# Neural Serotonin Receptors in Active and Hibernating Helicid Snails (*Helix lucorum*)

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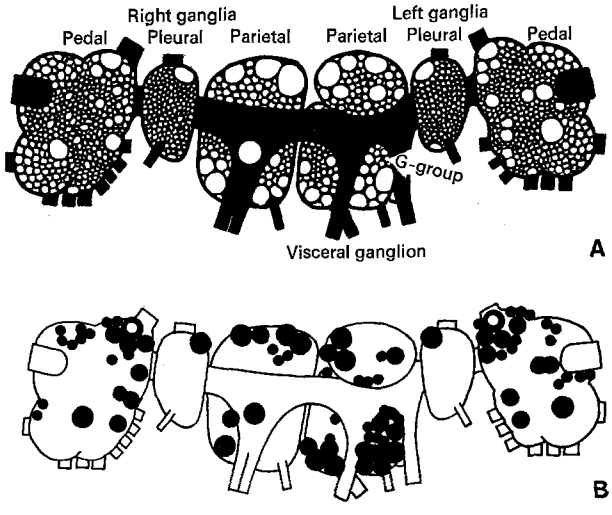
*Summary.* In hibernating snails *Helix lucorum*, the effect of 5-HT on neurons of an identified group is mainly hyperpolarization associated with a conductance increase, while, in active snails, the effect is depolarization and a decrease of the membrane conductance.

Serotonin(5-hydroxytryptamine, 5-HT) acts as a neurotransmitter in gastropods and, along with other transmitters, is able to mediate both excitation and inhibition[1]. Six types of neural 5-HT receptors are reported to be involved in these actions[2]. We present here results indicating that the complex pattern of 5-HT receptors varies at different stages of the life cycle of the snail.

Responses of neurons of the G-group (visceral ganglion) to serotonin

| Snails | No. of cells | Responses to 5-HT | | | Mean $E_c$[c] (mV) |
|---|---|---|---|---|---|
| | | Sign[a] | Membrane conductance | $E_{5-HT}$[b] (mV) | |
| Active | 36 | D (35) | Decrease | ~−80 | −38.3 |
| | | H (1) | — | — | — |
| Hibernating | 55 | D (14) | Increase | 0 or ~−30 | −41.2 |
| | | H (41) | Increase | ~−80 | −43.9 |

[a] D, depolarization; H, hyperpolarization; number of cells in parenthesis. [b] Reversal potential for serotonin (in some instances, estimated by extrapolation). [c] Threshold potential, i.e. critical level for action potential generation.




The investigated region of the CNS of *Helix lucorum*. Pedal commissures cut, the ring unfolded.
A) Diagram showing typical positions of large and small neurons. G-group occupies a lobule at the root of the anal nerve. B) Responses of neurons to 5-HT in active snails. Blackened, D response; white cored, H response. Each circle represents the results of a single experiment (smaller neurons) or those of several experiments (larger identifiable cells and the G-group; see the Table).

*Materials and methods.* Experiments were carried out on the isolated CNS of *Helix lucorum*, a land snail closely related to *Helix pomatia*. Pedal comissures were cut and the ring composed of 7 suboesophageal ganglia was unfolded and pinned with its inner surface uppermost (Figure 1A) in the bath chamber perfused with snail saline[3]. From 2 to 4 neurons were investigated in each preparation. The cell was impaled with 2 electrodes filled with potassium propionate, one for recording and another for passing current across the membrane. 5-HT was applied to the cell body by passing a current of 15–500 nA through a micropipette filled with 5-HT creatinine sulphate (braking current about 50nA). Conventional recording technique was used. To decide whether the cell is D or H (i.e. depolarized or hyperpolarized by the drug), we considered its response to 5-HT at threshold potential.

Snails were maintained in one of two experimental conditions. One group ('active snails') was kept in the laboratory at room temperature and occasionally fed. Animals of the other group ('hibernating snails') were kept at about 5 °C in a dry room, their peristome closed with a calcareous epiphragm; about 1 h before the experiments, snails were activated with warm water.

*Results and discussion.* In an earlier study we have found that neurons of an identifiable cluster (G-group, Figure 1A) produce mainly D responses to 5-HT in active snails and H responses in hibernating animals[4]. A priori, several possible mechanisms for a change of D-cells to H-cells could have been proposed. Results reported in the Table show that alterations in the ionic background of the responses are responsible for this change.

D responses in active snails and H responses in hibernating ones have a similar $E_{5-HT}$ which in both instances is more negative than the threshold potential; the former effect is, however, associated with a conductance decrease while the latter effect is associated with a conductance increase. By definition, this implies involvement of different receptors[2]. The Table shows further that D responses, which are recorded from 25% of neurons of the G-group in hibernating snails, are also different from D responses of these neurons in active snails, as far as ionic mechanisms are concerned. Thus, new species of 5-HT receptors appear on these neurons during hibernation. They have a relatively high sensitivity to 5-HT and are invariably associated with a conductance increase. At a later phase of such response, an increase of the input resistance of the membrane and a slow depolarization can be seen. It seems, therefore, that receptors characteristic

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of these neurons in active snails do not disappear during hibernation.

We did not pay special attention to neurons other than cells of the G-group. Surprizingly, in active snails we could find only 2 H-cells in the region outlined in Figure 1; other neurons are of D types (Figure 1B). D responses are mainly associated with a conductance decrease and are similar to D responses of neurons of the G-group in these animals. To elicit such response, a relatively strong current should be applied to the 5-HT pipette. The response slowly develops and lasts sometimes for minutes after stopping the 5-HT ejection. Many prominent neurons situated in other parts of the snail CNS have 5-HT receptors of this type, e.g. the paired giant metacerebral cells and the giant bursting neuron of the right parietal ganglion.

The 2 H-cells are most probably members of a symmetric pair. Each one occupies an anterior position in the pedal ganglion close to the cerebro-pedal connective (Figure 1B) and is usually the largest neuron of the area. An H response is sometimes preceded by a short D phase indicative of a composite reception of 5-HT. The H response is associated with a conductance increase and is $K^+$-dependent as the value of $E_{5\text{-HT}}$ shifts to a less negative region when the external $K^+$ concentration is increased. So far, we could not obtain H responses from pedal neurons of this area in hibernating snails. It seems therefore that 5-HT receptors of the 2 pedal H-cells are also affected by hibernation.

It was more than 15 years ago that KOSHTOYANTZ[5] suggested seasonal behavioral changes in land pulmonates to be closely connected with changes in central 5-HT mechanisms. In fact, considerable variations in the level of 5-HT in the snail brain have been demonstrated[6]; now we present evidence that changes in composition of 5-HT receptors occur as well. The changeable neurons of the snail may provide a simple model system for studying hormonally induced modifications in neural mechanisms underlying behavior.

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## A Simple Approach to the Toad's (*Bufo melanostictus*) Nerve Muscle Preparation

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*Summary.* A simple and modified dorsal approach method has been made in the toad's (*Bufo melanostictus*) sciatic gastrocnemius nerve-muscle preparation. This method incurs less blood loss, time consumption, nerve damage and visceral spoil compared to conventional ventral approach method.

Sciatic-gastrocnemius (SG) nerve muscle preparation (NMP) is commonly used as a basic tool for studying the proper nerve muscle function (NMF) experimentally. The previous procedure[1-3], which is generally advocated in the aforesaid NMP, has certain disadvantages over the present method.

The interrupted line shows the landmark for incision of the nerve muscle preparation. U, urostyle; F, skin fold.

Instead of ventral approach (VA) in the conventional method, the dorsal approach (DA) has been made. The object of this method is 1. minimum blood loss; 2. abdominal viscera is not disturbed and intestinal contents does not come out and spoil the nerve; 3. less time is required; 4. nerve is least damaged; 5. the same animal may be used in other experiment also; 6. study of NMF in vitro or in vivo with circulation or without circulation can be performed.

*Method.* The 10th vertebra, known as urostyle, was removed from pithed toad laid on the dissecting board with dorsal surface uppermost. Then the sciatic nerve (SN) on either side was exposed. A long longitudinal incision was made along the skinfold over the thigh caused by the triceps femoris and semi-membranous muscles (Figure). With the help of a glass seeker, the muscles, i.e. semi-membranous and triceps (femoral), were separated and SN along with its corresponding blood vessel was revealed. The SN was exposed by cutting through the pyriformis muscle and was traced with the help of glass seeker proximally upto their termination in the gastrocnemius muscle (GM). Then the vertebrae were bisected diagonally into 2 symmetrical halves without disturbing the root of the SN on either side. SN was

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