Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

How Do Microtubule Dynamics Relate to the Hallmarks of Learning and Memory?

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Review of Martel et al.

It is well known that the hippocampus plays an important role in learning and memory. In the fear conditioning procedure, whereas the amygdala is central to tone–shock association, the hippocampus is critical for contextual associations (Calandreau et al., 2005; Trifilieff et al., 2006). In particular, the dentate gyrus is critical for contextual memory formation and retrieval (Drew et al., 2013; Clemenson et al., 2015). Numerous molecular and cellular mechanisms underlie the role of the dentate gyrus in learning and memory. These include NMDA receptor activation, spinogenesis, and neurogenesis (Tsien et al., 1996; Hamilton et al., 2012; Drew et al., 2013; Kempermann et al., 2015). Notably, these processes may be linked: NMDA receptor activation in neural progenitor cells promotes neurogenesis (Deisseroth et al., 2004), and the survival of these newly generated neurons is competitively regulated by their levels of NMDA receptor activation as compared with the surrounding mature neurons. (Tashiro et al., 2006). Moreover, NMDA activation and the downstream activation of the transcription factor CREB contribute to maturation and

spinogenesis in newborn neurons (Silva et al., 1998; Vadodaria and Gage, 2014).

Microtubules play an essential role in many cellular processes, including transporting mRNA, organelles, and proteins (including NMDA receptors), and thus it is not surprising that proteins that regulate microtubule dynamics play critical roles in spine plasticity, neurogenesis, and memory formation. One such protein is a cytosolic phosphoprotein, stathmin, whose unphosphorylated form binds to tubulin heterodimers and prevents their polymerization. (Belmont and Mitchison, 1996; Shumyatsky et al., 2005; Uchida et al., 2014). When phosphorylated, stathmin releases tubulin, allowing microtubule formation (Belmont and Mitchison, 1996). Consistent with a role in memory, stathmin is expressed in neurons in the cortex, hippocampus, and amygdala, with little expression in glia (Shumyatsky et al., 2005). Mice lacking stathmin exhibit impaired long-term potentiation (LTP) at cortico- and thalamo-amygdala synapses, as well as impaired amygdala-dependent fear memory (Shumyatsky et al., 2005). However, these *stathmin* knock-out mice show normal hippocampus-dependent spatial memory, possibly because of low expression of endogenous stathmin in hippocampus (Shumyatsky et al., 2005). In addition, a nonphosphorylatable form of stathmin, *Stat4A*, (Kuntziger et al., 2001), has a very high affinity for tubulin heterodimers and destabilizes microtubules. Given the LTP impairments of stathmin knock-out mice, it is interesting that *Stat4A* knock-in mice ex-

hibit normal amygdala-dependent fear memory but have impaired hippocampus-dependent LTP and memory formation. The latter impairment involves reduced synaptic targeting of GluA2 after learning (Uchida et al., 2014).

In a recent article in *The Journal of Neuroscience*, Martel et al. (2016) proposed a mechanism that links NMDA receptor subunit trafficking, neurogenesis, spinogenesis, and specific aspects of learning to microtubule dynamics. The authors used an inducible bitransgenic mouse in which *Stat4A* was expressed only in the absence of the antibiotic doxycycline (Kuntziger et al., 2001; Uchida et al., 2014). Importantly, *Stat4A* expression was restricted to neurons in the dentate gyrus, lateral amygdala, and anterior cingulate cortex (Martel et al., 2016, their Fig. 2). The authors focused on effects in the dentate gyrus because it is a major site of adult neurogenesis (Kempermann et al., 2015).

Critically, Martel et al. (2016) demonstrated that *Stat4A*-expressing and control dentate gyrus had equivalent numbers of DCX+ neurons, neurons that presumably have the ability to proliferate (Martel et al., 2016, their Fig. 4B). However, using BrdU staining to label actively dividing neurons, the authors found that proliferation into the subgranular zone, survival in dentate gyrus, and dendritic complexity of immature (DCX+) neurons were reduced in *Stat4A*-expressing mice (Martel et al., 2016, their Figs. 3D,E, 4C,D). These data support the idea that the decrease in neurogenesis was not likely a result of depleted reserves of

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Figure 1. A summary of Martel et al. (2016) findings published in *The Journal of Neuroscience*. Mice that overexpress a constitutively active form of stathmin, *Stat4A*, exhibit changes from the molecular to behavioral levels. *Stat4A* expression destabilizes microtubules, therefore reducing synaptic targeting of NMDA receptors, spinogenesis, and neurogenesis. These mice also exhibit learning and memory impairments in contextual fear conditioning, NMDA-dependent memory tasks, and memory discrimination tasks. WT, Wild-type mouse; abDGC, adult born dentate granule cells.

neurons with the ability to proliferate but rather a decrease in the ability of these neurons to survive, proliferate, and mature.

Given that NMDA receptor signaling modulates aspects of neurogenesis (Deisseroth et al., 2004; Tashiro et al., 2006), Martel et al. (2016) tested whether *Stat4A* knock-in alters the composition of NMDA receptor subtypes. Compared with controls, *Stat4A*-expressing mice exhibited decreased GluN2A and GluN2B expression in the microtubule and synaptosomal fractions but no differences in whole-cell extracts, consistent with a role of microtubule dynamics in regulating NMDA receptor subunit transport to synaptic sites, but not in regulating subunit expression. Finally, the authors showed that *Stat4A* decreased CREB-dependent gene transcription. Importantly, doxycycline administration, effectively suppressing *Stat4A* transgene expression, in a separate group of mice reversed all of the alterations mentioned above.

Martel et al. (2016) verified that the molecular and cellular phenotypes produced by *Stat4A* expression were behaviorally relevant by demonstrating deficits in hippocampus-, NMDA-, and neurogenesis-dependent fear conditioning in *Stat4A*-expressing mice (Fig. 1). More specifically, these transgenic mice exhibited normal cued fear memory (amygdala-dependent) but impaired contextual fear memory (dorsal hippocampus and NMDA receptor-dependent). In addition, hippocampus-dependent, NMDA-independent memory

was spared in these mice (Hardt et al., 2009). However, the mice were unable to learn to discriminate two similar contexts: a deficit in pattern separation that critically depends on neurogenesis. Importantly, direct infusion of doxycycline into the dentate gyrus, but not into the lateral amygdala, rescued the impaired contextual discrimination (Martel et al., 2016, their Fig. 8).

One important consideration for interpreting these data are the nonphysiological role of *Stat4A*. Martel et al.'s (2016) *Stat4A* overexpression effectively reduced microtubule dynamics while leaving endogenous levels of stathmin unchanged. Therefore, the data reflect the role of microtubule dynamics in learning and memory rather than the role of stathmin *per se*. Under normal conditions, the four phosphorylation sites on stathmin are differentially regulated; for example, the level of stathmin phosphorylation increases on serine 16 and decreases on serines 25 and 28 after fear conditioning (Uchida et al., 2014). It would be interesting to explore the phosphorylation dynamics of stathmin during proliferation and survival of adult born dentate granule cells, dynamics that are surely regulated by a multitude of kinases and phosphatases in response to intracellular signals.

Martel et al.'s (2016) discoveries prompt further investigation into the molecules causally linking microtubule dynamics to learning and memory. In particular, Martel et al. (2016) show that

Stat4A knock-in leads to decreased synaptosomal GluN2A and GluN2B expression (without affecting the obligatory GluN1 subunit; Martel et al., 2016, their Fig. 10); however, it is known that GluN2B deletion in adult born dentate granule cells impairs contextual fear discrimination independent of neuronal proliferation and survival (Kheirbek et al., 2012). Therefore, *Stat4A* knock-in affects proliferation and survival through GluN2A or a mechanism independent of NMDA receptors, such as through cell-cycle arrest (Cassimeris, 2002). Furthermore, microtubule dynamics could regulate a multitude of downstream molecules. Rescue experiments of neurogenesis and of specific GluN2 subunits in adult born dentate granule cells in the same bitransgenic *Stat4A* mouse line used by Martel et al. (2016) could further elucidate the precise mechanisms by which *Stat4A* expression leads to the distinct cellular and learning phenotypes demonstrated by Martel et al. (2016).

The authors showed that *Stat4A* knock-in mice exhibit learning deficits associated with hippocampus rather than amygdala (Uchida et al., 2014; Martel et al., 2016). But global stathmin knock-out mice have impaired amygdala-associated memory with spared hippocampus-associated memory (Shumyatsky et al., 2005). These seemingly contradictory results may be due to differences between the gene expression profile of amygdala and hippocampus.

Hence, detailed analysis of brain-region-specific differences in protein expression and baseline kinase activity may supplement our understanding, complementing the importance of differential anatomical connectivity, of the mechanisms by which a given brain region (ie, amygdala, hippocampus, etc) specializes in a specific form of learning.

Martel et al. (2016) demonstrated a role for stathmin-mediated microtubule dynamics in GluN2A and GluN2B synaptic expression, neurogenesis, spinogenesis, and specific forms of fear conditioning (Fig. 1). Critically, suppressing the *Stat4A* transgene in the dorsal hippocampus rescues fear conditioning deficits. Moreover, the use of behavioral procedures to support the hippocampal, NMDA, and neurogenesis dependent nature of the learning deficits, and therefore molecular manipulation, can serve as a model for future phenotypical characterizations.

References

- Belmont LD, Mitchison TJ (1996) Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. *Cell* 84:623–631. [CrossRef Medline](#)
- Calandreau L, Desmedt A, Decorte L, Jaffard R (2005) A different recruitment of the lateral and basolateral amygdala promotes contextual or elemental conditioned association in Pavlovian fear conditioning. *Learn Mem* 12:383–388. [CrossRef Medline](#)
- Cassimeris L (2002) The oncoprotein 18/stathmin family of microtubule destabilizers. *Curr Opin Cell Biol* 14:18–24. [CrossRef Medline](#)
- Clemenson GD, Lee SW, Deng W, Barrera VR, Iwamoto KS, Fanselow MS, Gage FH (2015) Enrichment rescues contextual discrimination deficit associated with immediate shock. *Hippocampus* 25:385–392. [CrossRef Medline](#)
- Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC (2004) Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* 42:535–552. [CrossRef Medline](#)
- Drew LJ, Fusi S, Hen R (2013) Adult neurogenesis in the mammalian hippocampus: why the dentate gyrus? *Learn Mem* 20:710–729. [CrossRef Medline](#)
- Hamilton AM, Oh WC, Vega-Ramirez H, Stein IS, Hell JW, Patrick GN, Zito K (2012) Activity-dependent growth of new dendritic spines is regulated by the proteasome. *Neuron* 74:1023–1030. [CrossRef Medline](#)
- Hardt O, Wang SH, Nader K (2009) Storage or retrieval deficit: the yin and yang of amnesia. *Learn Mem* 16:224–230. [CrossRef Medline](#)
- Kempermann G, Song H, Gage FH (2015) Neurogenesis in the adult hippocampus. *Cold Spring Harb Perspect Biol* 7:a018812. [CrossRef Medline](#)
- Kheirbek MA, Tannenholz L, Hen R (2012) NR2B-dependent plasticity of adult-born granule cells is necessary for context discrimination. *J Neurosci* 32:8696–8702. [CrossRef Medline](#)
- Kuntziger T, Gavet O, Sobel A, Bornens M (2001) Differential effect of two stathmin/Op18 phosphorylation mutants on *Xenopus* embryo development. *J Biol Chem* 276:22979–22984. [CrossRef Medline](#)
- Martel G, Uchida S, Hevi C, Chévere-Torres I, Fuentes I, Park YJ, Hafeez H, Yamagata H, Watanabe Y, Shumyatsky GP (2016) Genetic demonstration of a role for stathmin in adult hippocampal neurogenesis, spinogenesis, and NMDA receptor-dependent memory. *J Neurosci* 36:1185–1202. [CrossRef Medline](#)
- Shumyatsky GP, Malleret G, Shin RM, Takizawa S, Tully K, Tsvetkov E, Zakharenko SS, Joseph J, Vronskaya S, Yin D, Schubart UK, Kandel ER, Bolshakov VY (2005) *stathmin*, a gene enriched in the amygdala, controls both learned and innate fear. *Cell* 123:697–709. [CrossRef Medline](#)
- Silva AJ, Kogan JH, Frankland PW, Kida S (1998) CREB and memory. *Annu Rev Neurosci* 21:127–148. [CrossRef Medline](#)
- Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH (2006) NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* 442:929–933. [CrossRef Medline](#)
- Trifilieff P, Herry C, Vanhoutte P, Caboche J, Desmedt A, Riedel G, Mons N, Micheau J (2006) Foreground contextual fear memory consolidation requires two independent phases of hippocampal ERK/CREB activation. *Learn Mem* 13:349–358. [CrossRef Medline](#)
- Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* 87:1327–1338. [CrossRef Medline](#)
- Uchida S, Martel G, Pavlowsky A, Takizawa S, Hevi C, Watanabe Y, Kandel ER, Alarcon JM, Shumyatsky GP (2014) Learning-induced and stathmin-dependent changes in microtubule stability are critical for memory and disrupted in ageing. *Nat Commun* 5:4389. [CrossRef Medline](#)
- Vadodaria KC, Gage FH (2014) SnapShot: adult hippocampal neurogenesis. *Cell* 156:1114–1114.e1. [CrossRef Medline](#)