Current Biology

CellPress

Dispatches

Cellular cognition: How single cells learn using non-neural networks

Deepa H. Rajan and Wallace F. Marshall*
Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94122, USA
*Correspondence: Wallace.Marshall@ucsf.edu
https://doi.org/10.1016/j.cub.2024.11.016

Single cells can perform surprisingly complex behaviors and computations, including primitive forms of learning like habituation. New work highlighted here uses mathematical modeling to show that relatively simple biochemical networks can recapitulate many features of habituation in animals.

How consciousness arises in biology is, in the words of David Chalmers, a ''hard problem''. One school of thought believes that cognitive processes arise as emergent properties of large networks of neurons. The power of neural networks, both biological and artificial, is beyond dispute. But there is growing evidence that even cells without a nervous system display certain forms of cognition and computation. As one example, single cells perform habituation, a fundamental type of learning. In habituation, an organism gradually stops responding to a repetitive stimulus. That's a pretty simple kind of learning, but given that it happens in a single cell, it's a bit like the proverbial dancing bear, for which the remarkable thing is that it can dance at all. But how can single cells learn or do other types of behaviors that we usually associate with cognition, despite not having neural networks? In fact, cells do contain networks, albeit made of signaling proteins rather than neurons. It has been proposed that biochemical networks inside cells may play a similar role as neural networks in the brain, and provide a basis for computation and learning[1,2]. A new study reported in this issue of *Current Biology* by Eckert *et al.* used a computational strategy to identify a set of abstract networks that can explain habituation in single cells, in particular in the giant ciliate *Stentor*[3].

Stentor is a unicellular pond-dwelling organism that exhibits habituation, a form of learning in which a behavioral response decreases following a repeated stimulus[4]. *Stentor* contract in response to mechanical stimulation, an apparent escape response from aquatic predators (Figure 1A). However, repeated low-force perturbations induce habituation, demonstrated by a progressive reduction in contraction probability[4] (Figure 1B). Habituated *Stentor* still contract if given a high-force mechanical stimulus[4,5] or photic stimulation[6], and repeated high-force stimulation precludes habituation[7]. These observations, which align with classic criteria for habituation[8,9], strongly suggest that the original contractile response decrement is due to learning rather than fatigue, ATP depletion, or sensory adaptation. But how can a cell learn without a nervous system?

Two abstract models have already been proposed for habituation in single cells like *Stentor*. One model, based on the observation that individual *Stentor* cells show a step-like response during habituation, proposes that a cell could be in one of two states, each with a different probability of responding to the stimulus, and with forward and reverse transition probabilities between the two states[10]. A second model for habituation that is applicable to single-celled learning is based on the idea of a filter which takes an input that varies as a function of time, and estimates statistical properties of that signal that serve to provide information about the outside world[11]. These abstract models help clarify our thinking about habituation in cells, but do not provide a direct link to molecular mechanisms.

One type of modeling framework that retains some advantages of abstract descriptions, but is more relatable to biochemical mechanisms, is the network model. Such models are drawn as a set of nodes connected by arrows, representing flow of information through a set of signaling proteins (Figure 1C). Nodes represent proteins, and arrows represent terms in a differential equation governing how the activity of each node changes over time[12]. Two features of this type of model make them directly linked to biology. First, the nodes represent actual proteins, even though their identity might not be known. Second, the mathematical functions represented by the arrows are chosen to reflect known biochemical regulatory mechanisms, such as Hill functions to represent cooperativity. Biological plausibility is ensured by construction. Another advantage of network models is the ability to enumerate over all possible network topologies, allowing an entire space of possible models to be systematically explored. This approach has revealed network models for a range of interesting functions, including adaptation[13], which is the same as habituation in terms of showing a reduced response to continued input.

The new study by Eckert *et al.* presents a model that not only shows response decrement but also exhibits several additional classic markers of habituation[8,9] not seen with conventional adaptation processes. Based on plausible biochemistry, the model features timescale separation and reversal behavior of memory variables as the foundation for habituation characteristics. To do this, they considered network motifs common in cell signaling, particularly negative feedback (NF) and incoherent feedforward (IFF) motifs. These motifs can give rise to a reduction in response after repeated stimulation[13,14], but by themselves these motifs do not exhibit frequency sensitivity, a common property of habituation in which higher frequency

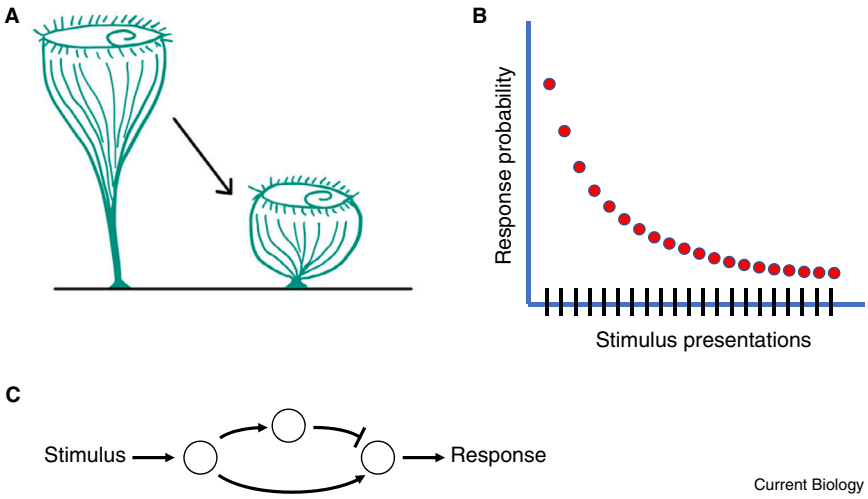

Figure 1. Modeling single-celled habituation.
(A) The giant ciliate *Stentor* contracts in response to mechanical stimulus. (B) The probability of a *Stentor* cell contracting decreases as the same stimulus is repeated, demonstrating habituation. (C) Network models like the incoherent feedforward network provide a modeling framework for molecular pathways.

stimulation results in both faster response reduction and faster response recovery time[15]. Inspired by the work of Staddon, who observed emergence of frequency sensitivity from concatenation of network motifs[15,16], Eckert *et al.* concatenated NF and IFF. These concatenated networks used regulatory functions based on the assumption that the state of nodes is regulated by a cycle of covalent modification such as by phosphorylation. To search parameters, they leveraged the growing body of experimental evidence on learning in single cells, especially the hierarchy of avoidance behaviors in *Stentor roeseli*[17,18] and habituation behaviors in *Stentor coeruleus*[4,7,10].

The model presented by Eckert *et al.* exhibits many of the habituation characteristics outlined by Richard Thompson and Alden Spencer[8,9] in a comprehensive survey of habituation in animals. Intensity sensitivity, a phenomenon wherein less intense stimuli result in faster habituation, and frequency sensitivity can be explained by timescale separation in decay rates of memory variables. Spontaneous recovery, or the restoration of response after the cessation of stimulus delivery, as well as subliminal accumulation, which is an improvement in memory retention when stimulus delivery continues even after habituation has reached asymptotic levels, emerge naturally from Eckert *et al.*'s model. Finally, potentiation, wherein habituation becomes more rapid after successive training bouts, and long-term habituation also result from this model. However, the model does not show a few other habituation hallmarks such as stimulus specificity, dishabituation (response recovery after presentation of a different stimulus), and habituation of dishabituation (attenuation of the response to the different stimulus after repeated presentation).

In the network model presented by Eckert *et al.*, nodes represent proteins but they are still abstract proteins and not directly relatable to known cellular pathways. In this sense, it is still a 'top down' model that starts from an abstraction which can then be brought down to the level of actual molecules. An alternative 'bottom up' strategy starts with known biomolecular processes, and builds a mathematical representation. Such a model has recently been reported for habituation in *Stentor*[5] which represents mechanoreceptors linked to ion channels coupled with an action potential threshold that, when crossed, leads to cell contraction. This model also includes activity-dependent receptor inactivation and degradation, known features of many receptor types. By simulating the interactions of these elements, it was shown that they are capable of performing habituation which matches many of the experimentally known features. In this case, degradation or inactivation of the receptor drives the reduction in response, while response recovery was driven by re-synthesis of receptors. As with the model of Eckert *et al.*, this biochemistry-based model could recapitulate many of the known features of habituation, as well as the outcome of new experiments that were carried out to test the model[5].

As demonstrated by Eckert *et al.* and other network models, as well as more abstract top-down models, there are several different ways to approach understanding habituation in single cells from a theoretical perspective. However, as the late E. O. Wilson wrote, "Nature first, then theory. Or, better, Nature and theory closely intertwined while you throw all your intellectual capital at the subject"[19]. In this vein, the model put forth by Eckert *et al.* motivates experiments in single cells to test the model predictions. Especially important will be experiments exploring the regimes and conditions for rate sensitivity of spontaneous recovery as well as long-term habituation, since these phenomena have not yet been demonstrated in single cells. Models play an important role by inspiring iterative experiments as well as revealing potential ways something can happen. In this case, the model illuminates the hard problem of how complexity akin to consciousness emerges in single cells.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Social insects: The waxy wonder of symmetry

Margaret J. Couvillon
Department of Entomology, Virginia Tech, 170 Drillfield Drive, Blacksburg, VA 24061, USA
Correspondence: mjc@vt.edu
https://doi.org/10.1016/j.cub.2024.10.038

A new study demonstrates that honey bee nests and their contents possess front-to-back symmetry, a design characteristic arising from a proximate thermal cue with an ultimate evolutionary benefit.

Humans like symmetry. We infuse symmetry into our buildings, art and proclivities. Aristotle wrote in his principal work *Metaphysics* that symmetry is a chief form of beauty. Leonardo da Vinci's Vitruvian Man, the drawing of a nude adopting different stances simultaneously, used the body within a square superimposed on a circle to depict the ideal — symmetric — human proportions. We created some of our grandest structures, such as the Taj Mahal and the Parthenon, to be symmetric. Numerous psychological studies have highlighted that humans find symmetrical faces more attractive and even more trustworthy than their asymmetrical counterparts. The ubiquity of symmetry in our human-created world mimics its pervasiveness in the natural world: countless animals and plants display bilateral symmetry, where each side of the form is a mirror image of the other. Additionally, many flowering plants and some Cnidarians and Echinoderms, such as jellyfish, anemones, sea stars, exhibit radial symmetry, where the organism is divided into equal parts around a central axis. However, until now, scientists had not yet found symmetry spanning across levels of biological organization, such as in the extended phenotypes, or adaptively beneficial structures, created by social insects. A new study in this issue of *Current Biology* by Michael Smith and colleagues[1] sheds light on how universal the affinity for symmetry might be: honey bees (*Apis mellifera*), superorganisms and insect pollinator extraordinaire, also prefer symmetry, and they organize, without template or centralized management, their wonderous wax comb nests in concordance to this law of form.

Wax comb is produced by worker bees, each of which possesses four secreting pairs of exocrine glands on the underside of their abdomen[2]. The energetic cost of synthesizing wax is incomprehensibly huge. Approximately 60,000 adult bees must consume 7.5 kg of honey, itself a literal liquid goldmine, to fuel the production of 1.2 kg of wax, which is the typical nest's wax weight[3]. The sheer energetic demands of this process are indirectly evident in swarming bees' nest site selections: the bees are more likely to choose a cavity with left-behind comb from previous tenants[4], a proclivity that allows the swarms to produce nearly twice as much honey compared to swarms that must set up house (i.e., build all comb from scratch) in an empty hive[5]. Honey is what bees eat to survive the winter, so more honey translates to increased winter survival. Efficiently and effectively creating comb can therefore make or break a colony. Not surprisingly, given its importance, comb creation begins as soon as swarms move into a new nest cavity[6], as every subsequent activity of consequence, like storing food (pollen and honey) and making babies, requires the presence of these multi-purpose storage bins. The hanging combs are planar, double-sided, and in parallel alignment, composed of tessellated hexagons of amazingly uniform size (Figure 1)[3,7].