just as important to salt-induced hypertension as the sodium sensor itself.

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# Suppressing Memories by Shrinking the Vesicle Pool

**Ethan B. Richman[1,2,3,*] and Liqun Luo[2,4]**
[1]Neurosciences Graduate Program, Stanford University, Stanford, CA 94305, USA
[2]Department of Biology, Stanford University, Stanford, CA 94305, USA
[3]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA
[4]Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA
*Correspondence: richman@stanford.edu
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**The cohesin complex regulates cellular functions spanning cell division and neuronal morphogenesis. Now, Phan et al. uncover a role for the cohesin complex in regulating memory acquisition and the size of the synaptic and dense-core vesicle pool.**

A powerful way to dissect the complexity of animal behavior is to isolate specific genetic, molecular, or cellular processes that control it. In this reductionist program, a researcher finds that the manipulation of a single gene might alter an animal's behavior. Then, she chases down the resulting molecular, cellular, and circuit changes caused by the manipulation to gain mechanistic understanding. This process often begins with developing a highly robust behavior of interest and examining one-by-one the effects of disrupting many different genes: a behavioral genetic screen. Due to unbiased choice of genes, behavioral functions may be found for the unlikeliest of genes. In this issue of *Neuron*, new work (Phan et al., 2018) uncovers the unexpected role of a component of the cohesin complex, best known for holding together sister chromatids prior to cell division, in regulating synaptic vesicle pool size and the acquisition of memories.

The fruit fly *Drosophila melanogaster* is a popular model organism for behavioral genetic screens due to its rapid reproductive cycle, hefty genetic toolkit, and relatively complex behavioral repertoire. Notably, *Drosophila* will acquire and express olfactory memories, in which a neutral odor (the conditioned stimulus) becomes appetitive or aversive following pairing during training with a reward or punishment (the unconditioned stimulus). These memories can persist for hours to days after they are acquired. By examining enhancements or suppressions in this memory assay, researchers have been highly successful in identifying the mechanisms underlying memory formation, consolidation, and retrieval (Tomchik and Davis, 2013).

In *Drosophila*, odors are detected by olfactory receptor neurons. These sensory neurons send olfactory information to the antennal lobe projection neurons, which subsequently relay to the mushroom body (Vosshall and Stocker, 2007). The intrinsic mushroom body (MB) neurons (also known as Kenyon cells) combine the olfactory information with unconditioned stimulus information delivered onto the mushroom body by dopaminergic (DA) neurons (Cognigni et al., 2018). A previous screen by the Davis

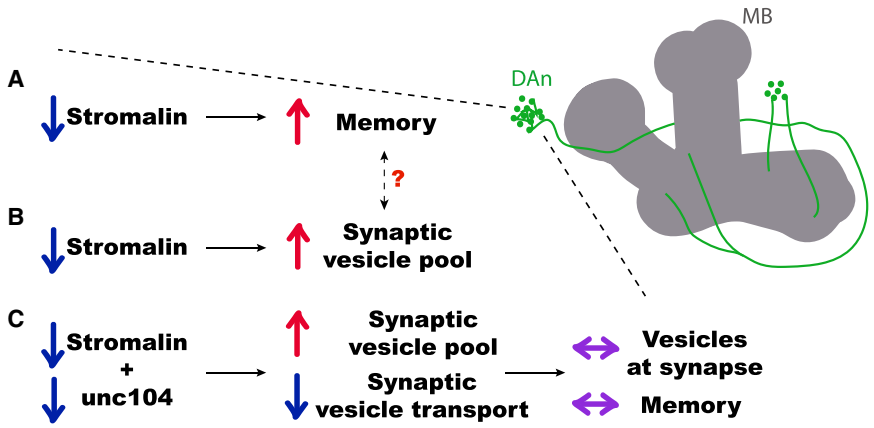


**Figure 1. Genetic Dissection of a Stromalin Role in Memory Suppression**
(A) Knockdown of Stromalin through the expression of RNAi in dopaminergic neurons (DAn) that innervate the mushroom body (MB) enhances olfactory memory.
(B) Stromalin knockdown leads to an increase in vesicle pool size, independent of apparent changes in neuronal morphology. Is this regulation of vesicle pool size responsible for the observed memory enhancement phenotype?
(C) Knockdown of *unc104*, a kinesin gene responsible for anterograde synaptic vesicle transport, cancels out the effects of a Stromalin knockdown, neutralizing the memory phenotype and suggesting that Stromalin regulates memory by setting vesicle pool size.

Lab, examining thousands of genes and fly lines, identified dozens of novel candidate genes whose knockdown by RNAi enhanced memory acquisition in a three-hour memory assay (Walkinshaw et al., 2015). One of these candidates was Stromalin, a member of the cohesin complex.

The cohesin complex is composed of Stromalin, SMC1, and SMC3 and forms a ring to hold sister chromatids together until the end of mitosis and is thus required during cell division for the proper separation of chromosomes (Nasmyth and Haering, 2005). The cohesin complex was subsequently identified in a genetic screen to have a post-mitotic function in the developmental axon pruning of MB neuron axons by regulating gene expression (Pauli et al., 2008; Schuldiner et al., 2008). In a careful series of mechanistic experiments spanning molecular, cellular, and circuit levels of analysis, Phan et al. (2018) suggest that Stromalin suppresses olfactory memory acquisition by specifically reducing the number of synaptic and dense-core vesicles. Gross changes in the size of the synaptic vesicle pool may parallel the effects on synaptic transmission and plasticity caused by changing synaptic vesicle availability (Alabi and Tsien, 2012). Intriguingly, Phan et al. did not find that the reduction of Stromalin affected other neuronal processes or morphology.

The initial screen that identified Stromalin as a memory suppressor gene reduced the expression of Stromalin by pan-neuronal expression of RNAi. Because DA and MB neurons are critical players in memory formation, Phan et al. examined the expression of Stromalin RNAi to these groups of cells, finding that disruption of Stromalin in each of these classes of cells was sufficient for generating the memory enhancement phenotype (Figure 1A). Therefore, the authors focused on DA and MB neurons to elucidate the mechanism by which Stromalin knockdown enhances olfactory memory. A key question is whether this reflects an extra function of Stromalin outside the cohesin complex or Stromalin acting as part of the cohesin complex to regulate memory. To determine this, the authors expressed RNAi against cohesin complex subunit SMC1, and found a similar memory enhancement phenotype to Stromalin knockdown, implicating the cohesin complex in the regulation of memory.

The beauty of a behavioral genetic screen is the firm handhold it provides into the molecular and cellular processes at work in complex behavior. Once Phan et al. had isolated the cellular players to DA and MB neurons, they began to test hypotheses regarding which cellular functions may be modulated in Stromalin knockdown animals. Since DA neurons provide critical valance information to MB neurons during the acquisition of memory, the authors questioned whether the communication between these neurons could be affected. In a series of technically challenging experiments, the authors found that Stromalin knockdown in DA neurons increased the transmission efficacy between DA and MB neurons *ex vivo*, and that DA neuron Stromalin knockdown inhibited the transmission and subsequent consolidation of valence information in MB neurons *in vivo*. What mechanism is responsible for this increased communication of the unconditioned stimulus information? Since previous work has shown that a complete deletion of Stromalin during development results in axon pruning defects of MB neurons (Schuldiner et al., 2008), the authors looked for neuroanatomical defects with Stromalin knocked down but failed to detect any changes in morphology, innervation of the MB lobes, or number of synapses or presynaptic sites. This is likely a difference between knockdown (current study) and more complete loss of function in a previous study (Schuldiner et al., 2008).

A useful tool in *Drosophila* neuroscience to label presynaptic terminals is Synaptotagmin:GFP (Syt:GFP), which is localized to synaptic vesicles. In examining Syt:GFP signals in DA neuron Stromalin knockdown animals, the authors found an increased synaptic fluorescence signal compared to controls. Intriguingly, this increase in synaptic fluorescent labeling was also found using a pan-neuronal driver, indicating that the cellular effect of Stromalin knockdown generalizes beyond DA neurons. Because the authors could detect no morphological or connectivity differences between knockdown and control animals, they hypothesized that Stromalin knockdown animals have increased numbers of synaptic vesicles. Using electron microscopy to specifically examine DA neurons with Stromalin knocked down, the authors found that synaptic and dense-core vesicle numbers were more than doubled without any change in their proportion (Figure 1B).

While the authors observed that Stromalin knockdown both enhanced memory acquisition and increased the vesicle pool size, the two phenomena could be independent of one another. Admirably, the authors substantially strengthened

the connection between synaptic vesicle increases in DA neurons and the memory enhancement phenotype in Stromalin knockdown flies by using an independent method of regulating synaptic and dense–core vesicles. The motor protein gene *unc104* is responsible for trafficking synaptic and dense-core vesicles to the axon terminals. By knocking down *unc104*, Phan et al. were able to reduce olfactory memory acquisition. The authors hypothesized that the reduced trafficking of synaptic (DA-containing) vesicles to the axon terminals might account for the memory suppression. Critically, when combining both the knockdown of *unc104* and Stromalin in DA neurons, the authors were able to counterbalance the vesicle pool size and revert the memory enhancement effects of Stromalin knockdown alone (Figure 1C). Taken together, these experiments lend support to the proposal that Stromalin suppresses memory by reducing the size of the synaptic vesicle pool, reducing the transmission of unconditioned stimulus information between DA neurons and MB neurons.

Finally, an intriguing finding by the authors somewhat complicates the interpretation of these results. By temporally restricting the expression of Stromalin RNAi to different time points from development to adulthood, the authors found a critical period for Stromalin action during development, specifically in the third-instar larval stage. Expression of Stromalin RNAi during adulthood had no detectable memory phenotype. The authors speculate that Stromalin may regulate gene expression during the larval stage, creating a genetic environment in the cell that persists into adulthood and regulates the synaptic vesicle pool size.

Future work will be required to establish what molecular processes connect changes in developmental Stromalin levels to changes in adult synaptic vesicle pool size. Most likely, an early component of this pathway is transcriptional, perhaps by directing interaction of the cohesin complex and genetic regulatory elements (Misulovin et al., 2008). While the authors' results strongly suggest that the Stromalin knockdown-induced memory enhancement phenotype was caused by an increase in the synaptic and dense-core vesicle pool size, it remains possible that some other not-yet-examined consequence of Stromalin disruption contributes to the behavioral effect.

The authors' experiments in this study were focused on a memory behavior and the neuron types responsible for it. As such, it is difficult to assess whether Stromalin has a specific role in memory acquisition, or whether the observed behavioral phenotype is simply a consequence of manipulations focused on the memory circuit. Given its pleiotropic functions that span cell division and morphogenesis in post-mitotic cells, the latter is more likely to be the case. Nonetheless, the results suggest that synaptic vesicle pool size is a critical parameter in the transmission of the unconditioned stimulus.

The findings of Phan et al. raise several exciting questions. How dynamic is this pathway for regulating vesicle pool size? While Stromalin is shown here to be involved during a limited developmental window, it is possible that yet-to-be-identified downstream components of this pathway may be regulated dynamically in the adult. What influence does the environment of the developing *Drosophila* have on its Stromalin levels and subsequent memory acquisition capacity? And, finally, what other circuit properties or behaviors require the precise regulation of synaptic vesicle pool size, and how can the criticality of this neuronal property help us to understand the cellular, circuit, and behavioral functions it regulates?

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