Journal Club

Editor's Note: These short reviews of a recent paper in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to mimic the journal clubs that exist in your own departments or institutions. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Butanone: The Memory of a Scent

Eiji Kodama and Paola Jurado

Laboratory of Molecular Neurobiology, Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan
Review of Torayama et al. (<http://www.jneurosci.org/cgi/content/full/27/4/741>)

The correlation of genes with animal behavior has always been a complicated and arguable issue. The matter is simplified in *Caenorhabditis elegans* with the unlimited population of clones, a fully sequenced genome, a repertoire of logical and predictable behaviors, and only 302 neurons with well mapped morphologies and connections. In addition to its simplicity, this small nematode responds to a number of stimuli (temperature, smell, taste, touch) with evidence of learned behavior.

Learning has been historically divided into associative and nonassociative types (Carew and Sahley, 1986). Habituation, a nonassociative paradigm, refers to a decrement in response after repeated presentations of a stimulus. The best example of this simple behavior in *C. elegans* is the “tap response.” Worms usually react to touch by changing their locomotion direction, but after repeated mechanosensory stimulation, the response decreases. Associative learning implies a relationship between two or more events. Stimuli sensed by *C. elegans* (conditioned cue), when coupled to bacteria as a source of food or to a harmful incentive (modulatory input), gives rise to a pattern comparable to associative learning. This behavior has been observed in several situations

(Table 1) (de Bono and Maricq, 2005; Bargmann, 2006).

In their *Journal of Neuroscience* article, Torayama et al. (2007) describe a novel behavioral associative plasticity, “butanone enhancement.” Worms learned and remembered a spatial association of food with the volatile attractant butanone. This increased the animal attraction toward butanone above that of naive worms [Torayama et al. (2007), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/27/4/741/F1>)]. Ten mutants defective in this behavior were newly isolated and two of them further characterized.

Olfaction in *C. elegans* is mediated by five pairs of ciliated amphid (head) neurons (Bargmann, 2006). Each expresses multiple chemosensory receptors. Two pairs of sensory neurons, AWA and AWC, are involved in attractive odor responses whereas the other three pairs mediate repulsive responses. Butanone is exclusively sensed by AWC. The AWC neuron pair differs in olfactory properties. The G-protein-coupled predicted olfactory receptor STR-2 is stochastically and asymmetrically expressed in only one of the two AWC neurons, designated as AWC^{ON} and AWC^{OFF} (Troemel et al., 1999).

The first mutant with defects in butanone enhancement, *olrn-1*, had a mutation in the gene encoding a novel transmembrane protein [Torayama et al. (2007), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/27/4/741/F4>)]. *olrn-1* expression was restricted to head and pharyngeal neurons, and its expression in AWC was strictly required for proper bu-

tanone enhancement. In *olrn-1*, the odorant receptor STR-2 was not expressed in AWC neurons (2AWC^{OFF}). A 2AWC^{OFF} phenotype could result from a structural deficiency in AWC, leading to a nonspecific cellular defect, or an alteration in AWC receptor distribution. Because *olrn-1* mutants sensed butanone and benzaldehyde, which are detected exclusively by AWC neurons, the second possibility is more likely. In this context, the authors examined the relationship of *olrn-1* with genes related to AWC^{ON/OFF} determination fate such as *nsy-1* [homolog of the human MAPKKK (mitogen-activated protein kinase kinase kinase) ASK-1] (Bargmann, 2006). *nsy-1* expressed STR-2 in both AWC neurons (2AWC^{ON}) and showed normal butanone enhancement. *nsy-1* mutation is epistatic to *olrn-1*, which means that *olrn-1* functions upstream of *nsy-1* in AWC^{ON/OFF} determination and butanone plasticity [Torayama et al. (2007), their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/27/4/741/F5>)].

Torayama et al. (2007) report that a series of mutants with 2AWC^{OFF} phenotype, or AWC^{ON} surgically ablated wild-type animals, consistently had defects on butanone olfactory learning. In contrast, mutants with 2AWC^{ON} neurons had normal butanone enhancement. These results suggest that at least one functional AWC^{ON} neuron is required for butanone enhancement. However, AWC^{OFF} is necessary for odor discrimination (Bargmann, 2006). Therefore, AWC left/right asymmetry seems to be significant for the contrast of different olfactory cues.

The second mutant isolated, *olrn-2*,

Received March 24, 2007; revised April 23, 2007; accepted April 23, 2007.

We are grateful to Dr. Mori, Dr. Kuhara, and all of the members from the Mori laboratory for critical comments and discussion.

Correspondence should be addressed to either Paola Jurado or Eiji Kodama, Laboratory of Molecular Neurobiology, Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan. E-mail: Opaula@bunshi4.bio.nagoya-u.ac.jp or z47741a@nucc.cc.nagoya-u.ac.jp.

DOI:10.1523/JNEUROSCI.1322-07.2007

Copyright © 2007 Society for Neuroscience 0270-6474/07/275267-02\$15.00/0

Table 1. Summary of *C. elegans* associative behavioral plasticity paradigms

Conditioned cue	Modulatory input	Response
Temperature	Food consumption/lack	Seeking/avoidance of cultivation temperature when previously paired/unpaired with food.
Smell (attractant diacetyl)	Smell (aversive acetic acid)	Avoidance of the chemoattractant diacetyl when previously paired with acetic acid.
Smell (bacteria)	Food consumption leading to visceral malaise	Avoidance of bacteria that previously caused malaise.
Taste (attractant NaCl)	Lack of food	Avoidance of the chemoattractant NaCl when previously paired with absence of food.
Taste (attractant Na ⁺ or Cl ⁻ ions)	Contact to aversive substance (garlic, copper)	Avoidance of the ions when each of them has been previously paired with garlic or copper.

was identical to *bbs-8*, a Bardet-Biedl syndrome gene. The expression of STR-2 in *bbs-8/olrn-2* was normal. Curiously, some of the phenotypes exhibited by Bardet-Biedl syndrome in humans include *situs inversus*, a left–right axis determination defect associated with cilia malfunction during development (Blacque et al., 2007). BBS-8 is involved in intraflagellar transport between the dendrite of the sensory neuron and the cilium. In *bbs-8/olrn-2* mutants, cilia are halved or have a perturbed distal end. Additional *bbs* mutants also showed a defective butanone enhancement. However, other cilia-defective animals had normal butanone plasticity although the naive attraction was seriously impaired in some cases [Torayama et al. (2007), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/27/4/741/F6>)]. This suggests that butanone enhancement requires *bbs* gene function rather than an intact cilia structure, and that weak sensitivity to the odor did not affect its proper association with food.

A previous case, to some extent related with the butanone enhancement phenomenon, was described in *C. elegans* by Nuttley et al. (2002). Pre-exposure to the

natural attractant benzaldehyde without food causes a decrease in the response toward the chemical. On the contrary, Nuttley et al. (2002) showed that coexposure with food and benzaldehyde led to a partial attraction, although never as intense as the naive level. Thus it is not straightforward to determine whether increased attraction to benzaldehyde after feeding is caused by association with food or just to a suppression of a habituation or adaptation mechanism. Although this “adaptation suppression” behavior was mostly dependent on serotonin, association of butanone and food seems to be at least partly independent.

Torayama et al. (2007) suggest that odors from two different sources (butanone and food), which seem to be sufficient to partially generate conditioning, can be sensed and associated in the same neuron. In the nervous system of higher animals, a specific brain area is thought to be necessary for such complex neural functions. We think that such an appealing suggestion deserves a deeper investigation and additional experiments with the aim to confine the elements necessary for food odor sensing to a single neuron.

References

- Bargmann CI (2006) Chemosensation in *C. elegans*. In: WormBook (The *C. elegans* Research Community, ed). Retrieved April 13, 2007, from <http://www.wormbook.org>
- Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, Badano JL, Mah AK, Beales PL, Davidson WS, Johnsen RC, Audeh M, Plasterk RH, Baillie DL, Katsanis N, Quarby LM, Wicks SR, Leroux MR (2007) Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev* 18:1630–1642.
- Carew TJ, Sahley CL (1986) Invertebrate learning and memory: from behavior to molecules. *Annu Rev Neurosci* 9:435–487.
- de Bono M, Maricq AV (2005) Neuronal substrates of complex behaviors in *C. elegans*. *Annu Rev Neurosci* 28:451–501.
- Nuttley WM, Atkinson-Leadbeater KP, van der Kooy D (2002) Serotonin mediates food-odor associative learning in the nematode *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 99:12449–12454.
- Torayama I, Ishihara T, Katsura I (2007) *Caenorhabditis elegans* integrates the signals of butanone and food to enhance chemotaxis to butanone. *J Neurosci* 27:741–750.
- Troemel ER, Sagasti A, Bargmann CI (1999) Lateral signaling mediated by axon and calcium entry regulates asymmetric receptor expression in *C. elegans*. *Cell* 99:387–398.