Learning and Memory, Part I: Brain Regions Involved in Two Types of Learning and Memory

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As presented in the previous two columns on fragile X syndrome,[1,2] the absence of fragile X mental retardation protein in fragile X disrupts the transport of messages (messenger RNAs [mRNAs]) along dendrites and their translation into proteins. This is because one of the functions of fragile X mental retardation protein is to normally repress local translation of mRNAs at sites adjacent to synapses, and in its absence, mRNAs are inappropriately translated. Researchers also found that activating a specific subtype of glutamate receptor (metabotropic glutamate receptor 5 [mGluR5]) is an important signal that initiates the translation of mRNAs at synapses. These two observations were put together to formulate a new hypothesis, called the "mGluR hypothesis of fragile X syndrome." It suggests that reducing mGluR5 signaling might reduce the excessive translation of synaptic proteins in fragile X. One target of therapeutic intervention therefore is to develop mGluR5 antagonists and try these drugs first in animal models of fragile X syndrome and eventually in patient populations.

This column and the next take the story further to discuss some of the molecular events that actually occur inside neurons during the development of synaptic plasticity and how these changes in synaptic strength can be measured in the laboratory. Among the topics that we cover are long-term potentiation and some of the mechanisms thought to explain why "neurons that fire together wire together."

We begin with a biking accident. Approximately 70 years ago, in a small town outside Hartford, Connecticut, a young boy was riding his bicycle when he fell off and hit his head. Soon thereafter, he developed a seizure disorder. The story of H.M. has been told and retold over the years and is only briefly summarized here. Initially, H.M. was successfully treated with anticonvulsant medications, but his seizures continued to get worse and became profoundly disabling over the next decade. He had difficulties finishing school and maintaining employment. In 1953, when H.M. was 27 years old, in an attempt to control the seizures, a surgical procedure was proposed to remove from within the medial temporal lobes what was believed to be sclerotic tissue that had formed after the head trauma. Dr. William Scofield performed bilateral medial temporal lobotomies that removed the hippocampi, the parahippocampal cortex, and parts of the amygdala.

After the surgery, H.M.'s seizures were much better controlled. However, it was soon apparent that he had also lost the ability to form new declarative memories. These types of memories are those that one is conscious of and can talk about. H.M. is still alive today and has met with the psychologist Dr. Brenda Milner for a hundred times, yet he retains no memory of her and has to be introduced each time they meet. He retains new information for only minutes at a time and has lost the ability to turn these short-term memories into long-lasting memories, a process called *consolidation*. It is important to realize that H.M. retains and can recall many memories formed before surgery and occasionally retains new information for longer periods but only with excessive practice.

These seminal studies by Scoville and Milner[3] established the role of the hippocampus in the consolidation of declarative memories. Scofield never performed the surgery again and, in fact, spent much of his time traveling the world to lecture about the dangers of this type of bilateral procedure. The basic

findings have now been confirmed in other accidental cases of hippocampal lesions in humans and in many animal studies. The advantage animal studies have is that they allow researchers to investigate systematically the underlying molecular events that are required for memory consolidation.

It was soon discovered that consolidation required stimulation of glutamate receptors and could be blocked by infusing glutamate receptor antagonists directly into the hippocampus. Both protein synthesis and gene transcription are also required and, in fact, must occur within a few hours of a learning session for short-term memories (which do not require protein synthesis or gene transcription) to be converted to long-term memories. This was discovered by infusing translation or transcription inhibitors bilaterally into the hippocampi. These infusions (and not into other brain regions) blocked the consolidation of declarative memories. It is important to realize that learning does occur under these circumstances. That is, the acquisition of short-term memories occurs, as can be demonstrated by testing retrieval within the first 3 hours of learning. These types of memories do not require protein synthesis or gene transcription.

Two additional questions must be addressed before we turn to the molecular events that occur within neurons during memory consolidation. First, where do declarative memories get stored once they are consolidated? Investigations are ongoing, but many researchers believe that memories are laid down in relevant cortical regions. For example, when one goes to a restaurant with friends, memories of music and the voices and clatter of dishes are consolidated in the auditory cortex. Memories of the restaurant's decor and the appearance of the food are stored in the visual cortex, whereas the smells are consolidated in the olfactory cortex, and tastes are consolidated in the insular cortex. Although the hippocampus is required for the consolidation of these declarative memories, it is thought that they are broken down into separate sensory components before their long-term storage in relevant cortical areas.

Second, why were only declarative memories disrupted after H.M.'s surgery? Dr. Milner showed that his nondeclarative memories were unaffected. One is often unaware of learning nondeclarative memories—such as the ability to ride a bicycle or tie one's shoes. The learning of these types of motor skills is sometimes called *procedural memory*. Another type of nondeclarative memory is exemplified by an individual with alcoholism or drug abuse who is flooded with the urge to drink or satisfy a drug habit when passing a particular bar or alleyway where this habit was satisfied in the past. These types of associations can be robust and long lasting, as exemplified by the relapse rates of these disorders. One type of nondeclarative memory, in which a loud noise is associated with a particularly unpleasant memory like a foot shock, is frequently used in animal models and is discussed below.

Declarative and nondeclarative memories can be hard to separate. You might have strong declarative memories of the birthday when you received your first bicycle and all of the times you fell as you tried to ride it that first day. These parts of the memories are declarative and, as mentioned, depend on an intact hippocampus. However, with some practice, you learned the new motor skill, and today, you are no longer aware of having to push your foot this way or lean to keep your balance—you just do it unconsciously. These types of motor skills are nondeclarative and do not depend on
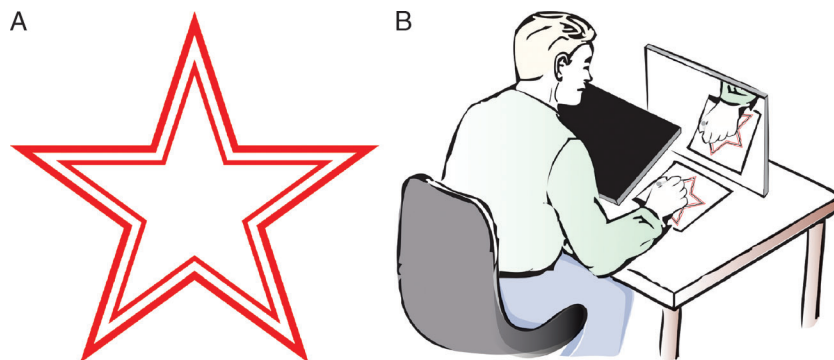

Fig. 1 A, Dr. Milner gave H.M. a test to examine one type of nondeclarative learning. The instructions here were to use a pen to draw a line that was within the border of the star. This is the star that was given to H.M. B, The task is made more difficult by requiring testees to use a mirror to see what their hands were doing.

the hippocampus but instead require portions of the basal ganglia and cerebellum.

Of the many tests Dr. Milner used with H.M., one is the nondeclarative task shown in Figures 1 and 2. The instructions are to keep the tip of the pen within the double lines. To make the task more difficult, you are instructed to see only your hands through a mirror. After practicing over the course of several days, the number of errors we make diminishes. Motor skills typically show gradual improvement with repetition and over time. Note that during this process, you are not aware of "learning" anything, but the skills improve, and fewer errors occur. H.M. performed as well as anyone else on this task, indicating that he was able to consolidate this nondeclarative memory, although formation of his declarative memory was permanently damaged. It is fascinating that, at the same time that he was learning the new motor skill, he formed no declarative memory of the testing situation: each day, he needed a new set of instructions as if he had never before seen the task or been told what to do.

One last example of nondeclarative memories is particularly relevant to several psychiatric disorders. Why do posttraumatic stress disorder and phobias occur in some individuals but not in others? These disorders are thought to rely on nondeclarative associations being formed, although in this case, declarative memories often are also formed. Many laboratories over the years have developed sophisticated paradigms to study the formation of nondeclarative memory in animal models.
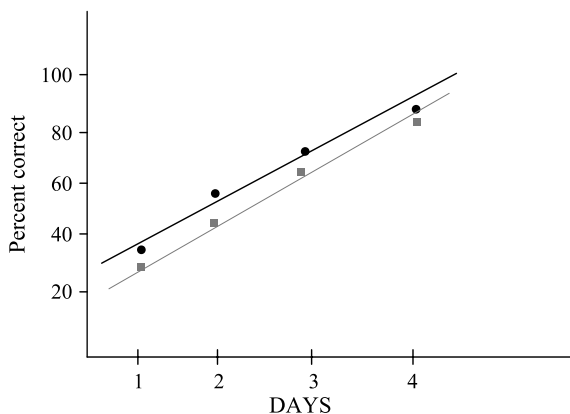

Fig. 2 Performance improves in normal individuals for several days (circles). H.M. also improved (boxes), indicating that his ability to learn this type of procedural nondeclarative memory was intact, despite the fact that he was unable to form declarative memories of the task. That is, every day, he had no memory of the tester or testing situation and required new instructions as to what the rules were.
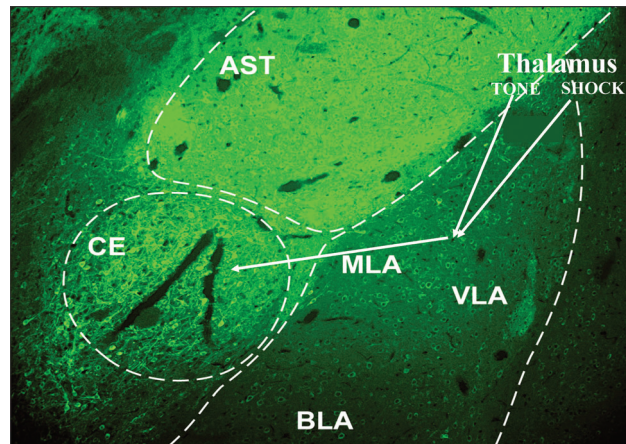

Fig. 3 The thalamus sends signals representing both the tone and shock to neurons of the lateral amygdala. If consolidation of the fear memory occurs, then this can be measured at a later testing time (24 hours) in which only the tone is presented to the test animal. If consolidation has happened, then the tone alone is sufficient to generate an action potential from these neurons, and this signal is passed along to the central nucleus (CE) and, from there, is transmitted to a number of deeper structures responsible for the behaviors observed. AST = amygdala striatal transition zone; BLA = basolateral amygdala; MLA = mediolateral amygdala; VLA = ventrolateral amygdala.

A form of this type of learning is called *fear conditioning*, and the consolidation of these fearful memories requires the amygdala. In a typical experiment, a rodent is placed into a small cage. A tone sounds, and after 20 seconds or so, a mild shock is delivered through the floor of the cage. Animals learn after only one or two sessions to fear the tone. This cued conditioning, with the cue being the tone, is distinguished from contextual conditioning, in which the rodent is placed into a cage that it can explore and then receives a mild shock. Lesion studies in rodents were able to establish the brain regions that are involved in these two types of learning. In the case of contextual learning, both the hippocampus and the amygdala are required, with the hippocampus involved in learning the spatial cues of the cage, whereas the amygdala is involved in the fear memories themselves. In contrast, only the amygdala is necessary for the consolidation of fear memories in the case of cued conditioning. (Obviously, other brain regions are involved, as we process, for example, the initial sensory cues; here, we are referring to the brain regions involved in the consolidation of fear memories.)

Let us dissect the cued condition training a bit further. The incoming sounds of the tone are detected and activate the auditory nerve that makes synaptic contact with the neurons of the cochlear nucleus. Several synaptic connections later, the signal arrives to

the thalamus, where part of the signal is projected to the lateral amygdala, whereas others move on to the auditory cortex for processing. It is the portion that arrives at the lateral amygdala that is of interest now. At the end of the period that the mouse is hearing the tone, the mild shock is given. It is initially sensed by nerve terminals in the paws and is passed up the spinal cord to reach the thalamus. Again, part of the incoming signal is passed to neurons that are found in the lateral amygdala, whereas another portion moves on for processing by the sensory cortex (Fig. 3).

Neurons of the lateral amygdala receive the synaptic inputs of the tone and the shock on their dendritic arbors. This is the critical event: the arrival of two synaptic inputs at the same time and to the same neuron and, in fact, to the same spine of this neuron. Something happens to the recipient spine when these two inputs arrive within milliseconds of each other. There is a strengthening of the connection for the signal representing the tone. Yesterday, the mouse would have heard the same tone and done nothing particularly dramatic. Once the association of the tone to the shock occurs and a fear memory is made, later, the mouse needs to only hear the tone to freeze in its tracks. It has "learned" that something bad happens when it hears this tone.

Following the pathway that leads to the freezing behavior is instructive. At the test session 24 hours after the fear-conditioning training, the mouse is exposed to the tone only. The tone is now able to depolarize the postsynaptic neuron in the lateral amygdala sufficiently for an action potential to be produced within

this neuron and passed on to the next relay station, a neuron within the central nucleus of the amygdala. Before the fear-conditioning training, neurons within the central nucleus would not have been activated by the mouse hearing the tone alone. Subsequent to the training where multiple pairing of tone and shock occurs, the synaptic effects from the tone are greatly increased. Neurons within the central nucleus now receive a strong synaptic input and are sufficiently depolarized to pass an action potential onto various nuclei within the hypothalamus and related structures. So, for example, wide pupils and paleness result from activation of neurons in the lateral hypothalamus, and elevated heart rate stems from activation of neurons within the dorsal motor nucleus of the vagus. Panting is the result of activation of the parabrachial nucleus, whereas increased vigilance comes from activation of the locus ceruleus. The facial expressions of fear are due to activation of the facial nucleus, whereas the freezing behavior stems from activation of the central gray. In reviewing some of the behaviors in this list, it is not hard to see the similarities with these behaviors and those that occur in posttraumatic stress disorder and other anxiety disorders (Fig. 4).

In the next column, we examine the molecular events that are responsible for the strengthening of synaptic connections when the inputs representing the tone and the shock arrive together on neurons in the lateral amygdala. As a preview, it should not be surprising that the strengthening of synaptic contacts requires protein synthesis and gene transcription in the postsynaptic neuron. These two events, in fact, provide the proteins

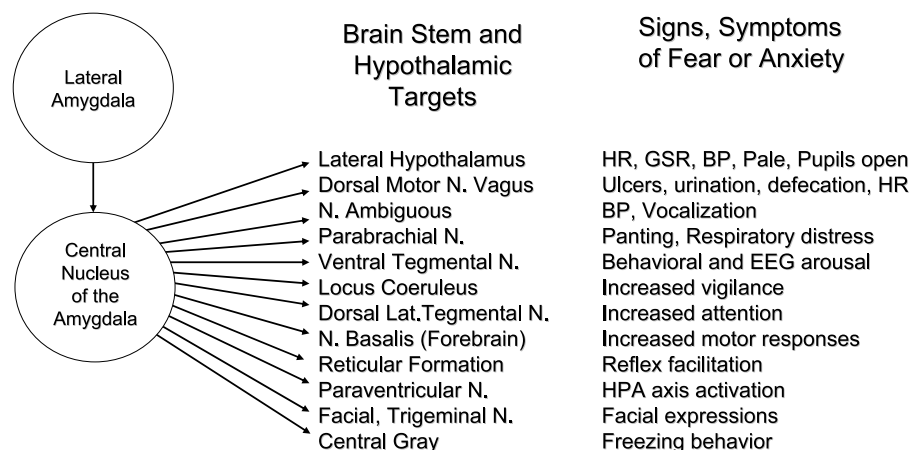|	Brain Stem and Hypothalamic Targets	Signs, Symptoms of Fear or Anxiety
Lateral Amygdala → Central Nucleus of the Amygdala	Lateral Hypothalamus	HR, GSR, BP, Pale, Pupils open
	Dorsal Motor N. Vagus	Ulcers, urination, defecation, HR
	N. Ambiguous	BP, Vocalization
	Parabrachial N.	Panting, Respiratory distress
	Ventral Tegmental N.	Behavioral and EEG arousal
	Locus Coeruleus	Increased vigilance
	Dorsal Lat.Tegmental N.	Increased attention
	N. Basalis (Forebrain)	Increased motor responses
	Reticular Formation	Reflex facilitation
	Paraventricular N.	HPA axis activation
	Facial, Trigeminal N.	Facial expressions
	Central Gray	Freezing behavior

Fig. 4 Schematic diagram indicating the connections between the central nucleus and a variety of target nuclei within the hypothalamus and brainstem that together are responsible for the symptoms of fear and anxiety. Reprinted with permission from the *Annual Review of Neuroscience,* Davis M. The Role of the Amygdala in Fear and Anxiety. *Annual Review of Neuroscience.* 1992;15:353–375. *www.annualreviews.org.*

that are necessary for the synaptic remodeling that occurs when synaptic plasticity develops. In addition, we have to somehow explain how the tone alone is now able to activate the postsynaptic neuron. The debate here is whether the changes are presynaptic or postsynaptic. For example, perhaps the action potential representing the tone now releases twice as much of the transmitter glutamate than before consolidation. This could explain the more robust response of the postsynaptic terminal: twice as much neurotransmitter would result in a larger depolarization. Another possibility is that there are twice as many glutamate receptors present on the postsynaptic terminal compared with the number before consolidation. Again, the increase in receptors might explain the increased postsynaptic response to the synaptic input. A third mechanism has also been proposed: that the same number of receptors exists but that the postsynaptic receptors are somehow more sensitive to the transmitter glutamate. They stay open for longer periods or allow for greater amounts of ions to pass through. This would explain the great depolarization that occurs with the same input from the tone. In fact, although the debate is not over, all three of these possible synaptic adaptations have been found to occur.

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REFERENCES

1. Lombroso PJ, Ogren MP. Fragile X syndrome: keys to the molecular genetics of synaptic plasticity. *J Am Acad Child Adolesc Psychiatry*. 2008;47(7):736–739.
2. Ogren MP, Lombroso PJ. Reversing the effects of fragile X syndrome. *J Am Acad Child Adolesc Psychiatry*. 2008;47(8):863–867.
3. Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. *J Neurochem*. 1957;20:11–21.

ADDITIONAL READINGS

Baddeley AD, Wilson BA, Kopelman M. *Handbook of Memory Disorders*. 2nd ed. New York: John Wiley & Sons; 2002.
Clark RE, Manns JR, Squire LR. Classical conditioning, awareness and brain systems. *Trends Cogn Sci*. 2002;6:524–531.
Corkin S. What's new with the amnesic patient H.M.? *Nat Rev Neurosci*. 2002;3:153–160.
Dudai Y. Molecular bases of long-term memories: a question of persistence. *Curr Opin Neurobiol*. 2002;12:211–216.
Eichenbaum H, Cohen NJ. *From Conditioning to Conscious Recollection: Memory Systems of the Brain*. New York: Oxford University Press; 2000.
Schacter D, Buckner RL. Priming and the brain. *Neuron*. 1998;20:185–195.
Squire LR, Kandel ER. *Memory: From Mind to Molecules*. New York: WH Freeman; 1999.
Squire LR, Zola-Morgan S. The medial temporal lobe memory system. *Science*. 1991;253:1380–1386.
Tulving E, Craik FIM. *The Oxford Handbook of Memory*. New York: Oxford Press; 2000.

WEB SITES

http://thebrain.mcgill.ca/flash/d/d_07/d_07_p/d_07_p_tra/d_07_p_tra.html
http://www.supermemo.com/english/links.htm
http://brembs.net/learning/
http://ocw.mit.edu/OcwWeb/Brain-and-Cognitive-Sciences/9–03Fall-2003/CourseHome/index.htm