Published in final edited form as:

Curr Biol. 2012 May 8; 22(9): R302–R304. doi:10.1016/j.cub.2012.03.034.

Behavioral Neuroscience: No Easy Path from Genes to Cognition

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Abstract

Two recent studies illustrate the limits of a strictly molecular approach toward understanding learning and memory.

About 15 years ago I attended a learning and memory meeting, where I heard a (then) young biologist describe a molecular model for long-term memory. The model, which featured a single neuron and centered on the cyclic AMP response element binding (CREB) protein pathway, was meant to synthesize insights from then-recent work on memory in *Drosophila, Aplysia*, and mouse. According to the speaker, the model could account for three different forms of learning that had been studied in these organisms, olfactory conditioning, sensitization, and spatial learning in the Morris water maze, respectively. I recall being struck by an implication of the speaker's claim, namely that the specific identity of the neuron in his model — whether a mushroom body Kenyon cell, an *Aplysia* sensory neuron, or a hippocampal CA1 pyramidal neuron — was more or less irrelevant, and that what truly mattered was the identity of the molecules engaged during each type of learning.

The above anecdote illustrates an ideology that appears, thankfully, to be waning in behavioral neuroscience. In particular, the notion that molecules possess an explanatory primacy in models of learned behavioral change appears far less attractive now than it did a decade ago. The main reason for the lessening attraction is an increased appreciation that knowledge of the specific ways in which the neural circuits that mediate a behavior are modified during learning is just as crucial as knowledge of the molecular changes triggered. The limits of the idea that merely identifying the molecular pathways engaged in a particular instance of learning is sufficient to explain the learning are nicely illustrated by two recent studies, one in the fruit fly [1] and one in the marine snail *Aplysia* [2].

The first study [1] examined habituation of olfactory avoidance in *Drosophila* [3]. Flies, like most animals, tend to avoid odors they find aversive. But, when given prior exposure to a moderately aversive odor, flies will habituate to it. This learning can be quantified by giving the flies a forced choice between two arms of a Y-maze, one arm that contains the training odor and one that contains air; flies previously given habituation training avoid the aversive odor less than do naïve flies.

By way of background, insect olfactory sensory neurons reside in the antennae; the axons of sensory neurons, each of which express only one (or a small number) of odorant receptor genes, project to glomeruli in the antennal lobe (homologous to the vertebrate olfactory bulb). Within the glomeruli the sensory axons synapse onto odor-specific projection neurons, as well as onto local multiglomerular inhibitory interneurons. The projection neurons relay information from the glomeruli to the mushroom bodies, which play an important role in olfactory associative memory [4].

Das *et al.* [1] found that four days of exposing flies to an aversive odor produced olfactory habituation that lasted several days. They also found that this long-term habituation depended on the strengthening of the synaptic connections between gamma-aminobutyric acid (GABA)ergic inhibitory interneurons and the projection neurons; and that the strengthening required cAMP signaling and the transcription of CREB within the interneurons specifically. But if the long-term habituation requires the activity of interneurons, the effects of which cross glomerular boundaries, how can odor specificity of habituation be maintained?

A key insight came from the discovery that the interneurons, besides releasing GABA, co-release glutamate. This, together with their additional finding that interneurons express *N*-methyl-D-aspartate (NMDA) receptors, led Das *et al.* [1] to conclude that prolonged exposure to the aversive odor leads to NMDA receptor-dependent long-term potentiation (LTP) of the interneuron-to-projection-neuron synapse; the LTP results from odor-induced depolarization of the projection neurons, via input from the olfactory sensory neurons (whose transmitter is believed to be acetylcholine [4]), coupled with glutamate release from the interneurons. Thus, although an odor stimulant causes release of glutamate onto projection neurons within several glomeruli, odor specificity of long-term habituation is achieved through potentiated inhibition only at interneuronal connections with projection neurons depolarized by the odor. (The authors further suggest that odor-induced LTP causes enhanced release of GABA from the interneurons via a retrograde signal, although the details of this part of the story, if correct, remain to be worked out.)

Strikingly, the plasticity-related molecules that are crucial for long-term olfactory habituation in fruit flies — cAMP, CREB, and NMDA receptors — are those prominently implicated in such disparate forms of learning as spatial learning in the Morris water maze [5–7] and fear conditioning [8–10]. But there is no conceivable way to understand how the joint activity of these molecules results in, for example, lessened avoidance of a funky odor by a fly, reduction in the time it takes to find a hidden platform in a tank of murky water by a mouse, or increased freezing by a rat to a tone that happened to precede an electrical shock, without a detailed understanding of the neural circuits that mediate each of these behaviors, as well as knowledge of the specific sites of learning-induced neural plasticity in each instance.

The second study [2] concerns a form of operant conditioning in *Aplysia*. Here, the authors examined the molecular basis of learning by the snails that food is inedible. It is difficult to convince an *Aplysia* that a morsel of seaweed cannot be ingested; but if the seaweed is presented in a plastic net to the animal, after repeated attempts to swallow the netted

seaweed the animal will eventually give up. Previous work by Michel and colleagues [11] had shown that this type of operant conditioning can exhibit short-term (lasting 30 min) and long-term (lasting 24 hours) forms, and that these two forms are mechanistically, as well as temporally distinct.

In their new study, Michel *et al.* [2] demonstrate a third, intermediate-term (lasting four to six hours) form of the gustatory learning, and mechanistically compare intermediate-term learning with the short- and long-term forms. The authors found that the memory for intermediate-term learning resembles long-term memory, and differs from short-term memory, in requiring protein synthesis; intermediate-term memory differs from long-term memory, however, in lacking a requirement for transcription.

These results resemble those previously reported for intermediate-term memory for behavioral sensitization of the defensive withdrawal reflex, a simpler, non-associative form of learning, in *Aplysia* [12–14]. Additionally, Michel *et al.* [2] found that the induction and maintenance of the operant learning depended on protein kinase C (PKC). Through the use of inhibitors differentially selective for the various isoforms of PKC, the authors identified PKM, the constitutively active fragment of PKC, as the critical isoform necessary for the induction and maintenance of the intermediate-term memory for learning that the netted seaweed is inedible. This finding is suggestive in light of evidence that PKM also underlies the persistence of memory in the mammalian brain [15–17]. Furthermore, maintenance of both the intermediate- and long-term memory for sensitization in *Aplysia* depends on PKM as well [18,19]. But, puzzlingly, Michel *et al.* [2] determined that the maintenance of the long-term memory for operant learning did not require PKM activity.

This finding is not unprecedented; it has also been reported that memories for some forms of mammalian learning do not appear to be maintained by PKM [20]. Moreover, it is possible that the apparent lack of an effect of PKM inhibition on the maintenance of the memory for operant conditioning of feeding in *Aplysia* resulted from some quirk of methodology, although Michel *et al.* [2] performed extensive control experiments to rule out this possibility. Taking these new results at face value, it is difficult to comprehend why the molecules underlying maintenance of the long-term memory for behavioral sensitization and those underlying maintenance of the long-term memory for operant conditioning of feeding should differ, particularly when the molecular bases of these two types of intermediate-term memory are otherwise quite similar.

The answer to this conundrum will require detailed information about specific, conditioning-induced changes within the neural circuits that are recruited during the learning in *Aplysia*, like the information that Das *et al.* [1] provided for olfactory habituation in *Drosophila*. The take-home lesson from the two studies [1,2] discussed here is that knowledge of the key molecular players does not provide a short cut to understanding memory and cognition; behavioral neuroscientists aiming toward this goal still face a long, hard slog.

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