Serotonin modulates axo-axonal coupling between neurons critical for learning in the leech

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Abstract

S cells form a chain of electrically coupled neurons that extends the length of the leech CNS and plays a critical role in sensitization during whole-body shortening. This process requires serotonin, which acts in part by altering the pattern of activity in the S cell network. Lent (1982) observed serotonin-containing axons and varicosities in Faivre’s nerve where the S-to-S cell electrical synapses are located. To determine whether serotonin modulates these synapses, S cell action potential (AP) propagation was studied in a two-ganglion chain containing one electrical synapse. Suction electrodes were placed on the cut ends of the connectives to stimulate one S cell while recording the other, coupled S cell’s APs. A third electrode, placed en passant, recorded the APs near the electrical synapse before they propagated through it. Low concentrations of the gap junction inhibitor octanol increased AP latency across the two-ganglion chain and this effect was localized to the region of axon containing the electrical synapse. At higher concentrations, APs failed to propagate across the synapse. Serotonin also increased AP latency across the electrical synapse, suggesting that serotonin reduced coupling between S cells. This effect was independent of the direction of propagation and increased with the number of electrical synapses in progressively longer chains. Furthermore, serotonin modulated instantaneous AP frequency when APs were initiated in separate S cells and in a computational model of S cell activity following mechanosensory input. Thus, serotonergic modulation of S cell electrical synapses may contribute to changes in the pattern of activity in the S cell network.
Introduction

Electrical synapses, including axo-axonal synapses, are present throughout the CNS both during development and in adults, where they can be associated with plastic changes in the nervous system (Peinado et al. 1993; Peinado 2001; Schmitz et al. 2001). Gap junctions may play major roles ranging from synchronizing activity, mediating rapid, selective synaptic transmission, and providing pathways for passage of small regulatory molecules between cells (reviewed in Bennett and Zukin 2004; Connors and Long 2004). The neurons that make up such networks are known to be modulated by a variety of neurotransmitters. However, it is less understood how these neuromodulators alter electrical synapses, how these changes affect the activity of an entire network, and what effect these changes may have on behavior.

The nervous system of the medicinal leech (Hirudo medicinalis) is particularly well-suited to address these problems. The leech CNS contains a linear network of electrically coupled interneurons, the S cells (Fig. 9). Each segmental ganglion contains a single S interneuron with a bifurcating axon that projects anteriorly and posteriorly into the connective nerves. At the midpoint of each connective nerve, the axon forms an electrical synapse with the S cell axon from the adjacent ganglion, forming a chain that extends throughout the CNS. S cells are so strongly coupled to one another that action potentials arising in one cell reliably propagate in both directions throughout the entire chain (Bagnoli et al. 1972; Frank et al. 1975; Carbonetto and Muller 1977).

The S cell network plays a critical role in sensitization during whole-body shortening, a defensive withdrawal reflex (Sahley et al. 1994; Modney et al. 1997; Burrell et al. 2003). The S cell receives excitatory synaptic input from the sensory neurons that initiate shortening (Baccus et al. 2000; Muller and Scott 1981) and directly excites motor neurons that produce shortening (Gardner-Medwin et al. 1973; Magni and Pellegrino 1978). While the S cell is not required for the animal to shorten (Sahley et al. 1994; Shaw and Kristan 1999), it is required
for sensitization of the shortening reflex (Modney et al. 1997; Sahley et al. 1994). During this form of learning, the number of S cell action potentials fired during shortening as well as the contribution of S cell activity to the reflex increase (Sahley et al. 1994). Recent experiments suggest that there is also an increase in the firing frequency of the S cell network (Cruz et al. 2003; B.D. Burrell, unpublished observation). All of these changes, both behavioral and physiological, appear to be due at least in part to serotonergic modulation (Ehrlich et al. 1992; Burrell et al. 2001, 2002). Depletion of serotonin from the leech CNS eliminates sensitization of the shortening reflex (Ehrlich et al. 1992). Exogenously applied serotonin mimics the learning-induced changes in S cell network activity, most likely through several different mechanisms including an increase in S cell excitability (Burrell et al. 2001) that results in recruitment of additional S cells to initiate action potentials (Burrell et al. 2002).

Each segmental ganglion contains several serotonergic neurons including the Retzius cells which are most likely the primary source of serotonin (Marsden and Kerkut 1969; Rude 1969; Stuart et al. 1974). In addition, Lent (1982) observed serotonin-containing axons and varicosities in Faivre’s nerve where the S cell axon is located, and similar varicosities have been observed at many S-to-S cell electrical synapses (K. J. Muller, personal communication). These may be the terminals containing dense-core vesicles observed in an electron microscope study of S cell electrical synapses (Muller and Carbonetto, 1979). Taken together, these studies raise the possibility that serotonin also modulates the S-to-S cell electrical synapse.

In the present study, we examined whether serotonin modulates the S cell synapse by measuring S cell action potential propagation in a two-ganglion chain containing a single electrical synapse. We found that serotonin increased action potential latency across the total length of the two-ganglion chain, and this effect was localized to the region of axon
containing the electrical synapse. Because S cell action potentials are normally initiated in several different S cells following a mechanosensory stimulus (Baccus et al. 2001; Burrell et al. 2002), we initiated action potentials in two different S cells and found that serotonin changed the instantaneous frequency. In addition, we used a computational model in which action potentials were generated in several different S cells using a pattern of S cell action potential initiation frequency similar to that observed following mechanosensory stimuli (Baccus et al. 2001; Burrell et al. 2002). The model suggests that modulation of S-to-S cell electrical synapses may contribute to the observed learning- and serotonin-induced changes in S cell network activity.
Materials and Methods

Animals

Leeches (2-3 g) were obtained from a commercial supplier (Leeches USA, Westbury, NY) and maintained in artificial pond water (HIRUDO salt, Leeches USA) in a refrigerated incubator at 18-20°C. Animals were anesthetized by cooling at 4°C in artificial pond water.

Intracellular recording

Single ganglia were dissected and transferred to a recording chamber filled with leech saline which consisted of (in mM) 110 NaCl, 5 NaOH, 4 KCl, 1.8 CaCl₂, and 10 HEPES, at pH 7.4. Each cell was impaled with a thin-walled, glass microelectrode (Frederick-Haer) filled with 3 M potassium acetate and having a 20-40 MΩ resistance. Signals were amplified using a BA-1S amplifier (NPI), viewed on a storage oscilloscope and then converted for digital storage using Axoscope data acquisition software with a Digidata 1322A A/D converter (Axon Instruments). Stimulus pulses were delivered using an STG 1004 programmable stimulator (Multichannel Systems). Experiments were performed at room temperature (22-24°C).

To examine the effect of octanol on electrical coupling between Retzius cells, initial measurements of coupling and input resistance were made by injecting hyperpolarizing current pulses (1 nA, 500 msec) into one Retzius cell (R₁) and measuring the resulting changes in membrane potential in R₁ (∆V₁) and the contralateral Retzius cell (R₂, ∆V₂). Electrical coupling was defined as the ratio ∆V₂/∆V₁. The ganglion was then perfused for 20 min. with 0.8 mM octanol (Sigma) dissolved in leech saline followed by a second set of coupling and input resistance measurements. To test for recovery, the preparation was washed with leech saline for 30 min. and then a third set of measurements was made.

To examine the effects of octanol and serotonin on changes in S cell response properties, S cells were identified by their location on the ganglion ventrum in the central neuropil packet...
close to the Retzius cells, diameter of the soma (~10 µm), and rapid, overshooting action potential. Input resistance was measured by injecting hyperpolarizing current pulses (1 nA, 500 msec) and latency to first spike was measured by injecting depolarizing current pulses (1 nA, 500 msec) and measuring the interval from the beginning of the current pulse to the peak of the first action potential. Both of these measurements as well as resting potential and threshold were made before and after a 20 min. incubation in 0.5 mM octanol or 50 µM serotonin (Sigma) dissolved in leech saline. Threshold for action potential initiation was defined as the point at which the slope of the membrane potential increase equaled 20 mV/s (Bekkers and Delaney 2001).

**Action potential propagation between S cells**

Chains of two to four ganglia were dissected from the midbody region and transferred to a recording chamber filled with leech saline. In each experiment, the tissue was perfused with cold leech saline and the rate of perfusion was adjusted as needed to maintain constant temperature (± 0.2 °C). Temperatures across different experiments ranged from 17-20°C.

Stimulation and recording were done using extracellular suction electrodes, which are not invasive and therefore less damaging than intracellular sharp electrodes. It is not possible to make chronic intracellular recordings of the S cell for the entire duration of the experiment (~60 min) without damaging the neuron, probably due to the small size of the S cell soma and the elasticity of the leech CNS (Burrell and Sahley 2004). Controlled stimulus pulses were delivered using a Grass S88 two-channel stimulator with SIU5 stimulus isolation units. Signals were amplified with Grass P5 AC preamplifiers, viewed on a storage oscilloscope and then converted for digital storage using Axoscope data acquisition software with a Digidata 1322A A/D converter (Axon Instruments).
To examine modulation of the S-to-S cell electrical synapse, S cell action potential propagation was measured in a two-ganglion chain containing a single electrical synapse (Fig. 1). Suction electrodes were placed on the cut ends of the connective to stimulate one S cell while recording the other, coupled S cell’s action potentials, which were distinctive as the largest impulses in extracellular recordings (Laverack 1969; Bagnoli et al. 1972; Frank et al. 1975). In some experiments, a third suction electrode placed *en passant* recorded the S cell action potentials near the electrical synapse before they propagated through it. Propagation across the total length of the two-ganglion chain was measured as the difference between the time of the stimulus artifact and the arrival time at the suction electrode on the cut end (action potential latency). Conduction along the axon without the electrical synapse was measured as the difference between the time of the stimulus artifact and the arrival time at the *en passant* electrode. Propagation including the electrical synapse was measured by taking the difference between the arrival times at the two recording electrodes. In each experiment, initial measurements of action potential propagation were taken by delivering ~40 stimuli at 20 Hz, which is within the range of S cell firing frequencies following a mechanosensory stimulus (Baccus et al. 2001; Burrell et al. 2002). The preparation was then perfused for 20 min with drug dissolved in leech saline (either octanol or serotonin; Sigma) and a second set of action potential propagation measurements was made. To test for recovery, the preparation was washed with leech saline for 30 min and propagation was measured again.

The effects of serotonin on instantaneous action potential frequency were studied using chains of four ganglia. As described above, suction electrodes were placed on the cut ends of the connectives to stimulate and record S cell action potentials. However, the *en passant* electrode was used to stimulate and was placed so that there were two electrical synapses between the two stimulating electrodes (see Fig. 9). Action potentials were initiated in pairs such that the first action potential was initiated in one S cell and the second action potential
was initiated in a separate S cell with a 20 ms delay between stimulating the two sites. Instantaneous frequency was defined as the frequency of a given pair of action potentials. In each experiment, two initial instantaneous frequency measurements were made. For the first measurement, ~20 pairs of action potentials were initiated using the same temporal sequence for the two stimulating electrodes. For the second measurement, the temporal order of the stimulating electrodes was reversed and another ~20 pairs of action potentials were initiated. The preparation was then perfused for 20 min with 50 μM serotonin dissolved in leech saline, followed by a second set of instantaneous frequency measurements. To test for recovery, the preparation was washed with leech saline for 30 min, followed by a third set of frequency measurements. In control experiments, the stimulating electrodes were placed on the same S cell on both sides of the ganglion.

Compartmental modeling

To explore the possible functional consequences of serotonergic modulation of electrical synapses at the S cell network level, computational modeling was performed using version 8.0 of the simulation program SNNAP (Simulator for Neural Networks and Action Potentials; http://snnap.uth.tmc.edu; Ziv et al. 1994). The model has been added to the ModelDB database (http://senselab.med.yale.edu/SenseLab/ModelDB; Hines et al. 2004), where one may download and run the simulation as well as view all parameters and equations.

The model consisted of five S cells as diagramed in Fig. 10B. Each S cell axon was modeled by modifying an example simulation provided by SNNAP (SNNAP8/Examples/Compartment_models/Cable/ Cable_10.smu), which is a neuronal process with ten homogeneous compartments linked together in an unbranched “cable”. The geometry of the cable was adjusted to approximate that of a single S cell axon with length = 5
mm and diameter = 10 µm (S cell axon diameter ranges from 5-10 µm (Muller and Carbonetto 1979)). Adjacent S cells were linked by simulated, non-rectifying electrical synapses. Because the example simulation includes only passive membrane properties, it was necessary to add active conductances. However, since voltage clamp data is not available for S cells, voltage-dependent sodium and potassium conductances as well as a leak conductance were modeled using the Hodgkin-Huxley squid axon simulation provided by SNNAP (SNNAP/Examples/HH_type_neurons/Biophysics_01/Lab_01_Squid/Spike) with the temperature set to 19.0°C. In each simulation, the initial value of the membrane potential = -50 mV. Because the primary goal was to reproduce experimentally measured S cell action potential latencies and conduction velocity, the conductance for connections between S cell axon compartments = 0.196 µS, for the electrical synapse between S cells under control conditions = 0.098 µS, and for the electrical synapse between S cells after modulation by 50 µM serotonin = 0.064 µS.

Before using the model to explore S cell network properties, it was tested at various stages of refinement by examining its ability to reproduce experimentally measured action potential latencies. A version of the model consisting of two S cells connected by a single electrical synapse accurately reproduced the action potential latency measured across a two-ganglion chain at 19°C under control conditions (data not shown). This model also reproduced the serotonin-induced increase in latency measured across the entire two-ganglion chain as well as localization of this modulation to the region containing the electrical synapse (see experimental data in Figs. 6 and 7). A series of models with one, two, three, or four S cells approximated the dependence of the serotonin-induced change in latency on the number of electrical synapses (see experimental data in Fig. 8). The four-S cell model also reproduced the serotonin-dependent change in instantaneous frequency when action potentials were initiated in two different S cells (see experimental data in Fig. 9). Finally, the conduction
velocity of the simulated four-S cell chain was ~1.59 m/s, which is consistent with previously reported experimental values (Frank et al. 1975).

The stimulation protocol was based on experimentally measured S cell action potential initiation frequencies following mechanosensory stimuli (Baccus et al. 2001; Burrell et al. 2002). As diagramed in Fig. 10A and B, 11 action potentials were initiated at three different S cell initiation sites by brief current injection (2 nA, 0.5 ms). The resulting S cell network activity was recorded intracellularly at the anterior and posterior ends of the simulated S cell chain and the data were exported to Axoscope (Axon Instruments) for analysis.

Statistical analysis

Results are presented as the mean ± SEM. Statistical analysis was performed using one-way ANOVA as well as independent t tests using Statistica analysis software (Statsoft).
Results

Octanol decreases electrical coupling between Retzius cells

Before determining whether serotonin modulates the S-to-S cell electrical synapse, we wished to test the experimental approach of using action potential propagation to monitor modulation of the synapse by applying the gap junction inhibitor octanol. However, although octanol has been shown to decrease gap junctional conductance in many systems (Johnston et al. 1980; Johnston and Ramón 1981; Weingart and Bukauskas 1998; Adler and Woodruff 2000; Harris 2001), it has not been previously tested in the leech. Therefore, to determine whether octanol also reduces electrical coupling in the leech, we examined the effect of octanol on electrical coupling between Retzius cells. Each ganglion contains a pair of Retzius cells that are coupled by non-rectifying electrical synapses. Because of their large soma size (~50 µm), Retzius cells are more amenable to long-term physiological recordings than S cells (see Materials and Methods). As shown in Fig. 2A, 0.8 mM octanol decreased electrical coupling ($\Delta V_2 / \Delta V_1$) between a pair of Retzius cells from 0.49 ± 0.06 to 0.36 ± 0.04. These data are summarized in Fig. 2B which plots measurements of electrical coupling for each treatment. Comparison of octanol-treated Retzius cell pairs with Retzius cells from the saline group using a one-way ANOVA with repeated measures (n = 4) confirmed a significant treatment/trial interaction effect (P < 0.05) and post hoc analysis showed a significant difference in coupling between pre-treatment and treatment measures in the octanol group (P < 0.05), but not in the saline group. Because a decrease in input resistance can cause an apparent decrease in electrical coupling, we measured input resistance as well. As shown in Fig. 2C, 0.8 mM octanol had no significant effect on input resistance (n = 4). Thus, these data indicate that octanol decreases gap junctional conductance in the leech.
Octanol increases S cell action potential latency across a two-ganglion chain

Because octanol is an effective inhibitor of gap junctional conductance in the leech, we used it to test the experimental approach of using action potential propagation to monitor modulation of the S-to-S cell electrical synapse. As shown in Fig. 3A (left), 0.5 mM octanol increased S cell action potential latency across the total length of a two-ganglion chain, and this effect was readily reversible. Because repetitive firing can alter action potential propagation, we tested whether the stimulation protocol altered latency by repeating the experiment on a different preparation that was perfused with leech saline only. As shown in Fig 3A (right), the stimulation protocol had no detectable effect on action potential latency. These experiments are summarized in Fig. 3B, which plots the mean change in action potential latency for each treatment (Octanol, n = 4; Saline, n = 3). Compared to saline alone, 0.5 mM octanol caused a significant increase in action potential latency (P < 0.001).

The octanol-induced increase in S cell action potential latency is localized to the region of axon containing the electrical synapse

To localize the octanol-induced increase in S cell action potential latency, a third suction electrode was placed en passant to record S cell action potentials near the electrical synapse before they propagated through it (see Methods, Fig. 1). This allowed a direct comparison of action potential propagation between approximately equal lengths of axon with and without the electrical synapse. Consistent with previous experiments, 0.5 mM octanol increased latency across the entire two-ganglion chain, as shown in Fig. 4A. However, the drug had little effect on the latency of action potentials recorded with the en passant electrode, so octanol acted primarily on tissue between the two recording electrodes. These experiments are summarized in Fig. 4B, which plots the mean change in action potential latency for the total length of the two-ganglion chain, the length of axon without the electrical synapse, and
the length of axon with the electrical synapse (n = 3). One-way ANOVA showed significant differences in the change in latency across these three lengths of axon (P < 0.05). Post hoc analysis indicated that the increase in latency across the length of axon without the electrical synapse was significantly different from the increases in latency across both the total length of the chain (P < 0.01) and the length of axon with the synapse (P < 0.01). In contrast, there was no significant difference between the mean increases in latency across the entire chain and across the length of axon containing the electrical synapse. The small increase in latency in the segment of axon without the synapse was not significantly different from saline controls (data not shown). Thus, the octanol-induced increase in action potential latency is most likely localized to the region of axon containing the electrical synapse.

**Octanol has no significant effect on threshold or latency to first action potential**

Although the inhibitory effect of octanol is localized to the region of axon containing the electrical synapse, it is possible that octanol increases latency by increasing action potential threshold in this region of membrane. Therefore, we used intracellular recording to examine the effect of octanol on S cell threshold as well as latency to first action potential. We found that 0.5 mM octanol had no significant effect on threshold (Pre-treatment = -35.7 ± 1.6 mV, n = 3; 0.5 mM octanol = -36.7 mV ± 0.9, n = 3) or latency to first action potential (Pre-treatment = 80.7 ± 3.4 ms, n = 3; 0.5 mM octanol = 80.8 ± 2.5 ms, n = 3). We also found that octanol had no significant effect on resting potential (Pre-treatment = -53.0 ± 1.0 mV, n = 3; 0.5 mM octanol = -51.7 ± 1.5 mV, n = 3) or input resistance (Pre-treatment = 17.8 ± 0.9 MΩ, n = 3; 0.5 mM octanol = 20.6 ± 0.6 MΩ, n = 3). Taken together, these data suggest that the octanol-induced increase in latency is not due to an increase in threshold and that 0.5 mM octanol has no major untoward effects on the S cell.
At a higher concentration of octanol, S cell action potentials often fail to propagate across the electrical synapse

To see whether a higher concentration of octanol would reduce junctional conductance to the point that action potentials failed to propagate across the electrical synapse, a two-ganglion chain was incubated in 0.8 mM octanol. As shown in Fig. 5A, in the presence of octanol some of the action potentials recorded by the en passant electrode did not propagate to the suction electrode on the cut connective, suggesting failure to propagate across the synapse. Fig. 5B presents a summary plot (n = 3) showing that in 0.8 mM octanol, action potential failures were never observed at the en passant electrode, but ~ 67% of the action potentials failed to propagate across the electrical synapse and this effect was statistically significant (P < 0.05). At octanol concentrations greater than 0.8 mM, action potential failures were observed at the en passant electrode (data not shown), possibly due to sodium channel block (Swenson and Narahashi 1980). Thus, we were unable to examine the effects of octanol concentrations greater than 0.8 mM on the electrical synapse.

In summary, results from the octanol experiments are consistent with octanol as a well-characterized inhibitor of gap junctional conductance and suggest that modulation of the S-to-S cell electrical synapse can be studied by examining action potential propagation.

Serotonin also increases S cell action potential latency across a two-ganglion chain

As shown in Fig. 6A, 50 µM serotonin also increased S cell action potential latency across the total length of a two-ganglion chain, and this effect was largely reversible. Fig. 6B presents a summary plot (n = 10) showing that compared to saline alone, 50 µM serotonin caused a significant increase in action potential latency (P < 0.01). A previous study has shown that serotonin can either increase or decrease S cell excitability, depending on serotonin concentration (Burrell et al. 2001). Therefore, we examined the change in action potential
latency over a range of serotonin concentrations. Fig. 6C presents a dose-response plot of the mean increase in latency versus serotonin concentration (1µM, n = 3; 5 µM, n = 3; 10 µM, n = 3, 20 µM, n = 3; 50 µM, n = 10). One-way ANOVA showed a significant increase in the change in latency with increasing serotonin concentration (P < 0.05). Post hoc analysis indicated that, compared to the saline group, 5 to 50 µM serotonin caused significant increases in action potential latency (5 µM, P < 0.05; 10 µM, P < 0.05; 20 µM, P < 0.01; 50 µM, P < 0.01). The superimposed curved is a sigmoidal fit to illustrate the trend in the data. This effect appeared to be half-maximal at ~10 µM and maximal at ~50 µM. We did observe a very small decrease in mean latency at 1 µM serotonin, but this change was not significant.

The serotonin-induced increase in S cell action potential latency is localized to the region of axon containing the electrical synapase

To localize the serotonin-induced increase in S cell action potential latency, we recorded action potentials before and after they propagated through the electrical synapse, as described for the octanol experiments. Consistent with previous experiments, 50 µM serotonin increased latency across the entire two-ganglion chain, as shown in Fig. 7A. However, the drug had little effect on the latency of action potentials recorded with the en passant electrode, suggesting that serotonin acted primarily on tissue between the two recording electrodes. These experiments are summarized in Fig. 7B, which plots the mean change in action potential latency for the total length of the two-ganglion chain, the length of axon without the electrical synapse, and the length of axon with the electrical synapse (n = 7). One-way ANOVA showed significant differences in the change in latency across these three lengths of axon (P < 0.05). Post hoc analysis indicated that the mean change in latency across the length of axon without the electrical synapse was significantly different from the
increases in latency across both the total length of the chain (P < 0.05) and the length of axon with the synapse (P < 0.05). In contrast, there was no significant difference between the mean increases in latency across the entire chain and across the length of axon containing the electrical synapse. Thus, like octanol, the serotonin-induced increase in action potential latency is most likely localized to the region of axon containing the electrical synapse.

Unlike octanol, however, we found that serotonin did not cause action potential failures. This is most likely due to the fact that the serotonin-induced increase in latency saturates before failures occur.

It is possible that the serotonin-induced increase in action potential latency reflects an increase in passive membrane conductance rather than a decrease in junctional conductance. In an attempt to distinguish these possibilities, we used intracellular recording to examine the effect of 50 µM serotonin on S cell input resistance. We recorded from S cell somas in single ganglia with sufficient lengths of anterior and posterior connective nerves to include the flanking S-to-S cell electrical synapses, and found that serotonin had no significant effect on input resistance (data not shown). This suggests that changes in conductance at or very close to the electrical synapse cannot be detected when recording at the S cell soma which is typically 2-3 mm away.

Serotonin has no significant effect on threshold or latency to first action potential

As with the octanol experiments, localization of the serotonin-induced increase in latency to the region of axon containing the electrical synapse does not rule out the possibility that serotonin increases action potential threshold. Therefore, we used intracellular recording to examine the effect of 50 µM serotonin on S cell threshold as well as latency to first action potential. We found that 50 µM serotonin had no significant effect on threshold (Pre-treatment = -37.4 ± 1.1 mV, n = 3; 50 µM serotonin = -36.8 ± 1.1 mV, n = 3) or latency to
first action potential (Pre-treatment = 75.1 ± 4.5 ms, n = 3; 50 µM serotonin = 73.4 ± 2.7 ms, n = 3). We also found that serotonin had no significant effect on resting potential (Pre-treatment = -49.3 ± 2.0 mV, n = 3; 50 µM serotonin = -50.3 ± 2.0 mV, n = 3) or input resistance (Pre-treatment = 17.1 ± 2.2 MΩ, n = 3; 50 µM serotonin = 18.2 ± 1.5 MΩ, n = 3). Thus, these data suggest that the serotonin-induced increase in latency is not due to an increase in threshold.

The serotonin-induced increase in S cell action potential latency is independent of the direction of propagation

The S cell electrical synapse is non-rectifying in that current flows equally well in both directions (Frank et al., 1975; Muller and Carbonetto 1979). As a result, action potentials arising in one S cell reliably propagate in both directions throughout the entire chain (Bagnoli et al. 1972). To determine whether the same is true for modulation of the synapse, we compared the serotonin-induced increase in S cell action potential latency in 50 µM serotonin between two groups of two-ganglion preparations (n = 5 for each group) that differed only in the direction of action potential propagation. We found that there was no significant difference in the mean increase in latency between the two groups (anterior → posterior propagation, 1.0 ± 0.2 ms; posterior → anterior propagation, 1.1 ± 0.4 ms), suggesting that the serotonin-induced increase in S cell action potential latency is independent of the direction of propagation.
The serotonin-dependent increase in action potential latency increases with the number of electrical synapses

Since serotonin increases action potential latency at or very close to the electrical synapse, the latency should increase linearly with the number of electrical synapses. This appears to be the case, as shown in Fig. 8, which plots the mean change in latency induced by 50 µM serotonin versus the number of electrical synapses in progressively longer chains of ganglia (0 synapses, n = 4; 1 synapse, n = 10; 2 synapses, n = 5; 3 synapses, n = 12). The superimposed line represents a linear regression fit to the data with a slope of approximately 0.8. Although previous experiments using three suction electrodes indicated that serotonin has no significant effect on latency in segments of axon lacking the electrical synapse (Fig. 7), it is possible that this effect would become significant with a longer length of S cell axon. Because it is not possible to determine this experimentally, we estimated the serotonin-induced change in latency on progressively longer axons using data from single ganglia without the electrical synapse (even though this increase in latency was still not statistically significant). As shown by the dotted line in Fig. 8, this effect likely represents a minor component of the observed changes in latency, increasing with a slope of ~0.16. Thus, the ~4-fold greater slope of the observed serotonin-dependent changes in action potential latency is consistent with localization of the modulation to the region containing the electrical synapse.

Serotonin can change instantaneous frequency when action potentials are initiated in different S cells

The observation that the serotonin-dependent increase in action potential latency increases with the number of electrical synapses raises the possibility that serotonin alters instantaneous frequency when action potentials are initiated in different S cells, which occurs during an
elicited shortening response (see below). When a pair of action potentials is initiated in two different S cells (one action potential per cell), the action potentials differ in the number of electrical synapses through which they propagate (when comparing propagation in either the anterior or posterior direction). Because an action potential propagating through the greater number of electrical synapses may experience a greater serotonin-induced increase in latency, we hypothesized that such a differential modulation of latency could either increase or decrease the interval between the two action potentials (depending on the temporal order of initiation) and thus either increase or decrease the instantaneous frequency.

To test this hypothesis, action potentials were initiated in pairs such that the first action potential was initiated in one S cell and the second was initiated in a separate S cell, with two electrical synapses between the two stimulating electrodes (see Methods, Fig. 9). As shown in Figure 9A, we found that 50 µM serotonin either increased or decreased the interspike interval, depending on the temporal order of stimulation at the two sites. These experiments are summarized in Fig. 9B, which shows that compared to controls in which the two action potentials were initiated in the same S cell (by placing the two stimulating electrodes on either side of a single ganglion; n = 3), 50 µM serotonin significantly increased (P < 0.05, n = 5) and decreased (P < 0.01, n = 4) the interspike interval. While the order of stimulation determined the direction of change, it had no major effect on the magnitude of the change (1st spike at stimulating electrode 2: range = 0.8 to 3.4 ms; 1st spike at stimulating electrode 1: range = -1.2 to -2.5 ms). We did observe a small decrease in the interspike interval in control experiments. This effect was independent of the temporal order of stimulation and recovered following a 30 min wash in saline (data not shown). Fig. 9C shows that the observed changes in interspike interval when action potentials were initiated in two different S cells had very different effects on instantaneous frequency. Serotonin significantly increased frequency by ~12 Hz when the first action potential was initiated by stimulating electrode 1 (P < 0.01), but
had little effect on frequency when the first action potential was initiated by stimulating electrode 2. This differential effect on frequency is due to the different initial spike frequencies which were \( \sim 73 \) Hz (1\textsuperscript{st} spike at stimulating electrode 1) and \( \sim 38 \) Hz (1\textsuperscript{st} spike at stimulating electrode 2), since a given change in interspike interval duration will have a greater impact on frequency when the initial interval duration is relatively brief (and initial frequency is relatively high). Thus, these results suggest that serotonergic modulation of S cell electrical synapses can change instantaneous frequency and that the magnitude of this effect is greater at higher initial frequencies (see Discussion, Fig. 11).

A compartmental model of the S cell chain suggests that modulation of electrical synapses may alter the pattern of S cell network activity following a mechanosensory stimulus. The serotonin-dependent change in instantaneous frequency raises the possibility that modulation of S-to-S cell electrical synapses alters the overall pattern of S cell network activity in response to a mechanosensory stimulus. A mechanosensory stimulus typically initiates S cell action potentials in multiple adjacent segments (up to five), because each sensory cell innervates several neighboring S cells and each region of skin within a segment is innervated by sensory neurons from several segmental ganglia. As a result, a single mechanosensory stimulus produces a train of S cell action potentials which is the sum of staggered action potential initiations from several different S cells (Baccus et al. 2001; Burrell et al. 2002). However, it would be difficult to assess experimentally the effects of modulating the electrical synapses, because serotonin has other modulatory effects that can alter activity of the S cell network, such as increasing excitability of the S cell (Burrell et al. 2001) as well as relieving conduction block at central branch points in mechanosensory cells (Mar and Drapeau 1996). Therefore, we used a compartmental model of a five-S cell chain as diagramed in Fig. 10\textit{B}. As illustrated in Figs. 10 \textit{A} and \textit{B}, 11 action potentials were
generated in three different S cells using a pattern of S cell action potential initiation
frequency similar to that observed following a mechanosensory stimulus (Baccus et al. 2001;
Burrell et al. 2002). Figures 10C and D show the resulting S cell activity recorded at the
anterior and posterior ends of the simulated S cell chain under control conditions. (Changes
in the pattern of activity due to modulation of the electrical synapses cannot be seen on such a
slow time base). Figure 10E-H shows how “modulating” the electrical synapses by reducing
the conductance (see Methods) changed the interspike intervals and instantaneous frequencies
in the anterior (Figs. 10E and G) and posterior (Figs. 10F and H) recordings. In each
recording, with the exception of the first interval (both action potentials initiated at the central
site) and last interval (both action potentials initiated at the posterior site), all other interspike
intervals (action potentials initiated at two different sites) either increased or decreased, with
an average change of ~0.5 ms. However, only intervals three (indicated by *) and nine
(indicated by #) in the posterior recording showed significant changes in instantaneous
frequency, with increases of ~14 Hz and ~21 Hz, respectively. As discussed in the previous
section, such differential effects on instantaneous firing frequency are due to the fact that
small changes in the interspike interval only cause large changes in instantaneous spike
frequency when the initial spike frequency is relatively high. Thus, these modeling results
suggest that serotonergic modulation of S-to-S cell electrical synapses may in fact cause
significant changes in the pattern of S cell network activity following a mechanosensory
stimulus.
Discussion

In this study, we have shown that bath applied serotonin as well as the gap junction inhibitor octanol increased S cell action potential latency and this effect was localized to the segment of axon containing the S-to-S cell electrical synapse. The serotonergic modulation was independent of the direction of propagation and increased with the number of electrical synapses. When action potentials were initiated in different S cells, both experimentally and in a computational model, serotonin changed the instantaneous frequency. These results suggest that serotonin decreases electrical coupling between S cells and that this modulation may alter the overall pattern of activity in the S cell network.

Octanol modulation of the S cell electrical synapse

Localization of the octanol-induced increases in action potential latency and propagation failures to the segment of axon containing the electrical synapse suggests that octanol decreases electrical coupling between S cells. These results are consistent with octanol as a well-characterized inhibitor of gap junctions in many systems, both vertebrate and invertebrate (Johnston et al. 1980; Johnston and Ramón 1981; Weingart and Bukauskas 1998; Adler and Woodruff 2000; Harris 2001) as well as our data showing that octanol uncouples leech Retzius cells. Recent molecular studies suggest that gap junctions in protostomes (which includes the leech) and deuterostomes (which includes some invertebrates and all vertebrates) are encoded by three distinct families of gap junction genes which show very low sequence identities to one another (Phelan and Starich 2001; Tearle 2002; Alexopoulos et al. 2004; White et al. 2004). Protostomal species appear to have only innexin genes, while deuterostomes have both connexin and pannexin genes. Thus, octanol most likely exerts its widespread inhibitory effects through a relatively non-specific mechanism rather than by binding to a specific site. While the precise mechanism of action is not clear, octanol is
thought to partition into the membrane and alter the physical properties of the bilayer. In particular, experiments have shown that the inhibitory effects of octanol correlate with a decrease in membrane fluidity (Bastiaanse et al. 1993).

As with many drugs, octanol can exert effects on other channel proteins. In particular, octanol has been shown to inhibit certain sodium channels in both vertebrates and invertebrates (Hirche 1985; Poyraz et al. 2003; Adams and Gage 1979; Oxford and Swenson 1979; Swenson and Narahashi 1980). Thus, it is possible that our results are due to octanol inhibition of sodium channels that are co-localized with the gap junction proteins. However, this seems unlikely for several reasons: (1) These sodium channels would have to be a different subtype from those found in the rest of the S cell axon or soma since octanol (≤ 0.8 mM) had little or no effect on action potential propagation in lengths of axon that did not include the electrical synapse and 0.5 mM octanol had no significant effect on threshold or latency to first action potential. (2) Previous studies have shown that 1 mM octanol only partially reduces peak sodium current in crayfish and squid giant axons and in *Aplysia* neurons (Adams and Gage 1979; Oxford and Swenson 1979; Swenson and Narahashi 1980). Thus, it seems unlikely that the lower concentrations of octanol used in this study would cause a major reduction in sodium current. (3) Recovery of octanol-induced inhibition of sodium currents in invertebrates can be very slow (> 1 hr) or absent (Adams and Gage 1979; Swenson and Narahashi 1980), whereas we observed almost complete recovery following a 30 min wash.

In sum, these octanol data suggest that measuring action potential propagation across the S cell electrical synapse is a valid experimental technique for studying modulation of electrical coupling between S cells. Similar approaches have been widely used to study gap junctions in cardiac tissue where electrical coupling between cardiomyocytes plays a major role in propagation of the cardiac action potential (Rohr 2004).
Localization of the serotonin-induced increase in action potential latency to the segment of axon containing the S-to-S cell electrical synapse suggests that serotonin also decreases electrical coupling between S cells. However, as in the octanol experiments, it is possible that these results reflect modulation of ion channels that are co-localized with the gap junction proteins. Serotonin has been shown to inhibit certain sodium currents as well as to enhance some potassium currents (Carr et al. 2002; Acosta-Urquidi et al. 1989), either of which could slow action potential propagation. Again, this seems unlikely since these sodium and/or potassium channels would have to be different subtypes from those found in the rest of the S cell axon or soma, given that serotonin had no significant effect on action potential propagation in lengths of axon that did not include the electrical synapse as well as no significant effect on threshold or latency to first action potential. Another possibility is that the increase in action potential latency reflects an increase in passive membrane conductance in the region of the electrical synapse. However, this also seems unlikely since, as with the sodium and potassium channels, such “leak” channels would have to differ from those found in the rest of the S cell membrane given that serotonin has no effect on S cell input resistance in isolated ganglia.

Serotonergic modulation of electrical synapses, primarily between dendrites, has been demonstrated in several other systems. In the leech, serotonin also decreases electrical coupling between pairs of Retzius cells (Colombaioni and Brunelli 1988; Beck et al. 2002). Serotonin, acting through a G protein-coupled receptor, induces a calcium influx through calcium channels and the resulting increase in intracellular calcium concentration is thought to play a role in decreasing coupling. Although both protein kinase A and protein kinase C are two common signaling pathways for serotonin receptor-activated G proteins, neither pathway appears to mediate this calcium transient. In the lobster stomatogastric ganglion,
serotonin can either increase or decrease electrical coupling between neurons of the pyloric network, depending on the particular pair of neurons (Johnson et al. 1993; 1994).

Interestingly, under control conditions the electrical synapse between anterior burster (AB) and pyloric dilator (PD) neurons is non-rectifying. However, serotonin enhances PD → AB electrical coupling with no significant effect on AB → PD coupling. This is in contrast to our results, in which the serotonin-induced increase in latency was independent of the direction of propagation. Finally, in rat somatosensory cortex, serotonin reduces dye-coupling between developing pyramidal cells (Rösig and Sutor 1996). In this case, uncoupling requires IP<sub>3</sub> receptor-mediated release of calcium from intracellular stores as well as activation of protein kinase C.

While less common than dendro-dendritic electrical synapses, axo-axonal electrical synapses have also been shown to be modulated in several systems. In the lateral giant axons of the crayfish, the circulating hormone ecdysterone reduces gap junction sensitivity to changes in cytoplasmic pH (Moreno et al. 1991). In the retina of both turtles and carp, dopamine narrows the receptive field profile of H1 horizontal cells by decreasing the conductance of gap junctions between axon terminals through a cyclic AMP-dependent pathway (Teranishi et al. 1983; Piccolino et al. 1984). In the hippocampus, where Schmitz et al. (2001) recently demonstrated axo-axonal coupling between pyramidal cells, activation of both muscarinic acetylcholine receptors and dopamine receptors appears to decrease coupling (Velazquez et al. 1997) whereas application of the cAMP analog 8-Br-cAMP and the cAMP stimulator forskolin appears to increase coupling (Gladwell and Jefferys, 2001). Clearly, it will be important to determine which cellular mechanisms underlie serotonergic modulation of the S-to-S cell electrical synapse.
Serotonin-dependent changes in instantaneous frequency

The serotonin-dependent changes in instantaneous frequency observed in the dual initiation site experiments suggest that modulation of electrical synapses may alter the pattern of S cell network activity. In these experiments, the suction electrodes were placed such that there were two electrical synapses between the stimulating electrodes. However, a mechanosensory stimulus can initiate action potentials in up to five different S cells (Baccus et al. 2001). Thus, there can be as many as four electrical synapses between initiation sites. This suggests that if serotonin does modulate S-to-S cell electrical synapses *in vivo*, the effects on firing frequency may be even greater than that observed in this set of experiments.

We did observe a small increase in instantaneous frequency in control experiments in which the two stimulating electrodes were placed on the same S cell. This effect was likely due to serotonin since it was reversible following a saline wash. While the mechanism underlying this modulation is not yet known, one possible explanation is that serotonin decreases the slow afterhyperpolarization (AHP). A decrease in potassium conductance could increase the rate of depolarization by the second action potential in a pair which could in turn decrease the interspike interval. Preliminary results indicate that serotonin does decrease the magnitude of the slow AHP in S cells (B.D. Burrell, unpublished observation) and a recent study has shown that activation of serotonin receptors decreases the component of AHP mediated by SK channels in turtle motoneurons (Grunnet et al. 2004).

Compartmental modeling

The compartmental model extends the results of the dual initiation site experiments by demonstrating that modulation of S-to-S cell electrical synapses may alter the pattern of S cell network activity following a mechanosensory stimulus. The greatest change in instantaneous frequency occurred in the simulated posterior recording, with increases up to ~
21 Hz. This is consistent with a previous study showing that when S cell activity was initiated by skin stimulation, serotonin shortened the interspike intervals (Burrell et al. 2002). Furthermore, the greatest effect was also observed in the posterior recordings. While much of this decrease in interval duration was likely due to an increase in the total number of action potentials elicited in the S cell network, it is possible that some of the change was due to modulation of electrical synapses.

As in the experiments with dual initiation sites, the model produced the greatest increase in frequency when the initial frequency was relatively high. To explore further how the change in frequency varies with the initial frequency, we plotted the theoretical change in frequency versus initial frequency for changes in interspike interval of ±0.5 ms, ±1.0 ms, ±2.0 ms, and ±3.0 ms, which approximate the range of changes observed in the dual initiation site experiments. As shown in Fig. 11, a change in the interspike interval of ±2.0 ms could lead to significant changes in firing frequency with initial frequencies in the range of ~60-80 Hz. This is well within the range of action potential initiation frequencies in the S cell network following a mechanosensory stimulus (typically ~10-100 Hz; Baccus et al. 2001; Burrell et al. 2002).

Functional consequences of the increase in instantaneous frequency of the S cell network

Taken together, the dual initiation site experiments and the compartmental modeling suggest that serotonergic modulation of S-to-S cell electrical synapses may increase the instantaneous frequency within a train of S cell action potentials initiated by a mechanosensory stimulus. One possible consequence of the increased frequency may be to enhance chemical synaptic transmission by the S cell through temporal summation or synaptic facilitation, or both. In both the dual initiation site experiments and in the model, the interspike intervals that yielded the greatest increases in frequency were sufficiently brief to produce facilitation (Atluri and
Regehr 1996). Wang (1998) has shown that the S cell has excitatory input to the Retzius
cells, which are the primary serotonin-containing neurons in the leech CNS. Thus, an
increase in input to the Retzius cells could lead to the release of additional serotonin. The
increased action potential frequency could also enhance firing in neurons that the S cell
excites through electrical synapses, such as the L motor neurons, by temporal summation
with other excitatory inputs. Finally, increases in S cell action potential frequency could
enhance release of the neuropeptide myomodulin, a neuromodulator that is present in the S
cell and has been shown to increase excitability of the Retzius cells (Keating and Sahley
1996; Wang et al. 1999).

*Functional consequences of inhibiting propagation of S cell action potentials through
electrical synapses*

A previous study examining activation of leech motor neurons during whole-body shortening
found that the S cell network accounted for the shortest latency excitation of the L motor
neurons (Shaw and Kristan 1995). Other parallel interneuronal pathways that contribute to
shortening also excite the L cell, but up to ~50 ms later than the S cell. Thus, another
possible effect of the serotonin-induced decrease in coupling between S cells may be to slow
the train of S cell action potentials in order to improve synchronization with the other parallel
pathways and enhance the shortening response. This may be relevant to the S cell’s
contribution to expression of sensitization in this reflex. Under basal conditions, S cell
activity and intensity of the shortening response are not correlated. However, in the
sensitized state, S cell activity and shortening are strongly correlated (Sahley et al. 1994).
Thus, the serotonin-induced delay of the S cell action potential train so that its input to
shortening motor neurons coincides with input from parallel pathways may contribute, along
with an increase in S cell network activity, to the S cell’s functional recruitment into the shortening neural circuit during sensitization.

Conclusion

In summary, these results demonstrate that serotonin can reduce action potential propagation in the S cell network. The data are consistent with a decrease in electrical coupling between S cells; however, we cannot rule out modulation of other channel types. In either case, this modulation may change the pattern of S cell network activity in a manner that could enhance synaptic transmission to postsynaptic cells. In addition, the decreased coupling may increase the latency of S cell input to motor neurons in order to synchronize with input from parallel pathways. Clearly it will be important to determine the cellular mechanisms that underlie modulation of S-to-S electrical synapses as well as whether this modulation represents an additional mechanism for how the S cell contributes to learning during whole-body shortening. These studies will broaden our understanding of how neuromodulators alter electrical synapses, how these changes affect the activity of an entire network, and what effect these changes may have on behavior.
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Figure Legends

Fig. 1. Schematic diagram of the preparation to measure modulation of the S-to-S cell electrical synapse. S cell action potential propagation was studied in a two-ganglion chain containing a single electrical synapse. Suction electrodes were placed on the cut ends of the connective to stimulate one S cell while recording the other, coupled S cell’s action potentials. In some experiments a third suction electrode, placed \textit{en passant}, recorded the S cell action potentials near the electrical synapse before they propagated through it.

Propagation along the entire length of the two-ganglion chain was measured as the difference between the time of the stimulus artifact and the arrival time at the suction electrode on the cut end (\textit{Latency}). Conduction along the axon without the electrical synapse was measured as the difference between the time of the stimulus artifact and the arrival time at the \textit{en passant} electrode. Propagation including the electrical synapse was measured by taking the difference between the arrival times at the two recording electrodes.

Fig. 2. Octanol decreases electrical coupling between Retzius cells. \textit{A}: Representative intracellular recordings from two Retzius cells showing that 0.8 mM octanol decreased coupling between these two neurons. Injection of a negative current pulse into one Retzius cell produced hyperpolarization in both that cell ($\Delta V_1$, \textit{left}) and the electrically coupled Retzius cell from the same ganglion ($\Delta V_2$, \textit{right}). The level of hyperpolarization in $\Delta V_2$ decreased during octanol treatment due to a reduction in electrical coupling between the two Retzius cells. \textit{B}: Plot of the mean change in electrical coupling between Retzius cells due to octanol treatment. Compared to saline alone, 0.8 mM octanol significantly decreased electrical coupling relative to pre-treatment levels. \textit{C}: Plot of mean change in Retzius cell input resistance following octanol treatment. No significant change in input resistance was observed in either octanol- or saline-treated Retzius cells.
Fig. 3. Octanol increases S cell action potential latency across a two-ganglion chain. A: Representative extracellular recordings from the connective nerve showing that 0.5 mM octanol increased S cell action potential latency (left) and that there was no detectable change in latency when ganglia were incubated in saline only (right). In this and all subsequent traces, arrows indicate the S cell action potentials. B: Plot of mean change in action potential latency relative to pre-treatment values. Compared to saline alone, 0.5 mM octanol caused a significant increase in action potential latency.

Fig. 4. The octanol-induced increase in S cell action potential latency is localized to the region of axon containing the electrical synapse. A: Representative traces showing that 0.5 mM octanol increased latency across the entire two-ganglion chain, but had little effect on the latency of action potentials recorded with the *en passant* electrode. B: Plot of mean change in action potential latency for the total length of the two-ganglion chain, the length of axon without the electrical synapse, and the length of axon with the electrical synapse. There was no significant difference between the mean increases in latency across the entire chain and across the length of axon containing the electrical synapse. In contrast, the increase in latency across the length of axon without the electrical synapse was significantly different from the increases in latency across both the total length of the chain and the length of axon with the synapse. The small increase in latency in the length of axon without the synapse was not significantly different from saline controls (data not shown).

Fig. 5. At a higher concentration of octanol, action potentials often fail to propagate across the electrical synapse. A: Representative traces showing that in 0.8 mM octanol, some of the action potentials recorded by the *en passant* electrode were not detected by the suction electrode on the cut connective, suggesting failure to propagate through the electrical
synapse.  

B: Plot of percentage of action potential failures detected by the two different recording electrodes showing that compared to the *en passant* electrode, the electrode on the cut end detected a significant increase in the percentage of action potential failures in the presence of octanol.

Fig. 6. Serotonin increases S cell action potential latency across a two-ganglion chain.  

A: Representative traces showing that 50 µM serotonin increased S cell action potential latency across the entire two-ganglion chain.  

B: Plot of mean change in action potential latency relative to pre-treatment values. Compared to saline alone, 50 µM serotonin caused a significant increase in action potential latency. The saline control data is the same as that shown in Fig. 3.  

C: Dose-response plot for the serotonin-dependent increase in S cell action potential latency. The superimposed curved is a sigmoidal fit to illustrate the trend in the data, suggesting that the effect was half maximal at ~10 µM.

Fig. 7. The serotonin-induced increase in S cell action potential latency is localized to the region containing the electrical synapse.  

A: Representative traces showing that 50 µM serotonin increased latency across the entire two-ganglion chain, but had little effect on the latency of action potentials recorded with the *en passant* electrode.  

B: Plot of mean change in action potential latency for the total length of the two-ganglion chain, the length of axon without the electrical synapse, and the length of axon with the electrical synapse. There was no significant difference between the mean increases in latency across the entire chain and across the length of axon containing the electrical synapse. In contrast, the increase in latency across the length of axon without the electrical synapse was significantly different from the increases in latency across both the total length of the chain and the length of axon with the synapse.
Fig. 8. The serotonin-dependent increase in action potential latency increases with the number of electrical synapses. Serotonin-dependent change in S cell action potential latency plotted against the number of electrical synapses in the chain of ganglia. The dotted line shows the estimated increase in latency due to an increase in axon length only. This calculation was based on the serotonin-induced change in latency measured for single ganglia without the electrical synapse. Serotonin concentration = 50 µM.

Fig. 9. Serotonin can change interspike interval duration and instantaneous frequency when action potentials are initiated in different S cells. A: Representative traces showing that serotonin can change the interspike interval duration when action potentials are initiated in two separate S cells. B: Plot of mean change in interspike interval duration for each temporal order of stimulation. Compared to controls in which the two action potentials were initiated in the same S cell (by placing the two stimulating electrodes on either side of a single ganglion; not shown in the schematic diagram), 50 µM serotonin significantly increased and decreased the interspike interval. C: Plot of mean change in instantaneous spike frequency for each temporal order of stimulation showing the differential effects of changes in interval duration. When the first action potential was initiated by stimulating electrode 1, serotonin significantly increased frequency by ~12 Hz.

Fig. 10. A compartmental model of a five-S cell chain suggests that modulation of S-to-S cell electrical synapses may alter the pattern of S cell network activity following a mechanosensory stimulus. A: Temporal order of stimulation for the S cell initiation sites shown in panel B. Eleven action potentials were generated in three different S cells using a pattern of S cell action potential initiation frequency similar to that observed following mechanosensory stimuli. Intervals indicated by * and # showed the greatest change in
instantaneous frequency and correspond to those in panels D, F and H. B: Schematic diagram of the S cell model showing the spatial pattern of action potential initiation sites. C and D: Resulting S cell activity recorded at the anterior (C) and posterior (D) ends of the simulated S cell chain under control conditions. The interval numbers correspond to those in panels E-H. (Changes in the pattern of activity due to modulation of the electrical synapses cannot be seen on such a slow time base). E and F: Plots of changes in interspike interval duration versus interval number for the anterior (E) and posterior (F) recordings as a result of “modulating” the electrical synapses. G and H: Plots of resulting changes in instantaneous frequency versus interval number for the anterior (G) and posterior (H) recordings.

Fig. 11. Changes in interspike interval duration may significantly alter frequencies within the range of action potential initiation frequencies observed in the S cell network. Plot of the theoretical change in instantaneous frequency versus initial frequency for changes in interspike interval of ± 0.5 ms, ±1.0 ms, ±2.0 ms, and ±3.0 ms (approximates the range of changes observed experimentally). The frequency of action potential initiations in the entire S cell chain following a mechanosensory stimulus typically ranges from ~10-100 Hz.
Fig. 1

Stimulate

Synapse

Record

Stimulus artifact

Latency

S cell action potential

Stimulus artifact

Latency

S cell action potential
Fig. 2

A

$\Delta V_1$ Pre-treatment $\Delta V_2$

0.8 mM Octanol

B

![Bars](chart1.png)

C

![Bars](chart2.png)
A

Pre-treatment

0.5 mM Octanol

Saline

Wash

B

Δ Action potential latency (ms)

0.5 mM Octanol

Wash

Saline

Wash

Fig. 3
Fig. 4

A

Pre-treatment

0.5 mM Octanol

5 ms

B

Δ Action potential latency (ms)

0 0.5 1.0 1.5 2.0

Stimulate

Synapse

Record
Fig. 5

A

Pre-treatment

0.8 mM Octanol

Failure

Wash

5 ms

B

% Action potential failures

Control

0.8 mM Octanol

Wash

Control

0.8 mM Octanol

Wash
Fig. 6

A

Pre-treatment

50 µM Serotonin

Wash

B

\(\text{Δ Action potential latency (ms)}\)

\begin{align*}
\text{50 µM Serotonin Wash} & : 1.5 \\
\text{Saline Wash} & : 0.5
\end{align*}

C

\(\text{Δ Action potential latency (ms)}\) vs. Serotonin concentration (µM)

\begin{align*}
\text{Serotonin concentration (µM)} & : 0.1, 1, 10, 100
\end{align*}
Fig. 7

A

Pre-treatment

50 µM serotonin

B

Δ Action potential latency (ms)

[Graph showing data with bars and error bars]
Fig. 8

Number of electrical synapses

∆ Action potential latency (ms)

0 1 2 3

0 1 2 3

Number of electrical synapses
Fig. 9

A

Stim. 2

Stim. 1

Pre-treatment

50 µM Serotonin

B

C

First spike initiated at stimulating electrode 2

First spike initiated at stimulating electrode 1

First spike initiated at stimulating electrode 1

First spike initiated at stimulating electrode 2

Δ Interspike interval (ms)

Δ Instantaneous spike frequency (Hz)

Spikes initiated in different S cells

Spikes initiated in the same S cell