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5-Hydroxytryptamine (serotonin) causes a reduction in the afterhyperpolarization following the action potential in lamprey motoneurons and premotor interneurons

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The actions of 5-hydroxytryptamine (5-HT) on single neurons in the lamprey spinal cord have been investigated by intracellular recordings in vitro. Administration of 5-HT either by pressure ejection or by perfusion in the bath caused a reversible reduction of the late phase of the afterhyperpolarization (AHP) following the action potentials. This effect was antagonized by methysergide. A reduction of the late AHP was observed in lateral interneurons, motoneurons and some unclassified cells.

The lamprey spinal cord contains a midline column of 5-hydroxytryptamine (5-HT)containing cells located just ventral to the central canal[4,10,14], which form a bilateral ventromedial plexus[10,23] with a very high density of 5-HT varicosities (see also Fig. 1). Dendrites of segmental motoneurons and premotor interneurons ramify within this plexus in close proximity to the 5-HT varicosities[24,25] and consequently 5-HT is thought to affect motoneurons and premotor interneurons. In the present study 5-HT is applied extracellularly near the ventromedial plexus to investigate whether 5-HT affects neurons with dendrites within this plexus. The spinal 5-HT interneurons also contain CCK-like and tachykinin-like immunoreactivity[26,27]. The present results have been reported in abstract form[25].

Spinal cords of lampreys (*Ichthyomyzon unicuspis, Petromyzon marinus*) have been dissected and sections of 10–20 segments were maintained in vitro at 7–9 °C (cf. ref. 28). The spinal cord was mounted ventral side up in a Sylgard-lined dish and the meninx primitiva was removed. Neurons were impaled with microelectrodes under visual inspection (cf. ref. 20). Only cells with a membrane potential more negative than −50 mV and an action potential with a magnitude greater than 50 mV were accepted. The microelectrodes were filled with Lucifer Yellow (5% in 0.1–1.0 M LiCl) to allow a subsequent morphological characterization of the neuron[24,28] and to determine whether the recorded cells had ramifications within the 5-HT plexus. The spinal cords were fixed in paraformaldehyde (40 g/liter) in 0.16 μM phosphate buffer (pH 6.9) with picric acid (2 g/liter) for 1–2 h and then maintained in 5% sucrose[24]. Cryostat sections (14 μm) were processed for 5-HT immunofluorescence[21] with tetramethylrhodamine-isothiocyanate-conjugated second antibodies (cf. refs. 6, 12, 13, 24).

Multibarrelled micropipettes containing 5-HT (10 mM), methysergide (3.3–10 μM) and Ringer solution were used to administrate the drugs, which were ejected by pressure[22]. The pipettes were held over the fluid surface (see Fig. 1) and a droplet of known dimension (i.e. volume of 2–130 nl) was ejected, and the micropipette was then lowered to the fluid surface just above the 5-HT plexus (Fig. 1). The fluid surface was only 100–300 μm above the spinal cord thus minimizing the diffusion distance. Also, 5-HT (10–100 μM) or zimeldine (Astra; 3–10 μM) were administered by perfusion of the spinal cord with physiological solution containing these substances. The advantage of the droplet method over the perfusion method is that a known quantity can be administered at a defined instant.

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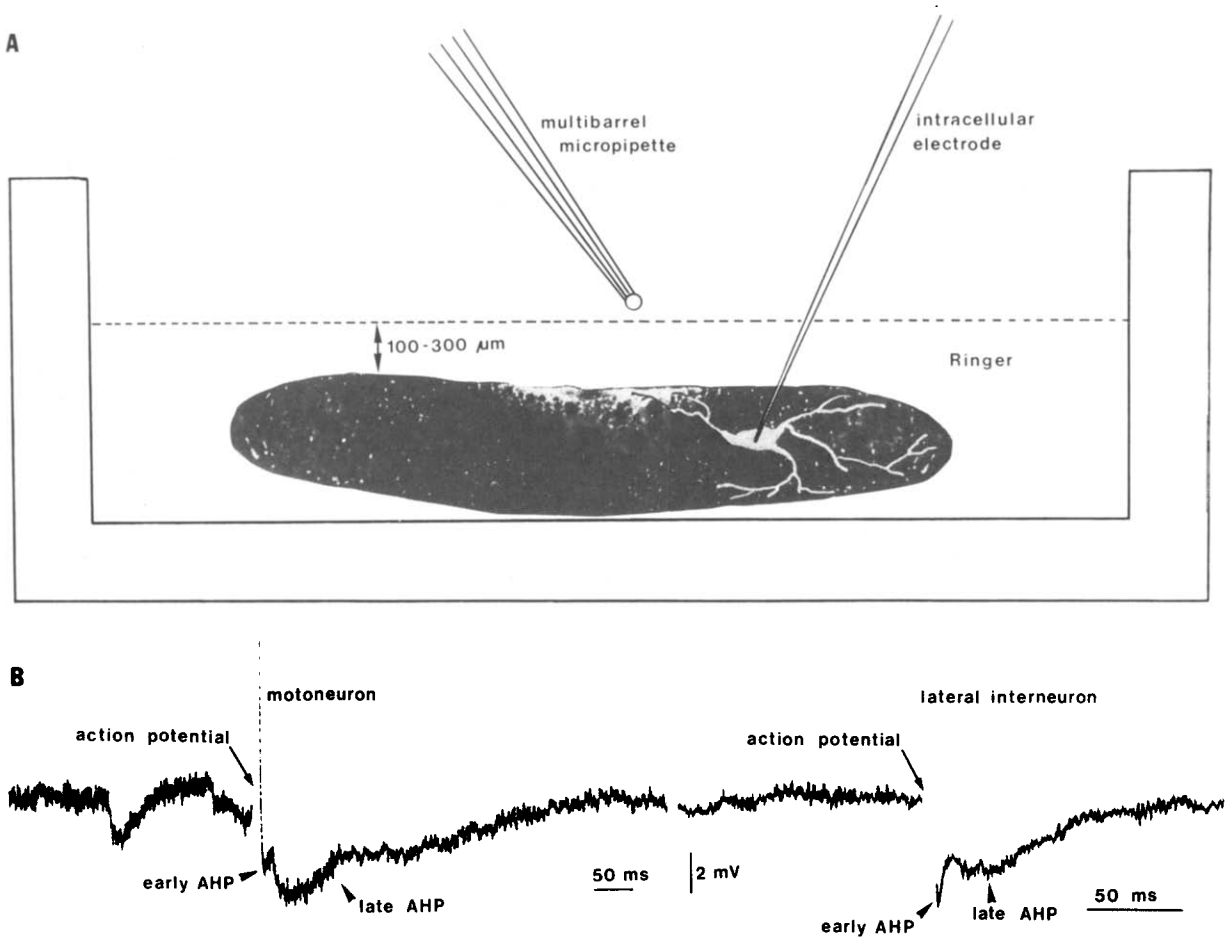

Fig. 1. A: schematic composite drawing of the arrangement of the recording electrode and drug-containing multibarrel micropipette. A montage of micrographs showing the spinal cord (transverse section, ventral side up) with the bilateral 5-HT plexus shown by 5-HT immunofluorescence is mounted in this drawing. The neuron is drawn in this micrograph. B: AHPs of a motoneuron (left) and lateral interneuron (right) showing a distinct early and late phase.

The application of 5-HT by ejection had for most cells (41 of 51) no effect on the level of membrane potential (Fig. 2A) and for the remainder the effects were unclear or inconsistent. All neurons were stimulated by short depolarizing current pulses (2 ms), each of which elicited an action potential, which was followed by an afterhyperpolarization (AHP) which had an early and a late phase (2–4 mV) (Fig. 1). In Fig. 2A–C it is shown that the late phase of the AHP is depressed following the ejection of 5-HT (2 nl). Fig. 2B shows sample records obtained before (B_1), just after (B_2) 5-HT application and during recovery (B_3). Fig. 2C shows the corresponding averaged records of 8 action potentials in each category (interrupted lines in Fig. 2A). Furthermore, the effect on the AHP only occurred after 5-HT administration, but not when similar amounts of the dissolving medium (Ringer) were ejected. The depression of the 'late' AHP seen with single pulses is also apparent when the late phase is potentiated to about 10 mV by using a short train of pulses as in Fig. 3. These effects of 5-HT were reversible (Fig. 2) and the action of 5-HT could then be repeated. The early phase of the AHP (cf. Fig. 1) on the other hand appears not to be affected (Fig. 2). The corresponding effects of 5-HT on the AHP have been observed on cells which discharge spontaneously. In addition they increase their discharge rate during the 5-HT application.

The amplitude of the early and the late phase of the AHP is reduced if the resting membrane potential becomes more hyperpolarized (for lamprey, see ref. 11). It is therefore important that for all cells re-

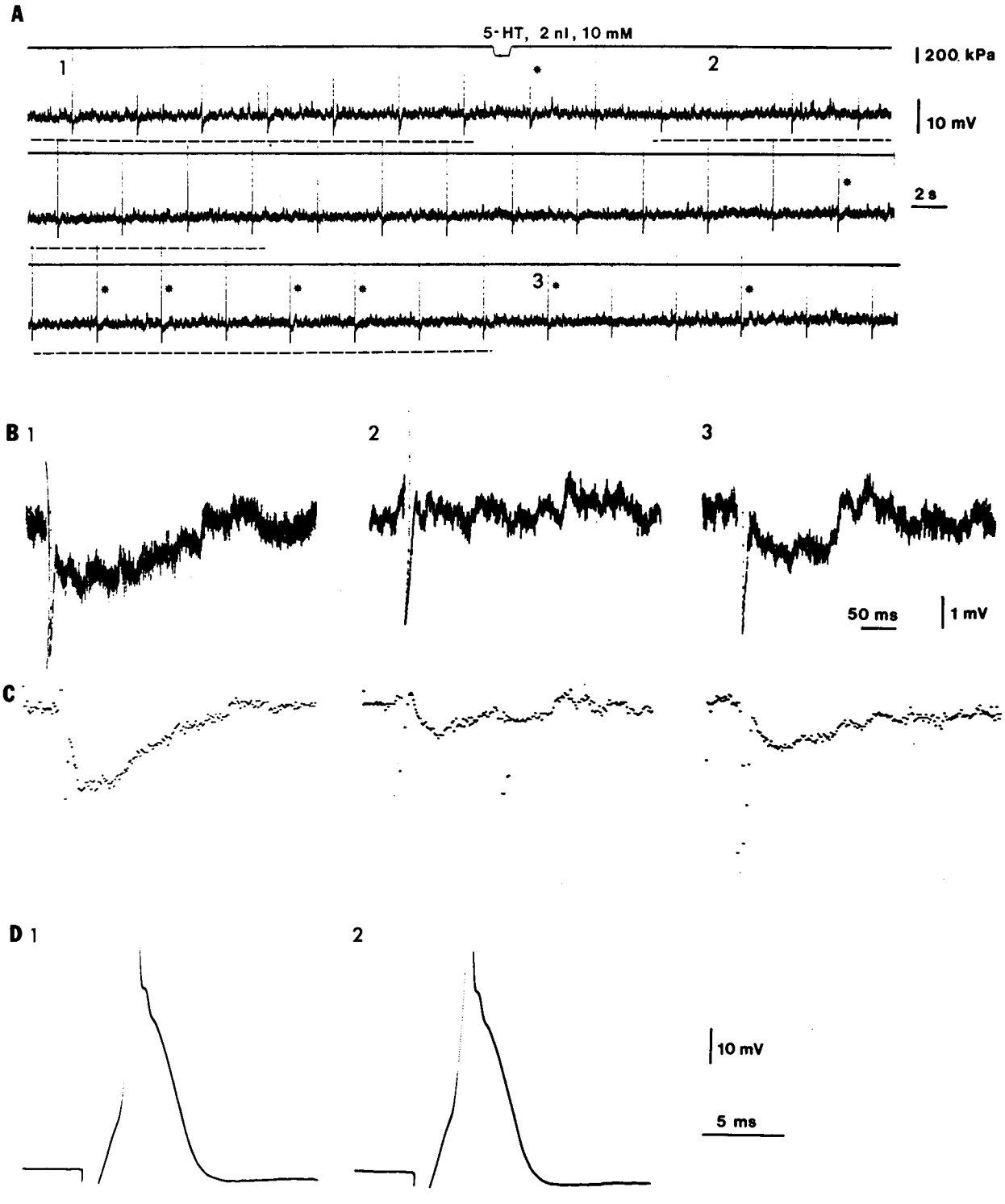

Fig. 2. Effect of administration of 2 nl of 5-HT (10 mM) on the afterhyperpolarization (AHP) following an action potential induced by a depolarizing pulse (2 ms). A: continuous recording for 150 s during which 5-HT was administered. Action potentials with discernable late phase of the AHPs after 5-HT administration are marked with asterisks and in all cases the early phase is seen. The action potentials indicated with 1, 2 and 3 in the continuous record are shown with higher resolution in B, in which the shape of the early and the late phase of the AHP is seen clearly. Note the abolishment of the late phase of the AHP 6 s after 5-HT administration in B_2, while the early AHP is not influenced. C: averaged AHPs of 8 successive action potentials; the intervals during which these averages were taken, are indicated in A by the dashed line. D: the shape of the action potentials elicited by short lasting depolarizing current pulses is shown at low sensitivity and high temporal resolution. The numbers 1 and 2 refer to the numbers in the continuous record; no difference could be detected between them. (This cell was not classified since it was not sufficiently filled with LY.)

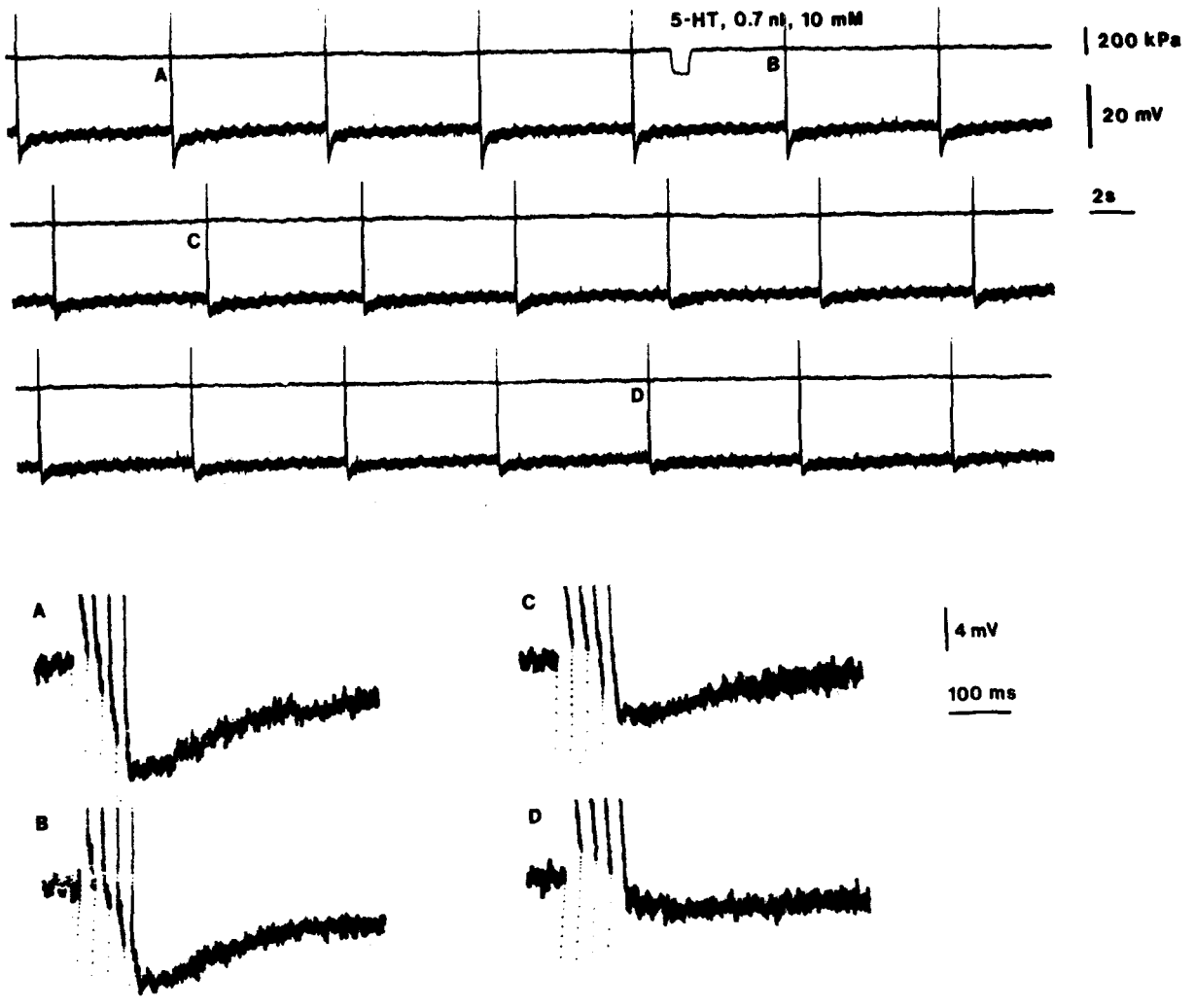

Fig. 3. Effect of administration of 0.7 nl of 5-HT (10 mM) on the enhanced AHP after 4 action potentials. Above: continuous record for 146 s. Below: individual AHPs at higher resolution; the letters at these AHPs refer to the letters in the continuous record. (This cell was not classified, since it was not sufficiently filled with LY.)

ported here the selective effect of 5-HT on the late phase of the AHP occurred with no observed effect of 5-HT on the level of membrane potential (Figs. 2–3). Moreover, if the effect encountered would have been due to changes in the level of membrane potential, the early phase of the hyperpolarization should also have been affected, but it was not (Fig. 2). These effects of 5-HT occurred in 24 cells out of the 41 tested in which there was no effect on the membrane potential. They include one type of premotor interneuron, the lateral interneuron (6 out of 8 cells), motoneurons (7 out of 15) as well as some unclassified neurons (11 out of 17). In most cases the unclassified neurons were not well filled with Lucifer Yellow (LY). All responsive cells had dendrites in the 5-HT plexus, but also 10 of the 17 unresponsive cells had dendrites in this region. The reason why 5-HT had no effect in cells with dendrites in the 5-HT plexus is unclear. It may be related to the diffusion distance of 5-HT to the dendrites of the cell recorded from and to the density of 5-HT receptors. Similar results (n = 8) were obtained in cells in which 5-HT was applied in the perfusion fluid.

To test if the effect of 5-HT was exerted through 5-HT receptors rather than being unspecific, the 5-HT receptor antagonist methysergide was adminis-

tered (7–15 nl) just prior (2 s) to a second application of 5-HT in 3 previously responsive cells. The 5-HT-induced reduction of the AHP was reduced with 50% or more, i.e. 5-HT had little effect if combined with methysergide. Hence the effect of 5-HT should be exerted on 5-HT receptors rather than being unspecific. To test whether the effects of exogenous 5-HT are similar to those of an endogenous release of 5-HT, a 5-HT uptake blocker was administered to the perfusion fluid (3–10 μM zimeldine[19]). In two of 11 trials a depression of the late phase of the AHP occurred. Such effects would be expected if a moderate spontaneous release of 5-HT would occur under the present experimental conditions.

We thus conclude that 5-HT can depress the late phase of the AHP in lamprey neurons. This phase is generally attributed to an opening of one type of potassium channel (K_{Ca}) by the Ca ions which enter the cells during the action potential[3,11,18]. The mode of action of 5-HT in the present case is unknown; it may be direct or via a second messenger. 5-HT might reduce the inflow of Ca ions during the action potential (note, however, the unchanged duration of the action potential (cf. ref. 11) in Fig. 2D or it might prevent the intracellular Ca from opening the K_{Ca} channels. The latter mechanism has been demonstrated for the action of noradrenaline in hippocampal neurons[9,17]. A 5-HT-induced reduction in the hyperpolarization following a train of action potentials has been found in leech touch cells[5]. In other systems 5-HT has been found to act via several different ionic mechanisms (see refs. 7, 16). 5-HT can potentiate motoneuronal activity in reptiles by inducing a change of membrane properties[15].

The amplitude and duration of the late phase of the AHP is one major determinant of the discharge frequency of a neuron during a steady depolarizing input[2,3,8], and a reduction of the AHP leads to an increased frequency and duration of the discharge during otherwise constant conditions[17]. In analogy it is likely that an activation of the present spinal 5-HT system will lead to a reduction in the AHP in the target neurons, which therefore will respond with an increase in the frequency of action potentials to depolarizing inputs of sufficient strength to evoke action potentials. The 5-HT interneurons are located in the midline and distribute branches to motoneurons and premotor interneurons on both sides of the spinal cord. This 5-HT system is strategically located to act as a gain control system for the motor output of the spinal cord. It will be interesting to learn what effect the probable release of the peptides which coexist in these 5-HT cells will have on the target neurons (refs. 26, 27; see above).

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