

This Week's Citation Classic

CC/NUMBER 28
JULY 12, 1982

Cowan W M, Gottlieb D I, Hendrickson A E, Price J L & Woolsey T A: The autoradiographic demonstration of axonal connections in the central nervous system. *Brain Res.* 37:21-51, 1972. [Dept. Anat., Washington Univ. Sch. Med., St. Louis, MO and Dept. Ophthalmol., Univ. Washington, Seattle, WA]

In this paper we described a technique for tracing connections in the brain that is based on the incorporation of isotopically labeled amino acids by nerve cells and the subsequent transport of the labeled proteins along their axons where they can be visualized autoradiographically. [The *SCJ*[®] indicates that this paper has been cited in over 745 publications since 1972.]

W. Maxwell Cowan
The Salk Institute
Post Office Box 85800
San Diego, CA 92138

February 26, 1982

"Until the early-1970s, the only effective way to trace connections in the central nervous system (CNS) was to map out the degenerative changes that occur when the region of interest was injured. For many purposes this approach is perfectly adequate and has revealed much of what we know about the connectivity of the brain. However, in many situations the results it gives are difficult to interpret, and it is of only limited use in the developing nervous system. An alternative approach is to label nerve cells with a radioactively labeled amino acid. Since amino acids are incorporated into protein only in the perikarya of nerve cells, and as many of the proteins so labeled are actively transported down axons to their synaptic endings, it is possible to map the distribution of the labeled fibers autoradiographically. A. C. Taylor and P. Weiss¹ had tried this approach in the amphibian visual system. A few years later R. Lasek, B.S. Joseph, and D.G. Whitlock² at the University of Colorado used it effectively to determine the central connections of spinal ganglia. But for some unknown reason (perhaps because it was thought that if labeled amino acids were to be injected directly into the CNS they would spread so widely that it would be impossible to label small, localized populations of nerve cells), apparently no one had tried it in the brain.

"About this time, Anita Hendrickson³ of the University of Washington was exploring the usefulness of axonal transport for studying the central connections of the retina at the electron microscope level. She and I began to study the changes that occur in axonal transport during development. Her finding that some proportion of the radioactively labeled proteins that are rapidly transported down axons is delivered to their syn-

aptic terminals was reported in a seminar she gave in St. Louis in the spring of 1970. This encouraged my colleagues, David Gottlieb, Joel Price, and Tom Woolsey, and me, at Washington University in St. Louis, to see if the same approach could be used in other parts of the CNS. We began by making injections of tritium-labeled amino acids into several regions of the brain whose connections we had previously analyzed with conventional neuroanatomical methods to see if we could reproducibly label the relevant connections and identify the sites in which they terminate. Our first experiments were fairly discouraging. The injections were far too large and resulted in so much spread of isotope that the developed autoradiographs were almost black. Fortunately, one of the systems of connections that was labeled was so distinctively organized that even in those early preparations the underlying pattern of the connections could be recognized. Thereafter, it was simply a matter of scaling down the size of the injections, refining the isotope delivery system, determining the optimum post-injection survival times, and appropriately modifying the autoradiographic procedure. Within a few weeks we had clear evidence in four or five different neuronal systems that the technique could be used to label entire fiber pathways in the CNS and to rather precisely define their modes of termination. We presented some of the results of our work at the annual meeting of the American Association of Anatomists in the spring of 1971. In view of the interest that was aroused, I suggested that we write a paper documenting the use of the method. Hendrickson spent a few days in St. Louis on her way back to Seattle. As I recall, the outline of the paper was written during a picnic in Forest Park across the street from the medical school.

"In retrospect, it seems that the paper was well received because at the time new techniques for tracing pathways in the CNS were sorely needed and the autoradiographic method clearly offered several advantages. It was uncommonly sensitive, it was not complicated by the labeling of fibers of passage or by species and age differences, it lent itself to certain types of developmental and quantitative analyses, and its use had been critically evaluated in a number of neural systems. In addition, since we provided a straightforward and detailed experimental protocol that others could follow, within a relatively short time it was adopted by several laboratories around the world. E. G. Jones and B. K. Hartman have published a recent review in this field."⁴

1. Taylor A C & Weiss P. Demonstration of axonal flow by the movement of tritium-labeled protein in mature optic nerve fibers. *Proc. Nat. Acad. Sci. US* 54:1521-7, 1965.
2. Lasek R, Joseph B S & Whitlock D G. Evaluation of a radioautographic neuroanatomical tracing method. *Brain Res.* 8:319-36, 1968.
3. Hendrickson A. Electron microscopic radioautography: identification of origin of synaptic terminals in normal nervous tissue. *Science* 165:194-6, 1969.
4. Jones E G & Hartman B K. Recent advances in neuroanatomical methodology. *Annu. Rev. Neurosci.* 1:215-96, 1978.