Solving the Mystery of Memory

By Paul Worley, M.D., and Marshall Shuler, Ph.D.

Editor's Note: The word "memory" is derived from the ancient Greek myth of Mnemosyne, the mother of the Muses, who was "said to know everything, past, present, and future." Memory is essential to our existence, and one of neuroscience's primary missions is to understand how the brain processes memory and to improve treatments for Alzheimer's disease, traumatic brain injury, drug addiction, and the many other afflictions associated with disrupted memory. Our article traces scientists' progress in understanding memory over the last 15 years.

In the 1980s, researchers developed experimental tools that showed enormous potential in helping us move toward a molecular understanding of memory, an enduring and ongoing challenge. This is where my own (P.W.) career in basic science research really began. As I read papers by leaders in the field, the goal and experimental approach seemed clear: To identify the molecules of memory and determine how they worked. All these years later, we have made great strides that can be appreciated in the story of a single protein in the brain that plays a key role in strengthening or weakening processes involved in memory.

There are several elements other than this protein that factor in (neurons, networks, genes, receptors) and, in explaining the complexities of how all the pieces fit together, it is best to start with synapses, which are chemical connections between neurons that are also thought to play a central role. Synapses contain both a presynaptic element that releases neurotransmitters and a postsynaptic element that includes receptors for neurotransmitters. Neurotransmitters are released in bursts and bind to receptors, which then become activated. Receptors function with other proteins to convert a signal from the activated receptor into changes in properties of the postsynaptic cell, in a process termed signal transduction. Synapses can be strong if they produce large changes in the postsynaptic cell, or weak if they produce little or no change. The strength of synapses can be modified by use, and this activity-dependent change in synaptic strength is a key to understanding memory.

The perception among neuroscientists is that specific synapses are activated as specific neurons are activated, and this results in enhanced connections between certain neurons, but not between others. If one of the resulting networks of interconnected neurons is activated, others are also likely to be activated, and this enhanced connectivity encodes information. Think of it as “neurons that fire together, wire together.” The mystery behind it all is how specific synapses are made to become weaker or stronger, and how these changes in synaptic strength are maintained for long periods of time.

Many diseases of the brain are associated with disrupted memory. This association is especially strong for Alzheimer’s disease, traumatic brain injury, and stroke. But concepts of memory are also central to understanding substance addiction, in which the drug usurps brain mechanisms that normally reward certain behaviors.[1] Indeed, drug addiction provides a window to an important frontier of memory research that involves understanding the relationship between reward and learning (reinforcement learning theory). Many of the following insights emerged from studies of cocaine addiction.

A Small but Vital Protein

More than 50 years ago, conventional studies showed that the synthesis of ribonucleic acid (RNA) and their translation into new proteins are required for animals to establish long-term memory.[2] In these studies, injection of chemicals that blocked RNA or protein synthesis blocked long-term memory. The finding might not seem surprising since neurons are made of proteins. However, the key observation is that the processes of RNA synthesis and subsequent expression of protein are required only during a brief window of time immediately following a significant experience. Various experiments determined that this time window is about three hours. This finding indicated that mRNAs and the proteins they generate within this time window make or allow memory to happen.[3] However, scientists still had to identify the mRNAs and determine how the proteins they encode function to facilitate memory.

The experimental tools developed in the 1980s allowed researchers to identify mRNAs that are rapidly induced in cells.[4,5] I (P.W.) was fortunate to be in the right place to develop animal models and molecular techniques to identify a set of genes that were rapidly increased for 1-3 hours during the time most critical for memory. These genes are termed cellular immediate early genes (IEGs), a term that recognizes their rapid and transient increase in cells. One of these IEGs encodes a protein named Homer1a. [6] This small protein is present in the cytoplasm of neurons and enriched in the neurons' dendrites, which receive electrochemical signals from other neurons. With help from Dr. Daniel Leahy of Johns Hopkins, we were able to visualize the molecular structure of Homer1a and determine that it encodes a single module for binding other proteins.[7] An obvious question was: How does this small protein, Homer1a, contribute to memory? Studies over the past 15 years support a model in which the Homer1a protein binds to a neurotransmitter receptor located at the synapse and changes its properties in a way that can enhance active synapses and suppress inactive synapses. This complex process reveals several concepts important to the study of memory, which we will describe more fully.

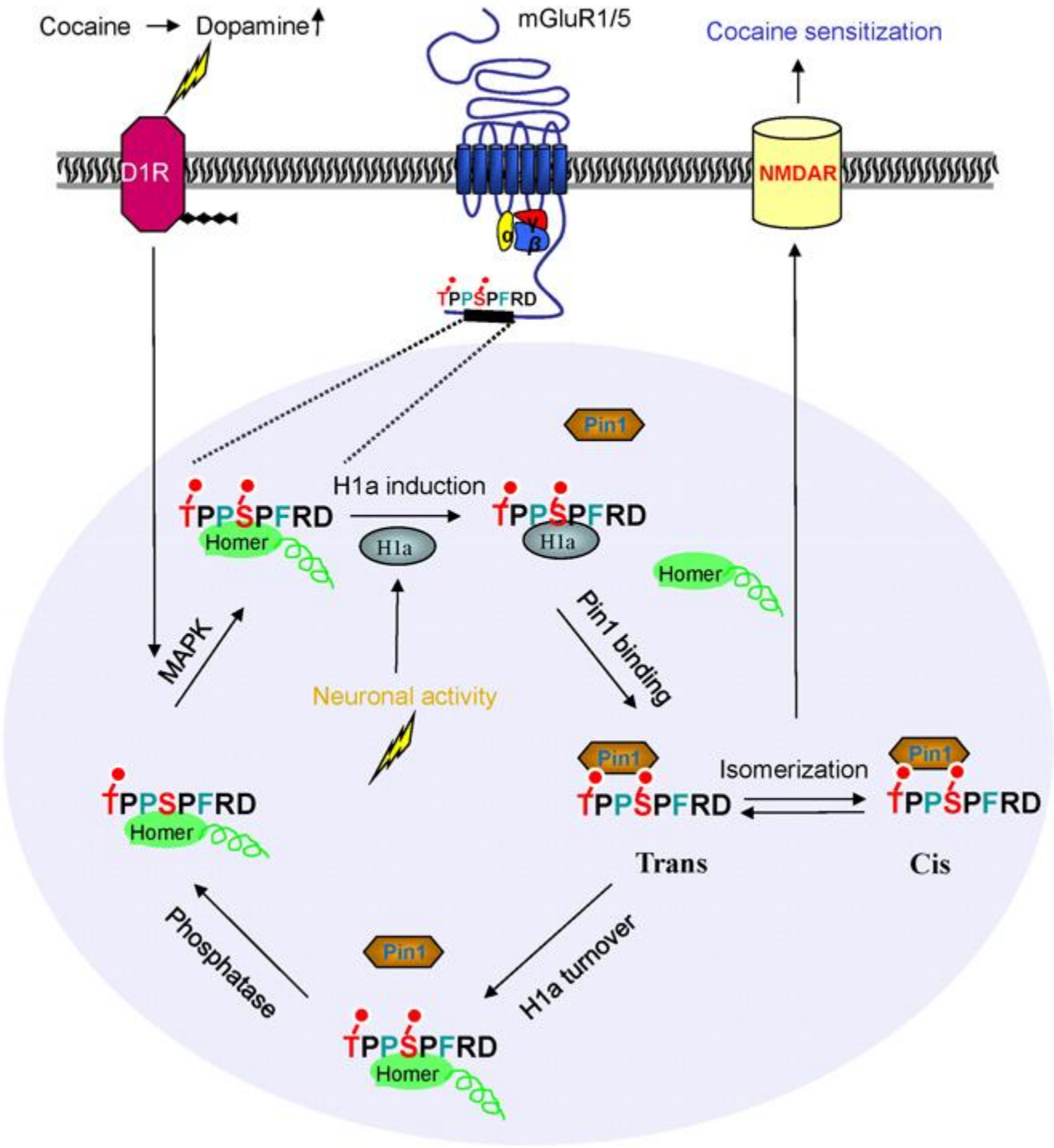
For the purpose of description, imagine a single neuron in an animal's hippocampus—a brain region that is important for memory. The neuron fires electrical discharges as the animal experiences its environment. This firing may happen as a consequence of finding food or some other event that is important for the animal, and it results from the activation of synapses onto the activated neuron. Electrical activation of the neuron induces transcription of the genetic code from the Homer1 gene to generate Homer1a mRNA. The Homer1a protein that is produced then moves from the nerve cell body to its dendrites, which are rich with synapses. This process takes about 45 minutes. Synaptic activity that induces generation of the Homer1a protein also produces long-lasting changes at the active synapse. Neurobiologists conceptualize these changes as a "tag" that makes them different from

synapses that are not active.[8] When the Homer1a protein arrives at synapses, it binds to a neurotransmitter receptor for the excitatory neurotransmitter glutamate, which is involved in learning and memory. The receptor is called metabotropic glutamate receptor type 5 (mGluR5). Homer 1a causes changes in synaptic function according to whether the synapse was tagged or not. If the synapse was not tagged, Homer1a causes weakening of the synapse. Inversely, if Homer1a binds to mGluR5 at a synapse that *was* tagged, it strengthens the synapse. The processes by which Homer1a weakens or strengthens synapses are very different and complex, as we describe in our next section.

A Protein's Role at Synapses

Homer1a reduces the strength of non-tagged synapses. The process begins as Homer1a protein increases in neurons that are strongly activated. In natural conditions, neurons are activated by synaptic input, but only a small percentage of the total number of synapses are activated. The majority of the synapses are quiet. At these quiet synapses, Homer1a protein binds to the mGluR5 receptor and activates this receptor by a process that does not require the neurotransmitter glutamate to be involved.[12] This is unusual for a neurotransmitter receptor, but is a phenomena that is part of a well-established pharmacology for mGluR5. The Homer1a-activated mGluR5 receptor then produces changes in signaling that results in the removal of a different class of glutamate receptor, called GluA2, from the synapse.[11] GluA2 is one of the major receptors that determine synaptic strength. Removal of GluA2, therefore, results in a persistent weakening of synapses. This can be considered a homeostatic process that prevents persistent increases of neuronal activity, which may be important to prevent damage to the neuron.

Graphical Abstract: Proposed model of mGluR5 signaling and cocaine plasticity in motor sensitization.



The abstract illustrates a role for mGluR5 in coupling dopamine receptor D1R activation to cocaine addiction. Critical events include phosphorylation of the Homer binding site in mGluR5, which creates a potential binding site for Pin1. The immediate early gene Homer1a is required for Pin1 binding, isomerization and enhanced activation of NMDA receptor. Enhanced NMDA receptor function mediates cocaine sensitization, an aspect of addiction. (Figure credit: Dr. Jia Hua Hu)

Homer1a can also strengthen synapses. This strengthening action occurs specifically at synapses that were activated during the process that increased neuronal activity and Homer1a expression, and occurs at the same time as a weakening of inactive synapses occurs. The process of synaptic strengthening is dependent upon changes of the mGluR5 receptor occurring at individual activated synapses. Dr. Anne Young's laboratory at the Massachusetts General Institute for Neurodegenerative Disease reported that the mGluR5 receptor is phosphorylated (undergoes a chemical change) at the same position, whereas Homer1a protein binds to it.[9] Phosphorylation often changes the way proteins function, and the fact that this occurred at the Homer binding site suggested that it might change the mGluR receptor in some important, though unknown way. Phosphorylation of mGluR5 is mediated by kinases (enzymes) that target the amino acids (building blocks of protein) serine or threonine that are adjacent to the amino acid proline (enzyme class is termed proline-directed kinase). When the receptor for the neurotransmitter dopamine (which is involved in the brain's reward system) is activated, the proline-directed kinase (termed mitogen-activated protein kinase MAPK; also termed ERK), increases phosphorylation of the mGluR5 receptor.[10] This process is important for understanding memory formation, since dopamine is a neurotransmitter that can change neuronal properties to enhance brain activity and memory (termed "neuromodulator"), and is released by special neurons in the brain in association with pleasurable events.

Addictive drugs such as cocaine and amphetamine also increase dopamine in the brain. One consequence of mGluR5 receptor phosphorylation is that the Homer1a protein binds more strongly. A second consequence is that it "creates" a binding site for an enzyme termed Pin1.[10] Pin1 is a prolyl isomerase that accelerates the rotation of the amino acid backbone of the phosphorylated serine-to-proline bond and changes the 3-dimensional structure of the protein. Pin1 binding requires phosphorylation of mGluR5, as well as Homer1a. Binding and isomerization are biophysical events that can be visualized at the level of single atoms using purified proteins and special techniques. This analysis reveals the relationship between binding of Pin1 and the motion of mGluR5. Together, these observations help us create an integrated model in which dopamine release in the brain increases rotational motion of the region of mGluR5 that connects with other proteins. In this way, dopamine changes the ability of mGluR5 to produce signal transduction in the postsynaptic neuron. But then what?

A Tie to Drug Addiction

Electrical recordings revealed that mGluR5 activation results in opening ion channels in the neuronal cell membrane. This opening results in a net movement of positive charge (sodium and calcium ions) into the neuron (inward current). Ion movement creates an electrical current that can be detected and recorded. This particular current is created by opening a third type of glutamate receptor termed the N-methyl-D-aspartate (NMDA)

receptor.[10] Details of this process are unknown, but we imagine that increased rotational motion of mGluR5 created by Pin1 causes the NMDA ion channel to open. The NMDA receptor is critical in this pathway, as it is known to produce changes in the postsynaptic neuron that increase the strength of synapses. Importantly, the ability of mGluR5 to activate the NMDA channel increases when mGluR5 is phosphorylated and both Homer1a and Pin1 are functional at the synapse. Mice whose mGluR5 gene has been mutated so that mGluR5 cannot be phosphorylated show altered responses to cocaine. Several experimental manipulations, including interruption of Homer1a induction, interruption of phosphorylation of mGluR5, or the reduction of Pin1 activity, all similarly reduce a cocaine-induced behavior linked to addiction.[10] This makes us believe that the biophysical events that mediate Homer1a-Pin1-mGluR5 potentiation at individual synapses are causal for cocaine addiction.

Enhancing Memory

How does the biochemical model of Homer1a-dependent synaptic modification work to enhance memory in the brains of animals? The role of Homer1a in memory is easiest to conceptualize in the hippocampus, an area for which we know a great deal about IEG expression and neuronal activity linked to memory. Recordings of hippocampal neurons, from conscious, behaving rats reveal that specific neurons are consistently activated in association with an animal's understanding of its position in space. These neurons are called place cells. Dr. Bruce McNaughton, then of University of Arizona, created electrodes (termed tetrodes) that could record from multiple neurons simultaneously,[13] and monitored activity during both active exploration and subsequent periods of rest in a rat's home cage. Dr. McNaughton and his then-postdoc Dr. Matthew Wilson (currently of MIT) discovered that patterns of neuronal activity that occurred during active exploration were "replayed" later while the rat was resting.[14] Subsequent studies by Drs. Wilson, David Foster, and others have shown that replay occurs during both rest and active exploration.[15] Moreover, aspects of the replay can be used to predict subsequent behaviors, as if we are listening in as neurons plan behaviors, in addition to reviewing significant daily events.[16]

Place cells and replay are also evident in the pattern of IEG expression in the hippocampus. Dr. John Guzowski, working with Drs. Carol Barnes and McNaughton, noted that mRNA transcripts of the IEGs Arc and Homer1a could be detected in place cells using an in situ hybridization method that distinguishes between mRNA that is present in the nucleus and mRNA that is present in the cytoplasm of neurons.[17,18,19] As predicted from in vivo recording data in the rat, specific neurons of the hippocampus showed place-specific activation that was consistent across time for the same place. Moreover, IEG activation occurred after the rat had returned to its home cage in the same neurons that were active during behavioral exploration.[20,21] This late re-expression of IEG expression is consistent with replay (as originally described). Interestingly, the size of the population of neurons that re-express IEGs is smaller

than the original place-cell population, as if the process that generates replay selects a subpopulation of place cells. This may represent selection of the rat's most important events for long-term storage.

Reinforcement Learning

Advances in understanding memory result from many different approaches. For example, methods used to identify brain IEGs have utilized viruses that naturally infect bacteria to store genetic information, and yeast cells whose growth can tell us about protein interactions. Investigators also use theoretical and computational approaches that are based on logic and systems or psychophysical data (the area of research that captured the imagination of author Marshall Shuler). In most cases, it is difficult to relate the empirical and the theoretical directly. But we can speculate on how certain theoretical properties of a learning model correspond to specific events or molecules from empirical data. We begin with a theoretical description of memory and definitions of critical events that must occur for memory storage.

We can define learning as relating outcomes based on behavioral import to preceding neural activity that engendered the goal-seeking behavior. This process occurs through the modification of synaptic strengths so as to make a given behavior more or less likely. Knowing which of the many synapses in a network (or indeed, in the brain) to modify poses a challenge, for the intricate pattern of activity preceding any outcome spans a spatially diffuse and vast array of synapses active at different moments in the past, not all of which are pertinent to the behavior to be learned. The attribution of "credit" to synapses that were causative in generating a behavior—the so-called spatiotemporal credit assignment problem—is therefore a central problem in neuroscience, addressed in reinforcement learning theory. In reinforcement learning, the relating of neural activity to future outcome requires an interaction between a signal conveying the success or failure of the behavior—the reinforcement signal—with a memory of which synapses were involved—referred to as an eligibility trace—in the stimulus-action response. The nature of the interaction between eligibility traces (that serve as memories of synapses' activity histories) and reinforcement signals is described as a synaptic learning rule. Such a rule, by predicating changes in synaptic strength on the presence of a reinforcement signal *and* eligibility traces, can resolve the credit assignment problem.[22] However, biological instantiations of the reinforcement signal, and particularly the putative synaptic eligibility trace, are unknown, as is the synaptic learning rule that governs their proposed interaction.

The processes theorized in reinforcement learning finds support in the molecular biology of the synapse. Imagine a neuron that communicates with many different neurons, and that communication with one particular neuron is important for behaviors that are rewarded, while other synapses and behaviors are not rewarded. In this scheme,

synaptic interactions that cause neuronal activation (spiking) are identifiable by increased NMDA receptor–mediated currents. This transient activation occurs similarly at synapses that are rewarded or not rewarded. For those behaviors and associated synapses that are ultimately (later) rewarded, the reinforcement signal—here proposed to be the neurotransmitter dopamine—must interact in some way with the preceding distribution of activated synapses. One possibility is that the biochemical synergy between NMDA receptor activation and dopamine receptor activation for triggering of the mGluR5 kinase MAPK described above may persist even if NMDA receptor is activated for some brief time (a fraction of a second to several seconds) before arrival of the dopamine receptor signal. In this scenario, MAPK would be preferentially activated at synapses that are associated with rewarded behaviors and create an eligibility trace in the form of phosphorylated mGluR5. Phosphorylated mGluR5 at specific synapses awaits the arrival of Homer1a from the nuclear transcription response. Together with Pin1, they cause a sustained increase of synaptic strength. In this manner, credit is assigned to a synapse in accordance to the degree in which its activity is predictive of future reinforcement, and is converted, albeit at a delay, to a change in synaptic strength as plasticity proteins subsequently arrive. NMDA-dependent signals at synapses that were activated with behaviors that were not rewarded will decay prior to arrival of Homer1a and be consequently weakened. This model rationalizes the time (45 minutes) requirement for IEG expression) as necessary to permit decay of non-rewarded synapses. By this speculative mechanism, synapses linked to rewarded behaviors are strengthened relative to other synapses, and this creates the substrate for long-lasting memories.

Conclusion

Important advances come with the integration of divergent ways of thinking. IEGs stand at the boundary between synaptic biology and systems physiology, and establish a framework for instantiation of reinforcement learning theory. Their selective expression in neurons that are engaged in information processing and storage affords a means to visualize and even to manipulate individual neurons or networks of neurons in order to understand their contribution to memory. IEG action in neurons reveals ways in which synapses are modulated as neurons and networks store information. Computational and theoretical models help place these molecular ideas into action and focus attention on the biochemical events that are most consequential, with the ultimate goal of understanding memory and preventing diseases that disrupt memory.

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Marshall G. Shuler

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