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Article Addendum

Olfactory learning and spike timing dependent plasticity

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Abbreviations: STDP, spike-timing-dependent plasticity; CS, conditioned stimulus; US, unconditioned stimulus; cAMP, cyclic adenosine monophosphate

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Hebbian spike-timing-dependent plasticity (STDP) is widely observed in organisms ranging from insects to humans and may provide a cellular mechanism for associative learning. STDP requires a millisecond-scale temporal correlation of spiking activity in pre- and postsynaptic neurons. However, animals can learn to associate a sensory cue and a reward that are presented seconds apart. Thus, for STDP to mediate associative learning, the brain must retain information about the sensory cue as spiking activity until the reinforcement signal arrives. In our recent study, we tested this requirement in the moth *Manduca sexta*. We characterized the odor responses of Kenyon cells, a key neuronal population for insect olfactory learning, and conditioned moths to associate an odor with a sugar water reward. By varying the amount of temporal overlap between odor-evoked spikes and the reward presentation, we found that the most learning occurred when spiking activity had no overlap with the reward presentation; further, increasing the overlap actually decreased the learning efficacy. Thus, STDP alone cannot mediate the olfactory learning in Kenyon cells. Here, we discuss possible cellular mechanisms that could bridge the temporal gap between physiological and behavioral time scales.

When animals learn to associate events, timing is critical. Free-flying honeybees, for example, can readily learn to associate the color of a flower with the sugary nectar it provides, but only if the timing is right. In an experiment in which color presentation (conditioned stimulus, CS) was timed, effective learning occurred only when the color was switched on while bees were approaching the sugar source (unconditioned stimulus, US).[1] However, if the color was presented only when the bees were feeding on or leaving the sugar source, the bees failed to learn the color-reward association. This study, like many others, illustrates that the brain must somehow retain information about a sensory cue that predicts a reward until the reward (or punishment) is given. What exactly is the neural representation that holds such information? Our recent study examined this issue.[2] Here, we further discuss our results.

It is often argued that the sustained neural representation of a stimulus consists of the persistent firing of single cells or reverberating firing within a neural network.[3] Persistent firings in single neurons have been observed in the prefrontal cortex of monkeys performing working memory tasks (reviewed in ref. 4). A theoretical study[5] proposed reverberating firing or a relay of neural firing in a recurrent network in the CA3 region of hippocampus of mammals engaging in trace conditioning to explain why the hippocampus is required for trace eyeblink conditioning.[6,7] Spike timing dependent plasticity (STDP), a form of Hebbian learning that requires millisecond-scale correlations in spiking between pre- and postsynaptic neurons, has been proposed as a mechanism for establishing these associations. But, is temporal contiguity between spike representations of CS and US always necessary for learning to occur?

To answer this long-standing question, one must first know the neural representation of the CS in a cell population in which learning is thought to occur in order to design behavioral experiments to test the importance of temporal contiguity between the CS and US for learning. To do this, we used the moth *Manduca sexta*,[2] focusing on the Kenyon cells in the mushroom body, a neuronal population in insects believed to be crucial for acquiring[8,9] and recalling[10,11] olfactory memory. Synapses between Kenyon cells and followers called β-lobe neurons undergo STDP.[12] Thus, Kenyon cells are a suitable cell population for testing the role of STDP in associative learning.[13] We found that long odor presentations reliably caused Kenyon cells to fire spikes, but only very briefly upon the odor's onset and offset; the spiking was not sustained. This response characteristic enabled us to separately test the importance of the onset and offset spikes in behavior experiments. By pairing an odor (CS) and a sugar water reward (US), we trained moths to extend their proboscises when prompted by an odor stimulus (the proboscis extension reflex[14]). We varied the amount of temporal contiguity between CS and US presentations by means of a computer-regulated stimulus delivery system. We found that moths learned to associate odor and reward best when onset spikes in Kenyon cells were followed a few seconds later by the reward. Maximizing the temporal overlap between the odor-evoked spikes in Kenyon cells and the US presentation actually

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reduced the amount of learning. Thus, in Kenyon cells, the mechanism of learning did not require the temporal contiguity of spiking between the neural representation of the CS and the neural representation of the US.

Learning may take place in other cells as well. To the extent that learning occurs in Kenyon cells, it does not require sustained spiking. We speculate that the very brief period of spiking elicited by odors in Kenyon cells may trigger biochemical representations that endure long enough to coincide with delayed reinforcement stimuli. Here we outline one such potential mechanism. Odor-elicited depolarization is known to elevate levels of calcium in Kenyon cells.[15,16] Elevated calcium could signal the presence of the CS, and activate enduring Ca^{2+}/calmodulin-sensitive adenylate cyclase dependent mechanisms that may underlie learning.[10,11] Indeed, many studies with mutants in *Drosophila* suggest that calcium-triggered, cyclic adenosine monophosphate (cAMP)-dependent mechanisms in the presynaptic terminals of Kenyon cells are critically important for olfactory learning. And, in Kenyon cells, reward pathways can also activate adenylate cyclase, here through octopamine receptors.[17] Thus, both conditioned and unconditioned stimulus signals could be detected and combined in Kenyon cells by a Ca^{2+}/calmodulin-sensitive adenylate cyclase. The fine-scale timing requirements for odor and reward pairing through adenylate cyclase are unknown, but some evidence indicates the effective temporal window for its synergic activation by a CS and US could be quite wide, at least on the order of seconds or tens of seconds.[18] Thus, cAMP-dependent plasticity could be triggered by a brief depolarization, yet provide a mechanism that encompasses behavioral timescales. Known molecular properties of adenylate cyclase could even explain the temporal requirement that the CS has to be presented before the US to cause learning.[18] This plasticity mechanism, like one initially proposed for *Aplysia*, and known as associative facilitation,[18,19] could occur largely through the presynaptic modulation of Kenyon cells and may not require Hebbian, temporally contiguous spiking in pre- and postsynaptic neurons at all.

This hypothesis, consistent with a recent theoretical study,[20] awaits testing. Other interesting theoretical frameworks for associative learning based on Hebbian plasticity also include slower biochemical processes such as the release of nitric oxide[21] or the global elevation of dopamine levels in the extracellular space.[22] We suggest it would be fruitful to look closely at the time course of biochemical processes triggered by transient spiking activity. This approach may illuminate mechanisms to bridge a large temporal gap between physiological and behavioral time scales in learning.

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References

1. Menzel R, Erber J. Learning and memory in bees. Scientific American 1978; 239:80-7.
2. Ito I, Ong RC, Raman B, Stopfer M. Sparse odor representation and olfactory learning. Nat Neurosci 2008; 11:1177-84.
3. Hebb DO. The organization of behavior: a neuropsychological theory. New York, NY: Wiley, 1949.
4. Galloway EM, Woo NH, Lu B. Persistent neural activity in the prefrontal cortex: a mechanism by which BDNF regulates working memory? Prog Brain Res 2008; 169:251-66.
5. Rodriguez P, Levy WB. A model of hippocampal activity in trace conditioning: where's the trace? Behav Neurosci 2001; 115:1224-38.
6. Solomon PR, Schaaf ERV, Thompson RF, Weisz DJ. Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. Behav Neurosci 1986; 100:729-44.
7. Bangasser DA, Waxler DE, Santollo J, Shors TJ. Trace conditioning and the hippocampus: the importance of contiguity. J Neurosci 2006; 26:8702-06.
8. Keene AC, Waddell S. *Drosophila* olfactory memory: single genes to complex neural circuits. Nat Rev Neurosci 2007; 8:341-54.
9. Krashes MJ, Keene AC, Leung B, Armstrong JD, Waddell S. Sequential use of mushroom body neuron subsets during *Drosophila* odor memory processing. Neuron 2007; 53:103-15.
10. Heisenberg M. Mushroom body memoir: from maps to models. Nat Rev Neurosci 2003; 4:266-75.
11. Davis RL. Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. Annu Rev Neurosci 2005; 28:275-302.
12. Cassenaer S, Laurent G. Hebbian STDP in mushroom bodies facilitates the synchronous flow of olfactory information in locusts. Nature 2007; 448:709-13.
13. Drew PJ, Abbott LF. Extending the effects of spike-timing-dependent plasticity to behavioral timescales. Proc Natl Acad Sci USA 2006; 103:8876-81.
14. Bitterman ME, Menzel R, Fietz A, Schäfer S. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). J Comp Psychol 1983; 97:107-19.
15. Wang Y, Guo H, Pologruto TA, Hannan F, Hakker I, Svoboda K, Zhong Y. Stereotyped odor-evoked activity in the mushroom body of *Drosophila* revealed by green fluorescent protein-based Ca^{2+} imaging. J Neurosci 2004; 24:6507-14.
16. Szyszka P, Ditzen M, Galkin A, Galizia CG, Menzel R. Sparsening and temporal sharpening of olfactory representations in the honeybee mushroom bodies. J Neurophysiol 2005; 94:3303-13.
17. Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. J Neurosci 2003; 23:10495-502.
18. Yovell Y, Abrams TW. Temporal asymmetry in activation of *Aplysia* adenylyl cyclase by calcium and transmitter may explain temporal requirements of conditioning. Proc Natl Acad Sci USA 1992; 89:6526-30.
19. Lechner HA, Byrne JH. New perspectives on classical conditioning: a synthesis of Hebbian and non-Hebbian mechanisms. Neuron 1998; 20:355-8.
20. Smith D, Wessnitzer J, Webb B. A model of associative learning in the mushroom body. Biol Cybern 2008; 99:89-103.
21. Montague PR, Sejnowski TJ. The predictive brain: temporal coincidence and temporal order in synaptic learning mechanisms. Learn Mem 1994; 1:1-33.
22. Izhikevich EM. Solving the distal reward problem through linkage of STDP and dopamine signaling. Cereb Cortex 2007; 17:2443-52.