RAPID COMMUNICATION

Cognitive Mechanisms of Memory for Order in Rhesus Monkeys
(Macaca mulatta)

Victoria L. Templer* and Robert R. Hampton

ABSTRACT: One important aspect of episodic memory is the ability to remember the order in which events occurred. Memory for sequences in rats and has been shown to rely on the hippocampus and medial prefrontal cortex (DeVito and Eichenbaum (2011) J Neuro 31:3169-3175; Fortin et al. (2002) Nat Neuro 5:458-462). Rats with hippocampal lesions were impaired in selecting the odor that had appeared earlier in a sequence of five odors but were not impaired in recognition of previously sampled odors (Fortin et al., 2002; Kesner et al. (2002) Behav Neuro 116:286-290). These results suggest that order is not represented by relative familiarity or memory strength. However, the cognitive mechanisms underlying memory for order have not been determined. We presented monkeys with lists of five images drawn randomly from a pool of 6,000 images. At test, two images were presented and monkeys were rewarded for selecting the image that had appeared earlier in the studied list. Monkeys learned to discriminate the order of the images, even those that were consecutive in the studied list. In subsequent experiments, we found that discrimination of order was not controlled by list position or relative memory strength. Instead, monkeys used temporal order, a mechanism that appears to encode order of occurrence relative to other events, rather than in absolute time. We found that number of intervening images, rather than passage of time per se, most strongly determined the discriminability of order of occurrence. Better specifying the cognitive mechanisms nonhuman primates use to remember the order of events enhances this animal model of episodic memory, and may further inform our understanding of the functions of the hippocampus. © 2012 Wiley Periodicals, Inc.

KEY WORDS: sequence; temporal; episodic memory; memory systems; temporal order

INTRODUCTION

Imagine that you hear a noise coming from under the hood of your car. Before making an appointment with the mechanic, you struggle to remember whether you first noticed it before or after you had the car in for a tune-up recently. Memory for the order of these events would help you decide whether to suggest that the mechanic may have caused the problem during the tune-up. Humans often remember the order in which events occurred (Eichenbaum, 2005; Tulving, 2005), probably using multiple cognitive mechanisms (e.g., Friedman, 2005; McColgan and McCormack, 2008).

A significant hurdle in the study of nonhuman memory is the fact that nonhuman animals cannot verbally report the rich details of private experience that often demonstrates human memory of personal events (e.g., Dere et al., 2006; Templer and Hampton, 2012). One successful approach has been to test for specific aspects of episodic memory in nonhumans, rather than attempting to capture all the properties of episodic memory with a single paradigm (e.g., Clayton et al., 2001; Fortin et al., 2002; Roberts and Roberts, 2002; Hampton and Schwartz, 2004; Dere et al., 2006; Clayton and Russell, 2009). The study of memory for the order of unique events appears to capture some aspects of episodic memory (Fortin et al., 2002; Kesner et al., 2002). In these studies, rats encountered five odors in sequence. Two odors from this list were then presented at test, and rats were rewarded for choosing the odor that appeared earlier in the list. Rats with hippocampal lesions were significantly impaired in reporting the order of odors but performed comparably to controls on recognition tests that required subjects to choose between an odor from the list and one that was not in the list. These results suggest that memory for order is dependent on the hippocampus and relies on cognitive mechanisms different from those responsible for recognition performance.

Fortin's and Kesner's experiments elegantly dissociate memory for order from recognition performance, but they do not unambiguously identify the cognitive mechanism by which order is remembered. Multiple memory processes, including relative familiarity and memory strength, may contribute to performance in both recognition tests (Charles et al., 2004; Tu et al., 2011) and tests of memory for order. Because memory fades over time, memory strength tends to correlate with order of presentation, with items presented earlier weakly represented compared to more recent items. Studies of memory for order conducted to date "do not provide definitive evidence that animals solve
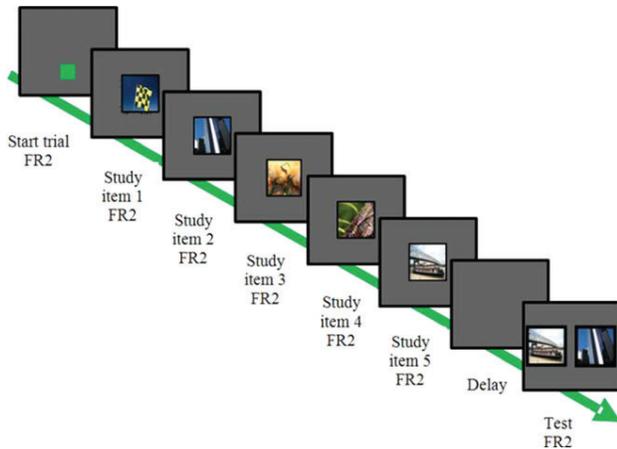
FIGURE 1.   Trial progression in Experiment 1. Monkeys initiated the study phase of each trial by touching the green ready box. They then saw and touched five images in sequence, separated by a 500-ms ISI. After a 500-ms delay after the fifth image, monkeys were rewarded for choosing the image that had occurred earlier in the sequence at test. In this example, the second and fifth images are presented at test and the image of the skyscrapers would have been correct. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

these tasks using a direct representation of the order of events (DeVito and Eichenbaum, 2011, pp. 3169)." This significant issue in memory representation necessitates closer examination of the cognitive mechanisms that underlie the ability to remember sequences. By directly manipulating multiple sources of information that might support memory for order, including memory strength, list position, intervening events, and temporal spacing, we evaluated several possible mechanisms for memory of the order of trial-unique sequences.

MATERIALS AND METHODS

Subjects

Six four-year-old male rhesus monkeys (*Macaca mulatta*) were used. Monkeys were pair-housed and kept on a 12:12 light:dark cycle with light onset at 7:00 am. Four of the monkeys were fed a full ration of food at the end of testing each day, and the other two were fed half of their food ration in the morning before testing and the other half of food after testing each day. Water was available ad libitum.

Apparatus

Subjects were tested on computerized touch-screen systems in their home cages. Each system consisted of a 15-inch LCD color monitor (3M, St. Paul, MN) running at a resolution of 1024×768 pixels, generic stereo speakers, two automated food dispensers (Med Associates, St. Albans, VT), and two food cups located below the screen.

General Procedure

Each monkey had access to his cage-mate at all times except during testing and during feeding at the end of the day. Immediately before testing, monkeys were separated by insertion of plastic dividers between cage-mates that allowed limited visual and physical contact, but prevented access to the cage-mate's testing equipment. Testing systems were locked to the front of each monkey's cage. Cage doors were then raised, giving subjects full access to the screen during testing. Food rewards were nutritionally balanced banana flavored pellets (Bio-Serv, Frenchtown, NJ). One to five test sessions were conducted daily between 10 am and 5 pm, 6 days per week.

Six thousand color photographs collected from public online digital image databases were used as memoranda. Images of humans were not used. Images were resized to 300×300 pixels.

Data Analysis

Proportions were arcsine transformed before statistical analysis to better approximate the normality assumption underlying parametric statistics (Keppel and Wickens, 2004, p. 155). All *t*-tests were two tailed.

Experiment 1

To prepare monkeys for a series of experiments designed to identify the cognitive mechanisms for memory for order, we trained them to identify which of two images from trial-unique sequences of five images had been presented first (Fortin, et al., 2002; Kesner, et al., 2002). Based on previous findings from monkeys (Petrides, 1991a,b, 1995) and rats (Fortin, et al., 2002; Kesner, et al., 2002), we hypothesized that monkeys would learn to select the earlier image.

Phase 1: Training on nonadjacent images. A green box appeared at the bottom of the screen and remained until the monkey touched it (FR2) to start a trial (Fig. 1). A photograph then appeared in the center of the screen on a gray background. The image was only sensitive to touch (FR2) after a required minimum study period of 250 ms. After the image was touched, it disappeared, a 500-ms interstimulus interval (ISI) occurred during which the screen was gray, and a second randomly selected image appeared in the same place as the first. This process repeated until five sample images were presented and touched. Following touches to the fifth image and a 500-ms retention interval, two test images from the list appeared to the left and right of the center of the screen separated by 400 pixels. Selection of the image that had occurred earlier in the sequence was rewarded with a positive auditory stimulus and a pellet food reward 100% of the time. Selection of the image that had occurred later in the sequence was followed by a negative auditory stimulus and a 10-s time-out during which the screen was black. The position of the correct and incorrect test images was pseudorandomized such that the correct image did not appear on the same side of the screen on more than four

TABLE 1.

Test Trial Types in Experiments 1 and 2

Normal nonadjacent trials	Normal adjacent trials	Novel trials
1+ vs. 3−	1+ vs. 2-	1 vs. novel
1+ vs. 4−	2+ vs. 3-	2 vs. novel
1+ vs. 5−	3+ vs. 4-	3 vs. novel
2+ vs. 4−	4+ vs. 5-	4 vs. novel
2+ vs. 5−		5 vs. novel
3+ vs. 5−		

Experiment 1 Phase 1 consisted of all normal nonadjacent trials. Experiment 1 Phase 2 consisted of both nonadjacent and adjacent trials. Experiment 2 consisted of normal nonadjacent trials and probe novel trials, on which monkeys received positive auditory reinforcement for either selection.

consecutive trials. After errors, the entire trial was repeated. If the subject erred again, a 1-s time-out was followed by appearance of only the choice images. This last phase of the trial, with the same images, was repeated as many times as needed until the subject selected the correct image. Trials were separated by a 500-ms intertrial interval (ITI) during which the screen was black. Six-hundred images were randomly drawn from the entire set of 6,000 for use in each session. Tests consisted of images from each of the six possible nonadjacent list positions. Each type of test was presented 20 times in a session, resulting in sessions of 120 trials (Table 1). Monkeys progressed to Phase 2 after achieving 80% correct in each of three consecutive sessions.

Phase 2: Transfer to adjacent images. All procedures were identical to Phase 1, except that all possible pairs of images were tested, including adjacent images, as shown in Table 1. The 10 test types were randomly intermixed in sessions of 120 trials, resulting in 12 tests of each type per session. Monkeys completed 10 sessions, and then progressed to Experiment 2.

Results and discussion. Monkeys took between 16 and 163 sessions to reach criterion in Phase 1 (mean: 76). Results from the 10 sessions of Phase 2 are shown in Figure 2. Symbolic distance refers to the number of images that intervened between test images in the study list. For example, a test using the first and second image from the study list had a symbolic distance of 0, whereas a test contrasting the first and fifth image had a symbolic distance of 3. Recency reflects the amount of time that passed since presentation of the earliest of the tested images. A test involving Images 1 and 5 is labeled low recency because 1 is the least recent image, whereas a test involving Images 4 and 5 is high recency because Image 4 occurred comparatively recently (Fig. 2).

Subjects were more accurate the greater the symbolic distance (Fig. 2, repeated measures ANOVAs and paired sample t-test: low recency (blue line): $F_{3,15} = 7.23$, $P = 0.003$; moderate recency (red line): $F_{2,10} = 53.1$, $P < 0.001$; high recency (orange line): $t_5 = 9.09$, $P < 0.001$). The symbolic distance effect suggests that discrimination of order resulted from a con-

tinuous representation of relative order rather than from image–image associations (e.g., Shettleworth, 2010). An image–image associative mechanism predicts the opposite pattern: subjects would perform better on test images that were closer together in the list because direct image–image associations would exist between adjacent pairs but do not between widely separated images (e.g., 2 vs. 3 would be an easier than 2 vs. 5).

Recent images were better discriminated than images that occurred earlier in the list (Fig. 2, stacking lines, repeated measures ANOVAs: symbolic distance 0: $F_{3,12} = 39.29$, $P < 0.001$; symbolic distance 1: $F_{2,10} = 33.50$, $P < 0.001$). Performances at a symbolic distance of 2 (1, 4; 2, 5 tests), however, did not differ significantly (paired sample t-test: $t_1 = -2.32$, $P = 0.068$). The fact that images were better discriminated when they had occurred later in the list indicates that memory for order decays with time. Similar recency effects are seen in human serial recall (Howard and Kahana, 2002; Oberauer and Lewandowsky, 2008).

While the symbolic distance effect we observed indicates that image–image associations are not the cognitive mechanism supporting memory for order, the presence of this effect does not discriminate among a variety of other candidate memory mechanisms. We conducted a series of tests that evaluate several different possible cognitive mechanisms. Each putative mechanism is described and operationalized in the introduction to the relevant experiment.

Experiment 2: Evaluating the influence of memory strength

In Experiment 1, monkeys remembered the order in which unique lists of images appeared. The pronounced symbolic distance effect observed suggests that the cognitive representation underlying performance is not an associative one. The symbolic distance effect is consistent with discrimination of order based on memory strength. Because memory decays with time, earlier
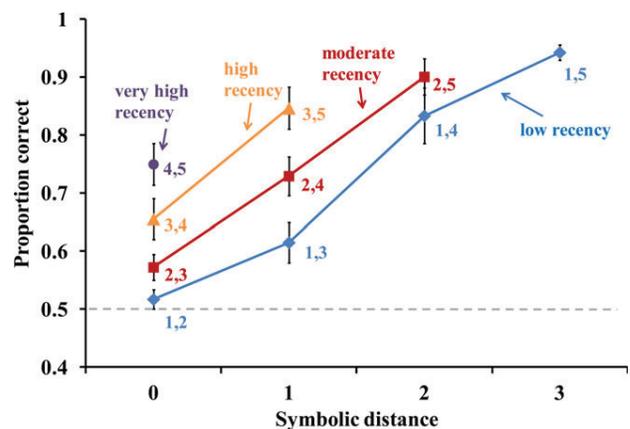

FIGURE 2. Proportion correct for all nonadjacent and adjacent test pairs in Experiment 1. The symbolic distance effect is evident in the upward trend of each colored line. A recency effect is shown by the fact that each of the colored lines stacks one above another. Error bars are standard errors of the mean. The dashed line indicates the chance rate of 50%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

images in a sequence will have weaker memory strengths, and monkeys may learn to choose the image with the weakest memory strength at test. In Experiment 2, we directly manipulated memory strength to determine the extent to which monkeys chose based on differences in memory strength. Images that had not been seen before and therefore had memory strength of zero were paired with one of the five images from the studied list in probe tests. If monkeys selected the image with the weakest memory strength in normal tests, they should choose the novel image significantly more often than any image from the studied list in these probe tests.

To prepare subjects for probe tests with no reinforcement, intermittent primary reinforcement was instituted. All correct trials were followed by positive auditory reinforcement but only 70% were also followed by food reward. After 10 adaptation sessions with all nonadjacent test pairs, monkeys received five 116-trial sessions with 20 randomly intermixed probe tests in which a novel image was drawn from a set of 100 never before seen images and presented at test with one of the images from the study list (Table 1). All responses on probe tests were reinforced with the positive auditory reinforcement but no food reward.

All other testing procedures were the same as in Experiment 1, except for image randomization. Study lists were generated using the same list of 6,000 images as before but images were selected by randomization without replacement. This ensured that monkeys saw all 6,000 images without repetition before the entire set of 6,000 images was reshuffled and used again. Monkeys worked at different rates, but it took on average about a week to use all 6,000 images, at which time they were rerandomized.

Results and discussion. Monkeys did not select the image with the lower memory strength. On novel image probe tests subjects chose the image from the list significantly more often than expected by chance, except in tests in which the novel image was paired with the fifth image from the list (Fig. 3; one-sample t-tests: 1 vs. novel: $t_5 = 7.51$, $P = 0.001$; 2 vs. novel: $t_5 = 6.15$, $P = 0.002$; 3 vs. novel: $t_5 = 3.90$, $P = 0.011$; 4 vs. novel: $t_5 = 3.79$, $P = 0.013$; 5 vs. novel: $t_5 = 0.76$, $P = 0.483$). If monkeys had learned to select the image with the weakest memory strength, they would have selected the novel image significantly above chance because the novel image has never been seen before and has memory strength of zero. Because we observed the opposite pattern, we conclude that choice of earlier images was not controlled by memory strength. It is likely that subjects did not choose image 5 significantly more often than expected by chance because the last image in the sequence was never correct and monkeys had learned to avoid selecting images that appeared in this position. Faced with a test involving two incorrect choices, one image that was not in the study list and the last image from the list, monkeys chose indiscriminately. Even in this case, monkeys did not select the novel image more than the image from the studied list. It is unlikely that monkeys' strategy changed between Experiment 1 and 2 because we observed the same pattern of performance in Experiment 1 and in normal trials that were administered concurrently with the probe trials in Experiment 2 (Fig. 3b). Furthermore, probes were infrequent, making it unlikely
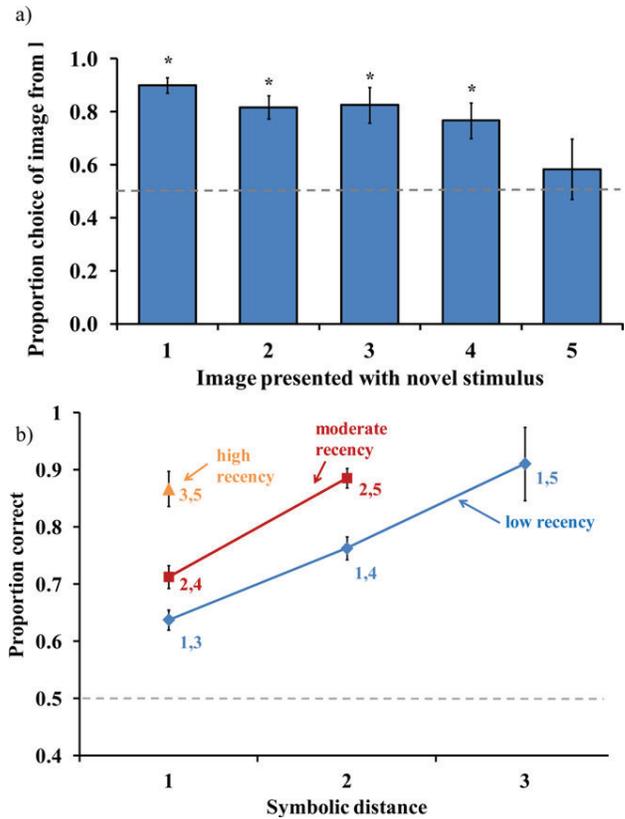

FIGURE 3. (a) Performance on novel image probe tests in Experiment 2. This figure displays the proportion of trials on which monkeys chose the image from the list rather than the novel image for each test pair type (Image 1 vs. a novel image, Image 2 vs. a novel image, and so on). Averages significantly above chance (50%, indicated by dashed line; $P < 0.05$, one-sample t-tests) are indicated by "*." (b) Performance on control tests in Experiment 2. This figure displays the proportion correct for all control test-pairs, which included all non-adjacent items (symbolic distance 0 items were not tested in Experiment 2; compare to Fig. 2). Error bars are standard errors of the mean. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that monkeys switched strategies depending on test type, especially because during study, it was not known which test would appear. The average amount of reinforcement, across both trial types was ~70%, the same as before normal and probe trials were intermixed.

A possible alternative explanation of the finding that subjects did not choose the novel item is that they treated it as the "sixth" and last image in the list, which should never be selected. This is unlikely because subjects had extensive history seeing and touching sample images in one particular location, and after a delay, touching one of two test items, in two different locations. They do not appear to have ever treated the test items as additions to the list. In fact, if subjects treated the novel test image as an additional list image to study they should have touched it, because this is what they have to do with all list images during study. Finally, if monkeys had treated the novel image as the sixth image in the list, performance on Image 5 versus novel tests should have shown the
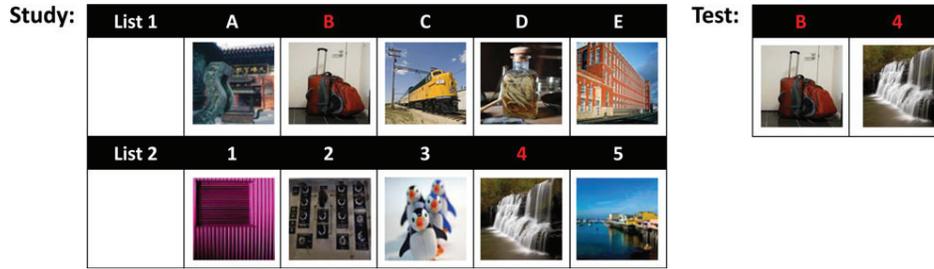
FIGURE 4.   Example of study and test phases of a between list probe test in Experiment 3. In this example, image B from the earlier list appeared with Image 4 from the most recent list. Different pairs of images were used in other probe tests. Sometimes ordinal position was congruent with temporal order, sometimes neutral with respect temporal order, and sometimes incongruent with temporal order. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

recency effect and would have been significantly higher, more like normal tests of Image 4 versus Image 5, but in fact, performance was close to chance (Fig. 3).

Experiment 3: Evaluating the influence of list position

Results from Experiment 2 indicate that memory strength does not determine image choice in tests of memory for order. Previous research with humans (Henson, 1998; Merritt and Terrace, 2011) and animals (Terrace, 2005; Scarf and Colombo, 2011) shows that, in some tests of memory for order, the underlying cognitive representation codes list position. For example, with extensive training monkeys learn to touch a set of five simultaneously presented and randomly arranged images in a predefined order. Performance in such simultaneous chaining paradigms transfers to so-called derived lists, consisting of pairs of images taken from separate previously learned sets. Thus, when presented with image B from one list and image D from a different list, monkeys reliably touch B first and D second, even though these two images were never seen together before (Terrace, 2005). Such performance indicates that monkeys represent the list positions of images in each learned list using a common code that applies across lists. We tested whether monkeys encode the list positions of images in the present task by presenting subjects with between-list probe tests, consisting of one image from the most recently studied list and one image from the previously studied list. Some of these cross-list tests were arranged such that the image with the lower list position occurred later in time. In these tests, list position and temporal order predict different choices, allowing us to determine which controls behavior.

We presented between-list probe tests in which one test image was from the previous list: A, B, C, D, and E, and the other was from the current list: 1, 2, 3, 4, 5 (Fig. 4). Sessions consisted of 120 trials: 96 normal trials, including all nonadjacent pairs, and 24 probe tests. Two blocks of 10 probe sessions were conducted. In the first block, half of the probe tests consisted of image E, the last image from the previous list and Image 1, the first image from the current list; on the other half of the probe tests, image C, the third image in the previous list

was presented with Image 3' the third image in the current list. In the second block of sessions, monkeys chose between B, the second image in the previous list, and 4, the fourth image in current list on half of probe trials. In the other half of probe trials, monkeys chose between D, the fourth image in previous list, and 2, the second image in current list (Table 2). All choices in probe trials were nondifferentially reinforced to prevent new learning. E versus 1 and C versus 3 probes were unreinforced, no matter which item was chosen; B versus 4 and D versus 2 probes were all reinforced with food and auditory feedback, no matter which item was chosen.

Because one entire list occurred before the other, choice of any image from the earlier of the two lists would be consistent with control of choice by temporal order. Each image also occupied a list position within its respective list, and this list position could be either congruent, neutral, or incongruent with respect to temporal order. In B versus 4 tests, selection of B would be consistent with both temporal order and list position. B occurred in the earlier list and in a lower list position within that list. In C versus 3 tests, C occurred before 3, but both images share the same list position within their lists. In D versus 2 tests, selection of D would be consistent with temporal order but not list position, because image D has a higher list position in its list than does 2. Finally, selecting E in E versus 1 tests would be consistent with temporal order, but inconsis-

TABLE 2.

Experiment 3 Probe Tests

Probe test	Temporal order	List position	Reinforcement	Distance
B vs. 4	B	B	Reinforced	6
C vs. 3	C	–	Unreinforced	4
D vs. 2	D	2	Reinforced	2
E vs. 1	E	1	Unreinforced	0

Between-list probe types are shown on the far left. Letters refer to images from the earlier list, numbers to the images from the most recent list. The second and third columns indicate the choice predicted by the temporal order and list position hypotheses, respectively. The symbolic distance, or number of images that intervened between test images during study, is indicated in the far right column.
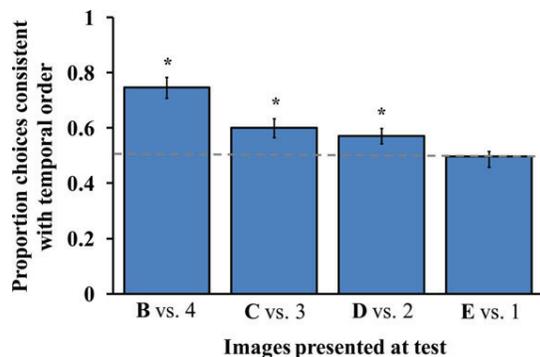
FIGURE 5.   **Performance on between list probe tests in Experiment 3. Images from two adjacent lists were tested (list one consisted of images, A, B, C, D, and E; list two of Images 1, 2, 3, 4, and 5). The temporal order hypothesis predicts that subjects would choose the image from the earlier list, because it occurred earlier in time: B over 4, C over 3, D over 2, and E over 1. The list position hypothesis predicts the opposite. Monkeys should choose the image with the lowest list position within its respective list, regardless of whether that list came first or second (Table 2). Bars with "*" indicate averages significantly above chance (50%, dashed line; $P < 0.05$; one-sample *t*-test). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]**

tent with list position. If choices were controlled by list position, monkeys should select the image with the lower list position in each test (e.g., 1 over E). See Table 2 for predictions based on list position and temporal order.

Results and discussion. Results of tests with images from two adjacent lists were consistent with control of choice by temporal order. Subjects predominantly chose the image from the earlier list, and the size of this effect varied along with temporal distance (Fig. 5; one-sample *t*-tests: B vs. 4: $t_5 = 5.85$, $P = 0.002$; C vs. 3: $t_5 = -2.89$, $P = 0.034$; D vs. 2: $t_5 = 2.59$, $P = 0.049$; E vs. 1: $t_5 = -0.19$, $P = 0.856$). Monkeys may not have chosen E significantly more than 1 because a temporal distance of 0 was not sufficiently salient. The chance-level performance on these tests is clearly inconsistent with list position because 1 occupies the lowest list position and E occupies the highest list position in their respective lists. If choice at test was controlled by list position, this test pair should have created the largest difference in favor of list position. It is possible that B was so strongly selected over 4 because of the congruence of temporal order and list position (Fig. 5; "far left bar"), however, this test pair also manifests the largest temporal separation (Table 2). Together, these results clearly favor control of choice by temporal order rather than list position.

Experiment 4: Temporal order: intervening images and intervening time

Experiment 1 revealed that memory for order is most accurate with largest symbolic distances or temporal separations. Experiment 3 showed a similar pattern: earlier images were selected most reliably when the temporal separation between probe test images was greatest. The evidence presented so far suggests that choice is controlled by temporal order, not by list position or memory

strength. However, temporal order itself can be characterized in different ways. At least two things change with temporal order in these experiments. First, temporal order can be characterized, as done in Figure 2, by the number of images intervening between to-be-discriminated test images. Second, larger differences in temporal order mean that the images are separated by longer intervals of time. To distinguish between these alternatives and to further characterize the control of choice in this task, we compared the effects of temporal spacing with those of intervening images on the accuracy of order discrimination in Experiment 4.

Experiment 4.1. We maintained temporal spacing while manipulating the number of images intervening between to-be-discriminated images by dropping either Image 1 or Image 3 from the study list on probe trials and inserting an unfilled temporal gap. Twenty probe trials were randomly scheduled among the 120 trials in a session. On half of the probe trials Image 3 was omitted, yielding the list: 1, 2, _, 4, 5, where "_" denotes an omitted image. The other half of the probe trials controlled for list length by omitting Image 1 from this study list (_, 2, 3, 4, 5), thus shortening the list to four images while maintaining the temporal spacing and placement of images in the remainder of the list. In place of the omitted images in both types of probes, a 550-ms unfilled interval was inserted during which the screen was black. This duration is the average latency for touching images in the studied lists. The test phase of all probe trials consisted of a choice between Images 2 and 4 from the studied list. Subjects were reinforced with 100% food and auditory feedback following correct responses. Negative auditory feedback and a time-out followed incorrect responses. The remaining normal test trials included all adjacent and nonadjacent images, and all other testing parameters remained the same. If accuracy of the order discrimination was controlled by the absolute amount of time intervening between images, there should be no difference in accuracy between the two probe types because the test images were separated by the same temporal interval in the study list. By contrast, if performance was controlled by the number of intervening study images, performance would decrease when Image 3 was omitted in the study list. Monkeys received 10 sessions resulting in 100 probe trials of each type.

Results and discussion. Monkeys were significantly less accurate on 2 versus 4 probe tests when Image 3 was omitted than they were when Image 1 was omitted (Fig. 6; paired-sample *t*-test: $t_5 = 3.76$, $P = 0.013$). This result indicates that the number of intervening images has a stronger effect on accuracy than does the duration of the interval separating study images. However, one possible concern is that poorer performance represents a generalization decrement caused by the novelty of this trial type. We attempted to control for this by comparing performance to control probe trials on which Image 1 was omitted, but it is possible that omitting Image 3 is more surprising than omitting Image 1. In the next experiment we controlled for this potential confound by more directly comparing the effect of increasing the time interval between study images with the effect of increasing the number of images intervening between to-be-tested study images.
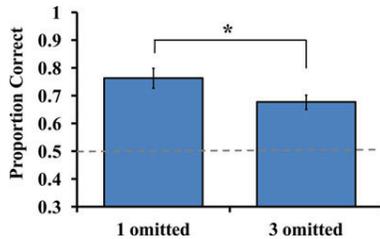
FIGURE 6. Performance on 2,4 probe tests in Experiment 4.1. In probe tests, either Image 1 or Image 3 was omitted, and a test of Images 2 and 4 always occurred. Both types of probes involved four image study lists, and the temporal spacing of the images was held constant despite the omission of an image. Error bars represent standard errors of the mean. The "*" indicates that the two conditions differ significantly from each other (*P* < 0.05; paired *t*-test). Chance is indicated by the dashed line. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Experiment 4.2. Probe trials with three images inserted between to-be-discriminated images, and probe trials with yoked elongated interstimulus intervals were pseudorandomly intermixed with normal trials in 120-trial sessions. Half of the probe tests were "image" trials in which three extra images appeared between Images 3 and 4. The other half of the probe tests were "time" trials in which a yoked extended ISI occurred between Images 3 and 4. The yoked ISI exactly matched the interval elapsed in the previous image trial between the offset of Image 3 and the onset of Image 4. All other procedures remained the same.

If it is the passage of time per se that accounts for the discriminability of images, monkeys should be equally accurate whether the interval between Images 3 and 4 was blank or filled with images. If however, the number of intervening images is more important for making the order of images discriminable; accuracy on "image" trials should be significantly higher than on normal 3 versus 4 tests.
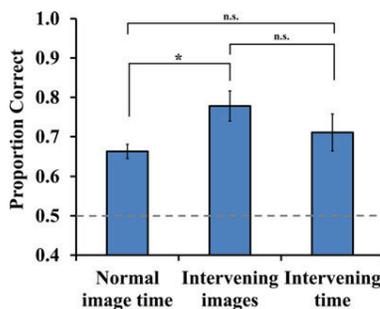

FIGURE 7. Performance on 3,4 tests in Experiment 4.2. In "intervening image" probe tests, three additional images were inserted between the to-be-discriminated Images 3 and 4. In "intervening time" probe tests, the interval between Images 3 and 4 in the study list was extended to match that which occurred in the previous "item" probe test. Error bars indicate standard errors of the means. The "*" indicates that the two conditions differ significantly from each other (*P* < 0.05; paired *t*-test). Chance is indicated by the dashed line. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Results and discussion. The discriminability of the order of images was affected more by intervening images than by the passage of time. Subjects performed significantly better on "image" probe trials than they did on normal trials, and performance on extended ISI trials was not significantly better than that on normal trials (Fig. 7; paired-sample *t*-tests: "image" probe trials versus normal: $t_5 = -4.06$, $P = 0.010$; "time" trials versus normal trials: $t_5 = -1.42$, $P = 0.214$). However, image and time probe trials did not differ significantly from one another ("image" probe trials vs. extended ISI "time" trials: $t_5 = 1.81$, $P = 0.130$). These results show that additional intervening images clearly enhance the discriminability of the order in which images occurred. Simply inserting an unfilled interval of the same duration as that required for inserting additional images did not have as strong an effect on discriminability. However, the absence of a significant difference between image and time trials, combined with the numerically better performance on time trials than on normal trials, suggests that the insertion of additional time did have some effect on discriminability. It is probable that even though no experimenter-generated images were presented during the unfilled interval in time probe trials, events that were not experimenter controlled still did occur in the interval, such as sights the monkeys saw, and calls or noises they heard. The occurrence of these events may have acted less strongly than the occurrence of additional images to enhance the discriminability of the order in which images occurred. Whether or not this is the case, it should be recognized that it is not possible to generate a pure manipulation of the passage of time per se, absent the occurrence of events of one kind or another. What is clear in these results is that the occurrence of salient events between to-be-discriminated images enhanced performance.

DISCUSSION

Representing the order of unique events is one of the defining functions of episodic memory and one that distinguishes it from semantic memory (Tulving, 1983). Here we studied the cognitive mechanism underlying memory for the order of unique events by establishing which features of events are critical for accurate performance. This work complements study of the neurobiology of memory for the order of events, and allows us to understand the relations between cognitive and neural mechanisms.

In Experiment 1, six monkeys learned to select, from among two choices, the image that had appeared earlier in sequences of five briefly studied images. Monkeys learned this discrimination with nonadjacent images and generalized these judgments to more difficult tests with images that had been adjacent in the studied list. We observed symbolic distance and recency effects suggesting that monkeys may form linear representations of the temporal order of the sequences. Charles et al. (2004) similarly found recency effects in a test where monkeys were trained to select the most recent item from a study list. To better evaluate the extent to which representations of temporal

order controlled choice, we conducted a series of experiments that ruled out alternative mechanisms and better defined the nature of the representations controlling choice. We found that the occurrence of intervening images exerted the strongest effect on the discriminability of the order in which images appeared.

To test whether relative memory strengths controlled responses at test, we directly manipulated memory strengths of test images in Experiment 2. When images from studied lists were paired with novel images, monkeys chose the image from the list. Had memory judgments been made on the basis of memory strength, subjects would have selected the novel image, which had the lower memory strength.

In Experiment 3, monkeys chose between test images from separate but adjacent study lists. Monkeys tended to select the images that had occurred in the earlier list, not the images that had occurred earliest in their respective list. This contrasts with the results from similar tests with mice, which found that animals did not prefer choosing the item from the earlier or later list (DeVito and Eichenbaum, 2011). Because subjects treated items that appeared between lists as unrelated, DeVito and Eichenbaum concluded that animals regarded each list as an independent temporally organized experience. Our monkeys seem to have done just the opposite. Two features of the DeVito and Eichenbaum study may account for this difference. Mice were exposed to the same sequences repeated 15 times over 5 days, which would favor treating specific lists as independent units; lists in the present study were trial-unique. Between-list tests in the mouse study occurred 3 days after training and presentation of the successive lists was separated by a 3-h delay; lists in the current study followed one another after just 500 ms making them much more like a continuous list.

Selection of items from the earlier list, as reported here, is consistent with choice by temporal order rather than choice by list position. Evidence from simultaneous chaining tests, in which subjects learn to respond to a fixed set of stimuli in a fixed order, indicates that monkeys choose on the basis of list position (e.g., Terrace, 2005). Unlike the training with repeating sequences used in simultaneous chaining, which causes monkeys to represent the list position appropriate for each image (Chen et al., 1997; Terrace et al., 2003; Terrace, 2005), the lists used in our study were trial-unique. Learning list positions may occur only after repeated presentations, which were not available to the monkeys in this study.

Experiments 2 and 3 identified temporal order as the most likely determinant of choice, so we attempted to better define what constituted temporal order in Experiment 4. We found that the order of events was better discriminated when intervening images occurred, compared to unfilled intervals of equivalent duration. Hence, it is unlikely that classic timing mechanisms that use an internal clock or oscillator to measure elapsed time (Roberts, 1981; Meck, 1983; Ortega et al., 2009) are responsible for discrimination of temporal order in this task. It may seem counterintuitive that performance would increase when there is more that happened, and thus more to remember, as when additional images intervene between target images. The occurrence of intervening events may serve to mark the passage of time, thereby increasing the subjective separation of two events.

Electrophysiological evidence suggests that the hippocampus represents changes in context. Recordings from rat hippocampal neurons show gradual changes in firing patterns over time that may be the basis of memory for order (Manns et al., 2007). These results are consistent with the idea that hippocampus is critical for keeping memories of similar events that occurred at different times distinct from one another (Hasselmo and Eichenbaum, 2005; Ross et al., 2009). Recently identified "time cells" in the hippocampus that encode successive moments (MacDonald et al., 2011) reinforce this position (Eichenbaum and Cohen, 2001).

Human neuroimaging studies (Mayes and Montaldi, 2001; Gelbard-Sagiv et al., 2008; Lehn et al., 2009) also demonstrate that the hippocampus supports memory for the order of events. This role of the hippocampus is also supported by the finding that the monkeys with lesions of the fornix, a major output pathway of the hippocampus, are impaired in recency judgments (Charles et al., 2004). These results, combined with the findings that rats demonstrated impaired memory for order performance but intact performance on recognition tests following hippocampal lesions (Fortin et al., 2002; Kesner et al., 2002), suggest that monkeys with hippocampal lesions would likely be impaired in our order task, but not on recognition tests. The prefrontal cortex (PFC) may interact with the hippocampus to store explicit memories (Ramus et al., 2007), including memory for order (DeVito and Eichenbaum, 2011). While the PFC has been implicated in memory for order (Petrides, 1991b, 1995; DeVito and Eichenbaum, 2011), the studies that tested memory for order in monkeys with PFC lesions were self-ordered working memory tasks (Petrides, 1995). Self-ordered tasks likely engage strategic use of working memory, potentially including planning. These capacities are probably not critical for memory for unique sequences of events that are experienced rather than generated. It would be particularly interesting to compare the performance of monkeys with PFC lesions to that of monkeys with hippocampal lesions on the current task to evaluate this hypothesis.

Researchers using rodent models have made significant progress identifying the neural basis of memory the order of events (Ramus et al., 2007) as distinct from the neural bases of recognition (Agster et al., 2002; Fortin et al., 2002; Kesner et al., 2002; DeVito and Eichenbaum, 2010; DeVito and Eichenbaum, 2011). Here, we have adapted techniques developed in rodents to study memory for trial-unique sequences of images in monkeys, and we have characterized the content of the cognitive representations responsible for accurate performance. Given that the performance of rats in odor order tasks showed similar symbolic distance effects, it is likely that rats represented sequences in the same way our monkeys did—as a temporal order dependent on the occurrence of events. Our monkeys appear to have represented the order of images with greater fidelity that rats were reported to represent the order of odors. Monkeys accurately discriminated images that were adjacent in the study list, whereas rats tested so far were unable to do this. Future neurobiological work testing whether memory for order in monkeys is also dependent on the hippocampus, and behavioral work in rats evaluating the cognitive bases of their performance will determine the

extent to which the mechanisms responsible for memory for the order of events is conserved across species.

Acknowledgments

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