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Ross Harrison's "The Outgrowth of the Nerve Fiber as a Mode of Protoplasmic Movement"

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Nature seems unaware of our intellectual need for convenience and unity, and very often takes delight in complication and diversity.—Santiago Ramón y Cajal, Nobel Prize Lecture, 1906.

Ross Harrison's "*The outgrowth of the nerve fiber as a mode of protoplasmic movement*" is likely the most important paper ever published in the *Journal of Experimental Zoology* (Harrison, '10). It is also among the most important papers published in the field of neuroscience during the first half of the 20th century. In a single stroke Harrison invented the method of tissue culture, and then used it to prove the neuron doctrine.

It is easy to dismiss the misguided ideas that dominated neuroscience at the turn of the 20th century. The field was at war with itself over the most basic of questions: whether the nervous system was composed of neurons. Although the prevailing theories of the time were eventually shown to be spectacularly wrong, they were nevertheless highly attractive to neuroscientists because of their apparent explanatory power. The idea was that the nervous system was not made up of distinct cells, but was a syncytium through which electrical current flowed. Although the syncytial or reticular theory was starting to lose credibility when Harrison published his paper in 1910, it was still widely accepted, and in modified form was to live on as late as the 1940s. It was plainly obvious to many physiologists that there could not be any gaps in the wiring of the nervous system any more than there could be gaps in the wiring of a house. The physics of electric current flow called for continuity. Opposing this view was the great neuroanatomist Santiago Ramón y Cajal, who argued that the nervous system was composed of discrete neurons, and that axons grew toward their targets behind motile structures called growth cones. This controversy raged on in the most public of possible venues: When Golgi and Cajal jointly received the Nobel Prize in Physiology in 1906, the two leading anatomists of the day took diametrically opposed views on the neuron doctrine, in lectures given from the same platform in Stockholm (Ramón y Cajal, '06; Golgi, '06).

The syncytial theory was first proposed by Victor Hensen in 1864 and was later elaborated upon by the embryologist Hans Held. Hensen thought that he had seen fine cytoplasmic threads connecting cells *after* they had divided. What if, he mused, all neurons retained threadlike "plasmodesms" with each other? The nervous system would begin development as a thoroughly interconnected meshwork. Functional wiring would involve deciding which threads mature into axons. Hensen imagined that this would be through *functional validation,* where the threads that linked electrically active neurons would selectively dilate into axons. In this way initially weak cytoplasmic threads would develop into larger axons with stronger current flow. In a single stroke Hensen and Held had solved the problem of how neural connectivity is established during development, how the strength of neural signaling is determined, and how electrical current could flow freely through the nervous system. It was a brilliant theory, but as Cajal would point out in his Nobel lecture, Nature had other ideas.

In addition to Hensen and Held's plasmodesmic theory there were other incorrect but popular views about nervous system organization. For example, Theodor Schwann had proposed that axons were secreted from chains of ensheathing glial cells—the Schwann cells of the periphery and the oligodendrocytes of the CNS. This strange hypothesis attracted many supporters, and still had proponents as late as the 1920s.

The neuron doctrine however, also had impressive support. In addition to Cajal it was defended by the physiologist Charles Sherrington, who argued that neurons made functional contacts at sites that he termed *synapses*. Neurons that were born in isolation and contacted each other during development would use synapses to communicate with each other. The problem of how electrical signals could be conveyed across a synaptic cleft

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was eventually solved by Otto Loewi, Henry Dale, John Eccles, and Bernard Katz, through their characterization of chemical neurotransmission at central and peripheral synapses.

Ross Harrison's insight was that the neuron doctrine could be tested experimentally by using microsurgery to progressively isolate neural tissues in amphibian embryos. In an early study he replaced the embryonic spinal cord with a cylindrical blood clot. The clot was subsequently penetrated by axons growing from the stump of the remaining brain stem, indicating that preformed structures were not needed for axonal growth. In a second experiment Harrison created conjoined twins, fusing an intact embryo to one lacking a nervous system. He found that nerves would grow into the denervated twin from its innervated partner, an observation that was at odds with Hensen's syncytial theory, but consistent with the neuron doctrine (Harrison, '06, '07). In other studies Harrison found that the nervous system could develop when electrical activity was suppressed using anesthetics, a serious blow to the functional validation model. Harrison was using microsurgery and pharmacology to create increasingly abstracted situations to study neural development. It was inevitable that he would try to grow neurons in isolation.

He began by culturing neural explants in hanging drops. This method had been used to maintain amphibian embryos after surgery, and Harrison reasoned that it would also work for isolated neural tissues. The cells lived, but did little else. Success came when he had the extraordinary insight of growing the cells in a protein matrix derived from clotted lymph. This is a technique that is reminiscent of the modern method of growing neural explants in collagen

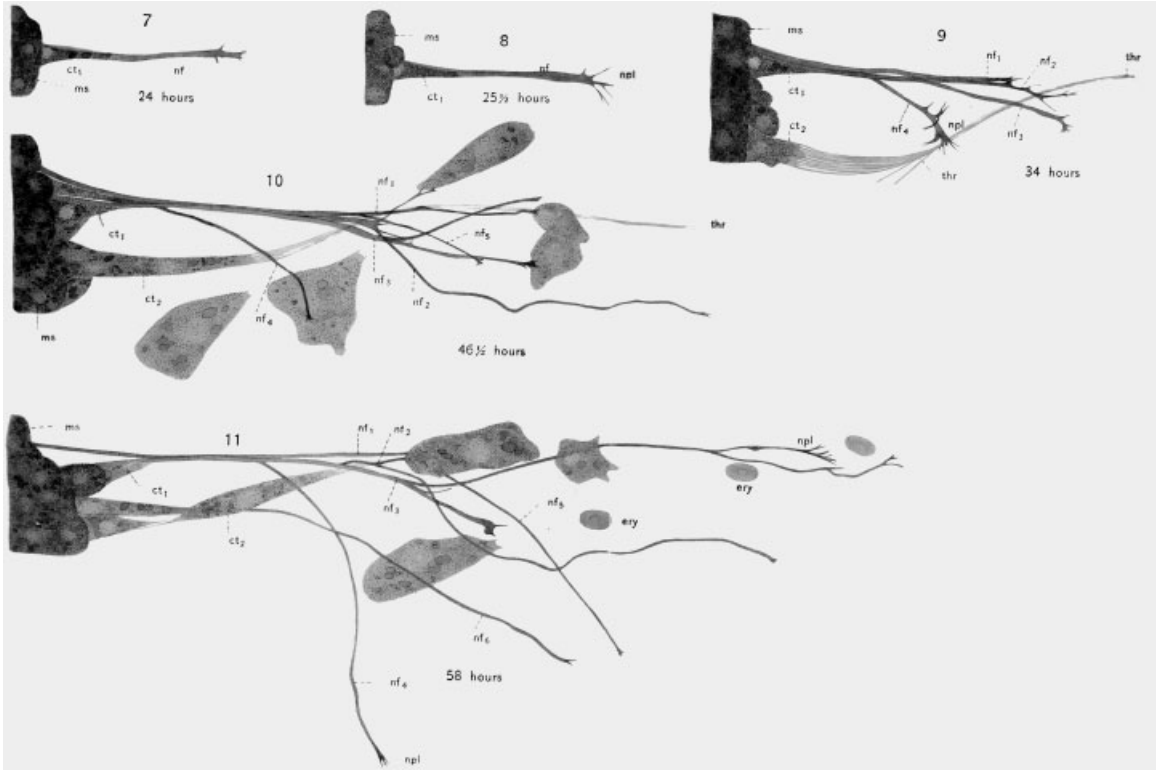

Fig. 1. Reproduction of Figures 7-11 from the original paper (Harrison, '10), showing the outgrowth and defasciculation of axons and growth cones in culture, as drawn by Ross Harrison. The original text:

''7. Apparently single fiber (*nf*) growing out from a pointed cell (*ct1*) which projects from a mass of cells (*ms*) one day after isolation of tissue. April 28, 1908, 12:25 p.m.

8. Same fiber, 2 p.m. Fiber is now clearly double.

9. Same group of fibers. 10.25 p.m. Four distinct fibers (nf_1-nf_4) are now visible. The fibrin filaments (*thr)* shown in this figure were present in the earlier stages but were omitted from the original sketches.

10. Same group. April 29, 11 a.m. nf_5 possibly a branch of nf_1.

11. Same group. 10:30 p.m. Continuation of nf_1 and upper branch of nf_2 , unfortunately left out of sketch. Note migration of cell (*ct2*). Identity of other isolated cells in figs. 10 and 11 is uncertain.''

matrix gels. The key to successful neurite outgrowth was to provide the cells with a *substrate*.

Harrison was the first person to observe live growth cones in time lapse as they extended from the explants (Fig. 1). He noted that motility involved the elongation of processes from the leading edge of the growth cone—a region known today to be rich in filamentous actin, and distinct from a less motile core region continuous with the axon, which is predominantly microtubular. In an uncharacteristically long discussion section to his 1910 paper Harrison tried to make sense of what he had seen. That axons could elongate in vitro proved that they did not need Schwann cells or pre-existing filamentous plasmodesms to form. The neuron doctrine was obviously correct. However, this meant that neuroscience now had to face the ''complication and diversity'' that Cajal had warned of a few years earlier. One reason Hensen and Held's syncytial model was so seductive was that it explained away the problem of neuronal connectivity. Now researchers had to deal with a seemingly intractable problem: if neurons grow to their targets, how do they find their way? To Harrison this meant that the intrinisic ''protoplasmic'' motility of the growth cone was under the control of extrinsic signals from the environment. Harrison thought that axons might be guided by mechanical features in the substrate, or by electrical fields. Unfortunately neuroscience would remain stalled in a speculative mode for nearly 70 years. Most of the key breakthroughs in our understanding of axon guidance have come from molecular biology, which made it possible to identify the extrinsic molecules that guide axons, and to define the intrinsic machinery, and especially the second messenger systems and molecular motors, that propel the growth cone.

In retrospect we recognize that there were elements of truth in the misguided ideas of the early neuroscientists. Schwann and his followers were certainly wrong in imagining that glia make axons, but today we know that glia are essential for axon guidance. The glia are in fact the source of many of the extrinsic cues that Harrison mused were acting on neurons. Hensen was equally wrong in thinking that the nervous system arose as a syncytium, but today we know that many neurons are effectively syncytial, electrotonically fused to each other through gap junctions. Similarly, the idea of functional validation through electrical activity has had a new lease on life as the basis for ''Hebb's hypothesis.'' Electrical coactivation by synaptic partners is today's prevailing model for how neurons refine their connections during development, and how they modify their synaptic strength during learning and memory.

Perhaps it is not surprising that our ''intellectual need for convenience and unity'' allows a plausible idea to mislead a field, as happened to neuroscience nearly a century ago. Attractive ideas sometimes take on a life of their own, and are particularly hard to excise. This adds to the respect we feel for a great experimentalist such as Harrison, who set neuroscience on the right track nearly 95 years ago with a brilliant paper published in the *Journal of Experimental Zoology*.

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