

# Why Emotional Memories Are Unforgettable

I have been fascinated with the distance between human genes and human behaviors for many years. One of the most interesting questions I have come across in my research concerns the link between emotions and learning. It is well known that memory traces tied to strong emotions are often retrieved with great clarity. Although the relationship is not perfect—emotions can as easily amend a memory as make one indelible—it has intrigued people like me for decades.

There may be many reasons for the link between emotion and recall. For one thing, we tend to internally repeat information that we find compelling, and what we find compelling is usually dripping in emotional competence. (Some researchers believe that emotions function as neurological Post-it notes, helping us to selectively pay attention to some sensory stimuli and not to others.) Since repetition schedules are the cognitive binders that paste memories to neurons, we tend to remember things better the more emotionally competent they become.

This relationship between emotional experience and memory has recently been described in molecular terms. In a remarkable series of experiments, researchers have shown that strong emotions potentiate learning via interactions between a known “memory” receptor and norepinephrine. That interaction is the subject of this month’s column. I will begin with a brief review of certain protein posttranslational modification processes, with mention of a process termed “long-term potentiation” (LTP). Later, I will discuss the interesting biology of a receptor involved in memory formation before moving to the results.

## Phosphorylation, LTP, and AMPA

There are 3 pieces of background information that I need to review. The first is molecular, the second is cellular, and the third is an interaction between the 2.

You may recall from undergraduate biochemistry class the importance of regulating protein function with the addition (or subtraction) of small subgroup molecules to specific amino acids within the protein. One of the most common forms of regulation is the addition or subtraction of phosphate groups. The enzymes responsible for such additions are called “ki-

nases” and “phosphorylases”; those responsible for the subtraction are called “phosphatases.” This reversible phosphorylation is so important to the overall regulation of the cellular environment that the Nobel Prize in physiology was awarded in 1992 to the 2 scientists who first figured out its role in the process—Edwin G. Krebs and Edmond H. Fischer.

The next bit of background information involves a phenomenon known as LTP, a topic I have covered in previous columns.<sup>1</sup> LTP is a type of synaptic plasticity that most researchers believe is involved in many aspects of memory formation. It was first observed in the mid-1960s when a group of researchers noticed that a brief burst of electricity given to a postsynaptic mammalian hippocampal neuron created a long-lasting increase in its overall transmission. Experimentally, the phenomenon could be observed as the temporal exposure of a primary and a secondary electrical pulse. When the primary pulse was given to a hippocampal postsynaptic neuron, a normal depolarizing signal was elicited.

If a second pulse was delivered to this neuron over a specific period, an increased electrical response was observed that lasted for hours. The neuron seemed to have “remembered” something, and the synaptic association was, at least temporarily, reinforced (the formal term is “synaptic strengthening”). If the neuron did not receive that signal within a specified time, no increase was observed. This gave researchers their first neural substrate capable of explaining not only the cellular mechanism behind memory but also the need for repetition to stabilize a given memory trace.

Since that time, a great deal has been learned about the molecules involved in mediating LTP. One critical mechanism for establishing LTP involves the synaptic insertion of GluR1 subunit-containing  $\alpha$ -amino-

3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (which I will mercifully simplify to “GluR1 receptors”) during the moment of learning (Figure). This insertion plays a major role in the synaptic strengthening mentioned above. AMPA receptors are an interesting group of proteins that play a role in many aspects of neural functioning. They are the classic ionotropic glutamate receptor. Glutamatergic neurons are the major fast excitatory neural substrates in the brain. AMPA receptors are composed of 4 individual subunits, GluR1 through GluR4. GluR1 is the major subunit involved in mediating hippocampal LTP.

The last piece of background information combines the addition of small groups with LTP. AMPA protein is regulated at several interior sites by the phosphorylation mechanisms described above. Indeed, phosphorylating this subunit regulates both the channel properties of the enzyme and its synaptic incorporation. If the subunit is phosphorylated at the appropriate site on the protein, LTP will commence. If it is not phosphorylated, LTP will not occur. Enzymes that are involved in this regulation include adenylyl cyclase, cyclic adenosine monophosphate-dependent protein kinase, and calcium/calmodulin-dependent protein kinase II.

This may sound like a lot of alphabet soup, but these proteins play a critical role in this story. I will now move on to some behavioral considerations regarding emotions and norepinephrine, which I will then relate to this mixture.

## Emotional arousal and memory

Although it is very difficult to define emotions as humans experience them, it is not difficult to show their effect on cognition. It has been known for some time that exogenously supplied amphetamine boosts retention scores of lists of words supplied in standard memory tests. The enhancing effect can be demonstrated whether the amphetamine is supplied before the words are memorized or after the learning has occurred. Animal studies first demonstrated that adrenergic systems and stimulation of the amygdala profoundly affected memory consolidation, a phenomenon that can now be observed in humans as well.

by John J. Medina, PhD



This behavioral effect may be most unambiguously seen with the use of  $\beta$ -adrenergic receptor antagonists. In one foundational experiment, 2 groups were exposed to an emotionally arousing story in the presence of pictures. One group was given the antagonist before the presentation; the other was given a placebo.

The group that was given the placebo remembered the pictures presented during the most emotionally salient part of the narrative best. This boost was wiped out in the group given the antagonist. The mechanism appears to be independent of age; a similar memory-inhibiting finding was observed in elderly persons who were exposed to the same experiment. Consistent with animal studies, administration of a  $\beta$ -adrenergic receptor can block the memory boost that emotional arousal normally supplies.

Activation of the amygdala is a key aspect of this boost. Noninvasive imaging technology (eg, positron emission tomography) has been used to evaluate amygdalar activity in the presence of emotionally competent stimuli—both noxious and pleasant. These studies demonstrated that the stability of long-term memory is associated with the amount of amygdalar activity that occurred in the initial encoding moments of the experience to be recalled. These data have been confirmed in patients who had selective lesions in the amygdala. They demonstrated no boost in long-term memory performance by emotionally arousing stimuli.

## What is the cellular biology of this phenomenon?

More recent data have begun to elucidate the cellular pathways associated with this memory boost. In the presence of emotionally competent stimuli, norepinephrine is released from neurons that arise from the lateral brain stem tegmentum and the locus caeruleus. This is a big deal. Neurons that originate from these areas project to an astonishingly wide variety of regions in the brain, including the hippocampus and the amygdala. After norepinephrine arrives at its target cells, the hormone binds to  $\beta$ -

(Please see Unforgettable Memories, page 16)

## Unforgettable Memories

Continued from page 14

adrenergic receptors.

But what happens next? And how do these events mediate a memory boost? The trail seemed to grow cold and, until a few months ago, that was the end of the story. Now we can relate a more detailed narrative.

### The research data

Groups of researchers performed a number of experiments on mice that allowed them to add a few more particulars to our understanding of the

process. The general idea was to examine well-characterized fear behaviors in these rodents and to observe the fate of the GluR1 protein during their fight-or-flight responses.

Mice have a natural fear reaction to the smell of fox urine. When mice were exposed to fox urine, researchers noted that the biochemical composition of GluR1 changed. Specifically, 2 phosphates had been added to the 2 sites mentioned previously, which are critical for its synaptic delivery. (This addition allowed GluR1 to be more easily incorporated into synapses involved in learning.) Spe-

cifically, the phosphorylation was found to lower the threshold for GluR1 insertion during LTP. Here, then, were the beginnings of an interaction. A well-characterized fear behavior was triggering a biochemical cascade known to be involved in memory formation.

The next set of experiments involved the direct injection of the stimulating hormone into the mouse. The researchers were again able to show a similar double phosphorylation in GluR1, and to familiarly demonstrate that this allowed GluR1 to become more easily incorporated into hip-

pocampal neurons (facilitating LTP). Whether one uses fear induction or just a sharp syringe, delivery of norepinephrine into the animal lowered the threshold for incorporation of GluR1.

Experiments were then conducted in mice whose GluR1 receptors had been genetically modified at the 2 phosphorylation sites. These mutant proteins were no longer capable of being phosphorylated. The mice were assessed for synaptic incorporation, and the mutant proteins remained unphosphorylated. Whether one uses fox urine or direct norepinephrine injection, the protein could not be inserted into the synapse.

But what about behavioral issues? Since LTP affects memory formation, did this double phosphorylation (followed by synaptic insertion) translate into improved hippocampal-mediated learning? There is a test commonly used to assess this type of memory formation in laboratory rodents, a modified contextual fear-conditioning protocol shown to require hippocampal involvement. Mice undergoing these experiments usually learn about contextual cues very quickly.

If the phosphorylation and insertion events lowered the threshold for GluR1 synaptic incorporation, learning should be potentiated in normal mice, whether one is using fox urine or the direct injection of norepinephrine. The mutant mice containing a GluR1 receptor that is impossible to phosphorylate should show no boost in learning, even in the presence of norepinephrine.

That is exactly what the researchers found. Mutant mice consistently performed poorly on the fear-conditioning test. When the results were taken together, the researchers were able to show that norepinephrine-stimulated phosphorylation of GluR1 assisted in the synaptic incorporation of the protein, lowered the threshold for experiencing LTP, and enhanced memory formation.

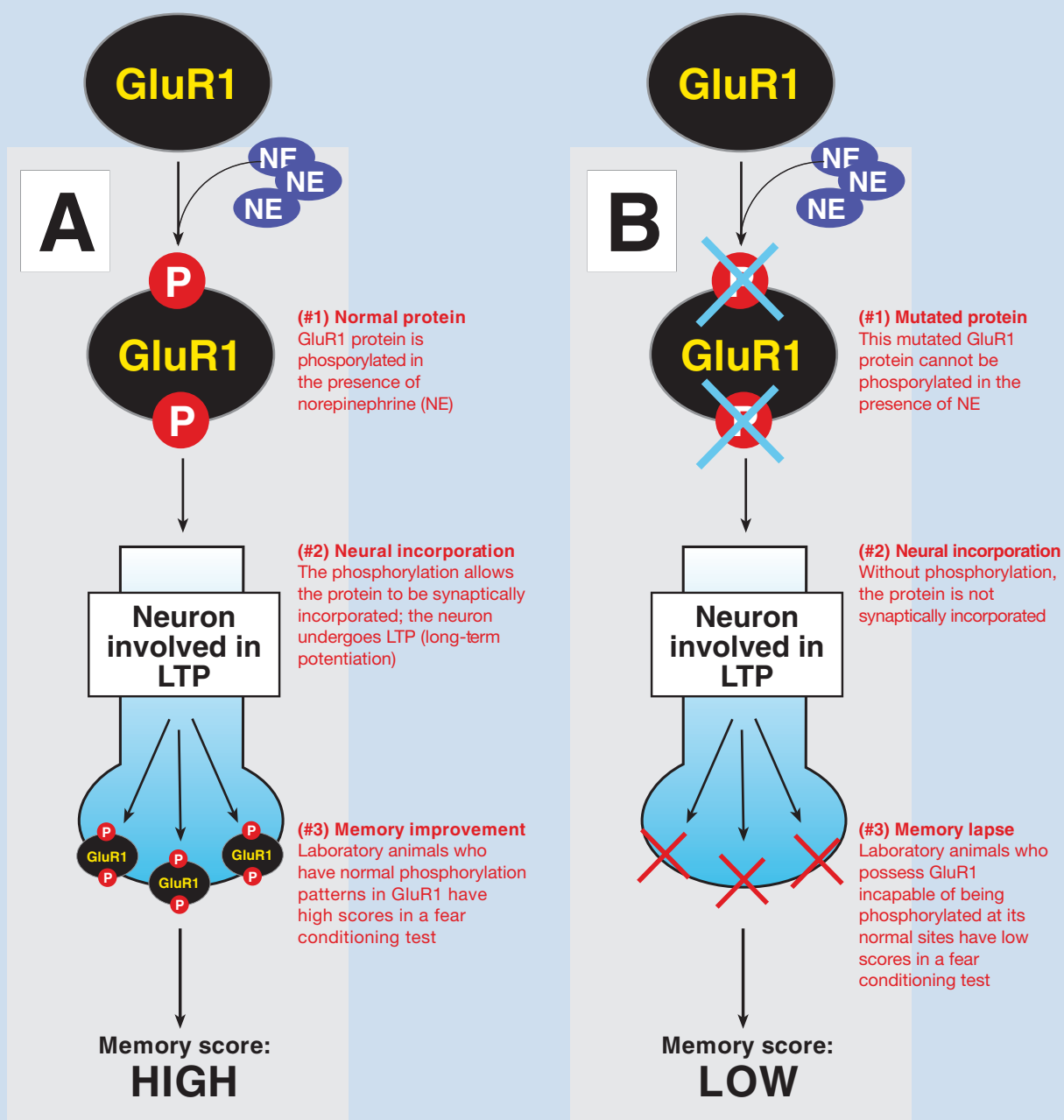
### Conclusion

These findings represent an important link between fear responses and the ability to trigger memory formation. They uncover a powerful molecular mechanism that demonstrates how emotions can enhance memory. Although the behavioral link has been known for many years, it is astonishing to think that this relationship can now so easily be described in terms of genes and proteins. These results generate as many questions as they do answers, and they may also trigger a certain grumpiness, especially when one thinks of their relevance to human biology.

Figure

## The relationship between memory and emotion

The molecular mechanisms undergirding the powerful role emotional arousal plays in memory formation have begun to be uncovered. Shown below is the relationship between exposure to norepinephrine and the phosphorylation of the protein GluR1. Panel A depicts experiments using normal, unmutated GluR1 protein. Panel B depicts experiments using GluR1 mutated in such a fashion that it cannot be phosphorylated.



Last month, I devoted an entire column to my reaction against willfully applying rodent data to human mental processes, a reaction that had equal parts caution and enthusiasm. Those same perspectives apply here. Mice are not humans, and great care must be taken when one attempts to jump the yawning chasm between the molecules supporting the psychological interiors of rodents and those supporting the psychological interiors of humans.

On the other hand, there is no need to be overly conservative. The great utility of laboratory animals is that they provide a “flashlight” service for people truly interested in human biology. With these phosphorylation experiments in hand, one now knows where to look. Humans have epinephrine and phosphorylation-prone AMPA receptors, and human neurons can undergo LTP just as rodent neurons can. Figuring out their molecular relationship using mouse data as a template is a responsible and grant-fetching thing to try.

It is quite an achievement to think we could finally begin to understand the relationship between strong emotions and strong memories at such an intimate level. It is one of the reasons why I find research trying to bridge the gap between gene and behavior so darned compelling after all these years. It’s enough to give an old scientist a norepinephrine spike!

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#### Reference

Medina J. The biology of memory extinction. *Psychiatr Times*. 2005;22(2):23-25. □