MEMORY RESEARCH

Retrieval practice protects memory against acute stress

Amy M. Smith,* Victoria A. Floerke, Ayanna K. Thomas

More than a decade of research has supported a robust consensus: Acute stress impairs memory retrieval. We aimed to determine whether a highly effective learning technique could strengthen memory against the negative effects of stress. To bolster memory, we used retrieval practice, or the act of taking practice tests. Participants first learned stimuli by either restudying or engaging in retrieval practice. Twenty-four hours later, we induced stress in half of the participants and assessed subsequent memory performance. Participants who learned by restudying demonstrated the typical stress-related memory impairment, whereas those who learned by retrieval practice were immune to the deleterious effects of stress. These results suggest that the effects of stress on memory retrieval may be contingent on the strength of the memory representations themselves.

The effects of experimentally induced stress on memory have been studied for more than a decade (1–7). The results support a robust consensus that stress impairs memory retrieval (8). These studies used a common method whereby participants learn words or images and return 24 hours later for a memory test. Before testing, psychosocial stress is induced. Critically, the memory test is administered ~25 min after stress introduction [for exceptions, see (5, 6)], when the stress hormone cortisol reaches peak poststress levels in the blood. Researchers have primarily examined memory after this delay because cortisol has been shown to affect brain regions that are implicated in memory retrieval (9).

Previous research on this topic has not been expressly concerned with the quality of encoding during initial learning. Before encoding, participants were typically instructed to "memorize" stimuli. However, the processes that take place at encoding influence memory representation and accessibility (10). Without guidance as to how to approach learning material, participants may choose ineffective encoding strategies, resulting in unstable memory representations. Many participants in these studies likely chose to learn by rereading, given that this method is often reported as the most popular study strategy (11). Rereading is a poor learning strategy, insofar as it creates relatively weak memory representations (12). Thus, it is unclear whether all memories are subject to the detrimental effects of stress, or whether only weakly encoded representations are vulnerable.

In our experiment, we addressed this by strengthening memory at encoding through the use of retrieval practice, the act of taking practice tests. Among a host of options for study techniques, we chose retrieval practice for two reasons. First, retrieval practice has consistently yielded long-term memory retention that is equal to or better than restudying (13–15) and a plethora of other learning strategies such as mental imagery (12), concept mapping (16), and the keyword mnemonic (17). Thus, we chose to use retrieval practice as an encoding technique because it had the most potential to create memories that were resilient to stress. Second, retrieval practice is an easily implemented learning strategy (12). We reasoned that if retrieval practice was successful at creating stress-resistant memories, our findings could be readily applied in real-world scenarios (e.g., test anxiety).

A second limitation of previous research on stress and memory concerns the timing of the final memory test. Researchers typically assessed memory 25 min after stress induction and found detrimental effects. However, contesting the consensus that stress generally impairs retrieval, recent research showed that participants who were tested immediately after stress induction exhibited memory performance that was better than or comparable to a no-stress control group (5, 6). Thus, a secondary aim of our study was to investigate the potentially facilitative effects of the immediate stress response in the context of a retrieval practice encoding manipulation.

In our experiment, 120 participants studied either 30 concrete nouns or 30 images of nouns, one item at a time. Half of the items in each list were of negative valence and half were of neutral valence. Whether words or images were studied first was counterbalanced. Sixty participants then engaged in study practice (SP), in which they restudied the 30 items. The other 60 participants engaged in retrieval practice (RP), in which they recalled as many items as they could remember. RP participants were not given feedback on the free recall test or on any subsequent tests. This procedure (item presentation followed by restudy or free recall) was then repeated for the 30 items of the other type. Afterward, SP participants restudied all 60 stimuli, whereas RP participants attempted to recall the words and images in any order. After a short distractor task, SP participants again restudied all 60 items, and RP participants attempted to recall all items.

Twenty-four hours later, 30 SP and 30 RP participants underwent stress induction, and 30 SP and 30 RP participants completed a time-matched nonstressful task. Our encoding and stress manipulations were fully crossed, so there were four between-subject groups: nonstressed SP, stressed SP, nonstressed RP, and stressed RP. During stress induction, participants gave extemporaneous speeches and solved math problems in front of two judges and three peers (18).
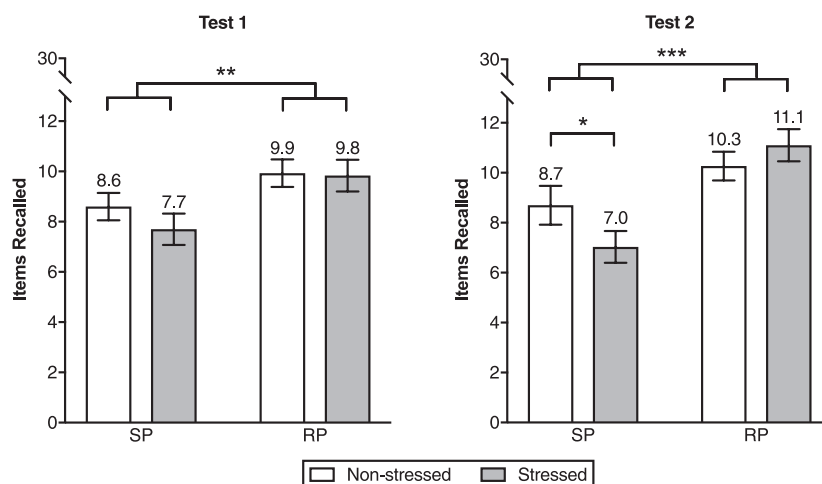
Fig. 1. Average number of items accurately recalled on tests 1 and 2. Test 1 was administered immediately after the onset of stress. Test 2 followed after a 25 min delay. Retrieval practice (RP) refers to the learning technique in which participants study stimuli and take three subsequent recall tests. Study practice (SP) refers to the learning technique in which participants study stimuli four times. Tests occurred on the day after learning. Error bars represent standard errors of the mean. *P < 0.05; **P < 0.01; ***P < 0.001.

Tufts University, 490 Boston Avenue, Medford, MA 02155, USA.
*Corresponding author. Email: amy.smith@tufts.edu

Measures of physiological arousal confirmed the effectiveness of the stress induction procedure (19). Five minutes into the stress induction or control task, participants completed test 1, in which they recalled either the words or images that were studied the previous day. Test 1 was given to examine memory during the immediate stress response. Twenty minutes later, participants completed test 2, in which they recalled the items that were not assessed on test 1. Test 2 was given to examine memory during the delayed stress response.

The results of our experiment are characterized by three key findings. First, on test 2, stressed SP participants recalled fewer items than nonstressed SP participants [Cohen's d effect size (d) = 0.44; 95% confidence interval (CI) = (0.03, 3.37)], whereas this difference was not evident for RP participants. As shown in Fig. 1, stress resulted in the memory impairment that researchers have repeatedly observed, but only for participants who encoded stimuli through SP. Not only did stressed RP participants outperform nonstressed SP participants [d = 0.61; 95% CI = (0.36, 4.37)], they demonstrated recall performance that was similar to nonstressed RP participants, as though stress had not been present.

Second, consistent with one of two recent studies that examined memory performance during both the immediate and delayed stress response (6), we found no difference in memory performance for stressed versus nonstressed participants on test 1. Stress neither impaired nor enhanced memory performance 5 min after the onset of stress (Fig. 1). Table 1 provides a more detailed report of test 1 and test 2 performances.

Third, we replicated the robust testing effect (13, 20). Participants who encoded through RP recalled significantly more stimuli than those who encoded through SP on test 1 [partial η^2 effect size (η_p^2) = 0.06; 95% CI = (0.45, 2.77)] and test 2 [η_p^2 = 0.13; 95% CI = (1.38, 3.98)]. SP and RP participants respectively recalled, on average, 8.2 and 9.9 items on test 1 and 7.9 and 10.7 items on test 2.

Our results call into question the growing consensus that stress generally impairs memory retrieval. We did not find this effect when stress acted on strong memory representations or when memory was assessed immediately after the onset of stress. Regarding the former, we showed that using a highly effective learning strategy to strengthen memory at encoding inoculated memory against the deleterious effects of the delayed stress response.

A combination of physiological evidence and cognitive theory helps to explain this finding. The delayed stress response is thought to impair retrieval through a physiological mechanism: Cortisol binds to glucocorticoid receptors in the hippocampus, impeding retrieval-related processing in this region (8, 9). Cognitive theories suggest that retrieval practice is a highly effective learning strategy because it creates multiple routes by which information can be accessed (14). When attempting to recall an item from memory, evidence suggests that associated (21–24) and/or contextual (14) information accompanies that attempt. More retrieval attempts thus create more distinct routes by which the same item can be accessed. Supporting this, a neuroimaging study found that, relative to study practice, retrieval practice increased hippocampal connectivity with other brain regions (25). In the case of our study, retrieval practice may have created multiple, contextually distinct retrieval pathways by which to access information. Although cortisol may have disrupted access to information by certain pathways, the robustness of the memory representation created by retrieval practice may have facilitated access to that information by alternate, undisrupted routes.

The ability for retrieval practice to strengthen memory against stress also has implications for real-world scenarios. For example, strong memory representations may reduce the retrieval failures that are common for students who experience test-related anxiety. Scenarios in which test anxiety impairs memory may thus be reconceptualized as scenarios that can be avoided when information is well encoded and accessible via many retrieval pathways.

Our finding that memory was unaffected when tested immediately after stress can likely be attributed to the biphasic nature of the physiological stress response. Immediately after the onset of stress, the body responds with two major hormonal changes: (i) the rapid and short-lived secretion of epinephrine and norepinephrine and (ii) the gradual and longer-lasting secretion of cortisol (26). The former response may facilitate neural processing (27), whereas the latter response impedes processing in memory-related brain regions (9). Thus, memory may be unchanged or even bolstered immediately after stress.

Several previous studies were unanimous in showing that memory, when measured after a poststress delay, was impaired by stress. Our results contest this robust finding. Whereas we did find memory retrieval impairment during the delayed stress response when information was encoded by restudying, that impairment was absent when information was encoded by retrieval practice. Thus, we argue that stress may not impair memory retrieval when stronger memory representations are created during encoding. Future research should be geared toward determining the cognitive mechanism by which retrieval practice protects memory against stress. The results of this line of research have the potential to fundamentally transform the way that researchers have viewed the relationship between stress and memory.

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Table 1. Average number of items recalled on test 1 and test 2 as a function of valence and item type. Tests occurred on the day after learning. Standard errors of the mean are given in parentheses.

	Negative	Neutral	Negative	Neutral
Test 1	**Words**		**Images**	
Nonstressed SP	4.4 (0.48)	2.9 (0.36)	5.0 (0.52)	4.9 (0.57)
Stressed SP	3.5 (0.54)	2.7 (0.36)	5.3 (0.69)	3.8 (0.45)
Nonstressed RP	4.5 (0.49)	4.1 (0.43)	6.2 (0.47)	5.4 (0.68)
Stressed RP	4.6 (0.58)	4.1 (0.36)	6.3 (0.79)	4.9 (0.29)
Test 2				
Nonstressed SP	5.3 (0.61)	3.9 (0.74)	4.5 (0.68)	3.8 (0.50)
Stressed SP	3.7 (0.61)	3.0 (0.61)	4.8 (0.47)	2.6 (0.36)
Nonstressed RP	5.1 (0.43)	4.2 (0.49)	5.6 (0.45)	5.4 (0.46)
Stressed RP	5.6 (0.37)	5.2 (0.69)	6.1 (0.50)	5.3 (0.60)

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BIOCATALYSIS

Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life

S. B. Jennifer Kan, Russell D. Lewis, Kai Chen, Frances H. Arnold*

Enzymes that catalyze carbon–silicon bond formation are unknown in nature, despite the natural abundance of both elements. Such enzymes would expand the catalytic repertoire of biology, enabling living systems to access chemical space previously only open to synthetic chemistry. We have discovered that heme proteins catalyze the formation of organosilicon compounds under physiological conditions via carbene insertion into silicon–hydrogen bonds. The reaction proceeds both in vitro and in vivo, accommodating a broad range of substrates with high chemo- and enantioselectivity. Using directed evolution, we enhanced the catalytic function of cytochrome c from *Rhodothermus marinus* to achieve more than 15-fold higher turnover than state-of-the-art synthetic catalysts. This carbon–silicon bond-forming biocatalyst offers an environmentally friendly and highly efficient route to producing enantiopure organosilicon molecules.

Silicon constitutes almost 30% of the mass of Earth's crust, yet no life form is known to have the ability to forge carbon–silicon bonds (*1*). Despite the absence of organosilicon compounds in the biological world, synthetic chemistry has enabled us to appreciate the distinctive and desirable properties that have led to their broad applications in chemistry and material science (*2, 3*). As a biocompatible carbon isostere, silicon can also be used to optimize and repurpose the pharmaceutical properties of bioactive molecules (*4, 5*).

The natural supply of silicon may be abundant, but sustainable methods for synthesizing organosilicon compounds are not (*6–8*). Carbon–silicon bond-forming methods that introduce silicon motifs to organic molecules enantioselectively rely on multistep synthetic campaigns to prepare and optimize chiral reagents or catalysts; precious metals are also sometimes needed to achieve the desired activity (*9–19*). Synthetic methodologies such as carbene insertion into silanes can be rendered enantioselective using chiral transition metal complexes based on rhodium (*11, 12*), iridium (*13*), and copper (*14, 15*).

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA.
*Corresponding author. Email: frances@cheme.caltech.edu

These catalysts can provide optically pure products, but not without limitations: They require halogenated solvents and sometimes low temperatures to function optimally and have limited turnovers (<100) (*16*).

Because of their ability to accelerate chemical transformations with exquisite specificity and selectivity, enzymes are increasingly sought-after complements to, or even replacements for, chemical synthesis methods (*17, 18*). Biocatalysts that are fully genetically encoded and assembled inside of cells are readily tunable with molecular biology techniques. They can be produced at low cost from renewable resources in microbial systems and perform catalysis under mild conditions. Although nature does not use enzymes to form carbon–silicon bonds, the protein machineries of living systems are often "promiscuous"—that is, capable of catalyzing reactions distinct from their biological functions. Evolution, natural or in the laboratory, can use these promiscuous functions to generate catalytic novelty (*19–21*). For example, heme proteins can catalyze a variety of non-natural carbene-transfer reactions in aqueous media, including N–H and S–H insertions, which can be greatly enhanced and made exquisitely selective by directed evolution (*22–24*).

We hypothesized that heme proteins might also catalyze carbene insertion into silicon–hydrogen bonds. Because iron is not known to catalyze this transformation (*25*), we first examined whether free heme could function as a catalyst in aqueous media. Initial experiments showed that the reaction between phenyldimethylsilane and ethyl 2-diazopropanoate (Me-EDA) in neutral buffer (M9-N minimal medium, pH 7.4) at room temperature gave racemic organosilicon product **3** at very low levels, a total turnover number (TTN) of 4 (Fig. 1A). No product formation was observed in the absence of heme, and the organosilicon product was stable under the reaction conditions.

We next investigated whether heme proteins could catalyze the same carbon–silicon bond-forming reaction. Screening a panel of cytochrome P450 and myoglobin variants, we observed product formation with more turnovers compared to the hemin and hemin with bovine serum albumin (BSA) controls, but with negligible enantioinduction (table S4). Cytochrome c from *Rhodothermus marinus* (*Rma* cyt c), a Gram-negative, thermohalophilic bacterium from submarine hot springs in Iceland (*26*), catalyzed the reaction with 97% enantiomeric excess (ee), indicating that the reaction took place in an environment where the protein exerted excellent stereocontrol. Bacterial cytochromes c are well-studied, functionally conserved electron-transfer proteins that are not known to have any catalytic function in living systems (*27*). Other bacterial and eukaryotic cytochrome c proteins also catalyzed the reaction, but with lower selectivities. We thus chose *Rma* cyt c as the platform for evolving a carbon–silicon bond-forming enzyme.

The crystal structure of wild-type *Rma* cyt c [Protein Data Bank (PDB) ID: 3CP5; (*26*)] reveals that the heme prosthetic group resides in a hydrophobic pocket, with the iron axially coordinated to a proximal His (H49) and a distal Met (M100), the latter of which is located on a loop (Fig. 1, B and C). The distal Met, common in cytochrome c proteins, is coordinatively labile (*28, 29*). We hypothesized that M100 must be displaced upon iron-carbenoid formation, and that mutation of this amino acid could facilitate formation of this adventitious "active site" and yield an improved carbon–silicon bond-forming biocatalyst. Therefore, a variant library made by site-saturation mutagenesis of M100 was cloned and recombinantly expressed in *Escherichia coli*. After protein expression, the bacterial cells were heat-treated (75°C for 10 min) before screening in the presence of phenyldimethylsilane (10 mM),

Science

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Protecting memories from stress

It is widely accepted that stress has a negative impact on memory retrieval. But specific approaches to learning can counteract this effect. Smith *et al.* found that when memory was tested immediately after the onset of stress, stress effects were reduced. Furthermore, when subjects learned novel material by using a highly effective learning technique involving practice tests, their memory was also protected against the negative effects of stress.
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