This Week's Citation Classic®

Steinbusch H W M. Distribution of serotonin-immunoreactivity in the central nervous system of the rat—cell bodies and terminals. *Neuroscience* 6:557-618, 1981.
[Department of Anatomy and Embryology, University of Nijmegen, The Netherlands]

The organizational principles of neural pathways that use serotonin as a neurotransmitter were described in this paper. The analysis was based on a novel immunohistochemical method and greatly extended results obtained by Swedish workers using histofluorescence or Canadian studies using autoradiography. [The *SCI*® indicates that this paper has been cited in over 720 publications.]

Serotonergic Neurons as Revealed by Immunohistochemistry

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In the late 1970s three techniques were available to visualize monoaminergic neurons: the formaldehyde-induced fluorescence (FIF), immunofluorescence with antibodies against the synthesizing enzymes, and autoradiographic methods. The FIF-method has a low sensitivity towards serotonin. It yields a yellow fluorescence that is difficult to differentiate from the more intense green fluorescence induced by high concentrations of catecholamines. The immunofluorescence method appeared ineffective for serotonin-synthesizing enzymes. The autoradiographic technique has contributed extensively towards our knowledge about the organization of the serotonergic system.

As a student, I had worked on a project with Albert Verhofstad on the development of antibodies against catecholamines, and during my PhD studies (at the Department of Anatomy and Embryology at the Catholic University of Nijmegen, with Professor Nieuwenhuys as supervisor) I tried to couple proteins directly with different monoamines, using formaldehyde for the conjugation procedures. We were fortunate to observe that formaldehyde was very effective in linking serotonin to a carrier protein. We also learnt that it was of utmost importance to use the same compound as coupling agent and as a fixative.[1] Later, it was shown that glutaraldehyde is much more suitable for catecholamines.[2,3]

One of the most exciting moments at that time was when we saw for the first time through the microscope the abundance of serotonergic nerve fibers in their full clarity. We were overwhelmed by the extent and richness of the serotonergic system in the brain. We decided to send a sample of the antibody to Tomas Hökfelt in Stockholm and asked him if he could confirm our observations. We received a swift and enthusiastic response. We then set out to make a detailed map of the anatomical distribution of the serotonin-immunoreactive neurons, fiber tracts, and terminal fields; this work appeared as the often cited paper. Hökfelt used the new antibody in his study demonstrating for the first time co-localization of serotonin with Substance P and TRH.

In retrospect there were several reasons for the frequent citation of this paper: the increasing interest in the neurobiology of the central serotonergic system; the specificity and sensitivity of the new method; the detailed character of the mapping, performed in the classical neuroanatomical tradition; the possibility of studying serotonergic neurons at the ultrastructural level; and, finally, it constituted a new development in the field of immunohistochemistry, i.e., the visualization of small molecule neurotransmitters (monoamines,[2-5] amino acids,[1] and second messengers[6]) by conjugation with a carrier protein.

1. **Storm-Mathisen J, Leknes A K, Bore A T, Vaaland J L, Edminson P, Haug F-M S & Ottersen O P.** First visualization of glutamate and GABA in neurones by immunocytochemistry. *Nature* 301:517-20, 1983. (Cited 50 times.)
2. **Steinbusch H W M, De Vente J, Wouterlood F G, Berkenbosch F & Bol J G J M.** Immunohistochemical localization of monoamines and cyclic nucleotides. Their application in quantitative immunofluorescence studies and tracing monoaminergic neuronal connections. *Acta Histochem.* 35(Supp.):85-106, 1988.
3. **Steinbusch H W M & Tilders F J H.** Immunohistochemical techniques for light-microscopical localization of dopamine, noradrenaline, adrenaline, serotonin and histamine in the central nervous system. (Steinbusch H W M, ed.) *Monoaminergic neurons: light microscopy and ultrastructure.* Chicester, England: Wiley, 1987. p. 125-66.
4. **Geffard M, Kah O, Onteniente B, Seguela P, La Moal M & Delaage M.** Antibodies to dopamine: radioimmunological study of specificity in relation to immunocytochemistry. *J. Neurochemistry* 42:1593-9, 1984. (Cited 30 times.)
5. **Steinbusch H W M, Verhofstad A A J & Joosten H W J.** Localization of serotonin in the central nervous system by immunohistochemistry: description of a specific and sensitive technique and some applications. *Neuroscience* 3:811-9, 1978. (Cited 330 times.)
6. **DeVente J, Steinbusch H W M & Schipper J.** A new approach to immunocytochemistry of 3',5'-cyclic guanosine monophosphate: preparation, specificity, and initial application of a new antiserum against formaldehyde-fixed 3',5'-cyclic guanosine monophosphate. *Neuroscience* 22:361-73, 1987.

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