

increasing uniformity, earliness, and yield of monoecious as well as gynoe-cious cultivars.

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#### References and Notes

1. V. A. Tiedjens, *J. Agr. Res.* **36**, 721 (1928).
2. J. P. McCollum, *Cornell (N.Y.) Agr. Exp. Sta. Mem.* **163**, 1 (1934).
3. L. M. Pike and C. E. Peterson, *Euphytica* **18**, 101 (1969).

4. G. Schneider, *Annu. Rev. Plant Physiol.* **21**, 499 (1970).
5. H. Harada, *Naturwiss.* **54**, 19 (1967).
6. The IT 3456, kindly provided by E. Merck AG, Darmstadt, Germany, was applied in 5 percent ethyl alcohol with 0.1 percent of the surfactant Tween 20 by spraying foliage to the runoff point.
7. R. W. Robinson, S. Shannon, M. D. de la Guardia, *BioScience* **19**, 141 (1969).
8. Aqueous solution of formulation 68-240 of Ethrel (125 ppm), provided by AmChem Products, Ambler, Pa., was applied at three weekly intervals commencing at the three-leaf stage.
9. Approved by the director of the New York State Agricultural Experiment Station as Journal Article No. 1843.

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## Serotonin: Two Different Inhibitory Actions on Snail Neurons

**Abstract.** Serotonin (5-hydroxytryptamine) inhibits snail neurons through two different mechanisms. Whereas on some cells it increases selectively the membrane permeability to chloride ions thus giving rise to a net influx of this ion, on other neurons it increases the permeability to potassium ions causing a net potassium efflux. The serotonin receptors involved in these two inhibitions are different; they also differ from the receptors involved in the excitatory action of serotonin previously described in snail neurons.

The function of serotonin [5-hydroxytryptamine (5-HT)] in the nervous system of both vertebrates and invertebrates is not fully known. A role as a synaptic transmitter has been postulated since the compound was first detected in these tissues (1). Its localization in neurons and synaptic endings (2) makes this hypothesis likely, but no crucial evidence linking 5-HT to a specific synapse has been obtained.

In gastropod mollusks, 5-HT has also been detected in nerve cells (3, 4) and it has been found recently to be taken up by nerve endings (5). Some gastropod neurons may be depolarized and excited by serotonin (6) through a selective increase of their membrane permeability to Na<sup>+</sup> (7). The response to 5-HT iontophoretically applied to these snail neurons can be blocked by concentrations (lower than 10<sup>-5</sup> g/ml) of *d*-tubocurarine (dTC), bromolysergic acid, lysergic acid diethylamide (LSD 25), tryptamine, and 5-HT (6-8). In spite of being blocked by dTC, the receptors to 5-HT of these cells (which will be called 5-HT A-receptors) appear to be different entities from receptors to acetylcholine (8). Neurons endowed with the A-receptors showed slow excitatory synaptic potentials (EPSP) which could be blocked by the same drugs which inactivate the A-receptors (7).

Recently Cottrell and Osborne (4) reported that the giant neuron of the metacerebral ganglion (GNMG) of the snail *Helix pomatia* appears to contain

5-HT. This neuron seems to synapse with another neuron located in the buccal ganglion which was depolarized and excited by low concentrations of 5-HT. Direct stimulation of the GNMG evoked EPSP's in the buccal ganglion neuron which were either blocked (9) or enhanced (10) by LSD 25. All these data could suggest that 5-HT is involved in the transmission of excitation to neurons endowed with the A-receptors.

I have recently observed that on two other groups of snail neurons, identifiable by both their localization and electrophysiological properties, 5-HT evoked inhibition by two different mechanisms; in a group of these cells 5-HT caused an influx of Cl<sup>-</sup>, whereas

Table 1. Effect of changes in the external concentration of Cl<sup>-</sup> or K<sup>+</sup> on the *E*<sub>5-HT</sub> of the responses to 5-HT of the neurons of the left pallial ganglion group. The values given in parentheses are the means and the standard errors.

Control	<i>E</i> <sub>5-HT</sub> (mv)	
	0 mM Cl <sup>-</sup>	15 mM K <sup>+</sup>
Normal saline		
-55	0	-55
-53	-8	-53
-54	-2	-56
-56	-2	-56
-59	-2	-54
-58	0	-57
-58	-1	-52
(-56.1 ± 0.74) (-2.3 ± 1.0)* (-54.7 ± 0.59)†		

\* The mean change between this value and the control in normal saline is statistically significant (*P* < .001). † The mean change between this value and the control in normal saline is not statistically significant (*P* > .05).

in the other group 5-HT produced an outflux of K<sup>+</sup>.

Central ganglia of the snail *Helix aspersa* were isolated and placed in a chamber containing a suitable saline solution (11). The naked somata of the neurons of the abdominovisceral ganglionic mass were impaled with double-barreled micropipettes filled with either KCl or potassium citrate. One of the barrels was connected to a d-c set for recording; the other barrel was used for driving the membrane potential to desired levels by passing currents across the cell membrane. Serotonin was applied iontophoretically to the neuronal somata from micropipettes filled with a 0.15M solution of a complex of 5-HT and creatine sulfate at a pH of 3.2. Blocking drugs were diluted in the saline and applied to the preparation by perfusion. All experiments were performed at room temperature (20° to 24°C).

Figure 1 shows the hyperpolarization caused by 5-HT when applied to one cell of a group of neurons located in the right pallial ganglion. These neurons are D neurons (12) presenting low resting potentials (-35 to -40 mv) and regular spike discharge. Long lasting (800 to 1000 mv) and relatively high intensity (5 × 10<sup>-7</sup> amp) iontophoretic currents were necessary to cause this inhibition, probably because the receptors are located far from the soma. Replacement of the Cl<sup>-</sup> content of the saline by sulfate ions affected neither the polarity nor the time course of the hyperpolarization (Fig. 1, A-C).

When current was passed inward through one of the intracellular barrels the cell was hyperpolarized to -90 mv or beyond (Fig. 1D). In this condition a diminution in the amplitude of the 5-HT hyperpolarization was observed, but not its actual reversal. When the external K<sup>+</sup> concentration was increased three times (from 5 to 15 mmole/liter) it became possible to observe a reversal around -50 mv (Fig. 1D). The factors that probably contributed to the failure to observe a reversal of 5-HT response in normal saline were on one hand anomalous rectification of the neuronal membrane beyond -75 mv, and on the other hand, the location of the receptor region far from the soma where the hyperpolarizing inward current was being injected. The increase in external K<sup>+</sup> concentration likely caused a shift of the reversal potential of the 5-HT hyperpolarization (*E*<sub>5-HT</sub>) to a level where the membrane potential was not affected by the rectification. Therefore it may be concluded

that 5-HT inhibited these neurons by causing a selective increase of  $K^+$  permeability, thus resulting in a  $K^+$  efflux.

Since 5-HT was probably acting on a spot located at some distance from the soma, it could be causing activation of interneurons or release of transmitter from nerve endings (or both). This possibility was ruled out because when either the whole content of  $Na^+$  and  $Ca^{2+}$  in the saline was removed, a condition known to abolish spikes in snail neurons (13), or when the whole content of  $Ca^{2+}$  was removed and the  $Mg^{2+}$  concentration was increased five times, which suppresses all transmitter release (14), there was no change in the hyperpolarization by 5-HT.

The receptors involved in this inhibition (called 5-HT B-receptors) were not at all affected by high concentrations of dTC (even  $10^{-3}$  g/ml), but they were blocked by LSD 25, tryptamine, and 5-HT, all in concentrations lower than  $10^{-5}$  g/ml.

The second group of neurons inhibited by 5-HT were H neurons (12)

Table 2. Comparative properties of 5-HT receptors in snail neurons. B, blocks; 0, no effect.

Compound	Receptor		
	A	B	C
<i>Physiological action</i>			
Excitation		Inhibition	Inhibition
<i>Ionic permeability</i>			
	$Na^+$	$K^+$	$Cl^-$
<i>Pharmacology</i>			
dTC	B	0	B
Prostigmine	0	0	B
LSD 25	B	B	B
Tryptamine	B	B	B
5-HT	B	B	B

located in the left pallial ganglion. The hyperpolarization caused by 5-HT in these cells could be easily reversed (Table 1) by artificially driving the membrane potential beyond  $-60$  mv. An average  $E_{5-HT}$  of  $56.1$  was measured in eight neurons. Replacement of the whole  $Cl^-$  content of the saline by sulfate ions caused a reversal of the response to 5-HT and the  $E_{5-HT}$  shifted to around  $-2$  mv (Table 1). Changes in the external  $K^+$  concentration did

not affect in a significant manner the control  $E_{5-HT}$  (Table 1).

Therefore, in this second type of inhibition 5-HT increased selectively the membrane permeability to  $Cl^-$  causing a  $Cl^-$  influx. The receptors involved in this inhibition which is dependent on  $Cl^-$  will be called 5-HT C-receptors. Like the A-receptors, they were easily blocked by low concentrations of dTC, but Prostigmin ( $10^{-6}$  g/ml) which did not affect either A- or B-receptors, readily blocked the C-receptors. On the other hand, C-receptors were also blocked by LSD 25, tryptamine, and 5-HT, all at concentrations of  $10^{-5}$  g/ml.

Since C-receptors occurred in H neurons having cholinergic inhibitory input dependent on  $Cl^-$  (12, 15), 5-HT could be acting by releasing acetylcholine from nerve endings. Saline, with high concentration of  $Mg^{2+}$  and a low concentration of  $Ca^{2+}$ , which suppressed all synaptic activity in the ganglia, did not alter the time course or the amplitude of the 5-HT hyperpolarizations.

These results demonstrate that 5-HT

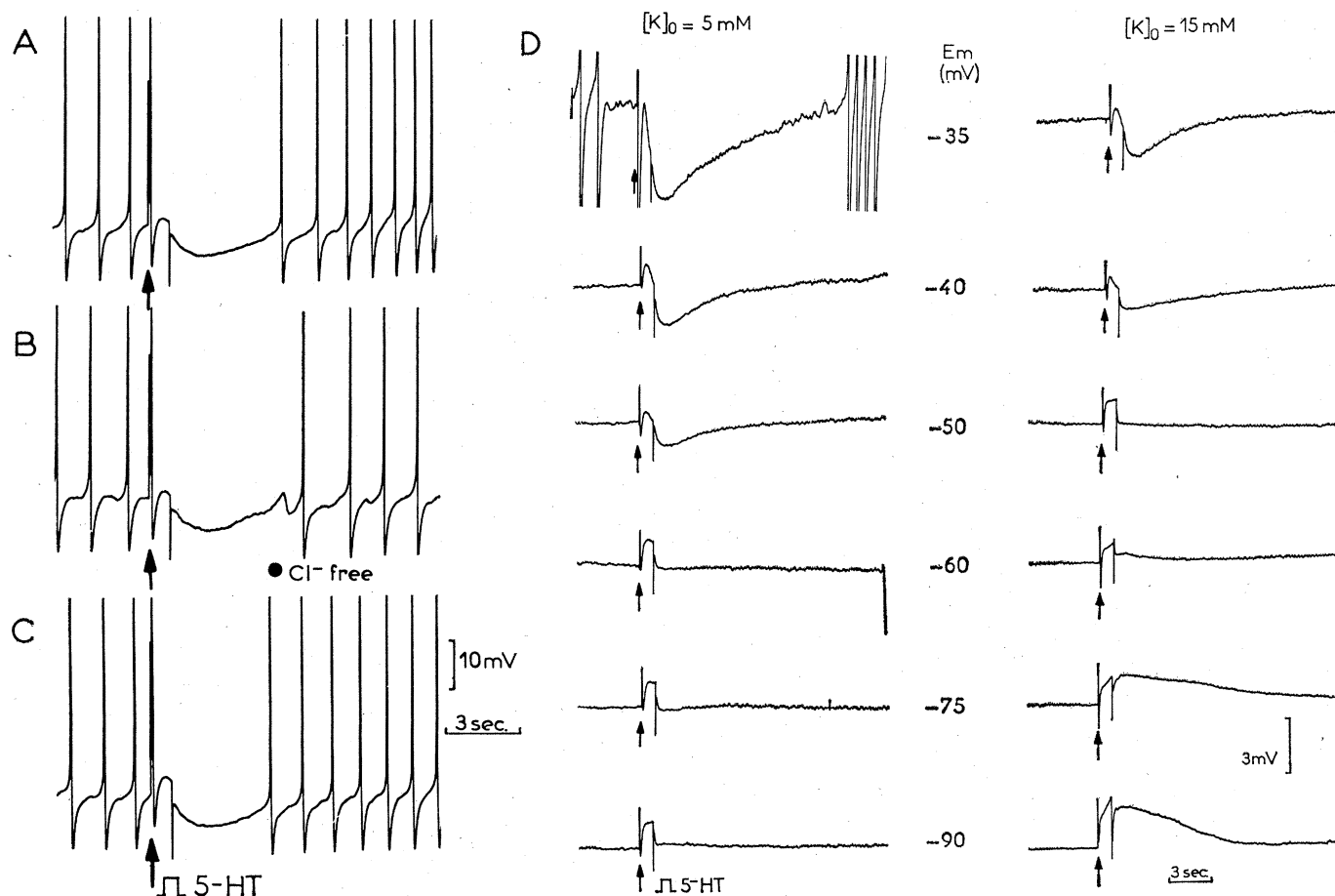


Fig. 1. Effect of ionic changes on the 5-HT hyperpolarization of the neurons of the right pallial ganglion group. (A) Control iontophoretic application ( $5 \times 10^{-7}$  amp, 1000 msec). (B) The same application in a saline free of  $Cl^-$ . (C) A new control after washing with normal saline. (D) Left column: Current injected inward. The membrane potential ( $E_m$ ) of another cell immersed in normal saline was driven to the values indicated in the middle column. The 5-HT hyperpolarization decreases in amplitude, but no clear reversal appears. Right column: When the outer concentration of  $K^+$   $[K]_0$  is tripled an actual reversal appears around  $-50$  mv (see text).

may inhibit snail neurons through two different ionic mechanisms, causing a selective increase of membrane permeability to either  $\text{Cl}^-$  ions or  $\text{K}^+$  ions. These data confirm that 5-HT possesses at least one fundamental property of chemical transmitters—the ability to alter selectively the membrane permeability to specific ions. Further work is still necessary to clarify whether 5-HT is actually involved in the synaptic inputs to the neurons endowed with 5-HT receptors.

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#### References and Notes

1. B. M. Twarog and I. H. Page, *Amer. J. Physiol.* **175**, 157 (1953); D. F. Bogdanski, H. Weissbach, S. Udenfriend, *J. Neurochem.* **1**, 272 (1956); B. B. Brodie and P. A. Shore, *Ann. N.Y. Acad. Sci.* **66**, 631 (1957); J. H. Welsh, *ibid.*, p. 618; — and M. Moorehead, *J. Neurochem.* **6**, 146 (1960).
2. A. Carlsson, B. Falck, K. Fuxe, N. A. Hillarp, *Acta Physiol. Scand.* **69**, 112 (1964); A. Dahlstrom and K. Fuxe, *ibid.*, **232**, suppl. 1 (1964); N. A. Hillarp, K. Fuxe, A. Dahlstrom, *Pharmacol. Rev.* **18**, 727 (1966); I. A. Michaelson and V. P. Whittaker, *Biochem. Pharmacol.* **12**, 203 (1963); L. M. Zieher and E. De Robertis, *ibid.*, p. 596.
3. E. Dahl et al., *Z. Zellforsch.* **71**, 489 (1966).
4. G. A. Cottrell and N. N. Osborne, *Nature* **225**, 471 (1970).
5. J. Taxi and J. Gautron, *J. Microsc.* **8**, 627 (1969).
6. H. M. Gerschenfeld and E. Stefani, *J. Physiol.* **185**, 684 (1966).
7. —, *Advan. Pharmacol.* **6B**, 369 (1968).
8. E. Stefani and H. M. Gerschenfeld, *J. Neurophysiol.* **32**, 64 (1969).
9. G. A. Cottrell, *Nature* **225**, 1060 (1970).
10. —, *J. Physiol.* **208**, 28P (1970).
11. D. J. Chiarandini, *Life Sci.* **3**, 1513 (1964).
12. L. Tauc and H. M. Gerschenfeld, *Nature* **192**, 336 (1961); H. M. Gerschenfeld and L. Tauc, *J. Physiol. (Paris)* **56**, 360 (1964).
13. V. D. Gerasimov, P. G. Kostyuk, V. A. Maiskii, *Byull. Eksp. Biol. Med.* **58**, 3 (1965); H. Meves, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **289**, R10 (1966); G. A. Kerkut and D. R. Gardner, *Comp. Biochem. Physiol.* **20**, 147 (1967); H. Meves, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **304**, 215 (1968); R. B. Moreton, *Nature* **219**, 70 (1968).
14. B. Katz, *The Release of Neural Transmitter Substances* (Thomas, Springfield, Ill., 1969), pp. 11–14.
15. G. A. Kerkut and R. C. Thomas, *Comp. Biochem. Physiol.* **19**, 819 (1966); D. J. Chiarandini and H. M. Gerschenfeld, *Science* **156**, 1595 (1967).
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## Compound Eyes: Localization of Two Color Receptors in the Same Ommatidium

**Abstract.** *The compound eye of the cockroach Periplaneta has receptors for ultraviolet light (maximum sensitivity at 365 nanometers) and green light (maximum sensitivity at 510 nanometers). Single photoreceptor cells in the compound eye were impaled, identified by spectral response, and marked with dye-filled microelectrodes. Using two different dyes, we showed that both types of receptors can be found in the same ommatidium.*

The retinas of arthropod compound eyes comprise hundreds or thousands of units called ommatidia (1). An ommatidium usually contains seven or eight elongate photosensory cells (retinular cells) whose cell bodies surround an attached axial structure known as the rhabdom. The rhabdom is formed by the juxtaposition or interleaving of masses of microvilli of the retinular cells and is the site at which photons are captured in the visual process. Generally each retinular cell sends an axon to the optic ganglia of the brain. The ommatidia are normally separated from one another by dense sleeves of screening pigment, and each possesses a small dioptric structure.

Behavioral tests show clearly that some arthropods have color vision (2). Certain other species are presumed to discriminate wavelengths because electrophysiological measurements reveal receptors with differing spectral sensitivities (3). Relatively little is known, however, about whether different color

receptors are present in the same ommatidium (4). We here present evidence, based on individually marked cells, that they can be (5).

These experiments were performed on a white-eye mutant of the cockroach *Periplaneta americana* (6). This mutant lacks all accessory screening pigments but is otherwise normal. On the basis of spectral responses, there are two kinds of receptor cells in the eye (Fig. 1B), an ultraviolet receptor with maximum sensitivity at 365 nm and a green receptor with maximum sensitivity at about 507 nm (7, 8). The sensitivity of the ultraviolet receptor is down 3 log units at 507 nm, whereas the sensitivity of the green receptor is down about 1.2 log units at 365 nm. Consequently it is a simple matter to decide which kind of cell has been impaled by examining the responses to green and ultraviolet test lights.

Isolated heads were sliced through the cornea so as to expose a row of ommatidia over their full length. The

preparation was mounted on soft wax in a short section of quartz tube and covered with artificial saline (8). Single cells near the surface of the cut were impaled with micropipette electrodes filled with aqueous solutions of the fluorescent anionic dyes Procion yellow (5 percent) or Procion red (2 percent) (9). The pipettes were pulled with several threads of fiber glass in them (to facilitate filling) and were filled from the shank with a hypodermic needle fitted with a 0.2- $\mu\text{m}$  Millipore filter. The procedure was generally to explore with a red-filled pipette until a cell sensitive to ultraviolet light had been impaled and identified. A series of 10- to 20-na pulses up to 100 msec long was then passed through the pipette for several minutes until the cell had visibly stained. By using the white-eye mutant and working close to the surface of the slice, we could usually see which ommatidium had been penetrated. Then a second electrode filled with yellow dye was advanced into the same or an adjacent ommatidium until one or more cells sensitive to green light were located and stained in similar fashion. Heads were fixed overnight and prepared for light microscopy by conventional means (10). The tissue was observed and photographed with a fluorescence microscope.

These experiments present technical difficulties. The retinular cells are about 7  $\mu\text{m}$  in diameter, and many of the pipettes that were small enough to enter them without producing appreciable damage would not pass sufficient current to stain the cells. This was particularly true of the pipettes filled with Procion red. Moreover, because the cells being studied were close to the surface of the tissue, their relationships to surrounding cells frequently became disrupted during preparation for microscopy. And finally, curvature of the ommatidia limited the number of cross sections that could readily be cut. We have nevertheless obtained slides from four different preparations which convince us that receptors for ultraviolet and for green light can be found in the same ommatidium.

An example is shown in Fig. 1A, which was traced from a projection of the original color transparency (11). The individual ommatidia are shown cut in cross section, with the images of the retinular cells arranged around the rhabdoms like the petals of flowers. The heavy lines connecting the centers of adjacent ommatidia have been added to the tracing to delineate ommatidial rows. Cellular debris from