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# Watching a memory form—VSD imaging reveals a novel memory mechanism

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**ABSTRACT**

Studies of the mechanisms underlying memory formation have largely focused on the synapse. However, recent evidence suggests that additional, non-synaptic, mechanisms also play important roles in this process. We recently described a novel memory mechanism whereby a particular class of neurons was recruited into the *Tritonia* escape swim network with sensitization, a non-associative form of learning. Neurons that in the naïve state were loosely-affiliated with the network were rapidly recruited in, transitioning from variably bursting (VB) to reliably bursting (RB). Even after the memory had faded some new neurons remained, and some original members had left, leaving the network in an altered state. Further, we identified a candidate cellular mechanism underlying these network changes. Our study supports the view that brain networks may have surprisingly fluid functional structures and adds to the growing body of evidence that non-synaptic mechanisms often operate synergistically with changes at the synapse to mediate memory formation.

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Understanding the neural basis of memory formation is a major goal of Neuroscience. While the field has focused for decades on the synapse, non-synaptic mechanisms such as neuronal excitability also play a crucial role in memory formation.[1,2] A current frontier in research on the neurobiology of learning concerns how neurons are chosen to join memory traces, a process called neuronal allocation.[3] We recently reported a novel mechanism for this in the sea slug *Tritonia*, where we found that certain neurons have characteristics that appear to predispose them for recruitment into the short term memory trace for sensitization of the animal's escape swim behavior.[4] These variably bursting neurons (VBs) have the interesting property of being loosely-affiliated with the swim network in the naïve state, where they participate in some but not all bursts of the rhythmic motor program, essentially switching in and out of the active network as it runs.[5] As the sensitization memory forms, many of these neurons begin bursting on every cycle, increasing the pool of reliably bursting neurons (RBs). This finding supports the perspective that networks may be more fluid in their functional structure than traditionally envisaged,[6] involving a core of reliably participating neurons and an associated group of loosely-coupled but context-appropriate neurons that can be rapidly recruited as needed, such as to participate in memory traces.

In our study we used a fast voltage-sensitive dye that allowed us to simultaneously record all action potentials in dozens of neurons in the animal's pedal ganglion, enabling us to monitor network activity as the sensitization memory rose with training and then dissipated with rest. Learning was monitored as a reduction in swim motor program onset latency across trials produced by motor program-eliciting nerve stimuli delivered at 2 minute intervals. Our initial result was that sensitization acted to rapidly expand the population of RBs: across preparations the second trial showed a 23%, and the third a 38% increase in the number of RBs over the first, naïve trial. Additionally, we found that neurons fired in a more correlated manner with sensitization, a factor that may also contribute to the enhanced escape swim behavior that we observed in the sensitized state.

Tracking neuronal commitment to the motor program across trials revealed that while with learning most VB neurons became more reliable participants, a smaller number typically moved in the opposite direction, shifting from RB to VB status. Interestingly, in control preparations a similar number of neurons moved into and out of the RB network, resulting in no net change in its size. Thus, the learning-related changes we observed were superimposed on a background of network fluidity. The

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trial-to-trial variation in the composition of the network in the absence of learning indicates that each instance of the motor program involves a shuffling of participants, such that a somewhat different group of neurons generates the motor program on each trial, a feature that has recently been noted in vertebrate motor, cognitive and sensory networks as well.[7-9]

Our study also tracked neuronal participation as sensitization faded. Unexpectedly, memory dissipation didn't involve all neurons that joined the RB group retreating back to their former variably-committed or non-bursting status. Instead, more than half of these new RBs remained behind as the active network returned to its naïve size, suggesting that vestiges of the sensitization memory might persist in the network long after evidence of learning, the reduction in motor program onset latency, has disappeared.

For several decades, research on the cellular mechanisms of memory storage has focused almost exclusively on synaptic plasticity. However, recent studies have established that certain properties of the neurons themselves can play an important role in determining who's in and who's out of the memory trace among the neurons activated by the training stimuli.[3] A recent Science perspectives piece on our Current Biology paper stated that our novel memory mechanism may be "simply too simple," apparently under the impression that we were suggesting this mechanism should replace synaptic plasticity as the major mechanism mediating memory formation.[10] To the contrary, we posed it as a complementary mechanism – supporting the view that learning likely involves a mix of synaptic and non-synaptic mechanisms that work in concert to create the memory trace. We further offered a possible mechanism driving the sensitization memory in *Tritonia*, prompted by previous studies showing that the serotonergic dorsal swim interneurons (DSIs) enhance both the synaptic strength and excitability of other swim network neurons.[11-13] In the present study we found that tonically driving just 2 DSIs in naïve preparations at the elevated resting rate they fire at during sensitization implants a false memory for sensitization (shortening of motor program onset latency). Furthermore, bath applying serotonin produces the same network changes seen in sensitization, including latency shortening and network expansion, mediated by the transition of variably- and non-bursting neurons to RB status in the swim motor program.

In summary, our finding represents a novel mechanism for neuronal allocation. Several studies have shown that neurons with elevated excitability just prior to training are more likely than their less active neighbors to be recruited into memory traces.[14,15] This has been shown to be associated with elevated levels of intracellular CREB, which is closely linked to activity level in neurons.[16,17] It makes sense that neurons more active around the time of training would be enlisted to participate in encoding context features of the experience. Our mechanism is different, being based not on the recent experience-dependent firing history of neurons, but on their being variably-coupled to the network in the resting state, regardless of recent experience. We argue here that this mechanism is well-suited to provide a pool of pre-positioned and context-appropriate participants for rapid recruitment into memories. Since neurons are likely typically loosely-coupled to multiple networks, a better understanding of how they can be nudged to commit more strongly to one network versus another could provide novel insights for improving function as networks break down with injury or aging.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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