Synthetic Biology in the Brain: A Vision of Organic Robots

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Abstract

Synthetic biology lies on the interface between natural and artificial life. It consists of the assembly of natural biological components into artificially configured biological systems. A main focus of synthetic biology has been the engineering of new gene circuits that can produce artificial cellular functions. I propose to scale up this approach to include, beyond single cells and gene circuits, also entire multi-cellular organisms and the brain circuits that regulate their behavior. Such synthetic biology in the brain will offer new ways for understanding how brain connectivity relates to brain function, and could ultimately lead to futuristic technologies such as neuronally-programmed organic robots or biologically-based brain repair. As a first step towards this ambitious goal I have developed a technique for genetically inserting new synaptic connections into the nervous system of the nematode worm *C. elegans*, enabling the manipulation of information flow in the nervous system and the reprogramming of whole animal behavior in this organism. This approach may be expanded and adapted to other genetic models, and opens the way to possible new forms of artificial life. Such technology, if practiced responsibly, could offer considerable benefits to science, industry and medicine.

Synthetic biology in the brain

Synthetic biology elegantly fuses the fields of engineering and biology, to accomplish the goal of designing and constructing new biological systems out of basic biological parts (Xie and Fussenegger, 2018). The rationale is that the creative process and the practical challenges faced when building a system can substantially contribute to understanding how that system works and to establish causal links between the system's organization and its operation. Synthetic biological systems can be considered as a special form of artificial life, which strongly hinges on natural life. On the one hand, they are composed entirely of organic matter and follow biological principles of operation. On the other hand, they are designed and constructed by human beings intended for human benefit. The organic nature of synthetic biological systems makes them self-reproducible, ecologically compatible with other organisms and the environment, and fully degradable. A unique combination of features that can rarely be found in other forms of artificial life.

Many synthetic biological applications focus on single cells and on the gene networks that control their function (Bashor and Collins, 2018). The potential outcomes are spectacular. For example, synthetic bacteria that could monitor, synthesize and regulate drug administration in a patient's body (Flores Bueso, et al. 2018); or engineered microalgae that could produce biofuels (Jagadevan, et al. 2018). I propose to expand synthetic biology beyond single cells or populations of single cells, to the realm of multi-cellular organisms. These modified animals exhibiting novel artificial behaviors could substantially enrich the repertoire of synthetic biology, producing more complex and farther-reaching forms of artificial life. Multi-cellular animals, just like single cells, are fundamentally governed by networks of gene interactions. However, the direct coordination and control of their overall behavior is produced by higher order networks of neurons and the synaptic connections that link between them. If it were possible to design and implement specific synaptic connections in the nervous system of an animal, then, in principle, new behaviors could be derived. At some point, an animal harboring an accumulation of such engineered connections, or perhaps new synthesized neural circuits, or, ultimately, an overall redesign of neural connectivity could arguably qualify as a form of artificial life.

Engineering synaptic connections in worms

Ceanorhabditis elegans is a 1 mm long nematode worm (Fig. 1a) that dwells in soil and compost, where it feeds on bacteria. Its nervous system consists of only 302 neurons, interlinked by a set of several thousand synaptic connections, which constitute its *connectome*. In fact, the *C. elegans* connectome is the first and, to date, only connectome of any animal to have been mapped. It is intriguing to consider the potential impact of editing the *C. elegans* connectome and forming within it new synthetic patterns of connectivity. Could new behaviors be programmed into the worm in this way? How would such novel behaviors coexist with native ones? To what extent could the entire lifestyle of the worm be reshaped through synthetic design of its neural circuits? Such synthetic biology at the level of the nervous system could help elucidate fundamental principles of brain structure-function relations.

One can think of various hypothetical ways to manipulate, modify and establish new patterns of synaptic connectivity in a live organism. One possible approach is to genetically insert new synthetic synapses into existing neural circuits. Like other metazoans, *C. elegans* uses both chemical and electrical synapses for neural communication. Chemical synapses are complex in structure and are thus challenging to construct artificially. Electrical synapses or gap junctions, in contrast, are considerably simpler. In vertebrates, gap junctions are composed of *connexin* proteins that assemble together to form hemi-channels embedded in the cell membrane. When two compatible hemi-channels contact each other, they fuse to from a gap junction, a physical channel that enables the passage of charged ions between the connected neurons (Fig. 1b). Invertebrates, like *C. elegans*, do not possess connexin proteins. Instead, they use *innexins* for constructing gap junctions, an independently evolved protein family. Ectopic expression of connexin in *C. elegans* neuron pairs could thus lead to the artificial formation of a gap junction, functionally and specifically linking the two neurons. I have found that connexin driven by cell-specific promoters readily expresses in *C. elegans* neurons in a punctate form, typical to synaptic proteins (Fig. 1c). Moreover, some of these puncta appeared in close apposition (Fig. 1c, inset), suggestive of putative gap junctions. To test the functionality of such presumed engineered electrical synapses, I expressed connexin in two sensory neurons (Fig. 1d). Normally, only one of these neurons responds to a certain stimulus (Fig. 1d, top). However, when, and only when, both neurons ectopically expressed connexin, the responses became equalized, consistent with a coupling of these neurons by a gap junction (Rabinowitch, et al. 2014).

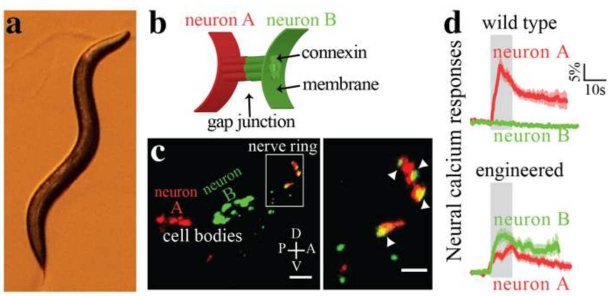

Figure 2: Genetic insertion of new synaptic connections between *C. elegans* neurons. (**a**) *C. elegans* is a 1mm long nematode worm. (**b**) Electrical synapses or gap junction are composed (in vertebrates) of connexin proteins expressed in two adjacent neurons. These form hemi-channels in each neuron and fuse together into a complete gap junction that electrically couples the neurons. (**c**) Ectopic expression of connexin fused to mCherry or GFP in two *C. elegans* neurons, A (AWC) and B (AIA). The rectangle, enlarged in the inset, marks the nerve ring, the region in which most synaptic contacts in *C. elegans* occur. Putative gap junctions are visualized as proximally localized puncta (▲). Scale bars: 5 µm and 2µm (**d**) Calcium responses of two (ASEL and ASER) neurons to a stimulus (salt removal).

Using the techniques described in Rabinowitch, et al. 2014 I also examined the capacity of engineered electrical synapses to reshape simple worm behaviors. For example, in *C. elegans*, the polymodal sensory neuron, ASH, is specialized for detecting noxious stimuli, which normally elicits a withdrawal response (Fig. 2a), resulting from the activation of premotor interneurons, such as AVA (Fig. 2b), and the inhibition of AVB, which otherwise drives forward acceleration. Strikingly, synthetically coupling of ASH to AVB caused worms to move forward, towards noxious stimuli, rather than escaping them by reversing (Fig. 2c), demonstrating a significant functional impact of a specific engineered synaptic connection. A similar principle enabled artificial switching of worm navigation towards a food-related odor, into avoidance of that odor, mimicking an effect that is otherwise attainable through training (Fig. 2d-f). I am currently applying these methods to generate completely novel behaviors in *C. elegans*, and am developing additional techniques for altering its connectome.

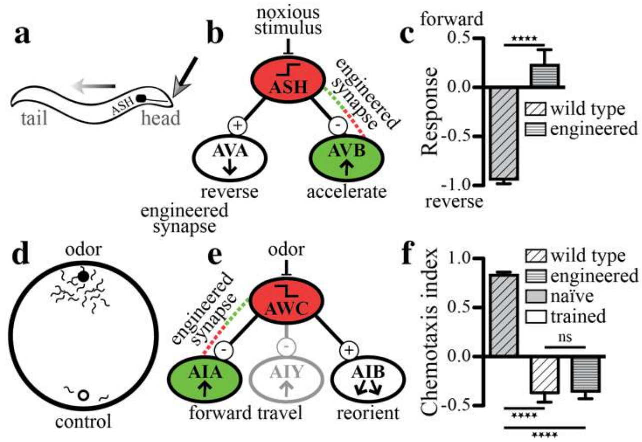

Figure 1: Engineered connections produce behavioral changes. (**a**) Worms reverse to escape noxious stimuli detected by the ASH sensory neuron. (**b**) Simplified circuit illustrating ASH connections to premotor interneurons AVA and AVB, which elicit reversing and forward acceleration, respectively. (**c**) An engineered ASH-AVB connection causes the worm to approach rather than avoid a noxious stimulus (unpublished). (**d**) Worms use chemotaxis to migrate towards attractive odors. (**e**) Simplified circuit shows the connectivity between olfactory sensory neuron, AWC, and downstream interneurons important for navigation. (**f**) An engineered AWC-AIA connection switches behavior from attraction to aversion, mimicking the effects of training (unpublished).

Future prospects

This work illustrates an encouraging step towards a long-term vision of extensive rewiring of the nervous system. Such advances could enhance our understanding of how neural structure determines brain function and could ultimately pave the way to the creation of small organic robots – a new form of artificial life, such as nematode worms programmed to distribute fertilizer among crops and hunt down pests, or to crawl into a patient, perform a medical procedure, and then leave. A long path awaits until such visions may become reality, and considerable ethical, safety and societal considerations will have to be weighed, but the potential gains for science and society are immense. Now is the time to start planning this journey.

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