gibbon, 1994; Crystal, 2002). For example, SRI and LRI can be discriminated by

resetting an interval timing mechanism when the rats are handled at the start of each trial. To establish evidence that an animal represents "when" (rather than how long ago) an event occurred, it will be necessary to rule out both interval-timing and circadian mechanisms. These hypotheses need further testing in order to evaluate potential timing mechanisms in the discrimination of WWW.

The observation that rats discriminate WWW contrasts with other recent reports. W. A. Roberts and S. Roberts (2002) assessed the ability of rats to remember the order in which they entered arms on a radial maze. The rats were unable to discriminate the first arm they encountered from the other arms. Bird, W. A. Roberts, Abroms, Kit and Crupi (2003) induced rats to carry food items (cheese and pretzels) from the center of a radial maze to boxes at the end of each arm; the rats were removed from the maze arm before the food was fully consumed. The rats hid and retrieved cheese preferentially over pretzels. However, the rats were insensitive to pilfering of the food. When Bird and colleagues degraded cheese at one delay interval but not the other, the rats did not selectively avoid locations with the degraded food. Taken together, the experiments by W. A. Roberts and colleagues provide evidence for knowledge of what type of food they hid and where they hid it, but no evidence that rats remembered when they hid it.

In contrast, Eichenbaum and colleagues (e.g., Fortin, Agster, & Eichenbaum, 2002) have documented that rats quickly learn the sequence of randomly ordered odors. After being presented with a sequential order, the rats learned to select an odor that had appeared earlier in the sequence. The rats were then divided into hippocampal lesion or sham groups. The rats with the hippocampal lesions performed as well as the control rats on an odor recognition task, but were impaired on tests of sequential order. These data are consistent with the hypothesis that the hippocampus is involved in memory for sequences of events. Eichenbaum and Fortin (2003)

argued that memory for the sequential order of unique events provides a model for episodic memory.

Episodic memory is the latest kind of memory to develop and the first to degenerate with age (Tulving, 2002). Patients with neurodegenerative diseases such as Alzheimer's disease (AD) show a loss of episodic memory. Development of a rodent model for episodic memory could lead to a better understanding of the neural, molecular, and behavioral mechanisms of episodic memory (Griffiths & Clayton, 2001). The availability of a rodent model could also permit the screening of putative pharmacotherapies for human memory disorders such as AD (Clayton & Griffiths, 2002) and is a possible step towards the treatment and prevention of diseases involving the loss of episodic memory.

CHAPTER 5

EVALUATING THE SELECTIVITY OF DISCRIMINATING WHAT, WHEN, AND WHERE

Experiments 1 - 3 investigated episodic-like memory in rats. Experiment 1 showed that rats could discriminate what, when, and where (Babb & Crystal, 2005). Rats were required to visit four randomly chosen locations, one of which was randomly selected to provide chocolate. I approached the issue of encoding unique events by providing rats with trial-unique objects in each arm of the radial maze. However, I found that rats navigated with respect to a representation of spatial locations and did not follow trial-unique cues (Babb & Crystal, 2003). In Experiment 1, the unique encoding event was the daily location of chocolate. The animals were later returned to the maze after either a short- or long-retention interval (SRI, LRI, respectively), with all 8 locations available. After the SRI, only the four locations not available in the first phase provided food; after the LRI, the four locations not available in the first phase, plus the chocolate location, provided food. The rats made more visits to the chocolate location after the LRI than after the SRI (Figure 1.1, top panel). Next, the animals received a taste-aversion treatment in their home cages, in which chocolate was paired with an injection of lithium chloride (LiCl). The animals were subsequently tested using the LRI (i.e., a condition in which the rats previously revisited the chocolate at a high rate). The rats made fewer revisits to the chocolate location after the lithium chloride treatment than in previous testing (Figure 1.1, bottom panel). The results from the LiCl treatment rule out the possibility that the rats were using a memory trace to revisit the

chocolate. LiCl pairing also rules out the use of relative familiarity; the animals could not solve the task using the previously learned rule. Therefore, the animals could not have reduced the rate of revisits to the chocolate location without knowledge of what, when, and where.

Experiment 1 demonstrated that rats possess what, when, and where components of episodic memory. This research was the first demonstration of episodic-like memory in rats, and it adds to the growing literature that suggests that animals may possess episodic-like memory (Clayton & Dickinson, 1998; Clayton & Dickinson, 1999a, 1999b; Clayton, Yu, & Dickinson, 2001, 2003). Although it may be impossible to demonstrate autonoetic consciousness in animals, relying on Tulving's (1972) definition of episodic memory may represent a critical strategy for testing episodic-like memory in rats.

In Experiment 1, there was the possibility that the rats could have been discriminating the "when" component using time of day, which would not require the animal to re-experience the previous event (Hampton, Hampstead, & Murray, 2005). Because in the previous study the rats were tested in the free-choice phase in either the morning or afternoon, the rats may have used a circadian oscillator, or time of day, (Mistlberger, 1994) to discriminate between the SRI and LRI test conditions. If the rats were discriminating "when" based on time of day, this would not be sufficient for the demonstration of episodic-like memory. Hampton et al. argued that the discrimination of "when" requires knowing how long ago the chocolate location was found, rather than what time of day is associated with the availability of the chocolate. Hampton et al. argue that encoding a rule about the availability of chocolate at different times of day is similar to semantic, rather than episodic, memory.

In Experiment 2, rats were given a SRI (1 hour) and a LRI (25 hours) that did not allow the use of a circadian oscillator to discriminate between the free-choice phases (Babb & Crystal,

in press). A separate study examined the longevity of spatial memory in rats and found that their accuracy in the radial maze was above chance after 25 hours (Babb & Crystal, *in preparation*). In Experiment 2, the inter-trial interval (ITI) was always at least 48 hours in order to reduce the similarity of the RI and ITI. After the animals had learned to selectively revisit the chocolate location (Figure 2.1, top panel), the chocolate was paired with lithium chloride. The rats were given a forced-choice phase, and during the 25-hr RI, they were fed chocolate and injected with lithium chloride (LiCl) before completing the free-choice phase (Figure 2.1, bottom panel). Therefore, the animals encountered chocolate while it was still good, and after encoding the location of chocolate they learned a new rule (i.e., chocolate is bad). The rats showed knowledge of what, when, and where both before and after the LiCl treatment. These results showed the rats were capable of flexibly updating their memory systems, and that the discrimination of what, when, and where is not based on time of day.

In Experiment 2, it was demonstrated that the rats learned their revisit strategy through experience, which is consistent with the explanation that rats were using episodic-like memory to solve the what-when-where task. The data from Experiment 3 suggested that the rats learned that a location replenished with a unique food, instead of their revisit strategy being specific to the chocolate food.

It is possible that in Experiments 1 and 2, the rats were not specifically encoding the content (“what”) of each location. The rats could have learned that a distinctive location replenished after the long retention interval without specifically encoding the content of each location. This kind of non-specific encoding would not be a demonstration of episodic-like memory. Also, after the chocolate was paired with LiCl, the rats could have switched from a

win-stay to a win-shift strategy with regards to the unique location. If the rats were using a win-shift strategy, this would not be an example of episodic-like memory.

In Experiments 1 and 2, satiation paradigms (Balleine & Dickinson, 1998; Clayton & Dickinson, 1999a; Colwill & Rescorla, 1985) using chocolate were not effective (i.e., even after exposure to large amounts of chocolate, the rats continued to prefer chocolate over regular food); therefore, taste aversion was used by pairing LiCl and chocolate. Unfortunately, unlike satiation, taste aversion is not reversible. A pilot study was conducted to determine that rats can be satiated to grape and raspberry food types (i.e., the rats switched their preferences to the regular pellets after repeated feedings of the unique food types). Therefore, after the rats have shown knowledge of what, when, and where with two food types, they were satiated to one, but not the second, food type. If the rats specifically encode the content of each location on the radial maze, they should selectively reduce revisits to the location that contains the devalued food type but continue to revisit the non-devalued location at a high rate. The two groups of rats were counterbalanced with respect to the order in which food types were satiated.

Experiment 4 investigated the selectivity of what, when, and where using the 8-arm radial maze. Two unique foods were presented during Phase 1, and the unique foods replenished after a LRI but not after a SRI. One of the unique foods was then devalued during the LRI using satiation, and the animals were then tested in the LRI to investigate whether they selectively reduced revisits to the devalued location while maintaining a high rate of revisits to the non-devalued unique location. The use of two foods and the selective devaluation of one food type during the retention interval would not allow the rats to solve this task without encoding the specific content at each location. In order to decrease revisits to the devalued unique location and simultaneously maintain a high number of revisits to the non-devalued unique location, the rats

would have to encode the content of each distinctive location, and recall the content of each location when deciding whether to revisit each unique location after the long retention interval.

EXPERIMENT 4

In Experiment 1, rats demonstrated knowledge of what, when, and where by revisiting the chocolate location more after the LRI than after the SRI. The rats then reduced revisits to the chocolate location in the LRI after chocolate was paired with LiCl. However, in this study the LiCl was administered outside of regular testing (i.e., before encoding, so there was the possibility that the rats did not encode the location of the chocolate). Because the test phase after the SRI occurred in the morning, and the test phase after the LRI occurred in the afternoon, there was also the possibility that the rats were discriminating the "when" component based on a circadian oscillator and were adopting different search strategies based on different times of day, which would not be considered a form of episodic-like memory.

In Experiment 2, time of day was controlled by administering retention intervals of 1 (SRI) and 25 (LRI) hours. Therefore, the test phase was always administered at the same time of day, and it was not possible to base a revisit strategy using a circadian oscillator. In this study, LiCl was administered during the 25-hr retention interval (i.e., after encoding). The rats continued to discriminate what, when, and where by revisiting the chocolate location more after the LRI than after the SRI, and they reduced revisits to the chocolate location after it was devalued during the LRI using LiCl. Experiment 2 also showed that this task was learned through experience. Experiment 3 showed the rats were able to transfer their revisit strategy

when grape replaced chocolate, which suggests that the revisit strategy was not specific to the chocolate food but rather to a unique location that replenishes.

The purpose of Experiment 4 was to evaluate the selectivity of discriminating what, when, and where in rats. In the previous experiments, there was the possibility that the rats were not recording the specific content ("what") of the location ("where"). There must be an integrated what-when-where representation in order for the animals to demonstrate episodic-like memory. It is also possible that in Experiments 1 and 2, after the chocolate was devalued using LiCl, the rats were simply switching from a win-stay to a win-shift strategy regarding the chocolate. In a standard radial maze task, the rats typically use a win-shift strategy (i.e., do not revisit a location after food is found there). In Experiments 1 - 3, the rats could have adopted a win-stay strategy with regard to the unique location after the taste-aversion treatment. If the rats were using a new strategy instead of actively avoiding the unique location after LiCl was introduced, this would not be an example of episodic-like memory.

Daily testing was divided into forced-choice (which always included both raspberry and grape) and free-choice phases, separated by a retention interval of either 1 hour (SRI) or 6 hours (LRI). The rats were trained to discriminate between the short and long retention intervals. After the long retention interval, the grape and raspberry replenished. After the short retention interval, the unique locations did not replenish. After the animals had learned to selectively revisit the unique locations following a long retention interval, one of the unique flavors was selectively devalued using satiation during the long retention interval. If the rats were encoding the content of the locations and if satiation was effective, the rats should selectively reduce revisits to the devalued location while maintaining a high number of revisits to the location containing the non-devalued unique food type.

The animals were then tested with new flavors (banana and chocolate) using the method described in Experiment 3. After demonstrating transfer to the new flavors, chocolate was paired with LiCl during the LRI. By selectively reducing revisits to the devalued location after LiCl treatment, the rats would demonstrate selective knowledge of what, when, and where.

If the rats can selectively reduce revisits to the devalued unique location while maintaining a high number of revisits to the non-devalued unique location, the data would indicate that rats specifically encode the content ("what") of each location ("where") and how long ago the unique foods were found ("when"). Using two unique food types does not allow the rats to selectively reduce revisits to one while maintaining a high number of revisits to the other without specifically encoding the content of each location.

METHOD

Participants

Twelve Long Evans rats (Harlan, Madison, WI) were individually housed in a colony with light onset at 0700 and offset at 1900. The rats were given unlimited access to 5001 Rodent Diet (Lab Diet, Brentwood, MO) for one week, and then given 20 g for one day and 15 g per day on subsequent days. Water was available at all times, except during brief testing periods. The rats were approximately 3 months old at the beginning of the experiment with an average weight of 300 g.

Apparatus

Testing was conducted in an eight-arm radial maze. The central hub (white polypropylene octagonal base [28.6 cm in diameter, 11.4 cm sides], metal walls [33.3 cm high],

and a clear polycarbonate lid, MED Associates, ENV-538) was equipped with eight computer-controlled guillotine doors (ENV-540). The arms (76.2 cm long, 8.9 cm wide with 17.5-cm high clear polycarbonate walls and topped with polycarbonate) radiated from the center hub with equal spacing between each arm. A food trough (ENV-200R1M) was located at the end of each arm. A photobeam (ENV-254, approximately 1 cm inside each food trough, 1 cm from the trough bottom) detected head entries. A 45-mg pellet dispenser (ENV-203) was placed behind each food trough. Additional photobeams were located in each arm at 3.8 and 5.1 cm from the guillotine doors.

The maze was positioned on stools 81.3 cm above the floor. White noise (67 db from a speaker located in the ceiling above the hub) masked outside noise. A 500-MHz computer in an adjacent room, running MED-PC for Windows (Version 1.15), controlled experimental events (guillotine doors and food) and recorded the data (photobeam breaks) with 10-msec resolution. A video camera in the ceiling above the center of the maze was used to observe the rats.

Procedure

Pretraining

Pretraining consisted of two days in which three pellets (PJA/I-0045, Research Diets Inc., New Brunswick, NJ) were placed along each arm, and one pellet was placed in each food trough to provide the animals with the experience of obtaining food in the maze. Each day one randomly chosen arm contained grape pellets (PJAI-G-0045, Research Diets Inc., New Brunswick, NJ) and one randomly chosen arm contained raspberry pellets (PJAI-R-0045, Research Diets Inc., New Brunswick, NJ).

Training

During initial training, the rats were individually placed in the central hub beginning at 0830; all 8 doors were then opened. The session ended when the animal had depleted all arms or 10 minutes had elapsed. Training was conducted once per day for 10 days. The arms containing regular food each dispensed one pellet; the arms containing the unique foods (grape and raspberry) each dispensed three pellets. The rats could revisit these unique locations up to five times, and receive 3 pellets during each visit; this procedure encouraged multiple revisits to the unique locations.

Subsequent training consisted of two shifts per day for each rat. The rats were individually placed in the maze beginning at 0830 for Phase 1 (forced-choice). Four doors (randomly chosen for each rat each day) were then opened, with the restriction that one of the arms dispensed three grape pellets, and one of the arms dispensed three raspberry pellets; all other arms dispensed one regular pellet (PJA/I-0045, Research Diets Inc., New Brunswick, NJ). A pellet(s) was delivered to each of the accessible food troughs contingent on the first head entry into the photobeam located in each of the troughs. The animals were later returned for Phase 2 (free-choice) in which all 8 doors are open; food was available at each of the arms not previously accessible in the forced-choice phase. The interval between the forced- and the free-choice phases served as a discriminative cue. On days with a short retention interval (SRI), the interval between Phases 1 and 2 was 1 hr, and the only arms that provided food in Phase 2 were the four arms not available in Phase 1. On days with a long retention interval (LRI), the interval between Phases 1 and 2 was 6 hr; the free-choice phase was identical to the SRI condition, except that the unique arms replenished (i.e., the grape and raspberry were available during the free-choice phase at the locations that provided each unique food type during the forced-choice phase). In Phase 2 after a LRI, a rat could revisit each unique location up to five times and receive 3 pellets

during each visit. The rats were required to visit each baited arm to collect the remaining pieces of food after a retention interval; the free-choice phase ended after the 4 or 6 different correct arms were visited in SRI or LRI, respectively. On any given day, either SRI or LRI (but not both) were tested.

Block Testing

The rats received alternating blocks of SRI followed by blocks of LRI sessions. Each block differed only in retention interval and consisted of 10-15 days of training. The rats received 3 blocks each of SRI and LRI sessions, for a total of 90 days of block testing.

Mixed Testing

The animals were then given 20 days of mixed SRI and LRI conditions. Mixed testing was the same as block testing, except the order of SRI and LRI was randomized across days.

Thirty minutes after a Phase 1, the rats were given 10 g of one of the unique food types (referred to as the *devalued food*) for thirty minutes, three times during the LRI. Although the rats did not eat all 30 g of food, pilot studies showed they would consume enough to become satiated to either the raspberry or grape. The rats were then returned to the maze to run a free-choice phase using the long retention interval. The LRI sessions after the satiation manipulation were identical to the LRI sessions in previous testing. The satiation was repeated in a later session using the opposite unique food for each rat. Each rat was tested with each food type as the devalued food, and the order of grape and raspberry was counterbalanced across rats. There were nine mixed sessions between the first and second satiation manipulations.

Transfer Test

The rats then received 9 trials of mixed SRI and LRI testing. The procedure was the same as the mixed condition in Experiment 1, except that banana (PJAI-Ban-0045, Research Diets

Inc., New Brunswick, NJ) and chocolate (F0299, Bio-Serv, Frenchtown, NJ) were used as the distinctive pellets (i.e., banana and chocolate replaced grape and raspberry, respectively). The first and second transfer trials were LRI and SRI, respectively.

Immediately after a Phase 1, the rats were given access to 15g of chocolate for 30 minutes, and approximately 10 minutes after removing the food they were injected with an isotonic solution of LiCl in distilled water (0.75 mol/L, 0.6-ml/100 g of body weight ip). The animals were returned to the maze 6 hours after Phase 1 for a LRI Phase 2 shift; the Phase 2 shift was identical to previous free-choices after LRI, except the rats were given 20 minutes to complete the session and the session was ended after the first four choices. One trial with LiCl was conducted.

Throughout the experiment, the arms of the maze were cleaned with Nolvasan (Fort Dodge Animal Health, Fort Dodge, Iowa) each day between forced- and free-choice phases. A plastic bag with holes and filled with all the food types used in the experiment was taped on the stool at the end of each arm beside the filled pellet dispensers (i.e., food odors were constant throughout all parts of the experiment).

RESULTS

Block training

During block training, the proportion of revisits to the unique location in the first four choices was .49 after the SRI and .96 after the LRI. Figure 4.1 shows that the discrimination of WWW was acquired through experience. The initial and terminal blocks consist of the first five trials in the first and last blocks, respectively, from SRI and LRI block training. There was a significant interaction between training and RI ($F(1, 11) = 47.34$, $p < .01$), reflecting a difference

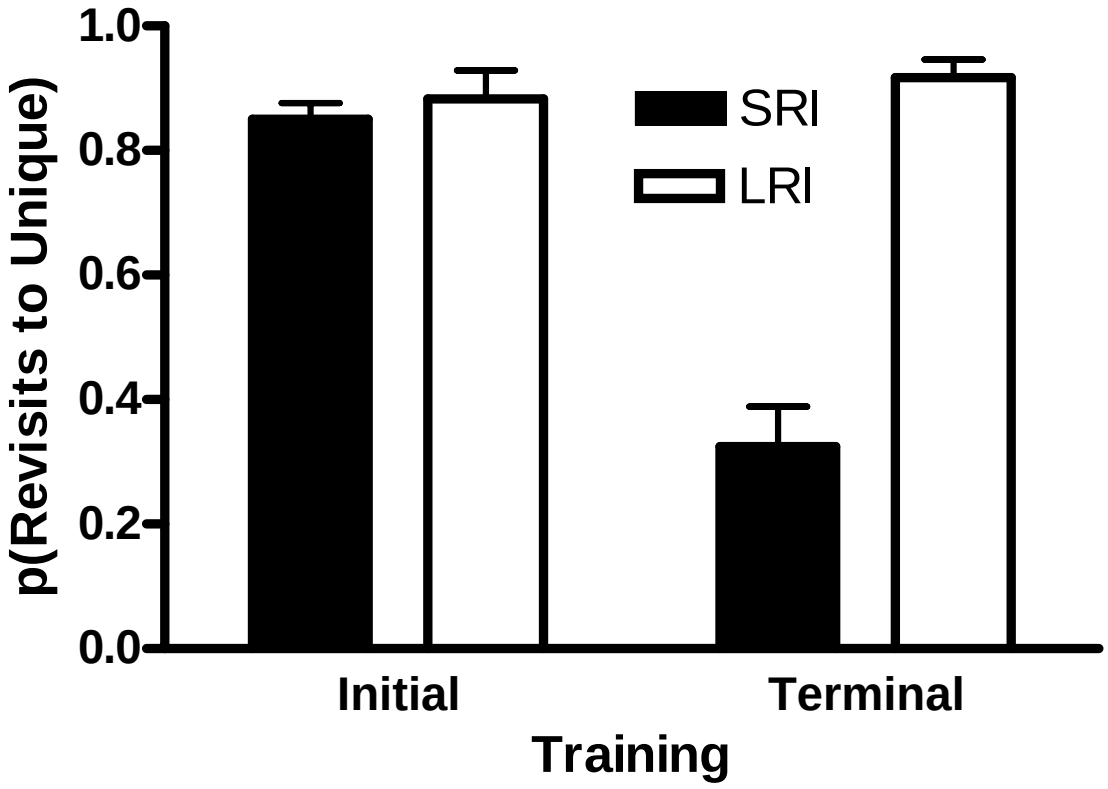

Figure 4.1

The discrimination of WWW was acquired through experience. The initial and terminal blocks consist of the first and last 5 trials, respectively, from SRI and LRI block training in Experiment 4. Error bars represent SEM.

between SRI and LRI in terminal ($t(11) = 8.19$, $p < .01$) but not initial ($t(11) < 1$) training. The proportion correct after the retention interval is shown in Table 3; the data shown in Table 3 is based on an analysis that was restricted to the first four choices among the six non-unique arms.

Mixed Training

The proportion of revisits to grape and raspberry during the first four choices following the retention intervals in mixed SRI and LRI is shown in Figure 4.2. The rats revisited the unique locations more after the LRI than after the SRI ($F(1, 11) = 120.61$, $p < .01$). There was no significant effect of food type ($F(1, 11) = 4.63$, $p > .05$), and no interaction between retention interval and food type ($F(1, 11) = .10$, $p > .05$).

Because the data in Figure 4.2 and Table 3 were derived from independent segments of the data (i.e., unique and non-unique arm entries, respectively), these data were used to compare error rates in the mixed condition. The probability of a revisit to a non-unique arm after a short retention interval (i.e., a baseline error rate) was .09 ± .02 (mean ± SEM). This baseline is lower than the probability of a revisit to a unique location after a short retention interval ($t(11) = 4.18$, $p < .01$). Therefore, after short retention intervals, the rats revisited the unique locations at a higher rate relative to the error rate for non-unique locations. After long retention intervals, the rats revisited the unique locations at a higher level than would be expected based on revisits to non-unique arms. In particular, the probability of a revisit to a non-unique arm after a long retention interval (i.e., baseline error rate) was .28 ± .02 (mean ± SEM). The probability of a revisit to a unique location after a long retention interval was significantly higher than this baseline error rate ($t(11) = 30.71$, $p < .01$). In summary, revisits to the unique locations occurred at a rate higher than expected from the baseline error rate after both long and short retention intervals.

Table 3

Mean (and SEM) of proportion correct in the first four choices excluding the unique locations after the retention interval in Experiment 4.

	Retention Interval	
Trial Type	Short	Long
Block Training	.86 (.01)	.72 (.01)
Mixed Testing	.91 (.02)	.72 (.02)
Satiation		.78 (.02)
Initial Transfer	.92 (.04)	.69 (.04)
Terminal Transfer	.93 (.02)	.84 (.03)

Note. The analysis of the first four choices was restricted to the six non-unique arms.

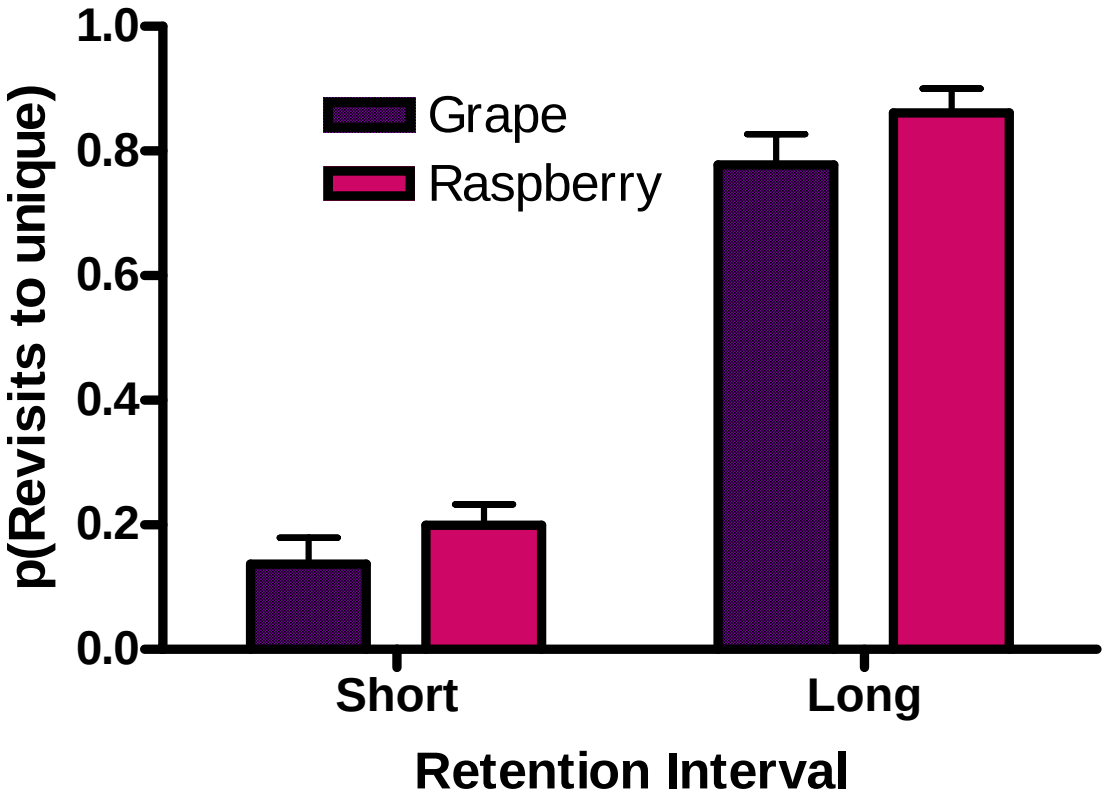

Figure 4.2

Mean proportion of revisits to the unique locations in the first four choices of Phase 2 in mixed testing is plotted as a function of retention intervals in Experiment 4. Error bars represent SEM.

Satiation

There was no difference between revisits to grape and raspberry after the LRI ($t(11) = 1.39$, $p > .05$); therefore, the proportions of revisits to the unique locations during mixed testing were combined into a single baseline score. The proportion of revisits to the devalued unique location and the non-devalued unique location after the satiation manipulation, compared with baseline performance, is shown in Figure 4.3 The rats revisited the devalued unique locations in the LRI less after the satiation manipulation relative to baseline ($t(11) = -3.79$, $p < .01$). Revisits to the non-devalued unique location were not different relative to baseline ($t(11) = -2.19$, $p > .05$).

The same conclusions were obtained when the analyses were performed separately on grape and raspberry satiation tests. Figure 4.4 shows baseline performance compared to performance during the grape satiation manipulation. The rats revisited the devalued grape location less after satiation relative to baseline ($t(11) = -3.18$, $p < .01$). Revisits to the non-devalued raspberry location were not different from baseline performance ($t(11) = -1.19$, $p > .05$). Figure 4.5 shows baseline performance compared to revisits to the raspberry location and the grape location in the raspberry satiation condition. Revisits to the devalued raspberry location declined, but only a trend toward significance was observed ($t(11) = -2.14$, $p = .06$), while revisits to the non-devalued grape location did not decline ($t(11) = -1.63$, $p = .13$).

Transfer Test

Figure 4.6 shows the rats exhibited complete transfer during the first LRI and SRI sessions. In the first two trials (LRI and SRI, respectively), the rats revisited the unique locations more after the LRI than the SRI ($t(11) = 3.32$, $p < .01$). In the remaining 7 trials, the rats

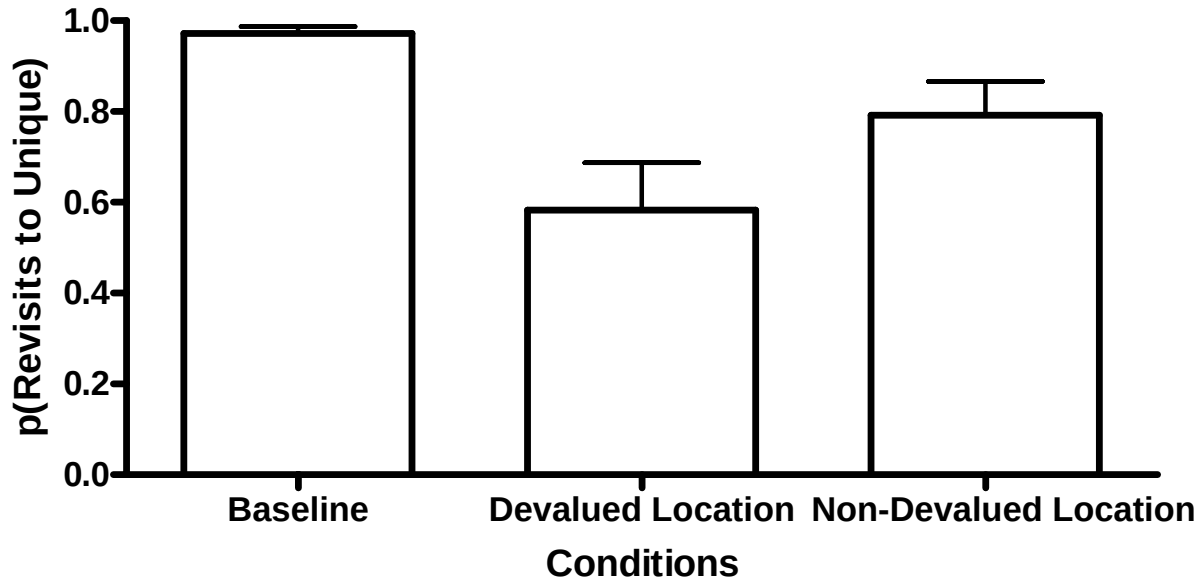

Figure 4.3

Mean proportion of revisits to the devalued location in the first four choices after satiation. Performance is compared to revisits in the first four choices in baseline and to the non-devalued unique location. Error bars represent SEM.

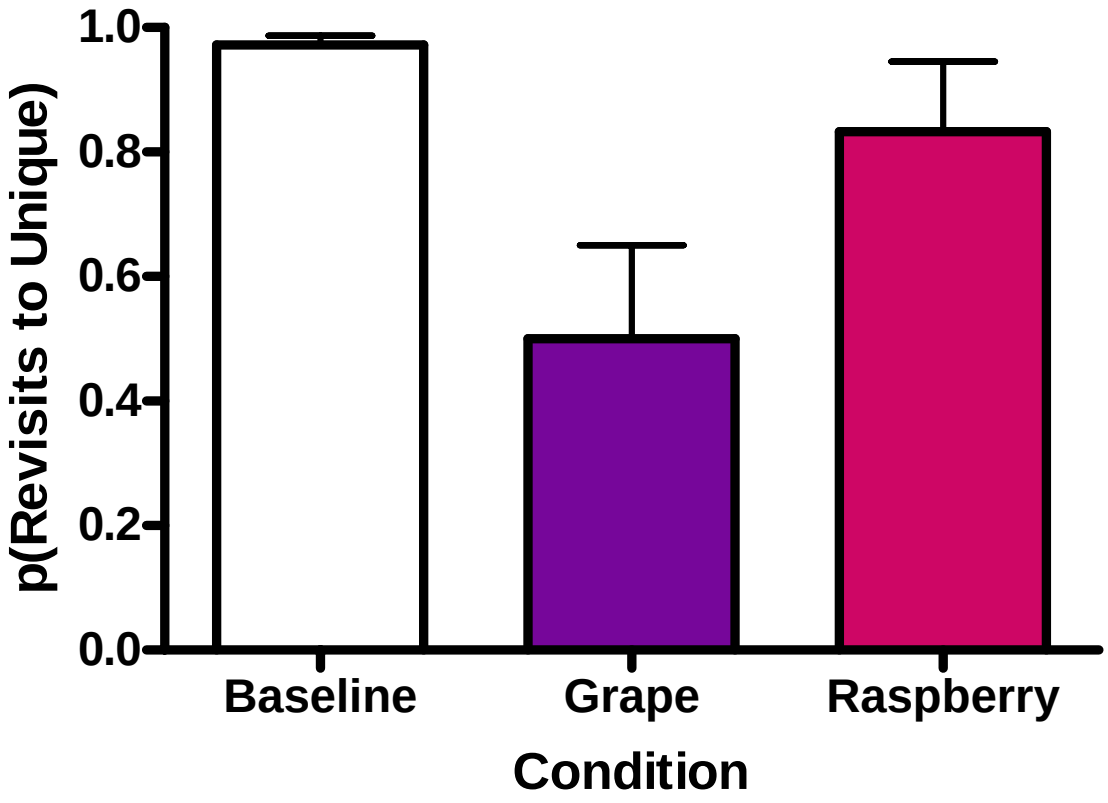

Figure 4.4

Mean proportion of revisits to the devalued grape location in the first four choices after satiation. Performance is compared to revisits in the first four choices in baseline and to the non-devalued raspberry location. Error bars represent SEM.

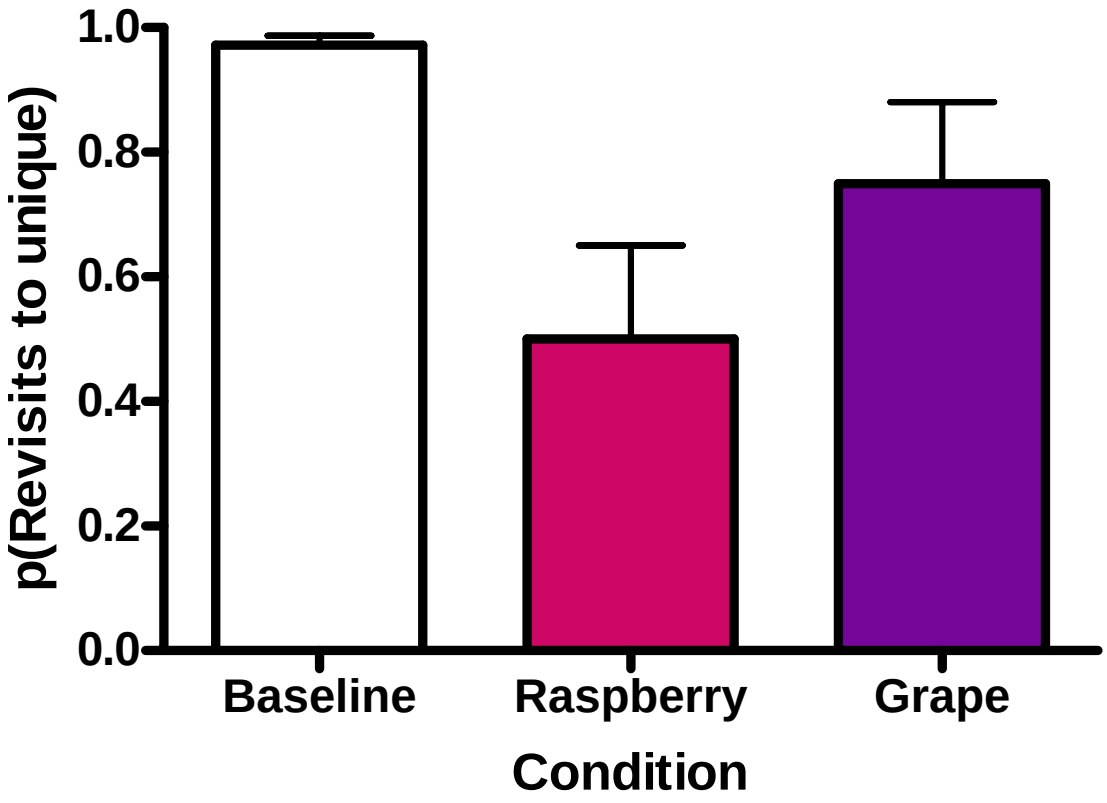

Figure 4.5

Mean proportion of revisits to the devalued raspberry location in the first four choices after satiation. Performance is compared to revisits in the first four choices in baseline and to the non-devalued grape location. Error bars represent SEM.

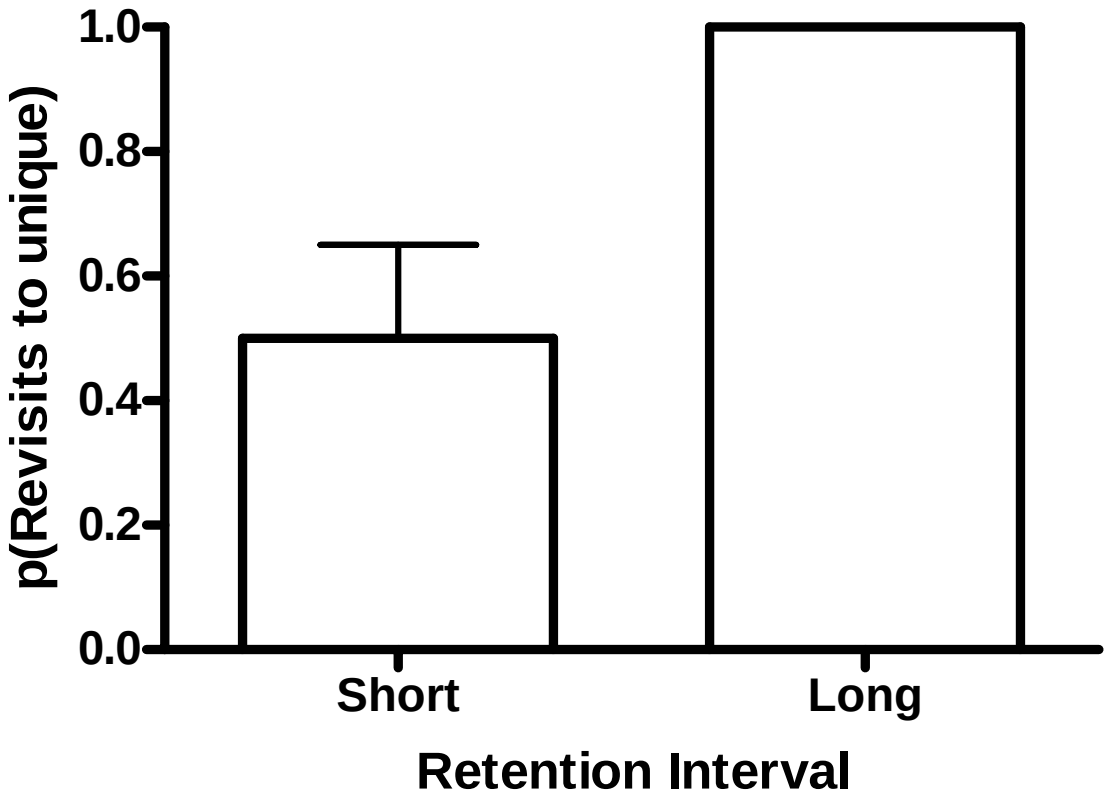

Figure 4.6

Mean proportion of revisits to the unique locations (banana and chocolate) during Phase 2 of the initial transfer test in Experiment 4. Error bars represent SEM.

continued to revisit the unique locations more after the LRI than after the SRI ($t(11) = 6.83$, $p < .01$; Figure 4.7).

LiCl Test

Performance from LRI in testing after the LiCl treatment is shown in Figure 4.8, together with the expected probability of revisiting banana and chocolate (i.e., LRI before LiCl treatment). There was a main effect of condition (i.e., pre-LiCl versus post-LiCl trials) ($F(1, 11) = 75.00$, $p < .01$). There was also a main effect of food type ($F(1,11) = 77.54$, $p < .01$). There was a significant interaction between pre-LiCl and post-LiCl conditions and food type ($F(1, 11) = 74.11$, $p < .01$). The rats reduced revisits to the chocolate location ($t(11) = 8.98$, $p < .01$) after the LiCl, but maintained a high number of revisits to the banana location ($t(11) = -1$, $p > .05$). There was no statistical difference between revisits to chocolate in the SRI before LiCl treatment and in the LRI after LiCl treatment ($t(11) < 1$).

A preference test was given immediately after the final LiCl-trial to evaluate the effectiveness of the taste-aversion manipulation using two bowls, each containing 50 chocolate or 50 regular pellets. After taste aversion the proportion of chocolate pellets consumed declined ($t(11) = 3.13$, $p < .01$) and the proportion of regular pellets consumed increased ($t(11) = 2.69$, $p < .05$).

In summary, the rats made more revisits to the unique locations after the long than after the short retention interval. The rats were then satiated to one of the unique food types, and revisits to the devalued location declined when compared to baseline performance, but revisits to the non-devalued location were not different from baseline. The rats were then transferred from grape and raspberry to banana and chocolate, and they showed complete transfer to both flavors. Next, chocolate was paired with lithium chloride during the LRI. The rats made fewer revisits to

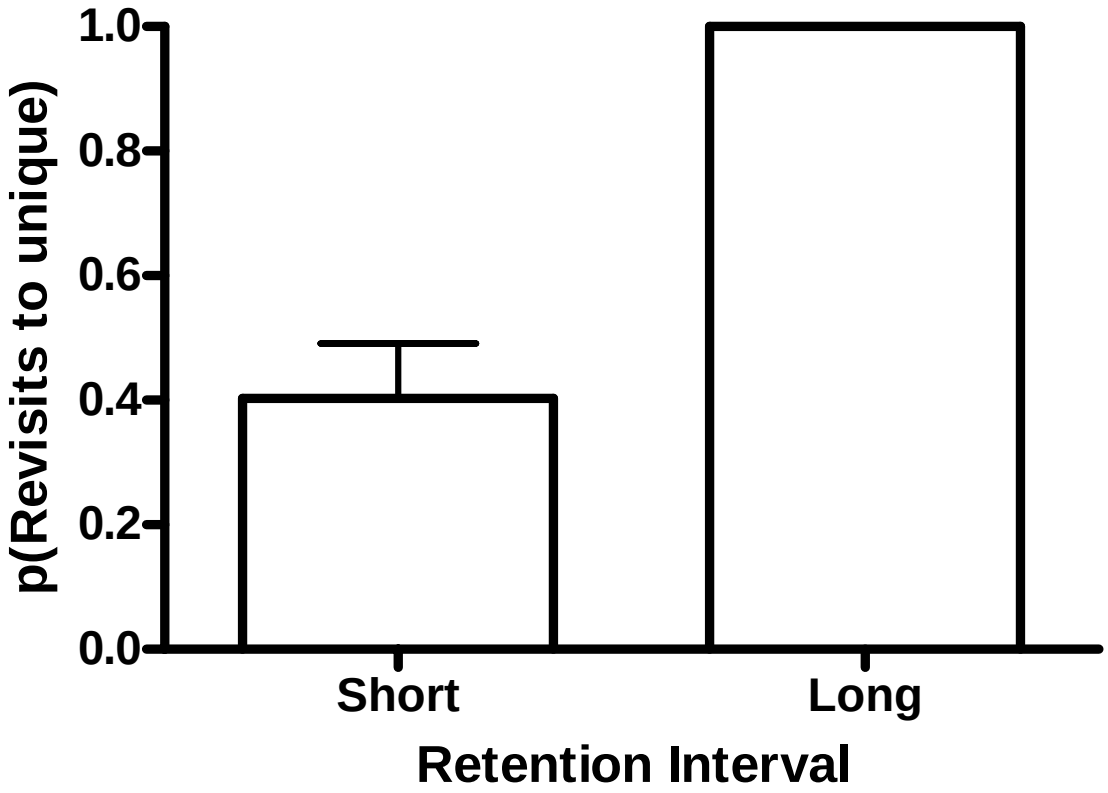

Figure 4.7

Mean proportion of revisits to the unique locations (banana and chocolate) during Phase 2 of the terminal transfer test in Experiment 4. Error bars represent SEM.

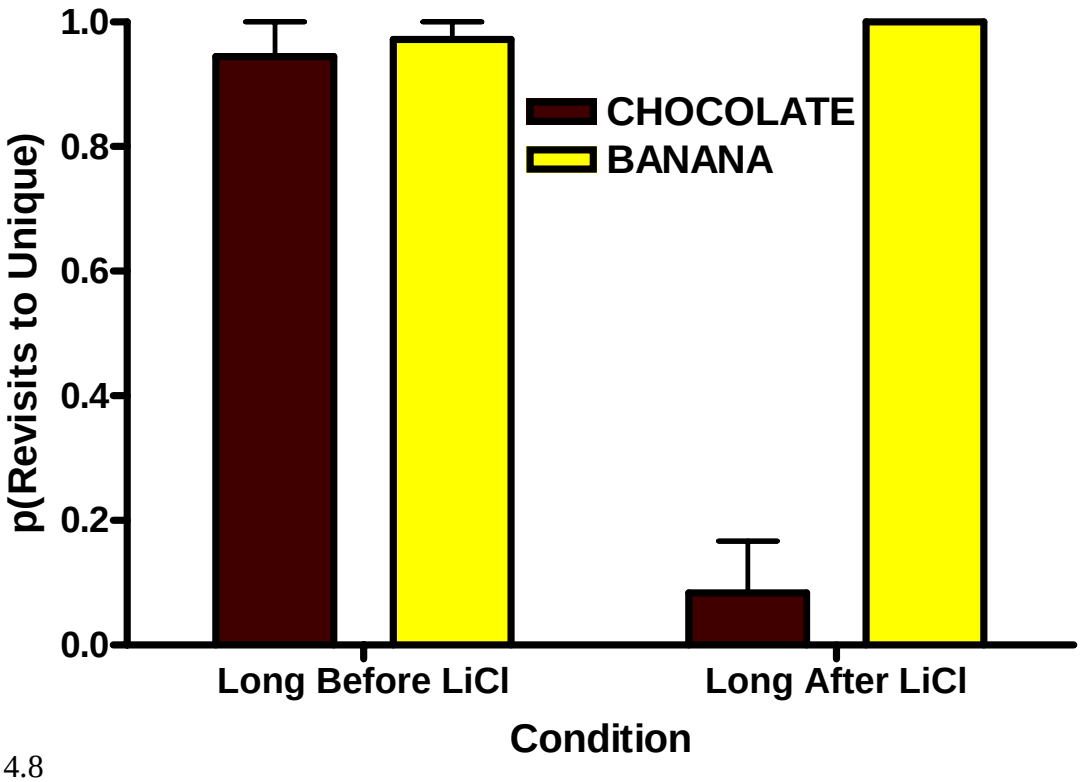

Figure 4.8

Mean proportion of revisits to the chocolate and banana locations in the first four choices of Phase 2 in mixed testing. Data is from testing before and after chocolate was paired with LiCl treatment, respectively (see text for details). Error bars represent SEM.

the chocolate location after the lithium chloride treatment than in previous testing. However, the rats maintained a high rate of revisits to the banana location after LiCl was paired with chocolate. The animals could not have selectively reduced the rate of revisits to the devalued location while maintaining revisits to the non-devalued location without specific knowledge of what, when, and where.

DISCUSSION

Rats were required to visit four randomly chosen, baited locations, two of which were randomly selected to provide unique flavors, during the study phase. Each of the unique locations provided 3 pellets, while the regular locations provided only 1 pellet. The animals were later returned to the maze after either a short or long retention interval, with all 8 locations available. After the SRI, the four locations not available in the study phase provided food; after the LRI, the four locations not available in the study phase, plus the unique locations, provided food. The unique locations replenished up to five times in each LRI session. The rats made more visits to the unique locations after the LRI than after the SRI.

The rats were then satiated to one of the unique food types during the long retention interval (i.e., after encoding the unique locations in the study phase). The rats showed a decrease in revisits to the devalued food type while continuing to revisit the non-devalued food type at a high rate.

The rats were then transferred from grape and raspberry pellets to banana and chocolate pellets. The rats exhibited complete transfer to the banana and chocolate distinctive pellets in both the first LRI and SRI trials and in the subsequent mixed training with short and long RIs.

Next, chocolate was paired with LiCl during the LRI (i.e., after encoding in the study phase). The animals were then tested in a free-choice phase, which was a condition in which they previously revisited chocolate at a high rate. The rats selectively reduced revisits to the location containing the devalued chocolate, but maintained a high rate of revisits to the location containing banana. The animals could not have selectively reduced the rate of revisits to the location containing the devalued food type while continuing to revisit the location containing the non-devalued food type without specifically encoding where each flavor was located, and when they had been encountered (i.e., knowledge of WWW).

In Experiments 1 and 2, there was the possibility that the rats were not specifically encoding the content of the unique location, and instead based their revisit strategy on a location that replenished after the LRI. By contrast, Experiment 4 showed that the rats encoded the specific food types at each unique location. The animals could not have selectively reduced visits to the unique food that was devalued during the long retention interval while maintaining a high rate of revisits to the non-devalued food if they had not specifically remembered the content of each location.

There was also the possibility that, in Experiments 1 and 2, the rats were shifting from a win-stay to a win-shift strategy with regards to the unique location. If the rats were revisiting the unique location based on this kind of strategy, this would not be evidence for episodic-like memory. However, if the rats were using a win-shift strategy in Experiment 4, they would have reduced revisits to both unique locations after the satiation and LiCl manipulations. Instead, they maintained a high number of revisits to the non-devalued location and reduced revisits to the devalued location, which rules out the win-stay/win-shift hypothesis.

In Experiment 4, satiation was used to devalue one of the unique food types. In Experiments 1 and 2, LiCl was the only method used to decrease revisits to the location containing the unique food after the LRI. Satiation is a preferred manipulation when compared with LiCl for two reasons. First, satiation is reversible—when a rat is satiated to a food type, the rat will resume eating the food as soon as a few hours after satiation, whereas a rat that has been fed a unique food type that was paired with LiCl will continue to avoid that food type long after the manipulation. Second, LiCl is also an aversive manipulation that involves a form of punishment. When the unique flavor is paired with LiCl, the flavor becomes aversive. Visiting a location with an aversive outcome is similar to punishing the response that produced the aversive outcome. By contrast, consuming a satiated flavor is not expected to punish the response that produced that flavor.

In Experiment 1, the rat encountered the aversive outcome in the study phase, which could have led to an encoding failure, and could lead to punishment if the rat revisited that location. In Experiment 2, the LiCl treatment occurred after encoding in the first post-LiCl trial. However, the problem described for Experiment 1 applies to the second post-LiCl trial. In Experiment 4, the punishment concern is eliminated because satiation would not lead to punishment. Moreover, only a single trial was conducted with LiCl, and the LiCl was presented after encoding the location of the to-be-devalued food type.

Experiment 4 met Clayton and colleagues' (2003) behavioral criteria of content, structure, and flexibility, and provides further evidence that non-human animals have episodic-like memory systems that Tulving (2002) claimed to be unique to humans. These findings may be an important step in the development of a rodent model of human episodic memory diseases and disorders.

CHAPTER 6

GENERAL DISCUSSION

These studies were among the first to demonstrate that rats discriminate what, when, and where (WWW) on the 8-arm radial maze. These experiments meet Clayton and colleagues' (2003) behavioral criteria for episodic-like memory in nonhuman animals.

Experiment 1 showed that rats discriminate what, when, and where using the 8-arm radial maze. In this experiment, there was the possibility that the rats were discriminating the "when" component based on time of day. Because the pairings of LiCl and the chocolate food took place outside of testing, there was also the possibility that the rats failed to encode the location of the chocolate, or that the rats adopted a win-shift strategy with regards to the chocolate location.

Experiment 2 demonstrated the rats acquired what, when, and where discrimination through experience, and that they did not discriminate "when" using a circadian oscillator. Pairings of LiCl and chocolate occurred during the LRI; therefore, the rats did not reduce revisits to the chocolate location due to a failure to encode the location of the chocolate.

Experiment 3 showed that the rats were able to transfer to a new unique food type (i.e., grape). These data point to an encoding of location, which raises the possibility that the rats did not encode a representation of both content ("what") and location ("where").

Experiment 4 demonstrated the rats selectively reduce revisits to a devalued unique food type, while maintaining revisits to a non-devalued unique food type. These data suggest the rats specifically encode content, location, and the point in time that these food types were found. The

use of two unique food types also rules out the hypothesis that the rats were switching from a win-stay to a win-shift strategy.

Tulving (1972) originally defined episodic memory as memory for what happened, where the event happened, and when in time the event occurred. Since this original definition, additions have been made to the concept of episodic memory, including subjective states of consciousness such as knowledge of self and mental time travel (Tulving, 1983; Tulving & Markowitsch 1998). However, these subjective states of consciousness are difficult, if not impossible, to test in non-verbal animals (Hampton & Schwartz, 2004; W.A. Roberts, 2002; Suddendorf & Busby, 2003; Suddendorf & Corballis, 1997). Therefore, these studies are based on Clayton and colleagues' (Clayton et al., 2003; Clayton, et al., 2000; Clayton, et al., 2001) argument that when studying episodic memory in nonhuman animals, we should focus on behavioral criteria instead of subjective states of consciousness.

In humans, episodic memory is the first kind of memory to degenerate with age, and the last to be acquired as a child (Tulving, 2002). Patients with neurodegenerative diseases such as Alzheimer's show a loss of episodic memory. The hippocampus plays an important role in the acquisition of new memories; it incorporates temporal information from the frontal lobes, thus providing a basis for "when" (Burgess, Maguire, & O'Keefe, 2002). The hippocampus has been implicated in spatial memory tasks, and may provide a basis for "where" (O'Keefe & Nadel, 1978). The right hippocampus processes spatial locations, while the left hippocampus is involved in episodic memory (Burgess, et al., 2002; Maguire, 2001). The left and right prefrontal cortices are implicated in encoding and retrieval of episodic memory, respectively (Tulving, 2002).

Development of a rodent model for episodic memory could lead to a better understanding of the neural, molecular, and behavioral mechanisms of episodic memory (Griffiths & Clayton,

2001). The availability of a rodent model could also permit the screening of putative pharmacotherapies for human memory disorders such as Alzheimer's (Clayton & Griffiths, 2002).

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