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Reminiscences of time spent with Amiram Grinvald

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Amiram Grinvald is one of the true pioneers of optical measurement of neuronal activity, particularly in the brains of primates. For forty years, he has used intrinsic and extrinsic optical methods to probe the workings of Sherrington's "great raveled knot." In this special section of *Neurophotonics*, others will honor his work by presenting new findings and by detailing many of Amiram's accomplishments. In this brief note, I shall do neither, but instead reminisce about collaborating with the young scientist and about how much fun we had.

I left Larry Cohen's lab at Yale in the fall of 1975, when Amiram had not yet appeared in New Haven. I had established my own lab at the University of Pennsylvania, but Larry's and my work on multiple-site simultaneous optical recording from neurons in the supraesophageal ganglion of the barnacle, *Balanus nubilus* was almost but not quite finished, and I returned to work with Larry at the Marine Biological Laboratory in Woods Hole in the summer of 1976. Amiram had come to Yale during the winter of 1975–76, and although I had met him earlier, when he first came to visit the lab and considered working there, I had never actually worked with him. I remember thinking that it was no surprise that this was the 30-km running champion of Israel!

That summer was an eye-opener! One was immediately struck by Amiram's intelligence, his drive, and his skill in the laboratory. At the end of the summer, I went back to Philadelphia with extensive plans for continued work developing optical recording techniques, and while I followed Amiram's and Larry's work from a distance, we had no direct interaction. By 1982, Amiram had returned to the Weizmann Institute in Rehovot, and Ana Lia Obaid, Hideaki Shimizu, and I had begun efforts to record activity from processes of leech neurons using intracellular staining with voltage-sensitive dyes.[1] The idea was simply to confine staining to the single neuron of interest but to have full spatial/temporal access to its activity, including that occurring in the dendritic spines that were inaccessible to microelectrode penetration. It turned out that Amiram, Ariel Agmon, Lili Anglister, Alan Fine, and Rina Hildesheim, all at the Weizmann, had exactly the same idea,[2] and briefly we found ourselves in competition. However, we quickly decided that it was better to collaborate than to compete, and Amiram invited me to come to Rehovot and work together. In 1984, I spent two-and-a-half weeks at the Weizmann, working with Amiram, Varda Lev-Ram, and Rina.

We had pretty much figured out the requirements for a successful molecular indicator for the purpose of recording membrane potential from *inside* a single cell:

> "[it] should be very sensitive to changes in membrane potential; should act from the inside of the cell; should diffuse readily, or be actively transported throughout the axoplasm of the fine processes of the neuron; should be retained within the cell without appreciable leakage; should not bind selectively to intracellular organelles; and should be without toxic effect. It should also have a large extinction coefficient, a very low quantum yield in aqueous media, but a high quantum yield when bound to the external [plasma] membrane, and, of course, a fast linear response to changes in membrane potential."[3]

Rina synthesized a series of styryl dyes for iontophoretic injection which were designed to meet these criteria, and we evaluated eleven of them. For our purposes, the best turned out to be RH-461, although we cautioned that several dyes should be tested for applicability to other preparations and that "fine tuning" of the molecular properties of the dyes would yield, for each preparation, the best compromise

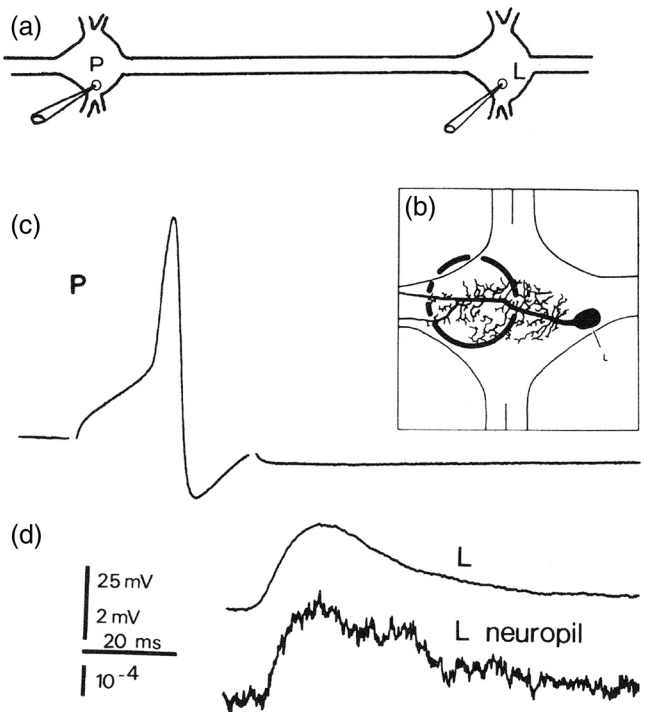

Fig. 1 Optical recording of the excitatory postsynaptic potential in the arborization of the L-motorneuron. (a) The experimental arrangement for interganglionic stimulation and recording. (b) The region of the neuropil that was monitored (after K. Muller and J. McMahon). (c) The microelectrode recordings of the presynaptic action potential in the P-sensory neuron. (d) The postsynaptic potential in the soma of the L-motorneuron (top trace) and the optical recording of the synaptic potential from the neuropil shown in the inset (bottom trace).

between hydrophilicity for fast diffusion, and hydrophobicity for greater voltage sensitivity. Over the several weeks that we worked together, Amiram, Varda, Rina, and I succeeded in recording action potentials from the 3-μm diameter axon of a lateral P (pressure) cell of the leech, *Hirudo medicinalis,* as well from numerous other leech neuronal processes. As we pointed out, the ultimate

> "test of the method of recording from fine neuronal processes by means of intracellular probes is the detection of small synaptic potentials from processes *in the neuropil* [my emphasis]. To accomplish this, we took advantage of the reliable interganglionic synaptic coupling of the lateral P cell to the contralateral L motorneuron [in Hirudo]."

As shown in Fig. 1 (Fig. 5 in Ref. 3), we were also able to obtain optical recordings of the excitatory postsynaptic potential in the arborization of the leech's L-motorneuron, in the midst of a complex neuropil. Thus, early work on intracellular recording of membrane potential in squid giant axons, by injection or perfusion,[4–7] was validated, but on a scale many orders of magnitude smaller.

This work was taken up, and vastly improved, by the Serbian connection of Zecevic, Antic, Popovic, and their collaborators.[8–11] They were able not only to record membrane potentials, using better voltage-sensitive dyes injected intracellularly but also to monitor action potentials and subthreshold events in the dendritic spines of neurons. But that is another story altogether.[12]

This work with Amiram was completed more than three decades ago, but one of the nonscientific memories that has stayed with me for all of this time is of a lunch at a Chinese restaurant in Rehovot. The menu was bilingual, but to my chagrin the two languages were Chinese and Hebrew! Great combination. I recall, with pleasure, ordering the moo shu white meat (pork), much to the consternation of some of the other diners. Itzak Parnas, an important Israeli neuroscientist, and great amateur Chinese cook, subsequently gave his approval!

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