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The Effects of Serotonin on Functionally Diverse Isolated Lamprey Spinal Cord Neurons

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The experiments reported here showed that application of serotonin (5-hydroxytryptamine, 5-HT) (100 μ M) did not induce any significant current through the membranes of any of the spinal neurons studied (n = 62). At the same time, the membranes of most motoneurons and interneurons (15 of 18) underwent slight depolarization (2–6 mV) in the presence of 5-HT, which was not accompanied by any change in the input resistance of the cells. Depolarization to 10–20 mV occurred in some cells (3 of 18) of these functional groups, this being accompanied by 20–60% decreases in input resistance. The same concentration of 5-HT induced transient low-amplitude depolarization of most sensory spinal neurons (dorsal sensory cells), this changing smoothly to long-term hyperpolarization by 2–7 mV. The input resistance of the cell membranes in these cases showed no significant change (n = 8). Data were obtained which provided a better understanding of the mechanism by which 5-HT modulates the activity of spinal neurons. Thus, 5-HT facilitates chemoreceptive currents induced by application of NMDA to motoneurons and interneurons, while the NMDA responses of dorsal sensory cells were decreased by 5-HT. 5-HT affected the post-spike afterresponses of neurons. 5-HT significantly decreased the amplitude of afterhyperpolarization arising at the end of the descending phase of action potentials in motoneurons and interneurons and increased the amplitude of afterdepolarization in these types of cells. In sensory spinal neurons, 5-HT had no great effect on post-spike afterresponses. The results obtained here support the suggestion that 5-HT significantly modulates the activity of spinal neurons of different functional types. 5-HT facilitates excitation induced by subthreshold depolarization in motoneurons and some interneurons, facilitating the generation of rhythmic discharges by decreasing afterhyperpolarization. In sensory cells, 5-HT enhances inhibition due to hyperpolarization, suppressing NMDA currents. The differences in the effects of 5-HT on functionally diverse neurons are presumed to be associated with the combination of different types of 5-HT receptors on the membranes of these

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types of spinal neurons.

Serotonin (5-hydroxytryptamine, 5-HT) is a biologically active substance present in a variety of tissues, including nervous tissue, in all vertebrates and invertebrate animals. The effects of disturbances in the effects of 5-HT – tremor, rigidity, and reciprocal limb movements – have long been known, though investigation of the effects of 5-HT on defined functional systems and individual nerve cells from vertebrate spinal and cerebral nuclei has demonstrated that this substance has a surprising variety of effects; great difficulties have been experienced in identifying the mechanisms of its actions.

5-HT is usually regarded as a modulator in the nervous system [54]. In vitro experiments based on local application of 5-HT to cells have shown that the effects of 5-HT are “smeared” over time, starting with a delay and continuing for some time after washing. The modulatory effects of 5-HT are also apparent where there are few terminals of 5-HT-containing fibers [56]. Most serotonergic cells in vertebrates are located in the cervical nuclei of the midbrain and medulla oblongata, and the fibers of these cells form the descending serotonergic system, which controls the activity of both sensory and motor neurons in the spinal cord, as well as the autonomic components of the thoracic segments [54].

The effects of 5-HT on individual nerve cells have been studied both on spinal cord and brain cells from mammals (rats, cats, guinea pigs, rabbits), as well as from reptiles, amphibia, and cyclostomata. The latter, like all vertebrates, have been shown to have both descending [15] and intraspinal [81] serotonergic systems, and the terminals of serotonergic neurons have been located in at dorsal and ventrolateral areas of spinal segments [80]. In complete agreement with such a wide morphological representation of the 5-HT fibers of segmental neurons of different functional types, 5-HT has been shown to influence the locomotor rhythm, which is generated by the segmental neural networks and which controls trunk movements in these animals [19, 31, 42]. Addition of 5-HT to the perfusing solution has been shown to produce dose-dependent decreases in discharge frequencies in ventral roots but to increase the duration of these discharges, by recruiting previously inactive motoneurons [31, 70, 85]. Discharges in the ventral roots are the output signal directed to the truncal musculature. These discharges follow on from intrasegmental processing of information arriving from centers in the brain and from the periphery. The main target cells for 5-HT in the spinal cord of the lamprey are premotor interneurons and motoneurons. The discharge frequencies of these and other cells (spontaneous or evoked by a depolarizing stimulus) increased in response to 5-HT [79]. In addition, 5-HT increased the duration of the depolarization plateau in oscillations induced by

NMDA in unidentified spinal neurons [83]. These facts are evidence that 5-HT potentiates excitation in motor and premotor cells. On the other hand, 5-HT-induced decreases in the amplitude of EPSP induced in motoneurons by stimulation of individual Mullerian (reticulospinal) axons and the hyperpolarization which has been observed suggest the possibility that 5-HT has inhibitory effects on the activity of these cells [18, 28]. There are significantly fewer serotonergic fibers and terminals in the dorsal part of the lamprey spinal segment than in the ventral part [32], which led to the suggestion that 5-HT has no effect on the activity of sensory cells (dorsal sensory cells) and giant interneurons [80].

Considering the small number of published reports (and their contradictory nature) on the effects of 5-HT on motoneurons, the absence of conclusive data on the effects of 5-HT on dorsal sensory cells, and the undoubted importance of 5-HT regulation of motor activity in cyclostomata, we elected to study the effects of 5-HT on the membrane properties, spike activity, (action potentials), and responses to the application of excitatory amino acids of different populations of lamprey spinal cord neurons, using completely isolated neurons identified from their morphological features. It was of interest to identify whether 5-HT modulates the electrical activity of isolated spinal neurons and to identify the nature of its modulation and its relationship to the presence in the membranes of isolated motoneurons of strictly postsynaptic receptors. The main study system consisted of motoneurons and interneurons, i.e., those cell populations which are sensitive to 5-HT in intact animals [42] and in in vitro isolated brain conditions [83]. Some of the data presented here have previously been published as a brief report [17].

Methods

Experiments were performed on adult *Lampetra fluviatilis* lampreys of length 25–30 cm, which were kept before experiments in large tanks with aerated water at temperatures of no greater than 10℃. A total of 222 lampreys, anesthet ized in 0.025% MS solution, were used for removal of part of the spinal cord from beneath the dorsal fin. These were treated with an enzymatic/mechanical method to obtain isolated cells. Enzymatic treatment consisted of incubating the cord in collagenase solution (1.6 mg/ml for 30 min) and then in pronase (0.5 mg/ml for 2.5 h). The method used for cell isolation has been described in detail elsewhere [1].

Enzyme-treated fragments were mechanically dissociated by repeated trituration with Pasteur pipettes with sequentially decreasing diameters, from 600 to 150 μ m. Each portion of the dissociated neurons contained numerous cells of different shapes and sizes. Studies used dorsal sensory cells and branched unidentified neurons. The former had the shape of slightly

flattened spheres of diameter 30–40 μ m and smooth surfaces. Branched neurons were multipolar cells with body diameters of 100–120 μ m. Unlike the dorsal sensory cells, these had 3–7 branched processes which were often 2–3 cell diameters long, reaching 300 μ m. Judging by the sizes and shapes of the bodies and the structures of the dendritic trees, these cells could be identified as motoneurons or large interneurons.

Mechanical isolation and subsequent maintenance of spinal neurons were performed using normal physiological saline containing 92.0 mM NaCl, 2.5 mM KCl, 2.6 mM $CaCl_2$, 2.4 mM $MgCl_2$, 20.0 mM HEPES-Na, 0.3 mM EGTA, 3.0 mM $NaHCO_3$, 0.75 mM NaH_2PO_4, 0.25 mM Na_2HPO_4, and 10.0 mM glucose, pH 7.4. Before use, solutions were aerated with oxygen and cooled to 6–10℃.

A whole-cell patch-clamp method was used [30]. Voltage-activated and chemoreceptive currents were studied in a clamped voltage regime; the effects of 5-HT on membrane potential and action potentials were studied in clamped current conditions. The solution within pipettes contained 110 mM KF, 20 mM Tris, 5 mM EGTA, and 10 mM glucose, pH 7.2. In some cases (the example in Fig. 10), the pipette solution contained 100 mM cesium glutamate, 20 mM $CsMeSO_4$, 4 mM $MgCl_2$, 8 mM TEA-Cl, 10 mM HEPES, 8.5 mM EGTA, 5 mM ATP, 1 mM GTP, 14 mM creatine phosphate, 50 U/ml creatine phosphokinase, 3 mM leupeptin, and 10 mM glucose, pH 7.3.

The experimental chamber consisted of two sectors: a large (2 ml) sector and a small (0.5 ml) sector, isolated from each other by a removable Plexiglas partition. The large sector was used for selection of cells and collection with a pipette, and the small for pharmacological tests. The effects of 5-HT on membrane potential and cell membrane resistance were studied by stopping the flow of solution through the small chamber and adding the desired concentrations of 5-HT from a Hamilton syringe. Traces of membrane potential were started before application of 5-HT, were taken continuously for 1.5–2 min, and were stored in a single file. During this time, measurements were made of membrane resistance by passing impulses of depolarizing subthreshold currents lasting 25 msec, with intervals of 75 msec. The Clampfit program was then used to determine the membrane resistance using the formula $R_m = (U)/ {}_{\Delta}I$), where R_m is membrane resistance, U is the amplitude of the potential recorded in response to an impulse of current, and ${}_{\Delta}I$ is the amplitude of the applied impulse current. Plots were then constructed of changes in membrane resistance before and during exposure to 5-HT (Fig. 1, *A*, *B*). After 2 min of exposure to 5-HT, the flow was continued for 5–10 min to wash the cells and restore initial

membrane parameters. Studies of the modulation of NMDA responses by 5-HT were performed as follows. At the first stage, the flow through the small chamber was stopped and a Hamilton syringe was used to add NMDA solution to the desired concentration; the response was recorded (depolarization or current). Cells were then washed for 3 min with normal solution. At the second stage, the flow was again stopped, 5-HT was added, and changes in membrane potential or current were measured. After one minute of exposure to 5-HT, NMDA was added to the chamber at the same concentration and the NMDA response in the presence of 5-HT was measured. Cells were then washed for 3 min, after which, at the third stage, the test with NMDA alone was repeated. A similar method was used to measure the effects of 5-HT on responses elicited by other excitatory amino acids. The effects of 5-HT on postspike afterresponses were studied by applying square-wave suprathreshold current impulses of duration 10 or 25 msec. Traces were made of several action potentials in normal solution, then after 30–60 sec of exposure to 5-HT, and again after 5 min of washing. Compensation was made for the capacitative component of the depolarizing current impulse [3]. The effects were demonstrated on plots by superimposing three different action potentials using the SigmaPlot program.

The experimental instrumentation included an Axopatch-D amplifier with a CV-4 head and a DigiData-1200 analog-to-digital converter (Axon Instruments, USA). Data were gathered using the Clampex and Fetchex programs from the pClamp 6.0 program suite (Axon Instruments, USA). Data were analyzed using the Clampfit and Fetchan programs from the same suite. Responses were recorded on the hard disk of an IBM PC 486 computer. Statistical data are presented as mean ± standard error.

Results

The Effects of 5-HT on Membrane Potential and Membrane Input Resistance of Spinal Neurons

Studies of the mechanism of action of any modulator on nerve cell activity require knowledge of how the membrane potential and cell membrane input resistance change in response to the agent of interest in the absence of any other influences. Application of 100 μ M 5-HT to the membrane of all the branched cells studied in this series (n = 10) resulted in depolarization lasting from 1 to 40 sec, after which the membrane potential returned to initial, as shown in Fig. 1, *A2*. The amplitude of evoked depolarization in most branched cells (7 of 10) was 2–6 mV. Changes in membrane input resistance were not detected (Fig. 1, *A1*). In some branched cells (3 of 10), depolarization was more significant, reaching 9, 14, and 25 mV. The

input resistance of these cells decreased by 12%, 20%, and 63% respectively on exposure to 5-HT.

The effects of 5-HT on the membranes of sensory cells (dorsal sensory cells) were significantly different from those on branched cells. Thus, depolarization was short-lived and of low amplitude in four of the eight cells studied. The subsequent hyperpolarization was more marked and had amplitudes of 2–7 mV and durations of 1–2 min (Fig. 1, *B2*). After washing for 6–10 min with physiological saline, membrane potential returned to baseline. The input resistance of sensory cells did not change in the presence of 5-HT. Only two cells showed insignificant increases in resistance, this being on the margin of the measurement error. One of these cells is shown in Fig. 1, *B1*. Long-lasting depolarization with amplitudes of 0.5–2.5 mV was seen in two dorsal sensory cells. Membrane potential showed no change on exposure to 5-HT in the other two cells.

The Effects of 5-HT on NMDA-Evoked Responses in Branched and Dorsal Sensory Cells

Glutamate, aspartate, and its derivative NMDA (N-methyl-D-aspartate) are presently regarded as possible excitatory mediators in the lamprey spinal cord [16, 29, 36]. Application of these amino acids or their derivatives to the membranes of lamprey spinal neurons has been shown to induced depolarization similar to excitatory postsynaptic potentials. We reproduced this test to identify any possible effect of 5-HT on the excitation of spinal neurons.

The effects of 5-HT on NMDA responses were studied in 18 branched cells. In all cases, NMDA induced dose-dependent depolarization, which reached maximum amplitudes of 40 mV at an NMDA concentration of 1.5 mM. The effects of 5-HT on NMDA responses were studied using intermediate NMDA concentrations of 0.5–0.8 mM. In all cells studied (n = 8), application of NMDA in the presence of 5-HT induced depolarization of higher amplitude than seen with NMDA alone. A typical example is shown in Fig. 2. After addition of 100 µM 5-HT to the stationary solution in the small chamber, the amplitude of the NMDA-induced depolarization was greater than the sum of the amplitudes of the depolarization induced by NMDA and 5-HT each used alone (Fig. 2, *C*). These results indicate that 5-HT potentiates NMDA responses. The effects of 5-HT on NMDA-induced currents were also studied in branched cells in conditions of membrane voltage clamping. In most neurons (7 of 10), 5-HT induced increases in NMDA currents (Fig. 3). It should be noted that application of 5-HT alone (100 µ M) more often failed to induce a specific current through the membrane of all types of cell (branched and dorsal). The more rarely appearing influx current was no greater than 300 pA (Figs. 3, *B*; 5, *B2*). At the same time, application of NMDA in the presence of 5-HT induced potentiation of the peak amplitude of the

NMDA current by an average of 92 ± 52%. There was a simultaneous increase in the duration of the slow component of the current, as shown in Fig. 3, *C*.

The effects of 5-HT on responses induced in branched cells by the application of other excitatory amino acids were also studied. Thus, application of 1 mM aspartate in the presence of 5-HT (*n* = 5) resulted in potentiation of the evoked depolarization by 50–80% of initial (Fig. 4). Similar results were obtained in four cells using kainate and quisqualate. The effects of 5-HT on depolarization and currents induced by application of glutamate were less marked. Thus, neither the depolarization nor the current showed any significant change due to addition of 5-HT in four of five cells (Fig. 5, *A*, *B*). One cell showed 30% increases in depolarization with subsequent recovery to initial after 7 min of washing with physiological saline. Summarizing these results leads to the conclusion that 5-HT behaves as a modulator of branched cells, facilitating excitation of these cells.

The effects of 5-HT on responses induced by excitatory amino acids in sensory cells were qualitatively different. Electrophysiological studies have shown that dorsal sensory cells from the lamprey spinal cord lack synaptic inputs [55]. Electron microscopic studies also failed to demonstrate synaptic structures on the surface membranes of these cells [20, 71]. However, these points do not exclude the possibility that presynaptic mediator receptors are present on the membranes of dorsal sensory cells, as we have demonstrated in relation to $GABA_B$ receptors [28]. In our experiments, application of glutamate and glycine to these cells evoked weak currents (0.5 nA or smaller). Kainate (0.5 mM) and quisqualate (1 mM) could evoke depolarization to 20 mV, and this was largely independent of the presence of 5-HT. NMDA (1 mM) acted the most effectively on the membranes of dorsal sensory cells, inducing high-amplitude depolarization and currents, and these responses were significantly modulated by 5-HT. In the presence of 5-HT, decreases in both the depolarization (Fig. 6) and current (Fig. 7) induced in dorsal sensory cells by this amino acid were seen more frequently. Decreases in responses to NMDA were seen in seven of 13 cells; responses showed no changes in two cells, and four showed small increases in responses by 10–13% of initial.

The Effects of 5-HT on Postspike Afterresponses in Branched and Dorsal Sensory Cells.

This part of the study was performed on 19 branched cells and 15 dorsal sensory cells. As in the first part of the experiments, studies of the effects of 5-HT on membrane input resistance of spinal neurons showed that most branched cells (11 of 19) depolarized in response to application of 5-HT. Membrane potential showed no change in two cells, while six cells showed weak hyperpolarization. Action potentials were induced by short and long impulses of

threshold current. The amplitude of this potential averaged 73.79 ± 23.4 mV. Postspike afterresponses were variable and could consist either of hyperpolarization or depolarization or could be of mixed nature. The dominant action potential afterresponse consisted of hyperpolarization. This was seen in 17 of 19 cells studied, and its amplitude averaged 15.5 ± 7.1 mV. 5-HT decreased the amplitude of afterhyperpolarization by 5.3 ± 4.2 mV (34.0 ± 27.0%), had no effect in two cells, and increased afterhyperpolarization in one cell. A typical example of the change in afterhyperpolarization is shown in Fig. 8. Four cells showed postspike depolarization. Afterdepolarization decreased in response to 5-HT (Fig. 9) and could induce a second action potential. When postspike responses were mixed in nature and were sufficiently clearly marked, 5-HT changed them as follows: hyperpolarization decreased, depolarization increased.

The effects of 5-HT on postspike reactions were studied in 15 dorsal sensory cells. During measurements of action potentials, the membrane potential averaged 77.8 ± 9.2 mV; action potential amplitude averaged 75.6 ± 4.7 mV. Application of 5-HT displaced the membrane potential towards hyperpolarization in nine of the 15 cells, while three showed depolarization and the three remaining cells showed no change in membrane potential. As in branched cells, postspike reactions consisted mainly of afterhyperpolarization (in seven of 15 cells). Afterdepolarization was seen in four dorsal sensory cells; afterresponses were of mixed character in one cell and completely absent in three cells. Unlike branched cells, postspike reactions of all types in dorsal sensory cells were less sensitive to 5-HT and their changes after application of 5-HT were insignificant. Figure 10 illustrates the absence of significant changes in the postspike reactions of dorsal sensory cells in the presence of 5-HT. As shown in Figs. 8 and 10, 5-HT also produced changes in the shape of action potentials – duration increased and the shape of the leading front sometimes changed.

Discussion

The effects of 5-HT were studied here in 82 isolated spinal neurons (62 cells in the present experiments and 20 branched cells in experiments with modulation of membrane potential oscillations [17]). These studies showed that all the cells studied were sensitive to 5-HT. At the same time, these results support the concept that in the absence of activity induced in both branched and dorsal sensory cells by any other factors, addition of 5-HT to the perfusing solution had little effect on the electrical parameters of the cell membranes. Thus, for example, application of 5-HT produced no significant changes in resistance or large currents through the membranes. The depolarization seen in many branched cells, although prolonged, was below

the threshold for inducing action potentials, while hyperpolarization was also small and did not prevent the induction of action potentials by depolarizing stimuli. These effects were induced by 5-HT at concentrations of 80–100 μ M. At lower concentrations, 5-HT had no effects at all. An increase in the concentration to 200 μ M increased the effects, though they disappeared rapidly and were only restored after prolonged washing; the instability of these effects prevented systematic investigation.

Other authors have also found that 5-HT alone has no effects in studies of the actions of this amine on motoneurons in isolated lamprey spinal cord preparations [18], on mechanosensitive cells in the medicinal leech [11], on motoneurons of neonatal rat spinal cord [10], and on motoneurons in the rat facial nerve [82]. However, it is well known that 5-HT significantly alters the parameters of already developed functional systems – it is involved in regulating the respiratory rhythm [13, 49] and it controls motor activity in vertebrates [54]. At the cellular level, 5-HT has been shown to increase the frequency of spontaneous and stimulation-evoked activity of preganglionic sympathetic neurons [22, 57] and rat red nucleus neurons [47] and increases the discharge frequency in motor nuclei of the rat spinal cord [89]. Vertebrate motoneurons are particularly sensitive to 5-HT, iontophoretic application of which can induce a depolarization plateau and rhythmic action potentials in the motoneurons of cats [38], tortoises [39], and rats [52, 76]. In rat spinal motoneurons, 5-HT also induced long-term changes in excitability, decreases in the thresholds of glutamate-evoked action potentials, and increases in discharge frequency in ventral roots [5, 56, 86].

Existing data on the effects of 5-HT on responses in motoneurons induced by application of excitatory amino acids are of special interest for understanding the results we have obtained. 5-HT has been shown to increase depolarization induced in frog motoneurons by application of glutamate, aspartate, NMDA, and quisqualate [35]. 5-HT is also known to potentiate responses to NMDA in cat [60] and rat [66, 67] neocortex neurons and to induce oscillations in spinal neurons which are “silent” when NMDA is applied alone [72, 74].

Our data on the effects of 5-HT on depolarization and currents induced by NMDA in branched cells are in complete agreement with the points discussed above. Preincubation of branched cells in solutions containing 5-HT increased NMDA-evoked depolarization by factors of 2–3, and also increased the frequency of action potentials occurring at the depolarization plateau. Similarly, 5-HT potentiated NMDA currents, which were increased by 5-HT by 100% and more. In our experiments, 5-HT also induced potentiation of responses to the application of other excitatory amino acids – aspartate, kainate, and quisqualate. Thus, the results obtained here

lead to the conclusion that that the major effect of 5-HT on motoneurons and other isolated lamprey spinal cord branched cells is to potentiate their excitation.

The modulatory effects of 5-HT on dorsal sensory cells consisted of minor inhibition of their activity. Addition of 5-HT to the perfusing solution produced small amounts of hyperpolarization in most of the cells studied. 5-HT suppressed the depolarization and currents induced in dorsal sensory cells by application of NMDA.

This type of 5-HT effect on vertebrate nerve cells has also been described many times in the literature. Examples include the suppression by 5-HT of responses to all excitatory amino acids and potentiation of GABAergic inhibition in cat cerebellar cells [44], inhibition of spontaneous activity induced by glutamate in cerebellar Purkinje cells [45], and potentiation of glycinergic inhibition in rat preganglionic sympathetic neurons [46]. Hyperpolarization and inhibition of activity due to 5-HT have also been seen in hippocampal neurons [41] and in unidentified dorsal horn neurons from the rat [24] and cat [27, 33] spinal cord.

Morita and Katayama [59] studied bullfrog dorsal ganglion cells and found that 80% of the cells studied responded to superfusion or iontophoretic application of 5-HT with hyperpolarization. A total of 16% of the cells showed rapid depolarization, which underwent a smooth transition to long-lasting hyperpolarization (compare with Fig. 1, *B*). The authors regarded rapid depolarization as the basis of presynaptic inhibition of activity in primary afferents. Hyperpolarization, also induced by 5-HT in the same cells, was regarded as able to increase the threshold for propagation of action potentials, decreasing action potential frequency, and thus able to suppress the release of mediator in preterminals. Another study, performed on the lamprey spinal cord, described contacts of the “tight junction” type between serotonergic fibers and the processes of labeled dorsal sensory cells; decreases in the amplitude of monosynaptic EPSP evoked in giant interneurons by stimulation of individual cells or the dorsal columns has also been described [26].

All these points are in agreement with the concept that 5-HT has the role of a modulatory or inhibitory mediator of descending serotonergic pathways, acting directly or indirectly (via interneurons) to increase inhibition in sensory neurons of the dorsal horn in vertebrates [14, 48].

As noted above, dorsal sensory cells do not have synaptic structures on their surface membranes. In our experiments, these cells generated very weak currents in response to application of glutamate, GABA, and glycine, which makes it doubtful that their membranes could have postsynaptic receptors for these amino acids. However, we have previously observed presynaptic $GABA_B$ receptors in dorsal sensory cells and their processes [2], and they evidently

also have presynaptic NMDA receptors, as the responses of these cells to NMDA described in the present study were clear, dose-dependent, and sensitive to blockade by the specific NMDA receptor blocker APVA. The presence of NMDA receptors on the processes of sensory cells at first glance appears unlikely, as activation is usually associated with functions not characteristic of sensory cells (memory, learning, and convulsive and pacemaker activities). However, NMDA receptors have recently been detected by immunohistochemical methods on the presynaptic terminals of rat dorsal root horn cells. These receptors were located in the immediate vicinity of active zone vesicles, which led to a conclusion about their possible functional role – activation of presynaptic NMDA receptors may facilitate release of mediator by increasing the Ca^{2+} current in the preterminal regions [51]. This suggestion has received experimental support [50] and it helps to understand the importance of our data.

Thus, we can conclude that 5-HT increased the excitability of motoneurons and weakly inhibits the activity of sensory cells of the dorsal region in the spinal segments of the lamprey. Attempts to identify the functional explanation of these conclusions are aided by the suggestion that, controlling the incoming afferent flows from the periphery to motoneurons, 5-HT can facilitate the transmission and analysis in segmental neurons of information arriving from brain centers, thus modulating motor activity.

The last part of the present study analyzed 5-HT-induced changes in the parameters of action potentials evoked by depolarizing stimuli in branched cells and dorsal sensory cells. Afterresponses (rapid and slow hyperpolarization, as well as depolarization) are known to play an important role in controlling the frequency of action potentials generated by neurons and propagated via nerve fibers [6]. Decreases in the amplitude of afterhyperpolarization lead to increases in the frequency and duration of action potentials [53]. In lampreys, regulation of discharge frequency plays the decisive role in organizing the locomotor rhythm and movements. The effects of 5-HT demonstrated in several studies on discharge parameters in the ventral roots, along with increases in the activity of locomotor generator cells, led investigators to assess 5-HT-related changes in action potential afterresponses. Our results – reversible decreases in afterhyperpolarization in branched cells (motoneurons and interneurons) and the 5-HT insensensitivity of the action potential afterresponses of dorsal sensory cells – are in complete agreement with previously published data obtained in other laboratories [79, 83, 84]. In our experiments, afterhyperpolarization in motoneurons decreased to 50% of initial, while afterdepolarization, conversely, increased (Fig. 9) and could induce repeat action potentials. These points provide further support for the idea that 5-HT has excitatory influences on

motoneurons and segmental interneurons, increasing their activity. Dorsal sensory cells, not within the locomotor generator, generate action potentials whose afterresponses were not changed by 5-HT.

It should be noted that action potential amplitudes in all the cells studied changed insignificantly in response to 5-HT – with increases or decreases – which we believe is associated with changes in the membrane potential. This same cause may underlie changes in the thresholds for action potential generation and in action potential leading edges. In addition, almost all the cells showed increases in action potential duration, mainly because of an altered time course in the descending and, in some cases, the ascending phase. These changes may be important in the conduction of sensory impulses from the periphery, especially in the case of high-frequency discharges from pain-perceiving sensory neurons. It is presently difficult to draw any conclusion regarding the mechanism of the 5-HT-induced changes in the kinetics of the currents underlying action potentials. There are two possible main explanations. The first is an increase in calcium entry into cells, as demonstrated in a number of studies [12, 73], which is often regarded as a possible mechanism of presynaptic facilitation [21, 37]. The second possibility is slowing of repolarization associated with suppression of the potential-activated efflux calcium current, which has also been demonstrated [40, 64, 83]. Since many investigators regard 5-HT in the spinal cords of lampreys and other vertebrates as an agent inducing presynaptic inhibition [18, 75, 89] associated with blockade of the calcium current [8, 25, 26, 68], and the data obtained here also provide evidence in favor of presynaptic inhibition in dorsal sensory cells, the second explanation seems to us to be in better correspondence with the results, though final resolution of the question of the reasons for the increase in action potential duration clearly needs further study. The very fact of changes in action potential parameters due to 5-HT is of interest as another possible mechanism for the modulation by 5-HT of the efficiency of synaptic transmission in the spinal cord.

Our results make it possible to regard 5-HT as a modulator which effectively influences the activity of different types of spinal neurons in the lamprey. This conclusion admits the following facts. Exposure to 5-HT alone produces weak and unclear effects. 5-HT has different effects on different cells of the same type. Thus, most of the branched cells showed depolarization, though there were also some cells which underwent hyperpolarization. The effects of 5-HT on the membrane potential of dorsal sensory cells and on NMDA-induced depolarization and currents in these and other cells were also variable. As in our own experiments, 5-HT has been seen to have effects in different directions in dorsal horn neurons

[77] and in the terminals of primary afferents in frogs [34], and to produce biphasic responses in rat hippocampal cells [4, 7] and cat motoneurons [90]. These facts could sometimes be explained by the use of different concentrations of 5-HT or by the presence of direct and indirect effects on the cells under study. In our experiments, both of these mechanisms were excluded and, moreover, we observed either only postsynaptic (in branched cells) or only presynaptic (in dorsal sensory cells) effects with 5-HT. There is one further possible explanation for the complexity of the effects of 5-HT. The concept that there is a multitude of 5-HT binding sites, or 5-HT receptors, is widely accepted [9, 43, 58, 61, 65, 78, 87]. This property is not characteristic for mediator receptors, but is associated with modulators. There is evidence that some of the 5-HT receptors are associated with second messenger systems [23]. The presence on spinal neuron surface membranes and their processes of several 5-HT receptor subtypes, which interact with many active regions of membranes, may be the cause of the wide variety of responses recorded by electrophysiological methods in brain preparations, in isolated cells, and on fragments of cell membranes.

As regards the lamprey spinal cord, studies in recent years have demonstrated that not only 5-HT, but also other endogenous amines and peptides, such as dopamine [69], tachykinin and substance P [62], and peptide Y and GABA [63] can modulate the activity of spinal segmental neurons, control the locomotor rhythm, and provide for adaptability and lability of the animals' movement behavior.

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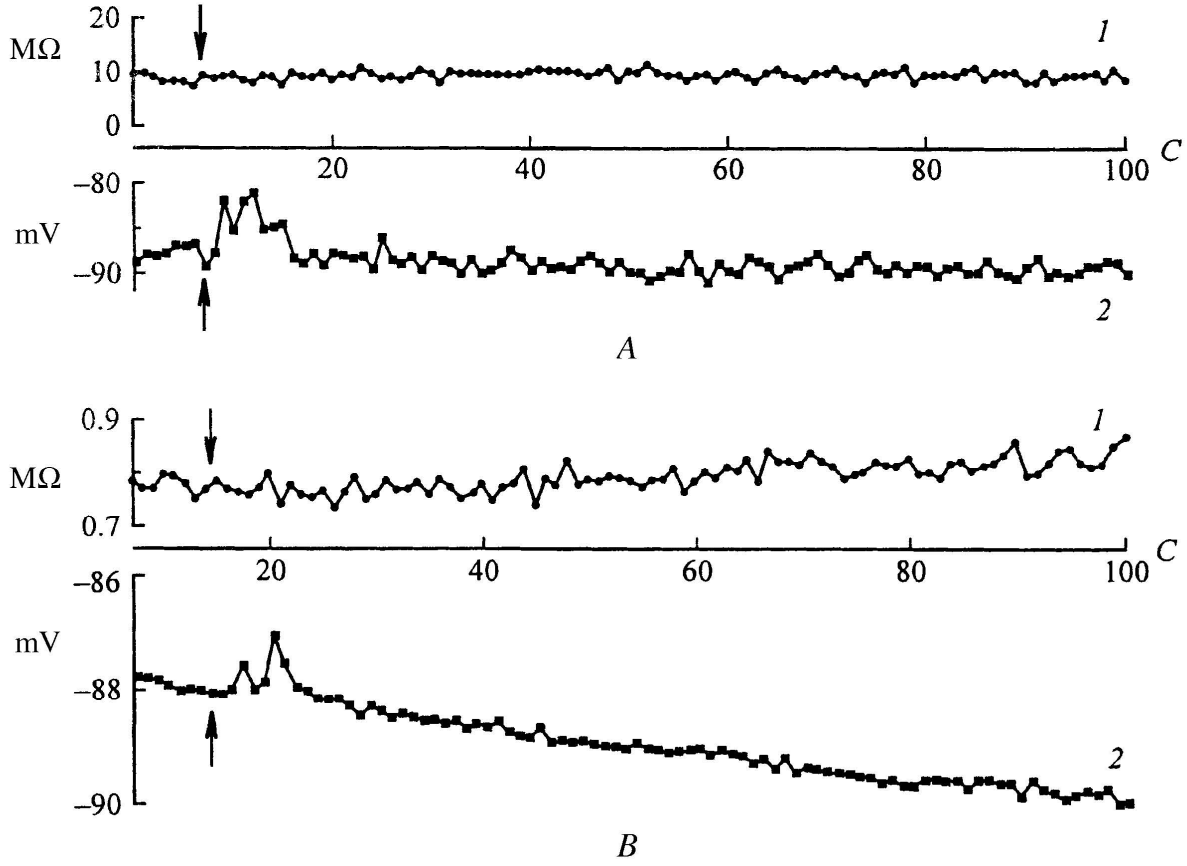
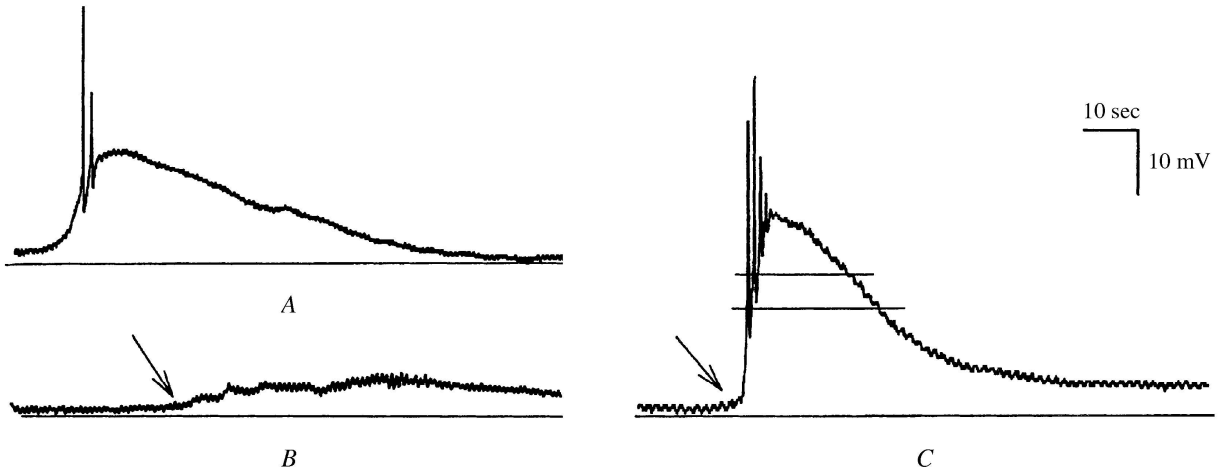
Appendix

Figure 1



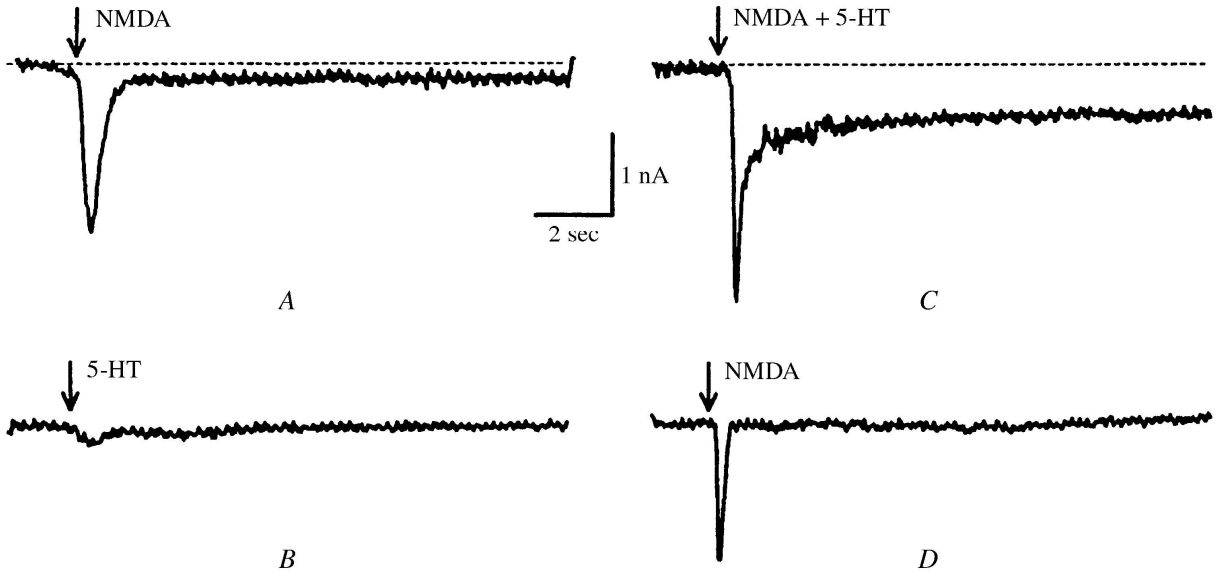
Changes in input resistance (*1*) and membrane potential (*2*) in response to 5-HT in a branched cell (*A*) and a dorsal sensory cell (*B*). The moment of 5-HT application is marked with an arrow.

Figure 2



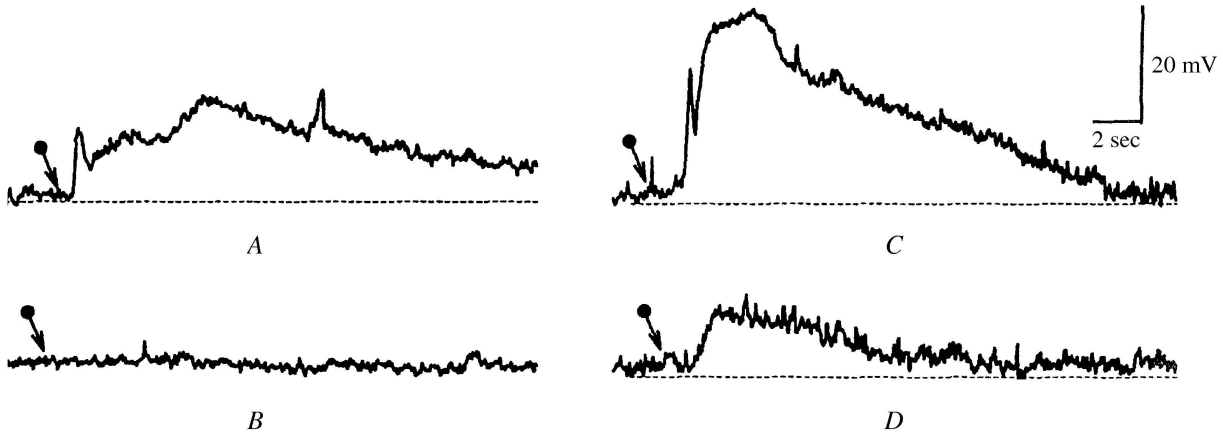
The effects of 5-HT on depolarization induced by NMDA in a branched cell. Depolarization induced by application of 0.8 mM NMDA (*A*), 100 mM 5-HT (*B*), and both agents (*C*). The double lines show the level of possible summation of responses to *A* and *B*. The initial stable membrane potential was –80 mV. Action potentials are visible on the ascending phase of the depolarization. The moment of 5-HT application is marked with an arrow.

Figure 3



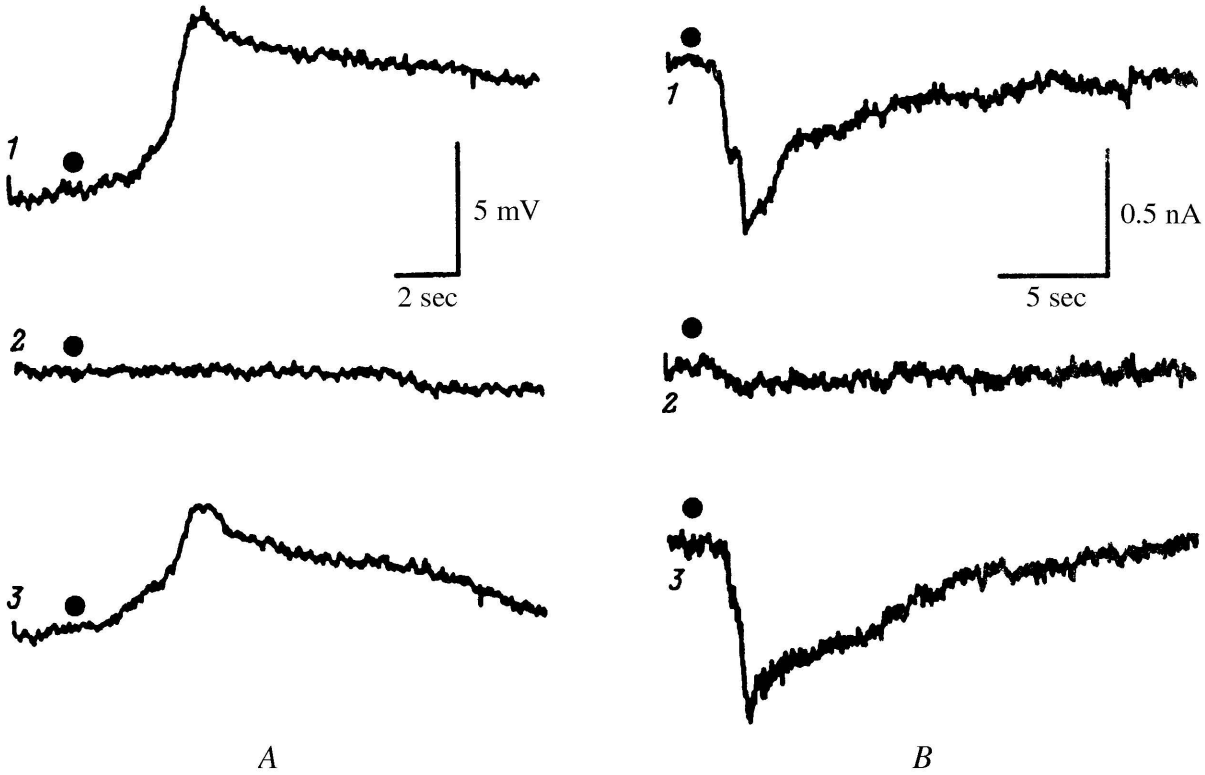
The effects of 5-HT on currents induced by application of NMDA in a branched cell. Current evoked by application of 1 nM NMDA (*A*), 100 mM 5-HT (*B*), NMDA + 5-HT (*C*), and NMDA alone after washing for 6 min in physiological saline (*D*). The moments of substance applications are marked with arrows.

Figure 4



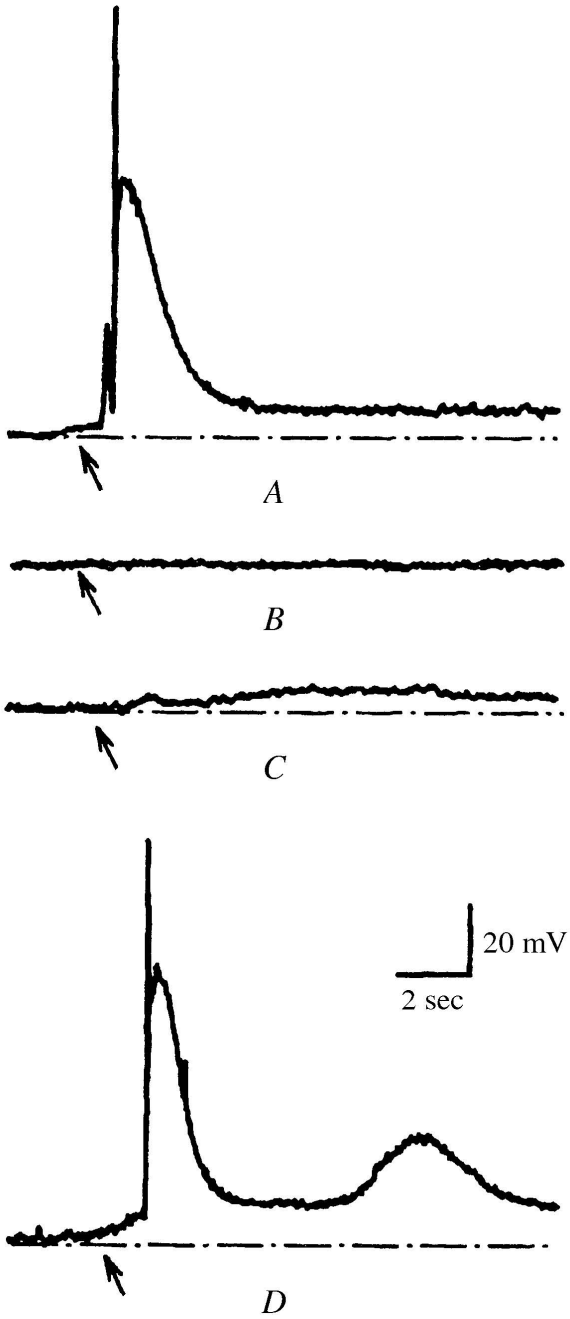
The effects of 5-HT on depolarization induced by aspartate in a branched cell. Depolarization induced by application of 1 mM aspartate (*A*), absence of response to 100 mM 5-HT (*B*), response to application of aspartate in the presence of 5-HT (*C*), and response to application of aspartate after washing for 6 min with physiological saline (*D*). The initial membrane potential was –80 mV. The moment of 5-HT application is marked with an arrow.

Figure 5



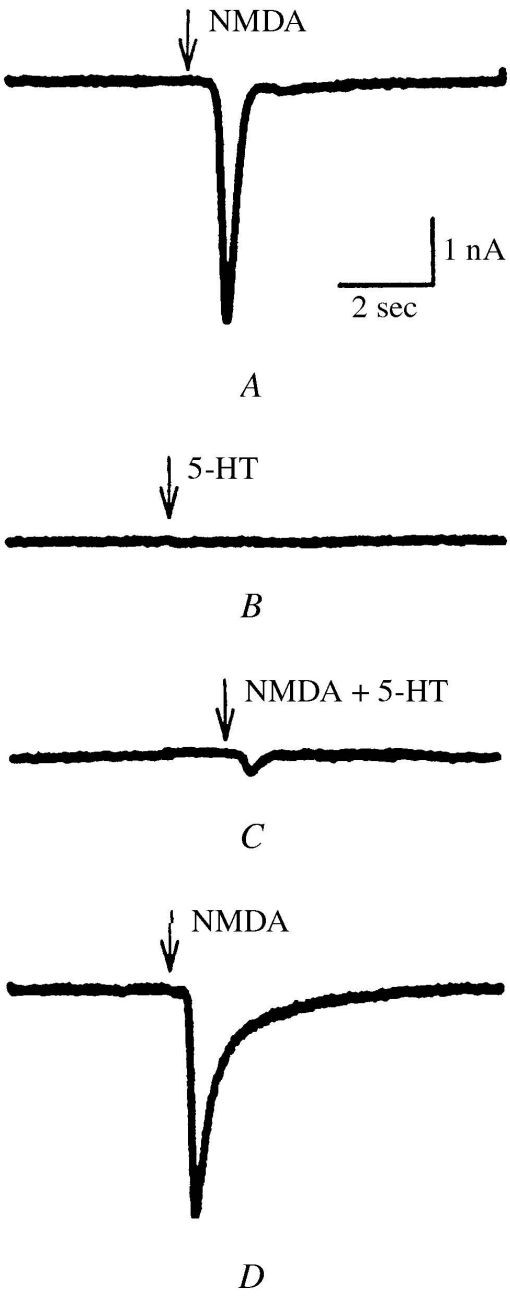
The effects of 5-HT on depolarization and currents induced in branched cells by application of glutamate. *A*) Depolarization induced by 1 mM glutamate (*1*), absence of response to addition of 100 mM 5-HT (*2*), and response to application of glutamate in the presence of 5-HT (*3*); *B*) current evoked in another cell by application of 1 mM glutamate (*1*), addition of 100 mM 5-HT (*2*), and application of glutamate + 5-HT (*3*). The moment of 5-HT application is marked with an arrow.

Figure 6



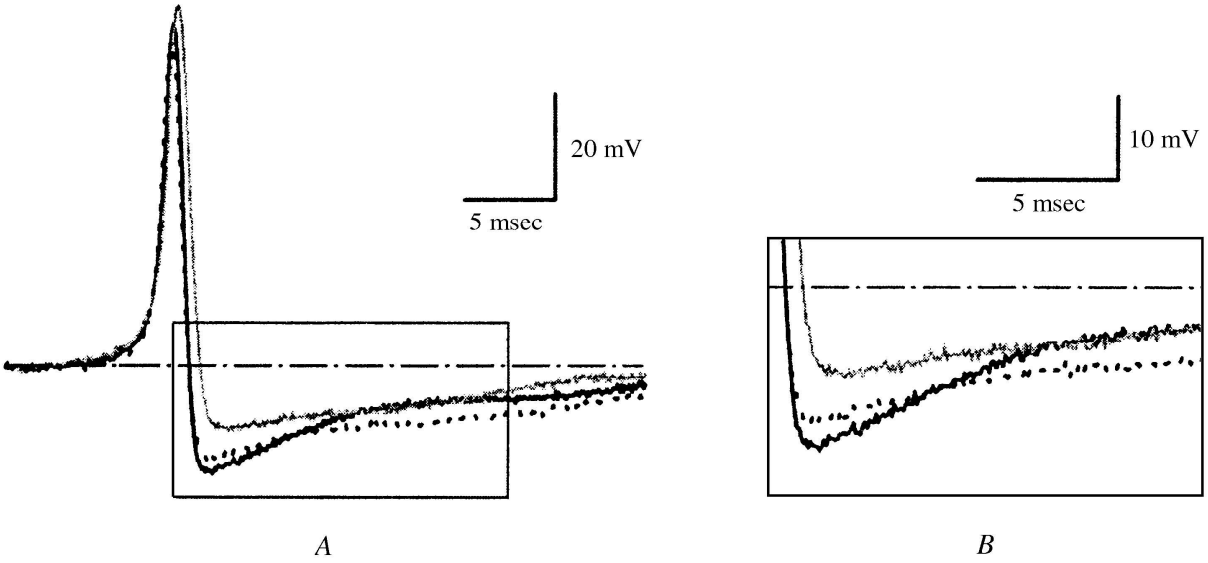
The effects of 5-HT on depolarization induced by NMDA in a dorsal sensory cell. Depolarization induced by application of 1 mM NMDA (*A*), absence of any change in membrane potential in response to application of 100 mM 5-HT (*B*), depolarization induced by NMDA after exposure to 5-HT for 1 min (*C*), and response to application of NMDA after washing for 10 min with physiological saline (*D*). The moments of test substance applications are marked with arrows.

Figure 7



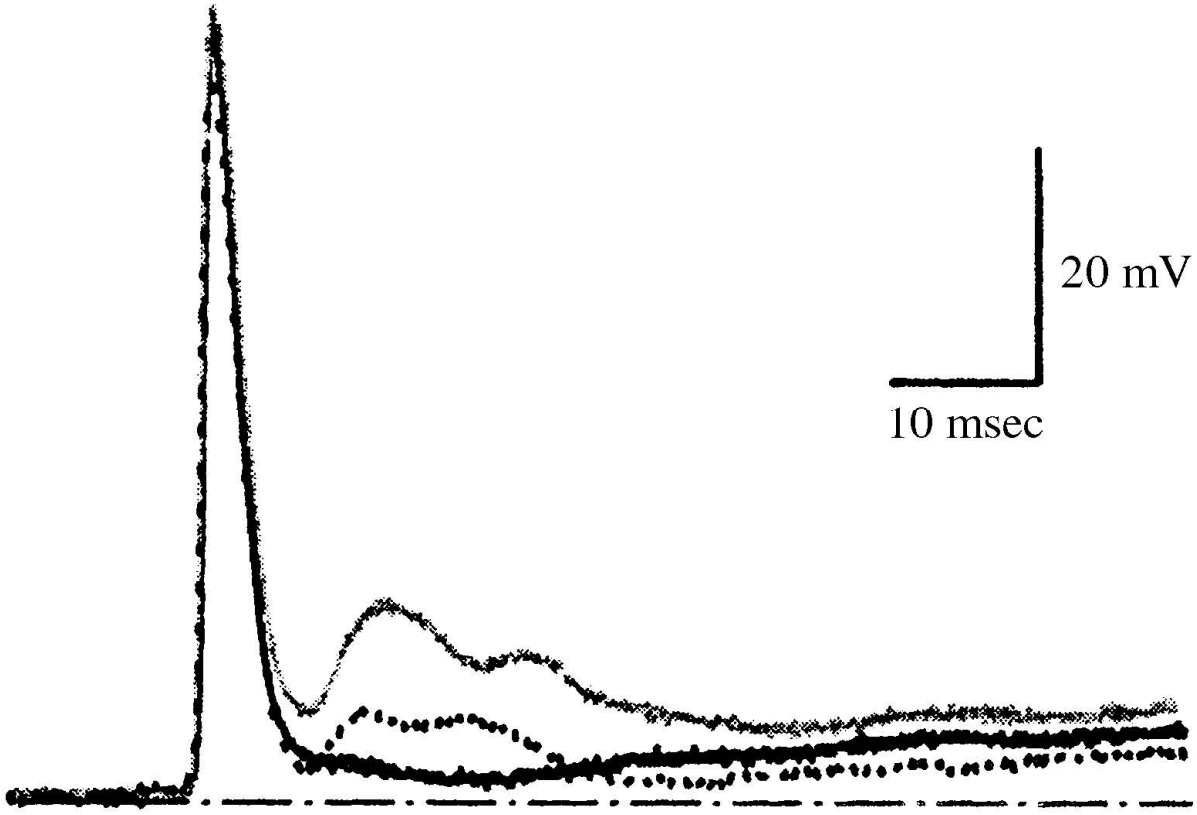
The effects of 5-HT on current activated in a dorsal sensory cell by application of NMDA. Current induced by NMDA alone (*A*); absence of a transmembrane current in response to application of 5-HT (*B*); NMDA in the presence of 5-HT (*C*); NMDA current after washing for 6 min with physiological saline (*D*). The moments of test substance applications are marked with arrows.

Figure 8



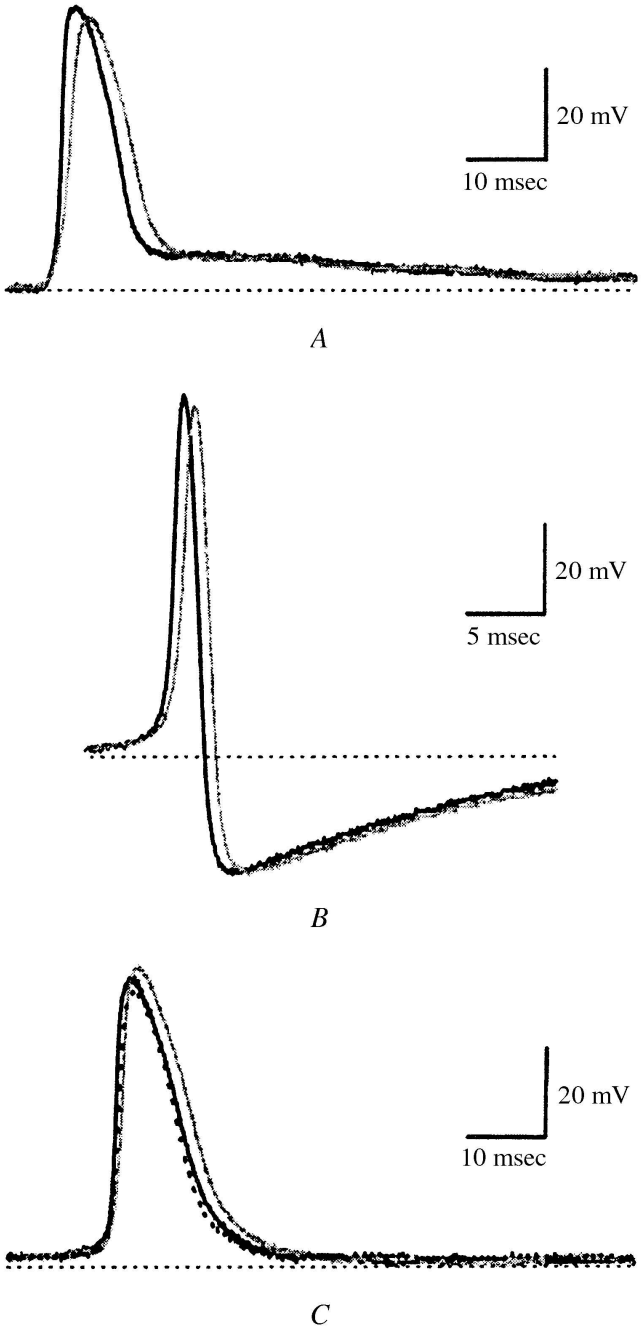
The effects of 5-HT on postspike hyperpolarization in a branched cell. *A*) Action potentials in normal solution (black line), after exposure to 5-HT for 1 min (gray line), and after 10 min of washing would normal physiological saline (dotted line); *B*) magnification of fragment of the plots shown in *A*. The dot-dash line shows the constant potential of –70 mV.

Figure 9



The effects of 5-HT on postspike depolarization in a branched cell. For further details see caption to Fig. 8. The constant potential (–70 mV) is shown by the dot-dash line.

Figure 10



Insensitivity to 5-HT of postspike afterresponses in a dorsal sensory cell. Types of postspike responses in three dorsal sensory cells (*A*, *B*, *C*). For further details see caption to Fig. 8. Constant potentials were –60 (*A*), –90 (*B*), and –70 (*C*) mV. The intrapipette solutions in the examples shown in *A* and *B* contained 100 mM Cs+.