Inserting new synaptic connections into damaged neural circuits: towards synapse therapy?

Ithai Rabinowitch*

Recovery from neural damage often requires reorganization of remaining neural tissue, including the formation of new synaptic connections between surviving neurons. Such rewiring may restore disrupted information flow caused by neuronal loss, and help adjust altered neural circuitry to reestablish diminished functionality. On occasion, proper rewiring may occur spontaneously, leading to successful recovery (Joy and Carmichael, 2021). However, in many cases natural rewiring is insufficient, and may even cause maladaptive deterioration due to miswiring (Nava and Röder, 2011). To overcome this, several interventional strategies have been developed to assist in the recovery process, by guiding synaptic rewiring in desirable directions (Su and Xu, 2020). For example, enhanced usage of the affected limbs or the impaired mental capacities can stimulate proper activity-dependent rewiring (Ganguly and Poo, 2013). Pharmaceutical targeting of specific molecular pathways (Joy and Carmichael, 2021) can bolster desirable plasticity processes and suppress unwanted ones. Cell-based therapy (Wechsler et al., 2018) can increase neurogenesis, replacing missing neurons and giving rise to new network connections. Brain-Machine Interfaces can serve as artificial communication pathways within the nervous system or between neurons and artificial effectors in order to bypass broken links in disrupted networks and establish new prosthetic neuronal connections (Wolpaw et al., 2020).

Since synaptic connectivity is in part determined genetically, it is conceivable that a complementary treatment approach, based on direct genetic insertion of new synaptic connections between target neurons in damaged neural circuits could, in principle, enable circuit recovery. But how can new specified synaptic connections be established in the nervous system? Where in the circuits should they be installed? Could they indeed enable recovery? In a recent study we have reported some initial steps taken to address these questions (Rabinowitch et al., 2021).

First, to simplify the problem, we focused on the relatively compact nervous system of the 1-mm long nematode worm *Caenorhabditis elegans* (*C. elegans*), which consists of only ~300 neurons interlinked by several thousand mapped synaptic connections. Moreover, the genetic power enabled by this model animal facilitates rational design and precise engineering of synapses. Thus, if it proves possible to overcome neuronal damage in *C. elegans* using genetically inserted new synapses then this might reflect on more complex systems, and it would allow us to at least move on to the next level of exploration on the path towards synapse therapy.

The small number of *C. elegans* neurons implies that damage to individual cells may lead to noticeable behavioral impairments. Indeed, in our study we removed a specific pair of interneurons (named AIA) from the worms' chemosensory neural circuit (**Figure 1**), and this caused a marked decline in the ability of the worms to navigate towards a source of an attractive odor. In particular, we showed that the removal of AIA neurons causes a break in information flow in the circuit, disrupting its ability to couple between ambient odor levels, detected by the AWC chemosensory neurons, and worm locomotion, regulated by various neurons, including AIA and AIB (**Figure 1A**), and thus undermining the capacity of worms to track potential sources of food (data not shown).

A potential synaptic approach for restoring the function of this damaged circuit could be to insert new synaptic connections between the remaining AWC and AIB neurons, thus reinforcing this surviving information route (**Figure 1B**). To do this, we applied a technique that we have previously developed (Rabinowitch et al., 2014), based on ectopically expressing the mammalian gap junction protein, connexin 36, in specific *C. elegans* neurons, causing new electrical synapses to form between these neurons. Moreover, since invertebrates, such as *C. elegans*, do not naturally form gap junctions from connexin proteins, but rather from a different protein family called innexins, the new inserted electrical synapses are expected to specifically link the target neurons. This method has proven to be effective in modifying neural circuit function and worm behavior in various neural circuits under diverse conditions (e.g., Rabinowitch et al., 2016; Hawk et al., 2018; Choi et al., 2020). Indeed, when we expressed connexin in AWC and AIB neurons we were able to observe a strong recovery in odor-guided navigation.

This encouraging result shows that direct artificial rewiring of a damaged neural circuit, using genetically-encoded natural synapses, could promote functional recovery of a simple neural circuit following neuronal loss. Our study revealed however, also a further unanticipated benefit of synthetic electrical synapses. We noticed that in addition to the intended AWC-AIB connections, new synthetic electrical synapses formed also between the two AWC neurons themselves and between the two AIB neurons themselves (many *C. elegans* neurons are organized in pairs), and that even AWC coupling alone (**Figure 1C**), or AIB coupling alone, was sufficient for behavioral recovery. This was likely due to a general tendency of lateral electrical synaptic connections to amplify weak signals in neural circuits (Szczupak, 2016). Thus, the dampening of neuronal responses following AIA removal could be offset by merely adding electrical synapses to groups of neurons along the disrupted pathway. We can therefore extrapolate that even in cases, in which the choice of patterns of artificial rewiring is not obvious or too complicated, simply inserting new electrical synapses between functionally equivalent groups of neurons associated with the damaged circuit could facilitate recovery, and in any case, may augment the effects of synthetic rewiring.

The path from this first demonstration of synapse-based recuperation of a lesioned neural circuit in *C. elegans*, to a future synapse therapy as a general means for restoring neural damage, is long and uncertain. To advance the possibility of synapse therapy, considerable work must be done. In particular, there still remains much to be learned in the relatively simple *C. elegans* system. For example, how generalizable is this approach? Could new synapses help bypass any form of circuit damage in any circuit? Are there particular patterns of disruption that are more amenable than others to synaptic treatment? How do inserted synapses affect other functions of the circuit and

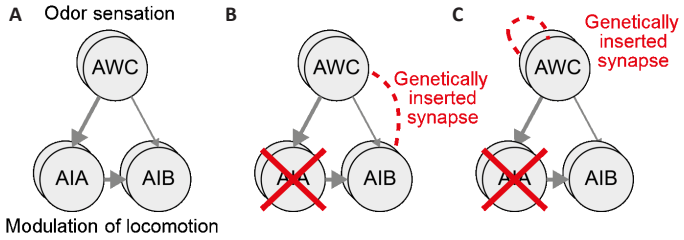

Figure 1 | **Circumventing neural damage in a *C. elegans* circuit for chemotaxis.**
(A) Simplified circuit diagram illustrates information flow in the circuit. The AWC chemosensory neurons respond to changes in ambient odor levels and transmit information in an AWC→AIA→AIB pathway as well as a direct AWC→AIB pathway. The AIA and AIB neurons participate in regulating locomotion. (B) Removal of the AIA neurons interrupts proper information flow in the circuit, and diminishes odor-guided navigation (not shown). To circumvent this damage, we reinforced the AWC→AIB pathway by genetically inserting a new electrical synapse between AWC and AIB. This led to behavioral recovery (not shown). (C) AWC-AIB synaptic insertion led also to the inadvertent formation of new synaptic connections between the two AWC neurons themselves (as well as between the two AIB neurons). We found that this synthetic electrical coupling in itself helped restore circuit function, due to the amplification of weakened signals in the damaged circuit. AIA, AIB and AWC are names of *C. elegans* neurons.

other aspects of behavior? How does the insertion of synthetic synapses influence natural processes of plasticity and rewiring? In parallel, it is not too early to examine the efficacy of synapse therapy in more complex invertebrate as well as vertebrate systems. In particular, as we have previously suggested (Rabinowitch et al., 2014), invertebrate innexin gap junction proteins could be used in vertebrate systems, in analogy to our use of vertebrate connexin proteins in *C. elegans*. These ectopic innexins could enable the construction of lateral networks within homologous neuronal populations or even bridge between two distinct populations if expressed in two different cell types. Furthermore, by combining different compatible innexin proteins, more refined features such as rectification could be implemented, enabling the study of genetically inserted synapses in conjunction with brain-machine-brain interfaces, and how they can offer different or combined bypasses for neural rerouting following damage.

Ultimately, we envision a day, in which the toolkit for brain repair will combine a diversity of powerful and complementary methods, including our proposed synapse-based therapy, to enable precise, personalized and optimal treatment of neural damage.

This work was supported by the Israel Science Foundation, grant No. 1465/20 (to IR).

Ithai Rabinowitch*
Department of Medical Neurobiology, IMRIC – Institute for Medical Research Israel-Canada, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel
***Correspondence to:** Ithai Rabinowitch, PhD, ithai.rabinowitch@mail.huji.ac.il.
https://orcid.org/0000-0003-0361-9055 (Ithai Rabinowitch)
Date of submission: February 25, 2021
Date of decision: March 13, 2021
Date of acceptance: April 29, 2021
Date of web publication: July 8, 2021

https://doi.org/10.4103/1673-5374.317964
How to cite this article: *Rabinowitch I (2022) Inserting new synaptic connections into damaged neural circuits: towards synapse therapy? Neural Regen Res 17(2):300-301.*

Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewers: *Bojun Chen, University of Connecticut Medical School, USA; John O'Brien, University of Texas McGovern Medical School, USA.*

References

Choi MK, Liu H, Wu T, Yang W, Zhang Y (2020) NMDAR-mediated modulation of gap junction circuit regulates olfactory learning in C. elegans. Nat Commun 11:1-16.

Ganguly K, Poo MM (2013) Activity-dependent neural plasticity from bench to bedside. Neuron 80:729-741.

Hawk JD, Calvo AC, Liu P, Almoril-Porras A, Aljobeh A, Torruella-Suárez ML, Ren I, Cook N, Greenwood J, Luo L, Wang ZW, Samuel ADT, Colón-Ramos DA (2018) Integration of plasticity mechanisms within a single sensory neuron of C. elegans actuates a memory. Neuron 97:356-367.

Joy MT, Carmichael ST (2021) Encouraging an excitable brain state: mechanisms of brain repair in stroke. Nat Rev Neurosci 22:38-53.

Nava E, Röder B (2011) Adaptation and maladaptation insights from brain plasticity. Prog Brain Res 191:177-194.

Rabinowitch I, Chatzigeorgiou M, Zhao B, Treinin M, Schafer WR (2014) Rewiring neural circuits by the insertion of ectopic electrical synapses in transgenic C. elegans. Nat Commun 5:4442.

Rabinowitch I, Laurent P, Zhao B, Walker D, Beets I, Schoofs L, Bai J, Schafer WR, Treinin M (2016) Neuropeptide-driven cross-modal plasticity following sensory loss in caenorhabditis elegans. PLoS Biol 14:e1002348.

Rabinowitch I, Upadhyaya B, Pant A, Galski D, Kreines L, Bai J (2021) Circumventing neural damage in a C. elegans chemosensory circuit using genetically engineered synapses. Cell Syst doi: 10.1016/j.cels.2020.12.003.

Su F, Xu W (2020) Enhancing brain plasticity to promote stroke recovery. Front Neurol 11:554089.

Szczupak L (2016) Functional contributions of electrical synapses in sensory and motor networks. Curr Opin Neurobiol 41:99-105.

Wechsler LR, Bates D, Stroemer P, Andrews-Zwilling YS, Aizman I (2018) Cell therapy for chronic stroke. Stroke 49:1066-1074.

Wolpaw JR, Millán JDR, Ramsey NF (2020) Brain-computer interfaces: definitions and principles. Handb Clin Neurol 168:15-23.

P-Reviewers: Chen B, O'Brien J; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y