nuclear yellow seems to diffuse more than fast blue or granular blue.
9. P. S. Goldman and W. J. H. Nauta, *Brain Res.* **122**, 393 (1977).
10. R. D. Lund, D. E. Mitchell, G. H. Henry, *ibid.* **144**, 169 (1978).
11. C. Shatz, *J. Comp. Neurol.* **173**, 497 (1977).
12. G. M. Innocenti and D. O. Frost, *Nature (London)* **280**, 231 (1979).
13. K. Fleischhauer and H. Wartenberg, *Z. Zellforsch. Mikrosk. Anat.* **83**, 568 (1967); J. Seggie and M. Berry, *Exp. Neurol.* **35**, 215 (1972).
14. P. I. Yakovlev, in *Regional Development of the Brain in Early Life*, A. Minkowski, Ed. (Blackwell, Oxford, 1967), p. 3.
15. H. P. Lipp and H. Schwegler, *Neurosci. Lett.* **20**, 49 (1980).
16. While this manuscript was in preparation, kittens DL15 and DL18 received, respectively, three and two injections of 5 percent fast blue in areas 17 and 18 on postnatal day 6. These animals were reinjected in areas 17 and 18 with 2 percent nuclear yellow on postnatal day 50 (DL15, two injections, 0.5 μl each) and 43 (DL18, three injections, 0.5 μl each). The results were similar to those described for DL7.
17. Supported by the Swiss National Science Foundation grants 3.319-0.78 and 3.628-0.80. I am grateful to F. Amaudruz, P. G. H. Clarke, D. O. Frost, M. Gaillard, L. Garey, M. Gissler, C. Vaclavik, and H. Van der Loos for their help at different stages of this work. Fast blue and granular blue were kindly provided by O. Dann, Institut für Pharmazie und Lebensmittelchemie der Friedrich-Alexander-Universität, D-8520 Erlangen.

10 September 1980; revised 29 December 1980

## Two Distinct Central Serotonin Receptors with Different Physiological Functions

Abstract. *Two distinct serotonin (5-hydroxytryptamine) receptors designated serotonin 1 and serotonin 2 bind tritium-labeled serotonin and tritium-labeled spiroperidol, respectively. Drug potencies at serotonin 2 sites, but not at serotonin 1 sites, predict their effects on the "serotonin behavioral syndrome," indicating that serotonin 2 sites mediate these behaviors. The limited correlation of drug effects with regulation by guanine nucleotides suggests that serotonin 1 sites might be linked to adenylate cyclase. Drug specificities of serotonin-elicited synaptic inhibition and excitation may reflect serotonin 1 and serotonin 2 receptor interactions, respectively.*

Serotonin (5-hydroxytryptamine) elicits both synaptic inhibition and excitation in the brain (*1–4*) and plays a role in numerous behavioral systems. Increased concentrations of serotonin in the brain result in a behavioral hyperactivity syndrome with head twitching, resting tremor, and hypertonicity (*5, 6*). A serotonin-sensitive adenylate cyclase in brain homogenates may mediate some serotonin responses (*7–9*). Serotonin receptors in the brain can bind tritium-labeled lysergic acid diethylamide (LSD) (*10*), serotonin (*11*), and spiroperidol (*12*). Recently, we demonstrated that [$^3$H]serotonin and [$^3$H]spiroperidol bind to physically distinct populations of serotonin receptors in the brain, whereas [$^3$H]LSD binds to both of these sites with similar affinity (*13*). The receptors that bind serotonin (designated serotonin 1 receptors) are regulated by guanine nucleotides, whereas sites that bind spiroperidol (serotonin 2 receptors) are not influenced by nucleotides (*14*). By comparing drug affinities for serotonin binding sites with drug potencies in physiologic functions, we now provide evidence that serotonin 1 receptors might be related to the serotonin-sensitive adenylate cyclase, whereas the behavioral syndrome resulting from central serotonin stimulation is mediated by serotonin 2 receptors. Moreover, drug specificities of serotonin-elicited synaptic inhibition and excitation reflect serotonin 1 and serotonin 2 receptor interactions, respectively.

Behavioral hyperactivity follows central serotonin stimulation with drugs such as 5-hydroxytryptophan, tryptophan plus a monoamine oxidase inhibitor, D-LSD, and quipazine (*5, 6*). The syndrome includes resting tremor, hindlimb abduction, splayed hindlimbs, snake tail, side-to-side head weaving, and head twitching. The head twitch is an easily monitored and reliable measure of the presence of the syndrome. Accordingly, we evaluated the potencies of a wide range of drugs in inhibiting hydroxytryptophan-elicited head twitches in mice (Fig. 1, A and B). A number of drugs—including classical serotonin antagonists such as cyproheptadine and metergoline, neuroleptics such as spiroperidol and pipamperone, and antidepressants (*15*) such as mianserin and amitriptyline—are capable of preventing head twitches induced by hydroxytryptophan. Although amitriptyline and mianserin are potent inhibitors of the induced head twitches, other antidepressants, such as desipramine and iprindole, are much weaker; thus, serotonin blockade is not likely to account for the therapeutic efficacy of these latter drugs. The butyrophenone neuroleptic spiroperidol is the most potent inhibitor of hydroxytryptophan-induced head twitches; a 50 percent inhibitory dose ($ID_{50}$) of 0.18 μmole per kilogram of body weight also inhibited apomorphine-induced stereotypy (*16*), a dopamine-linked behavior. Pipamperone ($ID_{50}$ = 1.73 μmole/kg) and chlorpromazine ($ID_{50}$ = 2.41 μmole/kg), on the other hand, are more than 100 and 8 times, respectively, more potent inhibitors of the serotonin than of the dopamine behavioral syndrome. Conversely, haloperidol ($ID_{50}$ = 4.26 μmole/kg) is approximately 10 times more potent in blocking the dopamine than the serotonin syndrome. Thus, while neuroleptic drugs are potent antagonists of the hydroxytryptophan syndrome, these effects are not mediated through the dopamine system.

Drug affinities for serotonin 1 receptors labeled by [$^3$H]serotonin do not correlate with head twitch blockade (Fig. 1A). By contrast, drug potencies in blocking induced head twitches closely correlate with affinities for serotonin 2 receptors labeled by [$^3$H]spiroperidol ($r$ = .98, $P$ < .001). Since in vivo blockade of head twitches correlates with in vitro receptor affinity, the drugs tested presumably differ little in their ability to reach target sites in the brain.

Several lines of evidence suggest that

Table 1. Comparison of drug potencies at serotonin receptors with physiological actions, means ± standard errors of three to six experiments, each performed in triplicate. The influences of drugs on microiontophoretic serotonin effects are from the literature (*1–4, 23–25*).

| Serotonin-related drugs | Affinity for serotonin 1 receptors: $K_i$ versus [$^3$H]-serotonin (n*M*) | Affinity for serotonin 2 receptors: $K_i$ versus [$^3$H]-spiroperidol (n*M*) | Effects on serotonin synapses | |
|---|---|---|---|---|
| | | | Inhibition | Excitation |
| Serotonin | 2.7 ± 0.55 | 2700 ± 400 | Agonist (*1–4, 23–25*) | Agonist (*1–4*) |
| D-LSD | 9.8 ± 1.0 | 8.9 ± 1.7 | Agonist (*3*) | Antagonist (*1–3*) |
| Lisuride | 6.2 ± 1.1 | 11 ± 3.6 | Agonist (*25*) | |
| Metergoline | 9.9 ± 2.1 | 2.1 ± 0.67 | Antagonist (*24*) | Antagonist (*4*) |
| Cyproheptadine | 1100 ± 120 | 2.4 ± 0.21 | No effect (*4*) | Antagonist (*4*) |
| Bromo-LSD | 100 ± 15 | 2.5 ± 0.41 | No effect (*1*) | Antagonist (*1*) |
| Methysergide | 150 ± 17 | 3.1 ± 0.91 | No effect (*1, 2, 4*) | Antagonist (*1, 2, 4*) |
| Methiothepin | 300 ± 27 | 4.1 ± 0.67 | No effect (*4*) | Antagonist (*4*) |
| Cinanserin | 1800 ± 540 | 15 ± 3.1 | No effect (*1, 4*) | Antagonist (*1, 4*) |


0036-8075/81/0515-0827$00.50/0 

serotonin 1 receptors are associated with the serotonin-sensitive adenylate cyclase. Serotonin 1 but not serotonin 2 receptor binding is regulated by guanine nucleotides (*14*), a phenomenon commonly reflecting a linkage to adenylate cyclase (*17*). Though guanine nucleotides are less potent in regulating serotonin receptors than some others, the specificity of the effect is the same as at other transmitter and hormone receptors. Of course some receptors regulated by guanosine 5-triphosphate have not been clearly linked to a cyclase (*18*). Kainic acid lesions of rat corpus striatum elicit parallel declines in serotonin 1 receptor binding and serotonin cyclase (*19*). Finally, drug potencies as inhibitors of the serotonin cyclase correlate with their affinities for serotonin 1 receptors (Fig. 1C) ($r = .92$, $P < .01$). By contrast, cyclase inhibition does not correlate significantly with drug affinity for serotonin 2 receptors (Fig. 1D). However, differences in ontogenetic development and drug sensitivity argue that serotonin 1 receptors and the serotonin-sensitive cyclase are distinct entities (*20*). The possible existence of multiple [$^3$H]serotonin-labeled receptors (*21*) might account for these these discrepancies as may the existence of distinct serotonin-sensitive adenylate cyclase complexes with different affinities for various drugs (*8, 9*).

Differential neurophysiologic actions

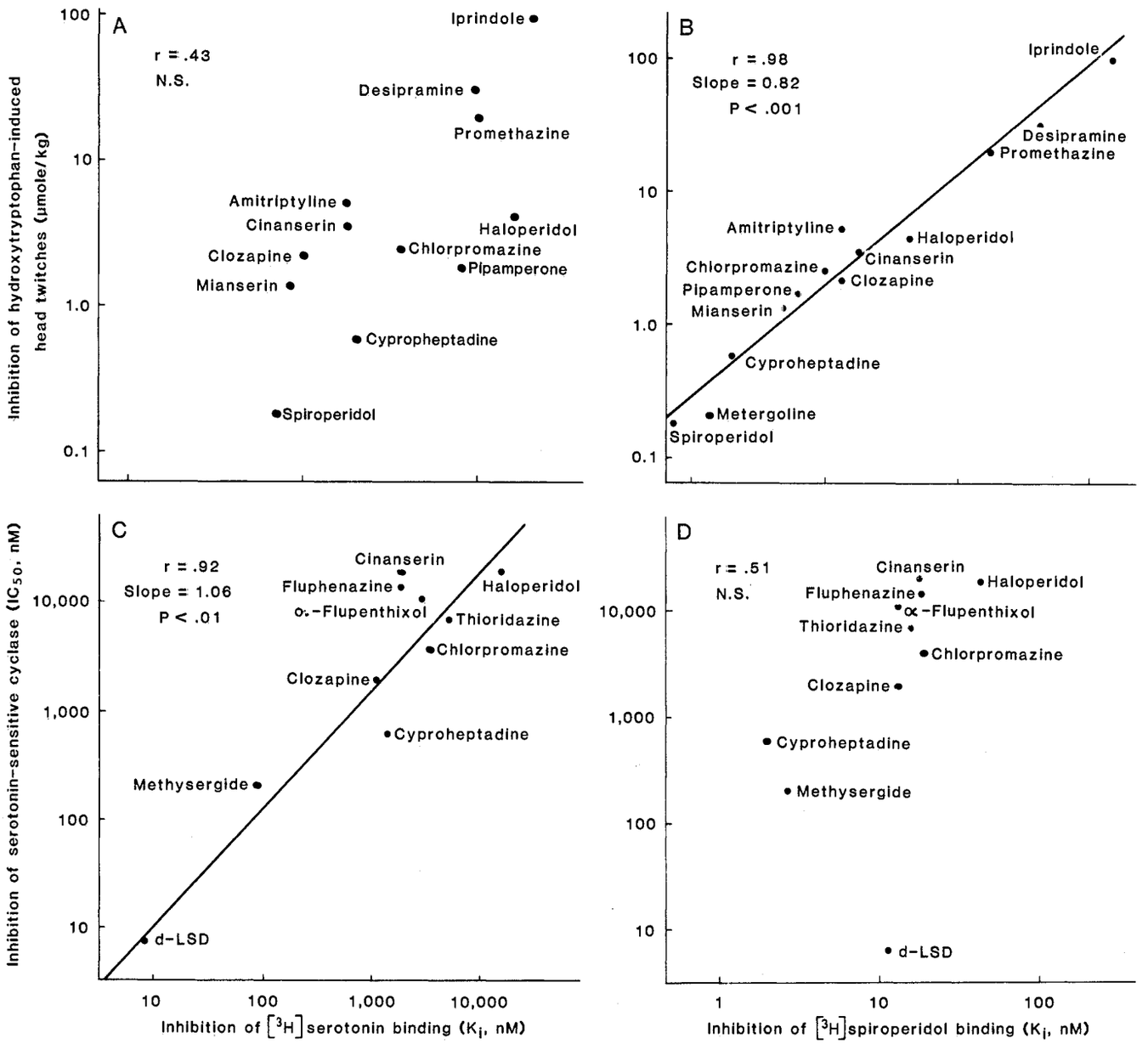


Fig. 1. Comparison of drug affinities at two serotonin receptors with drug inhibition of serotonin-related behavior. Binding assays were performed on cortical membrane homogenates prepared from freshly decapitated male Sprague-Dawley rats as described previously (*13, 14*). Behavioral studies were performed on mice after the injection of 300 mg of 5-hydroxytryptophan 30 minutes before a 2-minute observation period. Various drugs were injected 1 hour before the observation period (*6*). The number of head twitches were recorded at four or five concentrations, and $ID_{50}$ values were calculated for log-probit analysis. Slopes of these plots were parallel for all drugs. Standard errors did not exceed 15 percent of mean values. Drug inhibition values ($IC_{50}$'s of serotonin-sensitive adenylate cyclase) were derived from previously published reports (*8, 9*). Depending on brain region, species, and experimental conditions, absolute potencies of drugs vary at the serotonin-sensitive cyclase (*7–9*). Data depicted include results from all published studies on drugs at the serotonin-sensitive adenylate cyclase (*7–9*). A similar high correlation with affinities for [$^3$H]serotonin binding sites occurs regardless of the species and experimental conditions. Data were statistically compared by linear regression analysis.

of serotonin may also reflect discrete effects at serotonin 1 or serotonin 2 receptors (Table 1). Synaptic excitation but not inhibition by iontophoretically applied serotonin is antagonized by drugs such as cyproheptadine, bromo-LSD, methysergide, and cinanserin, which are 40 to 400 times more potent at serotonin 2 than at serotonin 1 receptors. Lisuride, LSD, and metergoline, which influence iontophoretic serotonin inhibition, are 15 to 200 times more potent at serotonin 1 receptors than the peripheral serotonin antagonists, which fail to inhibit serotonin. Metergoline, which blocks both inhibition and excitation of serotonin, displays low nanomolar $K_i$ values for both types of receptors. Since iontophoretic results are only qualitative, one cannot establish numerical correlations with drug affinities for receptor binding sites or behavioral potencies.

The data suggest that serotonin inhibition and excitation are mediated by serotonin 1 and serotonin 2 receptors, respectively. The excitatory actions of serotonin may reflect a facilitation of excitatory influences of other substances such as acetylcholine and glutamate (*22*). Since the serotonin behavioral syndrome appears to involve serotonin 2 receptors, it may reflect excitatory synaptic actions of central serotonin. The relationship between serotonin 1 receptors and both the serotonin cyclase and neural inhibition is less clear than the link of serotonin 2 sites and excitation. A major problem in evaluating serotonin 1 receptor binding, the serotonin-sensitive adenylate cyclase, and iontophoretic serotonin inhibition is the lack of specific, high-affinity antagonists. Whereas cyproheptadine has nanomolar affinity for serotonin 2 sites and a 500-fold predilection for serotonin 2 over serotonin 1 receptors, no such selective antagonist has yet been described for serotonin 1 receptors, the serotonin cyclase, or serotonin-mediated inhibition.

STEPHEN J. PEROUTKA
RICHARD M. LEBOVITZ
SOLOMON H. SNYDER*

*Departments of Neuroscience, Pharmacology and Experimental Therapeutics,* and *Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

### References and Notes

1. M. H. T. Roberts and D. W. Straughan, *J. Physiol. (London)* **193**, 269 (1967).
2. R. J. Boakes, P. B. Bradley, I. Briggs, A. Dray, *Br. J. Pharmacol.* **40**, 202 (1970).
3. G. J. Bramwell and T. Gonye, *ibid.* **48**, 3578 (1973); H. J. Haigler and G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* **188**, 688 (1974).
4. H. J. Haigler and G. K. Aghajanian, *J. Neural Transm.* **35**, 257 (1974).
5. D. G. Grahame-Smith, *J. Neurochem.* **18**, 1053 (1971); B. L. Jacobs, *Life Sci.* **19**, 777 (1976); R. S. Sloviter, E. G. Drust, J. D. Connor, *J. Pharmacol. Exp. Ther.* **206**, 339 (1978).
6. S. J. Corne, R. W. Pickering, B. T. Warner, *Br. J. Pharmacol.* **20**, 106 (1963).
7. K. Von Hungen, S. Roberts, D. F. Hill, *Brain Res.* **84**, 257 (1975).
8. A. Enjalbert, S. Bourgoin, M. Hamon, J. Adrien, J. Bockaert, *Mol. Pharmacol.* **14**, 2 (1978).
9. G. Fillion, J. C. Rouselle, D. Beaudoin, P. Pradelles, M. Goiny, F. Dray, J. Jacob, *Life Sci.* **24**, 1813 (1979).
10. J. L. Bennett and G. K. Aghajanian, *ibid.* **15**, 1935 (1974); J. P. Bennett, Jr., and S. H. Snyder, *Brain Res.* **94**, 523 (1975).
11. J. P. Bennet, Jr., and S. H. Snyder, *Mol. Pharmacol.* **12**, 373 (1976).
12. J. E. Leysen, C. J. E. Niemegers, P. J. Tollenaere, P. M. Laduron, *Nature (London)* **272**, 163 (1978); I. Creese and S. H. Snyder, *Eur. J. Pharmacol.* **49**, 201 (1978).
13. S. J. Peroutka and S. H. Snyder, *Mol. Pharmacol.* **16**, 687 (1979).
14. S. J. Peroutka, R. M. Lebovitz, S. H. Snyder, *ibid.*, p. 700.
15. K. Fuxe, S. Ogren, L. Agnati, J. A. Gustafsson, G. Jonsson, *Neurosci. Lett.* **6**, 339 (1977).
16. I. Creese, D. R. Burt, S. H. Snyder, *Science* **192**, 481 (1976).
17. M. Rodbell, M. C. Lin, Y. Salomen, C. Londos, J. P. Harwood, B. R. Martin, M. Rendell, M. Berman, *Adv. Cyclic Nucleotide Res.* **5**, 3 (1975); M. E. Maguire, E. M. Ross, A. G. Gilman, *ibid.* **8**, 1 (1977).
18. S. H. Snyder and R. R. Goodman, *J. Neurochem.* **35**, 5 (1980).
19. G. Fillion, D. Beaudoin, J. C. Rousselle, J. M. Deniau, M. P. Fillion, F. Dray, J. Jacob, *ibid.* **33**, 567 (1979).
20. M. Hamon, D. L. Nelson, A. Herbet, J. Glowinski, in *Receptors for Neurotransmitters and Peptide Hormones*, G. Pepeu, M. J. Kuhar, S. J. Enna, Eds. (Raven, New York, 1980), p. 223; D. L. Nelson, A. Herbet, A. Enjalbert, J. Bockaert, M. Hamon, *Biochem. Pharmacol.* **18**, 2445 (1980); D. L. Nelson, A. Herbet, J. Adrien, J. Bockaert, M. Hamon, *ibid.*, in press.
21. D. L. Nelson, N. W. Pedigo, H. I. Yamamura, *J. Physiol. (Paris)*, in press.
22. R. B. McCall and G. K. Aghajanian, *Brain Res.* **169**, 11 (1979); C. P. VanderMuelen and G. K. Aghajanian, *Nature (London)*, in press.
23. G. K. Aghajanian, H. J. Haigler, F. E. Bloom, *Life Sci.* **11**, 615 (1972).
24. B. S. R. Sastry and J. W. Phillis, *Can. J. Physiol. Pharmacol.* **55**, 130 (1977).
25. M. A. Rogawski and G. K. Aghajanian, *Life Sci.* **24**, 1289 (1979).
26. Supported by PHS grant DA-00266 and grants of the McKnight and John A. Hartford foundations. S.J.P. is a recipient of medical scientist training program grant 5T32GM07309 from the National Institutes of Health. S.H.S. is a recipient of PHS research scientist development award DA-00074. We thank D. C. Hanks for manuscript preparation.
* To whom requests for reprints should be sent.

11 August 1980; revised 21 October 1980

## Rod-Cone Interaction in the Distal Human Retina

Abstract. *During the rod-isolated phase of dark adaptation, b-wave implicit time of the human cone electroretinogram increased exponentially with a time constant corresponding to that for the regeneration of rhodopsin. In the presence of different photopically equated short-wave backgrounds, cone b-wave implicit time varied inversely with the scotopic brightness of the background. Taking into account the origin of the b wave, these measurements support the idea of a rod effect on cone function in the distal human retina.*

Convergence of rod and cone signals in the human visual pathway has been suggested from both psychophysical and electrophysiological studies. For example, psychophysical measurements have shown that a background light that is above rod threshold, but below cone threshold, can raise cone increment threshold (*1*), elevate dark-adapted cone threshold (*2*), and reduce the brightness of cone-detected stimuli (*3*). Interaction between rod- and cone-generated signals has been observed electrophysiologically in single-unit recordings from ganglion cells and the optic nerve of the rhesus monkey (*4*) and presumably occurs also in human ganglion cells and optic nerves. Intracellular recordings from the cat have demonstrated that light stimulation of both rods and cones produces a greater hyperpolarization of cones (*5*), cone-type horizontal cells (*5, 6*), and cone bipolars (*7*) than does stimulation of cones alone. Electron microscopic studies have revealed gap junctions between rod spherules and cone pedicles in humans (*8, 9*) and macaque monkeys (*9, 10*), raising the possibility of functional confluence in the outer plexiform layer of the retina. The present study demonstrates that rod-cone interaction can be detected in the b wave of the human electroretinogram (ERG) and, therefore, originates in the retina distal to ganglion cells.


Fig. 1. Dark-adapted ERG's from a normal observer in response to the 640-nm stimulus and a photopically matched 500-nm flash. Each tracing begins at stimulus onset and is an average of 16 responses. The lower response is generated by cones alone, lacking the slow b-wave component of the upper response (arrow) characteristic of the rod system. A pure cone ERG to the 640-nm stimulus was seen also in a second normal observer. The three remaining normal subjects showed in addition a very late, small rod oscillation to this stimulus (*12*).


0036-8075/81/0515-0829$00.50/0 