# This Week's Citation Classic ®

**Nelson D L, Herbet A, Bourgoin S, Glowinski J & Hamon M.** Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat. *Mol. Pharmacol.* 14:983-95, 1978.
[INSERM U 114, Collège de France, Paris, France]

Direct investigations on serotonin (5-HT) receptors by measuring the specific binding of [$^3$H]5-HT to brain membranes provided the first evidence for the heterogeneity of high affinity binding sites for the indoleamine in the central nervous system. In particular, the extensive degeneration of presynaptic serotonergic fibers and terminals due to an intracerebral administration of 5,7-dihydroxytryptamine in rats was associated with an up-regulation of high affinity 5-HT binding sites in the hippocampus but not in the striatum. [The *SCI*® indicates that this paper has been cited in more than 305 publications.]

## From Painting to Binding

**Michel Hamon**
**INSERM U288**
**Neurobiologie Cellulaire et Fonctionnelle**
**CHU Pitié-Salpêtrière**
**75634 Paris Cedex 13**
**France***

**Until 1976, studies on postsynaptic neurotransmitter receptors in the brain were rather indirect because they mainly consisted of looking for possible alterations in the presynaptic markers of neurotransmitter metabolism following the systemic or intracerebral administration of putative agonists and antagonists. Such studies were based on the hypothesis that stimulation of postsynaptic receptors might trigger the activity of neuronal loop(s) ending with a negative feedback control of the metabolic activity in presynaptic neurons producing the endogenous neurotransmitter acting on these receptors. Although other interpretations of such effects have since been proposed (notably through the involvement of presynaptic autoreceptors),[1] at that time, S. Bourgoin and I were using such protocols for exploring central 5-HT receptors in the laboratory of J. Glowinski, in Paris.**

**Then, two major events changed our way of thinking. First, J.P. Bennett, Jr., and S.H. Snyder,[2] and G. Fillion and coworkers[3] reported that 5-HT receptors could be directly investigated by measuring the specific binding of [$^3$H]5-HT to brain membranes. Second, we stopped working at the bench for one month for painting all the rooms in the laboratory. It was really a fantastic experience as it encouraged everyone in a free and informal exchange of ideas. No doubt physical activity is profitable to brain functions!**

**Indeed after this very stimulating episode, we set up a new research program using binding assays to explore 5-HT receptors in brain membranes, just as Bennett and Snyder, and the Fillion group, published a few weeks before. However, we soon observed that the preparation of membranes was critical as endogenous 5-HT sticks strongly to brain homogenates and may compete with exogenous [$^3$H]5-HT added for binding assays.**

**By using a highly sensitive radioenzymatic assay of 5-HT, we decided to control the removal of the endogenous amine at the various steps for the preparation of brain membranes, which allowed us to set up a relevant protocol yielding brain membranes with undetectable 5-HT entrapped. This protocol has since been adopted by many groups and adapted to the study of other neurotransmitter receptor binding sites.**

**At this stage, D.L. Nelson, a postdoctoral fellow from the University of Colorado, Denver, joined our group, and this was really the starting point of our investigations on central 5-HT receptors. We thus explored the pharmacological and regulatory properties of high affinity 5-HT binding sites in the rat brain and provided the first evidence for their heterogeneity. In particular, Hill plots of the inhibition of [$^3$H]5-HT binding by some agonists and antagonists yielded a slope significantly less than unity, and the extensive degeneration of presynaptic serotonergic fibers and terminals by the intracerebral injection of 5,7-dihydroxytryptamine produced an up-regulation of high affinity 5-HT binding sites in the hippocampus but not in the striatum.**

**Today, the demonstration of 5-HT receptor heterogeneity is definitively achieved with the well-accepted existence of four subtypes of high affinity 5-HT$_1$ receptors (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$) and three classes of low affinity 5-HT receptors (5-HT$_2$, 5-HT$_3$, and 5-HT$_4$). Indeed, the 5-HT$_{1A}$, 5-HT$_{1C}$, 5-HT$_{1D}$, and 5-HT$_2$ receptors have been cloned and sequenced,[4] and the heterogeneity that we reported in 1978 has been clearly understood since 1981 when Nelson and coworkers,[5] back in the US, at the University of Arizona, Tucson, showed that the hippocampus is enriched in 5-HT$_{1A}$ receptors whereas the striatum contains essentially 5-HT$_{1B}$ receptors.**

**In parallel with the increasing number of 5-HT receptor types discovered in the brain, the number of groups involved in this research also has increased considerably, so that people in the "5-HT field" who were meeting once or twice each 10 years in the 1970s have now set up a "Serotonin Club" with annual meetings. The recent evolution of the "5-HT field" has undoubtedly been one of the most spectacular in neurobiology, as only 10 years have passed since the publication of our paper on the possible heterogeneity of central 5-HT receptors and the development of highly selective ligands of the various 5-HT receptor subtypes with therapeutic value for the treatment of anxiety, depression, and migraine.**

1. **Vergé D, Daval G, Patey A, Gozlan H, El Mestikawy S & Hamon M.** Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT$_{1A}$ subtype. *Eur. J. Pharmacol.* 113:463-4, 1985. (Cited 110 times.)
2. **Bennett J P & Snyder S H.** Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors. *Mol. Pharmacol.* 12:373-89, 1976. (Cited 580 times.)
3. **Fillion G, Fillion M B, Spirakis C, Bahers J M & Jacob J.** 5-hydroxytryptamine binding to synaptic membranes from rat brain. *Life Sci.* 18:65-74, 1976. (Cited 70 times.)
4. **Hartig P R.** Molecular biology of 5-HT receptors. *Trends Pharmacol. Sci.* 10:64-9, 1989. (Cited 15 times.)
5. **Pedigo N W, Yamamura H I & Nelson D L.** Discrimination of multiple [$^3$H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* 36:220-6, 1981. (Cited 425 times.)

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