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# Stuck in Time Without a Nucleus: Theoretical Comment on Sangha et al. (2005)

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Is forgetting caused by the passage of time or by interference from new learning? S. Sangha et al. (2005) offer strong support for the latter idea by using the sea snail *Lymnaea*. Memory for inhibitory avoidance was prolonged from 3 days to 7 days by preventing the snails from making unreinforced conditioned responses (extinction) following training. Similar effects were obtained with posttraining ablation of the soma of the right pedal dorsal 1, the neuron necessary for consolidation, reconsolidation, and extinction in this task. Without the soma, *Lymnaea* was unable to retain any new learning or forget old learning, hence remaining "stuck in time." These findings elegantly demonstrate that transcriptional regulation of gene expression is essential for memory consolidation: Local protein synthesis is not sufficient. Furthermore, memory for conditioning and extinction can coexist in the same neuron.

*Keywords:* extinction, anisomycin, forgetting, transcription, translation

What is the nature of forgetting? Do memories passively decay with time, or are they actively replaced by new learning? These questions date back to early studies examining the effect of posttraining quiescence on memory retention in insects and fish (Minami & Dallenbach, 1946). However, because of disagreements between experiments involving proactive versus retroactive interference, no agreement was reached (Wixted, 2004). In the previous issue of *Behavioral Neuroscience*, Lukowiak and collaborators (Sangha et al., 2005) offer strong support for the role of new learning in forgetting, using the sea snail *Lymnaea*.

In order to breathe, *Lymnaea* can either extract oxygen from the water while submerged or float to the surface and open a breathing tube. Tapping the tube gently with a stick each time it is opened teaches the snail to refrain from aerial respiration. Prior work by Lukowiak and colleagues (Sangha et al. 2005) has shown that long-term retention of this avoidance learning is prevented by blockers of gene transcription, or translation, and requires the soma of a single neuron (right pedal dorsal 1; RPeD1), suggesting that this neuron is the essential site of plasticity (Sangha et al., 2004). Two conditioning sessions produce a memory lasting 2 days, but not 3 days. To determine whether forgetting depended on new learning, Sangha et al. (2005) examined the effect of submerging the snails under a barrier for 7 days following training. This prevented the snails from spontaneously surfacing and making unreinforced tube openings, which would extinguish the conditioned response. Previous work has shown that extinction is itself a form of learning that does not erase the original memory (Delamater, 2004; Quirk, 2002; Rescorla, 2004). Sangha et al. (2005) reasoned that this new learning may interfere with the original learning. In fact, they observed that snails that were prevented from surfacing showed excellent retention of the memory after 7 days, in support of the idea that new learning (in this case extinction) rather than the passage of time was responsible for the forgetting.

If new learning is responsible for forgetting, then blocking consolidation of that new learning should prevent forgetting. To test this, Sangha et al. (2005) exploited their invertebrate preparation in two ways. First, they observed that cooling the snails during the 7-day retention interval also prevented forgetting. Second, ablating the soma of the RPeD1 neuron 1 hr after training also prevented forgetting across the 7-day interval. Thus, they concluded that consolidation processes involving the nucleus are necessary for normal forgetting. However, these findings could also be interpreted as blockade of extinction learning because somatic ablation, or cooling, has been shown to block extinction in this system (Sangha, Scheibenstock, Morrow, & Lukowiak, 2003) and the snails were free to breathe aerially while in the home aquarium. One way to test whether the original learning was still intact would be to give several reinstating-conditioning trials after forgetting in an attempt to uncover the original learning.

Most interesting was the observation that snails without the RPeD1 soma were unable to retain any new learning or forget the old learning. When conditioned in a new context, RPeD1 soma-ablated snails learned but could not retain the memory for 24 hrs. The loss of the conditioned response at this time point cannot be attributed to expression of extinction because ablated snails are unable to consolidate extinction; it therefore represents true forgetting of the new training. When tested after an additional 7 days, the snails continued to show memory for the original preablation training given 15 days earlier. Thus, the ablated snails appeared to be "stuck in time," unable to learn new memories and unable to forget old ones. This is reminiscent of the famous neurologic patient H.M. who underwent a temporal lobectomy for intractable seizures (Scoville & Milner, 1957). To this day, H.M. is unable to retain any appreciable amount of declarative knowledge of events occurring after his surgery, yet his recall of premorbid declarative

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information is as good as controls, or sometimes better (Corkin, 2002). Of course the involvement of the hippocampus and related structures in consolidation of declarative knowledge is a systems-level process that is much more complex than nondeclarative associative learning in *Lymnaea*. However, the similarity in lesion deficits underscores the importance of the neuronal nucleus in consolidating new learning and updating existing memories.

More important, the findings with *Lymnaea* support findings from multiple species from flies to rodents showing that transcriptional regulation of gene expression is essential for memory consolidation (Bailey, Kim, Sun, Thompson, & Helmstetter, 1999; Calixto, Thiels, Klann, & Barrionuevo, 2003; Kida et al., 2002; Ohi, 1977; Pedreira, Dimant, & Maldonado, 1996; Squire & Barondes, 1970; Wustenberg, Gerber, & Menzel, 1998). There is additional interest in mechanisms either upstream of transcriptional regulation, such as DNA recombination (Peña de Ortiz & Arshavsky, 2001; Peña de Ortiz, Colon, Carrasquillo, Padilla, & Arshavsky, 2003; Wang, Ren, Perez, Silva, & Peña de Ortiz, 2003), or downstream of gene transcription, such as the regulation of mRNAs accumulated at specific synapses for immediate or delayed translation following learning (Perrone-Bizzozero & Bolognani, 2002; Schacher & Wu, 2002; Si et al., 2003).

The soma ablation procedure used by Sangha et al. (2005) leaves behind a functional neurite in which local protein synthesis can still occur. This means that local protein synthesis is not sufficient for consolidation of learning, extinction, or reconsolidation in this system. Thus, whereas short-term memory can occur in the absence of the nucleus, gene expression is essential for the formation of long-lasting memory. This raises another interesting question: How could memories formed prior to the ablation be maintained for up to 15 days in the absence of the nucleus? Most proteins would be expected to turn over within that time. The answer may lie in the fact that ablation of the RPeD1 soma occurred 1 hr after the end of training, which is sufficient time to induce the expression of immediate-early genes coding for proteins with synaptic function similar to *arc* (Lyford et al., 1995; Ramirez-Amaya et al., 2005) and *14–3–3 eta* (Kida et al., 2002). There could also be local translation of preexisting mRNAs accumulated in the remaining neurite. The issue of mRNA stability as a mechanism of plasticity in *Lymnae* warrants further study. For example, could the synaptic terminals (Giustetto et al., 2003) or dendrites (Steward & Worley, 2002) contain mRNAs with differing stabilities that would enable protein synthesis at different times after training?

This is not the first demonstration that extinction of conditioned avoidance requires the molecular machinery of the nucleus. In rats, extinction of inhibitory avoidance is blocked by transcription and translation blockers infused into the hippocampus (Vianna, Igaz, Coitinho, Medina, & Izquierdo, 2003; Vianna, Szapiro, McGaugh, Medina, & Izquierdo, 2001). Similarly, memory for extinction of cued fear conditioning is prevented by inhibitors of molecular cascades infused into the amygdala (Lin, Yeh, Lu, & Gean, 2003; Lu, Walker, & Davis, 2001; Walker & Davis, 2002) or the medial prefrontal cortex (Burgos-Robles, Santini, & Quirk, 2004; Hugues, Deschaux, & Garcia, 2004; Santini, Ge, Ren, Pena, & Quirk, 2004). Furthermore, extinction training activates the immediate-early transcription factor Fos in the medial prefrontal cortex (Herry & Mons, 2004; Santini et al., 2004). The dependence of extinction on molecular cascades suggests that extinction, like acquisition, involves synaptic potentiation (Milad & Quirk, 2002).

If extinction is not unlearning, then how do memories for conditioning and extinction coexist in the brain? The findings of Sangha et al. (2005) demonstrate that conditioning and extinction can coexist in a single neuron. This situation may reflect a necessary economy of space in a simple nervous system, but it is interesting to consider, given that the amygdala is necessary for both fear conditioning and extinction (Myers & Davis, 2002). The *Lymnaea* findings suggest that a single neuron possesses the capacity of accessing diverse forms of memory at different synapses, each expressing a specific repertoire of proteins. The specificity of gene expression at discrete synapses could depend on the selective trafficking of proteins and mRNAs to distinct synaptic targets in the cell. Such mechanisms for learning and memory have been proposed (Irwin, Baekelandt, Goritchenko, & Benowitz, 1997; Lyford et al., 1995; Steward & Worley, 2002) but remain to be demonstrated experimentally. Simple invertebrate systems, such as the sea snail *Lymnaea*, may hold the key to understanding these and many other questions related to the mechanisms of memory consolidation.

## References


Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mRNA in the amygdala. *Behavioral Neuroscience, 113,* 276–282.

Burgos-Robles, A., Santini, E., & Quirk, G. J. (2004). Blockade of NMDA receptors in the medial prefrontal cortex impairs consolidation of fear extinction. *Society for Neuroscience Abstracts, 328*.

Calixto, E., Thiels, E., Klann, E., & Barrionuevo, G. (2003). Early maintenance of hippocampal mossy fiber–long-term potentiation depends on protein and RNA synthesis and presynaptic granule cell integrity. *Journal of Neuroscience, 23,* 4842–4849.

Corkin, S. (2002). What's new with the amnesic patient H. M.? *Nature Reviews Neuroscience, 3,* 153–160.

Delamater, A. R. (2004). Experimental extinction in Pavlovian conditioning: Behavioural and neuroscience perspectives. *Quarterly Journal of Experimental Psychology: Journal of Comparative and Physiological Psychology, 57*(B), 97–132.

Giustetto, M., Hegde, A. N., Si, K., Casadio, A., Inokuchi, K., Pei, W., et al. (2003). Axonal transport of eukaryotic translation elongation factor 1alpha mRNA couples transcription in the nucleus to long-term facilitation at the synapse. *Proceedings of the National Academy of Sciences, USA, 100,* 13680–13685.

Herry, C., & Mons, N. (2004). Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. *European Journal of Neuroscience, 20,* 781–790.

Hugues, S., Deschaux, O., & Garcia, R. (2004). Postextinction infusion of a mitogen-activated protein kinase inhibitor into the medial prefrontal cortex impairs memory of the extinction of conditioned fear. *Learning & Memory, 11,* 540–543.

Irwin, N., Baekelandt, V., Goritchenko, L., & Benowitz, L. I. (1997). Identification of two proteins that bind to a pyrimidine-rich sequence in the 3′-untranslated region of GAP-43 mRNA. *Nucleic Acids Research, 25,* 1281–1288.

Kida, S., Josselyn, S. A., de Ortiz, S. P., Kogan, J. H., Chevere, I., Masushige, S., et al. (2002). CREB required for the stability of new and reactivated fear memories. *Nature Neuroscience, 5,* 348–355.

Lin, C. H., Yeh, S. H., Lu, H. Y., & Gean, P. W. (2003). The similarities and diversities of signal pathways leading to consolidation of conditioning and consolidation of extinction of fear memory. *Journal of Neuroscience, 23,* 8310–8317.

Lu, K. T., Walker, D. L., & Davis, M. (2001). Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *Journal of Neuroscience, 21,* RC162.

Lyford, G. L., Yamagata, K., Kaufmann, W. E., Barnes, C. A., Sanders, L. K., Copeland, N. G., et al. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron, 14,* 433–445.

Milad, M. R., & Quirk, G. J. (2002, November). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature, 420,* 70–74.

Minami, M., & Dallenbach, K. M. (1946). The effect of activity upon learning and retention in the cockroach. *American Journal of Psychology, 59,* 1–58.

Myers, K. M., & Davis, M. (2002). Behavioral and neural analysis of extinction. *Neuron, 36,* 567–584.

Ohi, S. (1977). Effects of actinomycin D on brain RNA synthesis and discrimination learning in the goldfish (*Carassius auratus*). *Physiology & Behavior, 19,* 261–264.

Pedreira, M. E., Dimant, B., & Maldonado, H. (1996). Inhibitors of protein and RNA synthesis block context memory and long-term habituation in the crab *Chasmagnathus. Pharmacology Biochemistry and Behavior, 54,* 611–617.

Peña de Ortiz, S., & Arshavsky, Y. (2001). DNA recombination as a possible mechanism in declarative memory: A hypothesis. *Journal of Neuroscience Research, 63,* 72–81.

Peña, de Ortiz, S., Colon, M., Carrasquillo, Y., Padilla, B., & Arshavsky, Y. I. (2003). Experience-dependent expression of terminal deoxynucleotidyl transferase in mouse brain. *NeuroReport, 14,* 1141–1144.

Perrone-Bizzozero, N., & Bolognani, F. (2002). Role of HuD and other RNA-binding proteins in neural development and plasticity. *Journal of Neuroscience Research, 68,* 121–126.

Quirk, G. J. (2002). Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. *Learning & Memory, 9,* 402–407.

Ramirez-Amaya, V., Vazdarjanova, A., Mikhael, D., Rosi, S., Worley, P. F., & Barnes, C. A. (2005). Spatial exploration-induced arc mRNA and protein expression: Evidence for selective, network-specific reactivation. *Journal of Neuroscience, 25,* 1761–1768.

Rescorla, R. A. (2004). Spontaneous recovery. *Learning & Memory, 11,* 501–509.

Sangha, S., Scheibenstock, A., Marten, K., Varshney, N., Cooke, R., & Lukowiak, K. (2005). Impairing forgetting by preventing new learning and memory. *Behavioral Neuroscience, 119,* 787–796.

Sangha, S., Scheibenstock, A., Morrow, R., & Lukowiak, K. (2003). Extinction requires new RNA and protein synthesis and the soma of the cell right pedal dorsal 1 in Lymnaea stagnalis. *Journal of Neuroscience, 23,* 9842–9851.

Sangha, S., Varshney, N., Fras, M., Smyth, K., Rosenegger, D., Parvez, K., et al. (2004). Memory, reconsolidation and extinction in *Lymnaea* require the soma of RPeD1. *Advances in Experimental Medicine and Biology, 551,* 311–318.

Santini, E., Ge, H., Ren, K., Pena, D. O., & Quirk, G. J. (2004). Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. *Journal of Neuroscience, 24,* 5704–5710.

Schacher, S., & Wu, F. (2002). Synapse formation in the absence of cell bodies requires protein synthesis. *Journal of Neuroscience, 22,* 1831–1839.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry, 20,* 11–21.

Si, K., Giustetto, M., Etkin, A., Hsu, R., Janisiewicz, A. M., Miniaci, M. C., et al. (2003). A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in aplysia. *Cell, 115,* 893–904.

Squire, L. R., & Barondes, S. H. (1970, February 14). Actinomycin-D: Effects on memory at different times after training. *Nature, 225,* 649–650.

Steward, O., & Worley, P. (2002). Local synthesis of proteins at synaptic sites on dendrites: Role in synaptic plasticity and memory consolidation? *Neurobiology of Learning and Memory, 78,* 508–527.

Vianna, M. R., Igaz, L. M., Coitinho, A. S., Medina, J. H., & Izquierdo, I. (2003). Memory extinction requires gene expression in rat hippocampus. *Neurobiology of Learning and Memory, 79,* 199–203.

Vianna, M. R., Szapiro, G., McGaugh, J. L., Medina, J. H., & Izquierdo, I. (2001). Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. *Proceedings of the National Academy of Sciences, USA, 98,* 12251–12254.

Walker, D. L., & Davis, M. (2002). The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacology Biochemistry and Behavior, 71,* 379–392.

Wang, J., Ren, K., Perez, J., Silva, A. J., & Pena de Ortiz, S. (2003). The antimetabolite ara-CTP blocks long-term memory of conditioned taste aversion. *Learning & Memory, 10,* 503–509.

Wixted, J. T. (2004). The psychology and neuroscience of forgetting. *Annual Review of Psychology, 55,* 235–269.

Wustenberg, D., Gerber, B., & Menzel, R. (1998). Short communication: Long- but not medium-term retention of olfactory memories in honeybees is impaired by actinomycin D and anisomycin. *European Journal of Neuroscience, 10,* 2742–2745.

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