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Pedigo N W, Jr., Yamamura H I & Nelson D L. Discrimination of multiple 'H-5HT binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **36**:220-6, 1981.
[University of Arizona Health Sciences Center. Tucson. AZ]

Inhibition-curve analysis for the neuroleptic, spiperone, provided the first direct evidence for multiple, high-affinity serotonin (5HT) binding sites. Spiperone showed 3,000-fold selectivity for a subpopulation of ^{3}H-5HT sites in rat brain, later designated $5HT_{1a}$ and $5HT_{1b}$ receptor subtypes, based upon their high- or low-affinity for certain neuroleptic drugs. [The *SCI*® indicates that this paper has been cited in more than 530 publications.]

5HT Binding Reveals New Receptors

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The pioneering work of S.H. Snyder and his many graduate and postdoctoral students, including H.I. Yamamura, established the utility of radioligand binding assays in characterizing neurotransmitter receptors. This simple but powerful technique stimulated an explosion of research into receptor regulation, drug design, and receptor dysfunction in human disease. Our use of ^{3}H-5-hydroxytryptamine (5HT) binding to identify novel subtypes of the serotonin receptor is but one example of the fundamental new discoveries made using this approach. One reason for the high number of citations of this paper is the expanding role of 5HT in human physiology, pathology, and behavior.[1]

The cooperative blending of researchers with different backgrounds and interests was crucial in this work. Yamamura was already a successful young investigator at the University of Arizona when I came to his lab in 1977. One of the first projects I joined was the study of brain dopamine receptors using the newly described Hgand^{3}H-spiperone. Initially thought to be highly selective for dopamine receptors, J.E. Leysen and colleagues[2] subsequently showed that ^{3}H-spiperone bound to SHT, not dopamine, receptors in brain regions other than striatum. This was confirmed in our lab where we noted two distinct ^{3}H-spiperone binding sites in striatum and frontal cortex, as well as regional differences in competition for these sites by 5HT and several antipsychotic drugs.[3]

At about this time, D.L. Nelson was completing his productive postdoctoral tenure under M. Hamon in the laboratory of J. Glowinski at the College de France in Paris. Hamon directed a research effort devoted to the study of brain serotonergic systems, including use of a radioligand binding technique adapted from J.P. Bennett and Snyder.[4] In their characterization of ^{3}H-5HT binding sites in rat forebrain, Nelson et al. noted several compounds that produced competition curves with slopes less than unity, suggesting either negative cooperativity or heterogeneity of serotonin receptors.[3] One such drug was the neuroleptic, spiperone.

The fusion of these diverse research endeavors started in the fall of 1979, when Nelson joined the University of Arizona as a junior faculty member. Yamamura generously offered temporary use of his facilities and introduced Nelson to his students. Our collaboration began when I learned ^{3}H-5HT binding from Nelson and we began to assess neuroleptic competition curves in discrete regions of rat brain.

The data we obtained were striking. Competition curves for spiperone plateaued for three orders of magnitude, indicating discrimination of multiple $5HT_1$ sites by this neuroleptic. Publishing this conclusion produced what is now a *Citation Classic*®. Although there was considerable resistance to the possibility of $5HT_1$ receptor subtypes, the seminal work by S.J. Peroutka and Snyder,[6] which first classified discrete5HTi and $5HT_2$ receptors, had established precedent for multiple serotonin receptors.

The enthusiasm, innovation, and dedication which prevailed in Yamamura's lab fired the crucible for this collaborative research effort. Although it is impossible to recognize the contributions of all involved, this work could not have been done without the help of three colleagues: J.Z. Fields, T.D. Reisine, and F. J. Ehlert.

Today, there are at least 6, and possibly as many as 15, serotonin receptor subtypes identified by pharmacological, receptor binding, and molecular biological techniques. Designing new, selective drugs at these sites and understanding their actions and potential therapeutic benefits represent a compelling, new challenge for modern pharmacologists.

1. **Osborne N N** *&* **Hamon M. N***euronal serotonin.* Chichester. England: Wiley. 1988. 555 p.
2. **Leysen J E, Niemegeers C J E, Tollenaere J P & Laduron P M.** Serotonergic component of neuroleptic receptors. *Nature* 272: 168-71, 1978. (Cited 545 times.)
3. **Pedigo N W, Jr.. Reisine T D. Fields J Z & Yamamura H I.** ^{3}H-spiroperidol binding to two receptor sites in both the corpus striatum and frontal conex of rat brain. *Eur. J. Pharmacol.* 50:451-3. 1978. (Cited 105 times.)
4. **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 620 times.)
5. **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-dihydroxy-tryplamine administration in the rat. *Mol. Pharmacol.* 14:983-95. 1978. (Cited 350 times.) [See also: **Hamon M.** From painting to binding. Citation Classic. *Current Contents®/Life Sciences* 34(12):9, 25 March 1991.]
6. **Peroutka S J & Snyder S H.** Multiple serotonin receptors: differential binding of (^{3}H)5-hydroxytryptamine. (^{3}H)lysergic acid diethylamide and (^{3}H)spiroperidol. *Mol. Pharmacol.* 16:687-99. 1979. (Cited 1.270 times.)

Received April 7. 1993