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Nonverbal working memory for novel images in rhesus monkeys

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Summary

Human working memory is greatly facilitated by linguistic representations, for example by verbal rehearsal and by verbal re-coding of novel stimuli. The absence of language in nonhumans raises questions about the extent to which nonhuman working memory includes similar mechanisms. There is strong evidence for rehearsal-like active maintenance in working memory when monkeys are tested with highly familiar stimuli, but not when tested with novel stimuli, suggesting that working memory depends on the existence of previously encoded representations. This difference in working memory for familiar and novel images may exist because, lacking language, monkeys cannot re-code novel stimuli in a way that permits active maintenance in working memory. Alternatively, working memory for novel images may have been present, but behaviorally silent, in earlier studies. In tests with novel images, the high familiarity of to-be-remembered stimuli compared to never before seen distractors may be such a strong determinant of recognition performance that evidence of working memory is obscured. In the current study, we developed a technique for attenuating the utility of relative familiarity as a mnemonic signal in recognition tests with novel stimuli. In tests with novel images, we observed impairments of memory by concurrent cognitive load, and by delay interval, that indicate actively maintained working memory. This flexibility in monkey working memory suggests that monkeys may re-code unfamiliar stimuli to facilitate working memory, and establishes new parallels between verbal human working memory and nonverbal nonhuman primate working memory.

ETOC Blurb

One might remember an unfamiliar stork by mentally rehearsing the words “large black and white bird.” Such linguistic recoding greatly increases the flexibility of working memory. Brady & Hampton report that monkeys also actively maintain information about novel images in working memory, indicating evolution of this capacity without language.

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AUTHOR CONTRIBUTIONS

Ryan J Brady contributed to experimental design, cognitive testing, data analysis, and writing the manuscript.
Robert R. Hampton contributed to experimental design, interpretation of results, and manuscript editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Keywords
Cognitive control; primate; active maintenance; rehearsal; novel stimuli

INTRODUCTION

Multiple memory systems have evolved with distinct computational properties, tailored to solve problems with distinct cognitive demands [1]. Some memory signals, such as familiarity, are advantageous for rapidly and automatically producing simple recognition of previously seen places, objects, or individuals, while consuming few cognitive resources [2,3]. By contrast, working memory, which requires active maintenance and consumes substantial cognitive resources, is adaptive when more flexibility is required such as when planning, when exerting self-control, or when making decisions that involve comparing alternative scenarios. Other memory systems have also been functionally dissociated from familiarity, for example in humans [5], monkeys [6], and rats [7]. Working memory likely evolved in addition to familiarity because it supports such flexibility despite incurring the costs of being slower, metabolically expensive, and cognitively taxing [4]. Because individual differences in working memory and active cognitive control correlate with intelligence among humans [8], differences in working memory among species may similarly account for some differences in intelligence among species. Thus, the diversity in intelligences among species may be partly explained by the degree to which active working memory processes, compared to automatic processes like familiarity, control behavior.

In both humans and nonhumans, the ability to retain information in working memory is dramatically affected by whether familiar or unfamiliar stimuli are used [6,9–12]. Evidence for active working memory in monkeys has been found in tests using highly familiar stimuli and not in those using unfamiliar stimuli [6,13–15]. Humans show more proficient working memory and stronger pre-frontal cortex involvement with familiar compared to unfamiliar stimuli [10,16–18]. Human working memory can engage with unfamiliar stimuli however, at least partly because language allows unfamiliar stimuli to be recoded into familiar words for rehearsal [19]. For example, humans can readily recode a novel stimulus, such as the sight of an unfamiliar animal, into the familiar words “black and white bird”. This recoding allows humans to engage working memory in virtually any situation, with any stimulus. The absence of language may severely constrain nonhuman working memory. Unless the to-be-remembered stimuli are highly familiar, nonhumans may have to rely on less flexible mnemonic processes, even in situations where performance would benefit from active working memory.

While familiar stimuli may be more readily processed in monkey working memory, as is the case in humans [16,17], reported failures of working memory for unfamiliar images may appear more complete than they are. Most studies of working memory in monkeys have employed some version of a recognition paradigm, requiring monkeys to identify a recently seen image presented among distractors (Figure 1, regular study). A previously studied image can be recognized at test for at least two reasons, either because it produces a strong familiarity signal, relative to the distractors, or because it was held in working memory.
between study and test [5,6]. When memory tests are conducted with a small set of images that repeat trial after trial, all of the images become highly familiar in just a few trials, making it difficult to identify the most recently studied sample on the basis of relative familiarity. When a large set of images is used, the images used on each trial do not repeat until the whole set of images has been used, and each test therefore contains a sample image that became familiar during study, presented among relatively unfamiliar distractors. Thus, in tests with a large set of stimuli, accurate choice can be controlled by familiarity alone, and accuracy may be near ceiling, preventing manipulations that attenuate working memory from impairing accuracy. When the delay interval is increased to reduce accuracy away from ceiling, the delay may be so long that working memory is no longer effective, and again manipulations that impair working memory may not manifest in decreased accuracy. As a result, the current literature may have led to false conclusions about the types of stimuli that can be actively maintained in monkey working memory. Working memory may be available but behaviorally silent in tests with novel images.

We developed a study procedure that attenuated the utility of familiarity as a mnemonic signal in tests with unfamiliar images. This procedure allowed us to study the processes contributing to memory for unfamiliar memoranda, when accuracy was away from ceiling, and at delays short enough to permit working memory. When using this procedure, we observed impairments of memory by concurrent cognitive load, and by delay interval, indicative of actively maintained working memory for unfamiliar and novel images.

RESULTS

The familiarization study procedure impaired the utility of familiarity as a mnemonic signal

In Experiment 1a, we evaluated a familiarization study procedure in which we presented both the sample and distractor images that would appear at test during the study phase, and indicated with a cue which of these images was the sample and should be selected at test (Figure 1, image familiarization). As a result, all of the images presented at test had been seen recently, and were familiar. Our aim in using this familiarization procedure was to reduce the utility of relative familiarity as a mnemonic signal. If effective, this manipulation would then allow us to better determine the extent to which monkeys actively maintain information about novel images in working memory. We used a 2 × 2 within-subjects experimental design to test monkeys with both a regular study condition and the familiarization study condition, comparing the effect on memory on images drawn from both a small set of four repeating images and a large set of 1280 images. We hypothesized that if familiarization of both sample and distractor images at study attenuated the utility of familiarity as a mnemonic signal, then the difference between trials with the regular study condition and the familiarization study condition would be greater in tests using the large set of images. This is because the familiarity of images in the small set is largely saturated, and additional familiarization should have little effect. By contrast, familiarization of the distractors drawn from the large set should greatly attenuate the difference in familiarity between the sample and the distractors.

Familiarization of distractor images at study attenuated the utility of familiarity as a mnemonic signal at test. Monkeys were more accurate overall with the large set of images
(M = 99% correct) than with the small image set (M = 89%) in regular study trials. When
the familiarization study procedure was applied, it caused a significantly larger decrease in
accuracy in tests using the large set of images than in tests using the small set of images
(Figure 2; two factor repeated measures ANOVA, main effect of familiarization: $F_{1,5} = 80.31, p < 0.001$, main effect of image set: $F_{1,5} = 10.67, p = .02$; interaction: $F_{1,5} = 39.37, p = .002$). These results support the idea that with a small set of images where images repeat
every trial, the familiarity of all images is nearly maximal, making familiarity much less
useful as a means of discriminating the sample from distractors. Additional familiarization
during study has little effect. However, an alternative to the account that the familiarization
procedure attenuated differences in familiarity between the sample and distractors is that the
familiarization procedure altered processing of the sample in some way during study. For
example, the familiarization study procedure takes longer, requires the monkey to touch
more images, and may in other ways be a more difficult study condition. We next assessed
whether the effect of the familiarization procedure was specific to the images presented as
distractors or was due to changes in processing of the to-be-remembered sample image at
study.

The effect of familiarization is specific to the familiarized images

In Experiment 1b, we compared accuracy following familiarization trials with that following
"false- familiarization" trials in which the study procedure was exactly the same as that used
in Experiment 1a, except that the distractor images used at test were different than the ones
made familiar at study, thereby maintaining the higher familiarity of the sample, relative to
the distractors, at test. If the effect of the familiarization study procedure was specific to a
decrease in the discriminability of the samples and distractors based on familiarity, then we
should observe a significant decrement in accuracy on familiarization trials but not false-
familiarization trials. If the effect is due to the familiarization study procedure being more
difficult, then we should observe the same effect following both familiarization and false-
familiarization.

The decrease in accuracy caused by the familiarization procedure was not due to a change in
the difficulty of the study phase of familiarization trials, but rather to attenuation of the
utility of familiarity as a mnemonic signal at test. We used accuracy in standard matching
trials from Experiment 1a as a baseline in statistical analyses re-plotting these data with the
new results in Figure 3. Familiarization again significantly decreased accuracy compared to
regular trials, and false-familiarization did not have a significant effect (Figure 3; one way
ANOVA main effect of study type: $F_{2,10} = 73.91, p < .001$; post-hoc comparisons
bonferroni corrected alpha = .017; regular study vs. familiarization: $MD = .238, p = .001$;
regular study vs. false-familiarization: $MD = .018, p = .345$). Accuracy in familiarization
trials was significantly lower than false familiarization trials (familiarization vs. false-
familiarization: $MD = -.220, p = .001$). It is clear from these results that there is a strong,
specific, negative effect on accuracy of using of the familiarized images as distractors at test.

Working memory for both familiar and unfamiliar images.

Having established the familiarization study procedure as a method for attenuating the utility
of familiarity as a mnemonic signal, we could then more clearly test whether working
memory was engaged with images in the large set. Changes in illumination during a memory interval substantially reduced memory performance in monkeys [20–22] suggesting the operation of working memory that is vulnerable to distraction. More recent work has found that the extent of the memory impairment is a function of the amount of concurrent cognitive load, causing loss of working memory above and beyond any effect of changes in illumination [6]. This more recent work confirms the presence of working memory in monkeys because effective working memory requires access to limited cognitive resources that are made less available under concurrent cognitive load [6,23,24]. Thus, in Experiment 2, we tested for the presence of working memory by manipulating concurrent cognitive load. To induce concurrent cognitive load, monkeys were required to complete a previously learned image classification task during the delay interval. Monkeys were presented with an image of a bird, fish, flower or human, drawn randomly from a set of 600 possible exemplars, and were required to classify the image according to previously learned associations with the four category icons (Figure 4). Because the familiarization study procedure attenuates the utility of familiarity as a mnemonic signal, we hypothesized that if active working memory is engaged with unfamiliar stimuli, then the effect of concurrent cognitive load would be evident in tests with the large set of images and with the familiarization study condition, but less so, if at all, on trials without familiarization. Because previous work has shown that working memory is engaged in tests with a small set of images, we expected concurrent cognitive load to impair matching accuracy in all tests with the small set of images.

Active working memory was engaged with unfamiliar images from the large set. Concurrent cognitive load reduced accuracy in tests with the large set of images only when familiarization occurred during study. Concurrent cognitive load reduced accuracy in tests with the small set of images with and without familiarization (Figure 5 left panel; three factor repeated measures ANOVA, set size x familiarization x concurrent cognitive load three way interaction: \(F(1,5) = 23.74, p = 0.005\)). Overall, concurrent cognitive load affected accuracy with familiar images more than accuracy with unfamiliar images (concurrent cognitive load x set size interaction: \(F(1,5) = 103.3, p < .001\); main effect of concurrent cognitive load: \(F(1,5) = 37.01, p = 0.002\); main effect of set size: \(F(1,5) = 95.88, p < .001\)). Post hoc tests confirmed that concurrent cognitive load significantly decreased accuracy in the familiarization study condition with the large set of images, but not in trials with the regular study procedure (post-hoc paired samples t-tests, bonferroni corrected alpha = 0.025, large set familiarization concurrent cognitive load vs. empty delay: \(t(5) = 6.20, p = .002\); large set regular study concurrent cognitive load vs. regular: \(t(5)=2.01, p = 0.10\)). By contrast, concurrent cognitive load significantly decreased accuracy in both study conditions with the small set of images (post-hoc paired samples t-tests, bonferroni corrected alpha = 0.025, small set familiarization concurrent cognitive load vs. empty delay: \(t(5)= 8.07, p < .001\); small set regular study concurrent cognitive load vs. empty delay: \(t(5)=6.53, p = .001\)). Finally, the familiarization procedure affected accuracy with unfamiliar images more than with familiar images, replicating the findings of Experiment 1 (Figure 5, left panel, set size x familiarization interaction: \(F(1,5) = 223.6, p < .001\), main effect of familiarization: \(F(1,5) = 102.96, p < .001\)).
To further evaluate these conclusions, we also analyzed the effect of concurrent cognitive load expressed as the accuracy difference between concurrent cognitive load and no load conditions. This analysis produced convergent results. Concurrent cognitive load significantly reduced accuracy in tests with the large set of images only when the familiarization study procedure was used, while it had a similar effect on accuracy in tests with the small set of images irrespective of study condition (Figure 5 right panel; large set regular study vs. familiarization: $t_{(5)} = -5.84, p = .002$; small set regular study vs. familiarization: $t_{(5)} = 0.61, p = .57$).

These results represent a double dissociation of working memory and familiarity. First, concurrent cognitive load made it difficult for monkeys to maintain information in working memory. This effect was more apparent with images from the small set than with images from the large set, replicating previous findings [6]. Second, pre-exposure, during study, to the images that would appear as distractors at test negatively affected accuracy with images from the large set than with images from the small set, replicating Experiment 1. Most importantly, we found that concurrent cognitive load negatively affected performance with images from the large set, but only after pre-exposure to the images that would appear as distractors. Taken together, these results suggest the contributions of working memory are greatest with repeating images, and when familiarity is not a reliable signal for discriminating the sample from distractors.

**Working memory for novel images**

We found evidence of working memory for unfamiliar images in Experiment 2, but this was not found in earlier work [6]. This difference in results is likely due to the fact that the familiarization procedure attenuated the utility of familiarity as a mnemonic signal in this experiment, unmasking the operation of working memory. However, an alternative interpretation is that due to repeated use of the large set of images across experiments 1 and 2, the images became familiar, like the images from the small set. One possibility is that exposure to the large set of images across experiments 1 and 2 was enough to create long-term memories of the images that facilitated working memory maintenance. Such long-term memories would not be available with novel images.

In order to more clearly test the extent to which novel stimuli can engage working memory in monkeys, we repeated Experiment 2 but with a completely novel set of 10,000 clip art images that the monkeys had never seen, and that were never repeated across trials. Additionally, the order of small set and large set testing sessions was reversed from Experiment 2 to account for any order effects. If long-term memories of images used as memoranda are required to engage working memory, then we should not observe an effect of concurrent cognitive load when testing is conducted with novel images. In contrast, if monkeys can either hold novel images in working memory, or recode novel images in terms of existing long-term memories, such as “red” or “round,” then we should continue to observe an effect of concurrent cognitive load consistent with working memory in Experiment 3.

Novel images engaged active working memory. Experiment 3 replicated, with novel images, the double dissociation found in Experiment 2. First, we found that concurrent cognitive
load decreased accuracy for familiar images more than novel images, (Figure 6 left panel; three factor repeated measures ANOVA, set size × concurrent cognitive load interaction: $F_{(1,5)} = 103.3, p < .001$; main effect of concurrent cognitive load: $F_{(1,5)} = 77.3, p < .001$; main effect of set size: $F_{(1,5)} = 80.51, p < .001$). The effect of concurrent cognitive load in tests with novel images was only present on trials with the familiarization procedure (Figure 6 left panel; separate two factor repeated measures ANOVA for novel images, main effect of concurrent cognitive load: $F_{(1,5)} = 27.4, p = .003$; post hoc paired samples t-test bonferroni corrected alpha = .025, large set familiarization concurrent cognitive load vs. empty delay: $t_{(5)} = 5.40, p = .003$; large set regular study concurrent cognitive load vs. empty delay: $t_{(5)} = 3.03, p = .029$). For familiar images, concurrent cognitive load decreased accuracy in both conditions (Figure 6 left panel; separate 2x2 ANOVA for small set, main effect of concurrent cognitive load: $F_{(1,5)} = 65.92, p < .001$; post hoc paired samples t-test bonferroni corrected alpha = .025, small set with familiarization: $t_{(5)} = 8.09, p < .001$; small set with regular study procedure: $t_{(5)} = 6.5, p = .001$). Second, we further replicated the findings of Experiment 1 that the familiarization procedure negatively affected accuracy in tests with novel images more than accuracy in tests with the small set of images (Figure 6 left panel; set size × familiarization interaction: $F_{(1,5)} = 223.6, p < .001$; main effect of familiarization: $F_{(1,5)} = 124.22, p < .001$). To further test these conclusions, we also analyzed the results using a difference score as in Experiment 2, again obtaining convergent results (Figure 6 right panel, large set regular study vs. familiarization: $t_{(5)} = -2.78, p = .039$; small set regular study vs. familiarization: $t_{(5)} = -0.76, p = .48$).

Concurrent cognitive load decreased accuracy after short delays more than long delays.

Susceptibility to concurrent cognitive load is a strong indicator of working memory [6,23], but it is only one diagnostic feature. Another indicator of working memory is a relatively steep forgetting function [25–27]. Although working memory representations could theoretically be maintained indefinitely if rehearsal was sustained, in practice it is not possible to maintain attention, as demanded by rehearsal, over long periods of time. Monkeys typically show substantial deterioration of working memory between five and thirty seconds [6,28]. In Experiment 4, we further tested whether working memory was engaged with unfamiliar stimuli by assessing the effect of concurrent cognitive load after both five and thirty second retention intervals. We hypothesized that if the effect of concurrent cognitive load seen in familiarization trials is indicative of working memory, then we should observe a larger decrease in accuracy from concurrent cognitive load at the short delay, when working memory should make the greater contribution to performance.

Concurrent cognitive load reduced accuracy more at the shorter retention interval, consistent with impairment of working memory (Figure 7 left panel; two factor repeated measures ANOVA, delay × concurrent cognitive load interaction: $F_{(1,5)} = 8.68, p = .032$; main effect of concurrent cognitive load: $F_{(1,5)} = 31.02, p = 0.003$; main effect of delay: $F_{(1,5)} = .057, p = .82$). To further evaluate these conclusions, we also analyzed the effect of concurrent cognitive load expressed as the accuracy difference between concurrent cognitive load and no load conditions. This analysis produced convergent results (Figure 7 right panel; paired samples t-test, short delay vs. long delay: $t_{(5)} = 2.92, p = .033$).
These results of manipulating delay interval provide converging evidence for the hypothesis that active working memory maintenance was engaged by novel stimuli. Because working memory is an active process that requires constant cognitive control, a long memory interval should decrease the probability that control will be sustained and working memory representations will be maintained. At long delays concurrent cognitive load is less likely to decrease accuracy because working memory has already begun to fail and makes a reduced contribution to accuracy.

**DISCUSSION**

Monkey working memory can be engaged by novel stimuli, and does not require the existence of long-term memories of the specific images studied to function. Because familiarity is an automatic and powerful process serving recognition, it can mask the presence of working memory for novel images in traditional recognition tests. We attenuated the utility of familiarity as a mnemonic signal with the familiarization study procedure established in Experiment 1 and revealed the operation of working memory. These observations of active memory maintenance with unfamiliar and novel images expand the range of conditions under which monkey working memory is known to be engaged. Although human working memory is strongly associated with language, these results raise the intriguing possibility that the mechanisms supporting working memory maintenance emerged well before the development of language. Linguistic representations almost certainly facilitate working memory in humans, but our experiments show that linguistic representations are not required for active working memory, even in the case of novel material for which monkeys have no specific long-term memories.

When monkeys remember familiar images drawn from a small set of repeating images, working memory is critical. Performance cannot be supported by familiarity because all of the images are similar in familiarity due to constant re-use across trials [6,12,29]. In contrast, when tested with unfamiliar stimuli that do not repeat frequently, differences in familiarity provide a clear basis for discriminating the sample from distractors, and the presence of working memory is masked. However, when familiarity is made a poor mnemonic signal by the familiarization procedure described here, the presence of otherwise behaviorally silent working memory becomes evident. Absent use of the familiarization procedure, and at longer delay intervals over which working memory is likely to fail, familiarity is the dominant basis for identification of the sample in tests using large sets of images. Thus, in many memory tests with rhesus monkeys, working memory may be present, but may not control choice.

One previous study found a directed forgetting effect consistent with the presence of working memory when the same two stimuli were used repeatedly, but found no such effect when novel images were used [14]. This result is consistent with our findings that working memory is more readily engaged by familiar than by novel images, and that relative familiarity often controls choice in memory tests with novel images, masking working memory. Similarly, another previous study did not find evidence of rehearsal when monkeys were asked to remember lists of 6 to 10 items drawn randomly from a set of 32 images [30]. Because the images were drawn randomly on each trial, and the delay between the sample
and test was sometimes as long as 24 seconds, it is likely that recognition performance was supported by familiarity, again masking working memory.

The current results support the account that we unmasked the presence of working memory by attenuating the utility of familiarity. According to this account, working memory is engaged by novel stimuli, whether or not it contributed to choice at test. However, another possibility is that experience with our procedure trained the monkeys to use working memory with novel images even though they did not do so initially. According to this account, the failure to respond correctly on trials with the familiarization procedure induced the engagement of working memory maintenance, thus overcoming the lack of differences in relative familiarity. In at least some circumstances, nonhumans demonstrate flexible control of active memory maintenance in so-called “directed forgetting” paradigms (e.g. monkeys [14,31], and pigeons [32,33]). When an experimental cue predicts an upcoming memory test, active maintenance is engaged, but not so when the cue predicts a test that does not require memory for the sample. While the current set of experiments does not discriminate between the unmasking and the induction accounts, our results do show that monkey working memory is engaged in at least some conditions by novel images. Further work will be required to refine our understanding of the conditions that control the engagement of working memory in monkeys. An important consideration in the interpretation of the present results is that the six monkeys used in these experiments have had years of experience with cognitive testing generally, and specifically with memory tests thought to require active cognitive control. Thus, the findings of these experiments show that rhesus monkey working memory can be engaged by novel stimuli, but the engagement of working memory found here may not be typical of naive monkeys.

One parallel between human and nonhuman working memory that is reinforced by our findings is that working memory maintenance is facilitated by stimulus familiarity. In humans, the prefrontal cortex is more active when subjects remember familiar images compared to novel images [10], working memory capacity is greater for familiar stimuli that exist as long-term representations [16,34,35], and familiar representations consume fewer working memory resources than unfamiliar representations. Language grants human working memory an advantage in maintaining unfamiliar stimuli in mind, through the ability to transform unfamiliar stimuli into familiar stimuli though verbal re-coding[19]. For instance, when a participant is presented with a completely novel image to hold in mind, they can recode the unfamiliar image into familiar words (e.g. red house, green field) and then rehearse the results of recoding. The value of such recoding is evident experimentally when human participants struggle to remember complex geometrical figures or fractals that are difficult to recode, or when memory suffers under articulatory suppression [16,19].

These findings that monkey working memory is engaged by novel stimuli raises the interesting question of whether monkeys, like humans, somehow recode novel images into a familiar abstract code. Nonhumans do not have access to linguistic representations, but may have other abstract codes that can be similarly employed. For example, monkeys readily “categorize” images [current results, 9,29,31,34] and such categorization may represent recoding. Monkeys may spontaneously recode stimuli in various ways, for example according to biological relevance [39], animacy [40], and kinship [41]. While such recoding
is sophisticated, it falls far short of the complexity and flexibility of human linguistic representation. This difference in the sophistication of recoding may represent an important limitation on monkey working memory and cognition generally.

Human children undergo a dramatic increase in working memory capacity concurrent with the development of language, and score better on measures of cognitive control as language develops [42,43]. Furthermore, children who are raised bilingual score higher on measures of cognitive control compared to children who are mono-lingual, and children with language learning difficulties perform worse on working memory tasks and other measures of cognitive control [42,44]. This literature suggests that language is critical for working memory proficiency. The results of the current study support a more nuanced view that monkeys and humans share many properties of cognitive control in working memory, independent of language, and that language has elaborated these capacities in humans. It may be that the ability to recode stimuli is an underlying critical skill for cognitive control, and that language is a particularly strong example of such recoding.

In sum, our results demonstrate active memory maintenance in monkeys that extends beyond familiar stimuli. These results suggest that it is likely that mechanisms supporting the maintenance of unfamiliar memoranda in working memory evolved before language, and at least 32 million years ago when a common ancestor of humans and rhesus monkeys lived [45,46]. Working memory affords an organism many functions that other memory systems cannot, for example allowing for high priority representations to be temporarily kept in mind, and serving as a mental workspace for information to be further processed and integrated with existing representations [27,47]. Because working memory plays a central role in determining human intelligence [8,48], comparing working memory between human and nonhuman primates may provide information we need to account for differences in intelligence across species. The current series of experiments, combined with the accumulating literature documenting monkey working memory [13,15,31,49], continue to develop our understanding of the mechanisms responsible for differences between human and nonhuman primate cognition.

**STAR METHODS**

**Contact for Reagent and Resource Sharing**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ryan J Brady (ryan.brady@emory.edu).

**Experimental Model and Subject Details**

Six adult male rhesus monkeys (Macaca mulatta) were used in all the experiments reported here. Monkeys, received a full food ration daily, and had ad libitum access to water. Monkeys were trained and tested in their home cage using a portable testing rig consisting of a 15-inch color LCD touch-sensitive screen (Elo TouchSystems, Menlo Park, CA) operating with a resolution of 1024 X 768 pixels, and two automatic food dispensers (Med Associates, Inc., Stl. Albans, VT) that delivered nutritionally balanced primate pellets (Bio-Serv, Frenchtown, NJ) into food cups below the screen. Each screen was mounted on the front of
the monkey’s cage. Testing was controlled by a personal computer with a custom program written in Visual Studio 2013 (Microsoft Corporation). For each monkey, the calories from pellets earned during testing were subtracted from their total food ration, and they were given the balance of their ration in primate chow at the end of the day. This work was approved by the Emory University Institutional Animal Care and Use Committee (YER-2003537-062419GA). All procedures complied with the approved guidelines. The monkeys used in this study had extensive experience with recognition memory tasks, as well as categorizing images, e.g [6,50]. The monkeys had previous experience with a flashing square stimulus that indicated a to-be-remembered target (Brady & Hampton, 2018), but had never been trained on the familiarization study procedure used here.

Method Details

**Experiment 1a Methods**

**Stimuli:** 1284 clip art images (200 × 200 pixels) were collected (Hemera® Clip-Art Set, 2003). From these, 4 were randomly selected to constitute the small set of repeating images, the remaining 1280 constituted the large set of images.

**Experiment 1a Procedure:** Experiment 1a consisted of initial training with the familiarization procedure, followed by two experimental phases. In the first experimental phase, monkeys were tested with a small set of images comparing performance with and without the familiarization study procedure. In the second phase, we repeated this comparison with a large set of images.

Familiarization trials began by touching a green ready square twice in a row (Fixed Ratio2, hereafter FR2), which was followed by the presentation of an image in one of four locations in the center row of the screen. Monkeys touched this image (FR2) which caused it to disappear and the next image to come up in one of the other possible study locations. This repeated for image three and image four. After the fourth image was touched, all touched images appeared again in the same locations in the middle row of the screen, and the sample image was highlighted with a white blinking frame. When monkeys touched this highlighted image (FR2), an empty delay occurred until test. At test, the four images appeared, one in each of the four corners of the screen. Selecting the previously highlighted image (FR2) resulted in a positive auditory reinforcement as well as food reward. Selection of an image that was not the previously highlighted one resulted in negative auditory reinforcement and 5 seconds was added to the normal inter-trial interval of 3 seconds.

**Initial Training:** Monkeys were trained in familiarization in 100 trial sessions with the small set of 4 repeating images with a 5 second delay. Monkeys moved to Experiment 1 when they achieved an overall proportion correct of 80 percent or higher in two consecutive sessions. If monkeys did not achieve criterion in 15 sessions, they were moved to a remedial phase in which only one image was presented during study with the blinking square around it. Once criterion was met in the remedial phase, they progressed to another remedial phase consisting of two images during study. Once achieving criterion in this phase, they repeated the initial training with 4 images for another 15 sessions or until achieving criterion.
Small set testing: Monkeys received 8 sessions of 80 trials, consisting of half regular study trials, and half trials with the familiarization procedure. On each trial, the same four images were used. Each image served as a distractor or a sample the same number of times across trials. The location of the sample during study and test was pseudorandomly counterbalanced within each session such that each location was used equally often. Similarly, the order of trial-type was pseudorandomly counterbalanced such that 4 of each trial type occurred every 8 trials. A 5 second delay occurred between touching the sample and presentation of the test (Figure 1).

Large set testing: Monkeys were required to score 80 percent or higher for two consecutive sessions with the large set of images and the familiarization procedure. Images were not repeated until all 1280 images had been used. After achieving criterion, monkeys received 8 sessions of 80 trials with half regular study trials, and half familiarization trials. Both trial types had a 5 second empty delay between touching the sample and presentation of the test. Four of 6 monkeys met criterion within the first 15 sessions of familiarization training. The two remaining monkeys met criterion after passing through the remedial phase.

Experiment 1b Methods:

Subjects: The same six monkeys were used.

Stimuli: The same large set of 1280 images used in Experiment 1a was used in Experiment 1b.

Experiment 1b Procedure: Familiarization and false-familiarization trials were pseudorandomized and counterbalanced such that 4 of each trial type occurred every 8 trials. All trials had a five second delay between touching the sample and presentation of the test. Images were not repeated until all 1280 had been used. The monkeys completed 8 sessions of 80 trials.

Experiment 2 Methods:

Subjects and Stimuli: The same subjects and testing equipment used. The same small and large sets of images were used. The concurrent cognitive load was a categorization task using a set of 600 images consisting of 150 each images of birds, fish, flowers, and people [6].

Experiment 2 Procedure:

Initial Training: Monkeys repeated the initial training phase described in Experiment 1 to ensure they were performing the recognition memory task at criterion accuracy.

Category retraining: Each trial began with touching the green ready square at the bottom of the screen (Figure 4). Then a picture from one of the four categories (bird, fish, flower, or person) appeared in the middle of the screen (400 × 300 pixels). Monkeys touched this image (FR1) causing the four corresponding category symbols (200 × 200 pixels) to appear in the four corners of the screen. Upon touching the correct symbol, monkeys received
auditory and food reward reinforcement. If the incorrect symbol was touched, no food reward was given, a negative auditory reinforcement sounded, and a 5 second delay was added to the normal 3 second inter-trial interval. Training sessions consisted of 100 trials in which 25 trials were from each category. The monkeys repeated these sessions until they had completed at least 5 sessions and scored 80 percent correct or higher in the most recent two sessions.

Experiment 2 employed a $2 \times 2 \times 2$ design such that trials began with either a regular or a familiarization study phase, included either a concurrent cognitive load or an empty delay interval, and used either the small or the large set of images. Monkeys were first tested with a small set of repeating images, followed by the large set of images.

**Small set testing:** Monkeys had to meet a criterion of 80 percent correct or higher in two consecutive sessions on regular match to sample trials with the small set at a 5 second delay. Once achieving this, they received 8 sessions of 80 trials, in which trials with concurrent cognitive load and the familiarization study procedure were fully crossed (each session consisted of 20 trials with familiarization and concurrent cognitive load; 20 trials with familiarization only; 20 trials with normal sample phase and concurrent cognitive load; 20 trials with normal sample phase and no concurrent cognitive load), resulting in 160 trials for each condition after 8 sessions. All trials had a 5 second delay between touching the sample and presentation of the test. If the monkey responded incorrectly in the category task, or took longer than the five seconds allotted within the delay interval to complete the categorization task, the same type of trial was immediately repeated but with a new sample, new distractors, and a new category task. Aborted trials were omitted from analysis.

**Large set testing:** Phase 2 was the same procedure as Phase 1, except the large set of images was used such that images did not repeat until all 1280 images had been used.

All monkeys completed category re-training within 10 sessions.

**Experiment 3 Methods:**

**Subjects:** The same subjects and testing equipment were used.

**Stimuli:** The same small set of four images used in Experiment 1 was used in Experiment 3. A new set of 10,000 clip art images was used for the large set. The same set of 600 category images, and corresponding symbols, were used for concurrent cognitive load.

**Experiment 3 Procedure:** Experiment 3 procedures were exactly the same as Experiment 2 with the exception that the order of Phase 1 and 2 were reversed such that the large set data were collected first. This was to ensure any effects were not due to the order of testing the small and large sets of images.

**Experiment 4 Methods:**

**Subjects and Stimuli:** The same subjects and testing equipment used in Experiment 1 were used in Experiment 4. The same large set of 10000 images used in Experiment 3 was used in Experiment 4. For concurrent cognitive load, the same set of 600 category images, and
corresponding symbols, were used. The "short" delay used was a five second interval, and
the "long" delay was a thirty second interval.

Experiment 4 Procedure: In Experiment 4 all trials began with image familiarization, and
all trials used the large set of 10,000 images. There were four conditions psuedorandomly
distributed across sessions of 80 trials such that each condition occurred equally often. The
four conditions were No concurrent cognitive load-Short delay, No concurrent cognitive
load-Long delay, Concurrent cognitive load-Short delay, and Concurrent cognitive load-
Long delay. There were 8 sessions of 80 trials, resulting in 160 trials for each condition.

Quantification and Statistical Analysis

For all experiments reported, proportion correct scores were arc sin transformed prior to
analysis [51]. All statistical testing was completed using IBM SPSS 25 statistical software.
Statistical details for each experiment can be found in the results section of each experiment.
For all statistical tests, significance was determined as having ap value less than .05, which
was adjusted using a bonferroni correction for post-hoc comparisons. Each results section
details the specific statistical test and design used for the respective experiment.

Data and Software Availability

Raw data file will be provided as appropriate

ACKNOWLEDGEMENTS

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HIGHLIGHTS:

• Rhesus monkeys actively “hold in mind” information about novel images
• Humans recode novel images into familiar words, monkeys lack language
• The ability to recode novel images in working memory likely evolved before language
• Our new methods distinguished contributions of familiarity from working memory
Figure 1: *Familiarization* study procedure (left column) and regular study phase (top right). On familiarization trials, the monkey touched each image in turn before they all appeared together and the sample was indicated with a blinking square. At test, the sample was presented with the other images just seen during the study phase. In regular study trials, one image appeared at study with the blinking square around it. At test, the sample was presented with the other three images from the small set as distractors, or with three unfamiliar images from the large set. In all cases, the 5 second delay began when the monkey finished touching the sample image.
Figure 2: Image familiarization attenuated the utility of familiarity as a mnemonic signal. Data are represented as mean proportion correct ± SEM. The image familiarization study procedure attenuated accuracy most strongly in tests using the large set of images. These results indicate that familiarity is more important for accurate choice with the large set of images than with the small set of images. The image familiarization procedure is thus a tool to attenuate the utility of familiarity as a mnemonic signal.
Figure 3: The effect of the familiarization procedure is specific to the images that appear as distractors at test. Data are represented as mean proportion correct ± SEM. Because performance is not affected when the familiarized images are not used as distractors at test, the results of Experiment 1a are not due to changes in the processing of the sample at study. Instead, the detrimental effect of the familiarization study procedure results from a decrease in the discriminability of the sample from the distractors based on familiarity.
Monkeys touched the green ready square to view an image that they then categorized as a bird, fish, flower, or human (Basile & Hampton, 2013). Monkeys categorized the image by touching the corresponding symbol. This task was presented during the delay intervals on concurrent cognitive load trials. Monkeys had to categorize correctly to proceed to the memory test. Because working memory requires cognitive resources, the concurrent cognitive load is expected to impair working memory, if present.
Figure 5: Double dissociation of familiarity and working memory in recognition accuracy.
The familiarization procedure impaired accuracy with the large set more than with the small set (left lines represent mean proportion correct ± SEM), and concurrent cognitive load impaired accuracy with the small set of images more than with the large set (right bars represent a mean difference score ± SEM). Note that larger scores on the right bars indicate poorer performance under the concurrent cognitive load condition. Taken together, these results suggest first that with images from the small set, the utility of familiarity is already highly attenuated so the familiarization procedure does not have an effect. Second, these results suggest that the contribution of working memory is robust in tests with the small set of images, regardless of whether the familiarization procedure was used. By contrast working memory contributes to accuracy in tests with the large set of images less robustly overall and only when the utility of familiarity as a mnemonic signal was attenuated by the familiarization procedure. The presence of an effect of concurrent cognitive load in tests with the large set of images after familiarization suggests that working memory is normally obscured through the very powerful control of choice by relative familiarity.
Figure 6: Double dissociation of familiarity and working memory in recognition of novel images. The left lines represent mean proportion correct ± SEM. The bars on the right represent a mean difference score ± SEM. These results replicate those of Experiment 2 and also indicate that novel images engage working memory in monkeys.
Figure 7: Concurrent cognitive load decreased accuracy most at short delays.
All trials began with the familiarization procedure. Accuracy was lower on long delay trials with no concurrent cognitive load, and was less negatively affected by concurrent cognitive load than on short delay trials. The left lines represent mean proportion correct ± SEM. The right bars represent the mean difference score ± SEM. Note that higher scores on the right indicate greater decrements in performance from concurrent cognitive load. The combination of sensitivity to concurrent cognitive load and to delay provides strong evidence for the operation of working memory.
## KEY RESOURCES TABLE

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*Curr Biol. Author manuscript; available in PMC 2019 December 17.*
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**Experimental Models: Organisms/Strains**

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**Software and Algorithms**

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