

Dynamic neuromodulation of synaptic strength intrinsic to a central pattern generator circuit

Paul S. Katz*, Peter A. Getting† & William N. Frost*

* Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77225, USA

† Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242, USA

MOTOR circuits are often thought to be physically separate from their neuromodulatory systems^{1,2}. We report here a counter example, where neurons within a circuit appear to modulate synaptic properties of that same circuit during its normal operation. The dorsal swim interneurons (DSIs) are members of the central pattern generator circuit for escape swimming in the mollusc *Tritonia diomedea*³. However, DSI stimulation also rapidly enhances the synaptic potentials evoked by another neuron in the same circuit onto its follower cells. This modulatory action appears to be mediated by serotonin (5-hydroxytryptamine); the DSIs are serotonin-immunoreactive⁴, and bath-application of serotonin mimics and occludes the effect of DSIs. These results indicate that during the escape swim, circuit connection strengths are dynamically controlled by the activity of neurons within the circuit itself. This 'intrinsic neuromodulation' may be important for the animal's initial decision to swim, the generation of the swim motor programme itself, and certain types of learning.

The DSI neurons (DSI-A, B and C) are essential elements of the central pattern generator (CPG) circuit for escape swimming in *Tritonia* (Fig. 1a). Through their synaptic connections with the ventral swim interneurons (VSI) and C2 cerebral neurons,

they participate in the generation of the swim motor programme⁵ (Fig. 1b); brief hyperpolarization of several DSIs can reset the rhythm³, whereas depolarization of individual DSIs can prolong the motor pattern⁵. In previous considerations of this network, no other role was suggested for the DSIs and no heterosynaptic changes of synaptic efficacy were known to occur during the behavioural response. We show here that the DSI cells have an additional powerful neuromodulatory role, increasing the gain of specific network synapses, during the normal operation of the circuit⁶.

Stimulating individual DSIs at rates similar to those observed during a swim episode (Fig. 1b) enhanced the strength of synapses made by the CPG interneuron C2 onto its postsynaptic followers in the swim motor network, both inside and outside the CPG (Fig. 2A, B). Within the CPG circuit, C2 evokes a dual-component synaptic potential in DSI-B (Fig. 2A, a), consisting of an initial excitatory component followed by a prolonged inhibitory potential⁷. When the C2-DSI connection was examined either during or immediately after stimulation of any one of the other DSI neurons, the size of the inhibitory synaptic potential was significantly enhanced (Fig. 2A, a). DSI stimulation increased the amplitude of the inhibitory component an average of $71 \pm 16\%$ (Fig. 2A, b). The excitatory component was also enhanced in some, but not all, cases.

DSI stimulation also increased the synaptic strength of other C2 synapses onto neurons within the same motor circuit, but outside the CPG. DSI stimulation increased the amplitude of the excitatory postsynaptic potential (e.p.s.p.) evoked by C2 in the dorsal flexion neuron (DFN) by an average of $198 \pm 21\%$ (Fig. 2B).

The modulation of C2 synaptic strength was quite dynamic, arising within seconds of the onset of DSI stimulation and decaying within a few seconds of its cessation (Fig. 2C, D). In three animals for which identical stimulus protocols were used, modulation of the C2-DFN e.p.s.p. decayed to a half-maximum value 5.7 s after the end of the DSI spike train (5 Hz for 10 s).

Previous work reported that the DSI neurons are immuno-

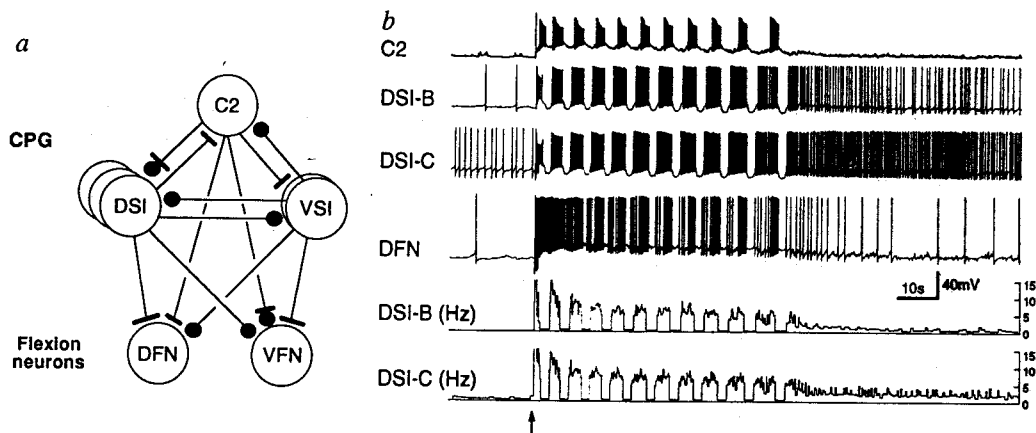


FIG. 1 The organization and neural firing pattern of the *Tritonia* swim motor network. a, The central pattern generator (CPG) circuit⁷, consisting of the dorsal swim interneurons (DSI), ventral swim interneurons (VSI), and cerebral neuron 2 (C2), generates the swim behaviour^{3,5,7,9,24}. The CPG interneurons synapse on two classes of efferent neurons: the dorsal flexion neurons (DFN) and the ventral flexion neurons (VFN) which drive the muscle movements²⁵⁻²⁷. There are two types of DFN (DFN-A and DFN-B); only DFN-A will be discussed here. The monosynaptic connections in the CPG between the three DSIs (DSI-A,B,C), the two VSIs, and the single C2 are shown for one side of the brain⁷. Excitatory connections are denoted by bars, inhibitory by circles, and multicomponent synapses by combinations of the two. C2 makes a biphasic excitatory/inhibitory connection onto DSI-B and -C, but is purely inhibitory onto DSI-A (our unpublished data, and ref. 7). b, The swim motor pro-

gramme was elicited in an isolated brain preparation by brief stimulation of pedal nerve 3 (10 Hz, 1 s at arrow). Simultaneous intracellular recordings are shown from all of the neurons described in this study (C2, two DSIs and a DFN) using glass microelectrodes (10–20 M Ω) filled with 4 M potassium acetate. All neurons were identified on the basis of soma location and coloration, synaptic interactions, and activity pattern during the swim motor programme^{3,25}. Instantaneous spike frequency profiles are shown in the bottom two traces for the DSI neurons. Note that the spontaneous spike activity of both DSIs following the swim was greater than that before the swim. Normal saline composition (in mM): 420 NaCl, 10 KCl, 10 CaCl₂, 50 MgCl₂, 10 HEPES (pH 7.6), 11 D-glucose. Animals were collected from four different locations in the coastal waters off California, Washington and Vancouver Island.

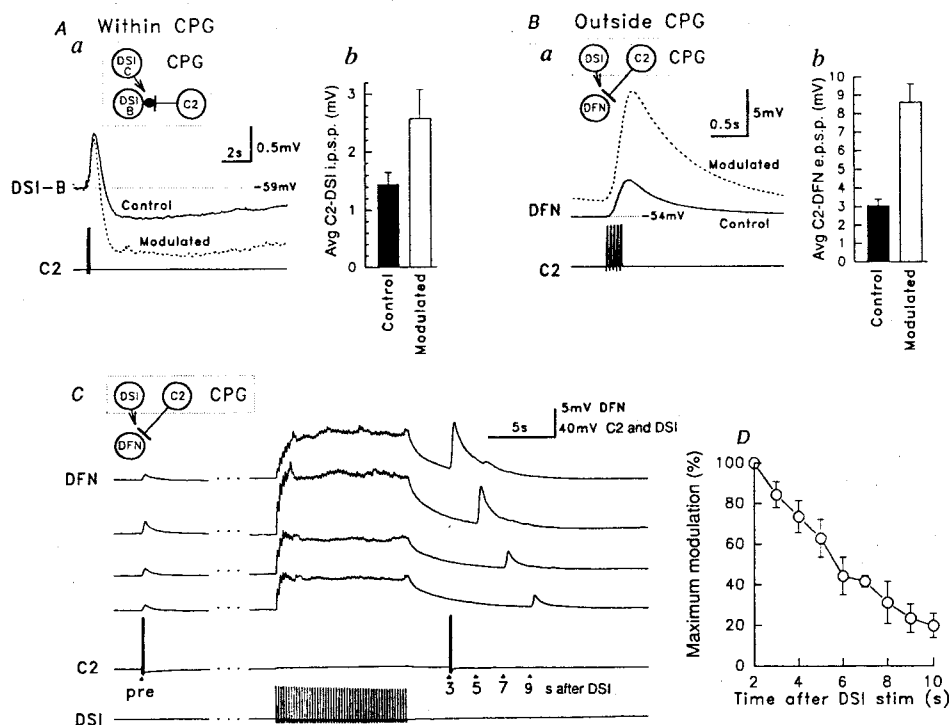
reactive for serotonin (5-HT)⁴. We have replicated this finding (Fig. 3a, b) and have obtained further evidence suggesting that DSI may release 5-HT to produce its modulatory effects. Like DSI stimulation, bath-application of 5-HT (100 μ M) increased the amplitude of the synaptic potentials evoked by C2 (Fig. 3C-E). This 5-HT-evoked enhancement of C2 synapses was not additive with the effect of DSI stimulation, and in fact completely occluded the modulatory action of DSI in each of the three experiments in which it was tested (Fig. 3F). These data, together with other data on the effects of 5-HT antagonists and precursors⁸, are consistent with the hypothesis that DSI produces heterosynaptic facilitation of C2 synapses by releasing 5-HT.

The function of this intrinsic neuromodulation is not yet known, but there are many intriguing possibilities. For example, it may be involved in the animal's decision to swim; previous work has established that, at rest, these neurons are components of a reflex withdrawal circuit which rapidly reconfigures into a CPG circuit in response to an appropriately strong stimulus^{9,11}. As this CPG is a network oscillator dependent upon synaptic properties to produce its rhythmic output^{5,9}, the rapid modulation of C2 synapses within the CPG may be an important component of the reconfiguration mechanism. Modulation of the C2 connections outside the CPG may also be important in the overall reconfiguration mechanism, enhancing the effectiveness of the CPG at driving the efferent neurons. In addition, the rapid onset and decay of the modulation indicates that it may rise and fall cycle by cycle during the swim itself, suggesting a possible role in the actual mechanics of rhythm generation. Finally, the modu-

lation may have a role in non-associative learning; following a swim episode, the spontaneous firing frequency of all DSIs remains elevated for several minutes (Fig. 1b). As a single DSI firing at frequencies as low as 2 Hz is able to enhance C2 synapses (data not shown), this prolonged elevation in the spontaneous DSI firing rate may contribute to behavioural changes observed in sensitized animals, such as lowered threshold and onset latency for swimming^{12,13}.

In central pattern generator circuits, all the established sources of neuromodulatory input arise extrinsic to the circuits being modulated^{1,14,16}. The system we have described constitutes an alternative source of neuromodulatory input, intrinsic neuromodulation, where the neuromodulatory neurons are integral components of the circuit being modulated. Although neuromodulation has not previously been shown to be intrinsic to pattern-generating circuits, other components of motor systems have been shown to evoke neuromodulatory effects in addition to their synaptic actions¹⁷. For example, motor neurons, using peptide cotransmitters, modulate neuromuscular properties in a number of systems^{18,20}. Mechanosensory neurons also evoke neuromodulatory effects in reflex circuits^{21,22}. Furthermore, some modulatory neurons, extrinsic to a circuit, receive strong synaptic input on their terminals from that same circuit, making them functional members of the circuit that they are modulating²³. These studies together with our data indicate that intrinsic neuromodulation may be a more general property of circuits than previously appreciated. It provides a mechanism by which circuits can alter their own properties rapidly and dynamically during normal operation. \square

FIG. 2 DSI stimulation enhanced the synaptic potentials evoked by C2 inside and outside the CPG. **A**, a, C2 stimulation (5 spikes; 20 Hz) evoked a biphasic synaptic potential in a DSI-B neuron (control). Stimulation of another DSI, in this case DSI-C (5 Hz for 10 s; not shown), ending 3 s before the C2 train, increased both the amplitude and duration of the inhibitory component of the synaptic potential (modulated). **b**, The average C2-DSI i.p.s.p. increased in amplitude from 1.4 ± 0.2 mV to 2.6 ± 0.5 mV (paired Student's *t*-test, $t=3.37$, $P<0.01$, $n=9$) following DSI stimulation (5–7 Hz, 10–20 s). On this and other graphs, error bars represent standard error of the mean. **B**, a, C2 stimulation evoked an e.p.s.p. in the DFN (control). DSI stimulation enhanced the amplitude of this e.p.s.p. (modulated). **b**, On average, DSI stimulation (5–7 Hz, 10–20 s) increased the amplitude of the C2-DFN e.p.s.p. from 3.1 ± 0.3 mV to 8.8 ± 1.0 mV (paired Student's *t*-test, $t=7.40$, $P<0.001$, $n=31$). **C**, C2 was stimulated (5 spikes, 20 Hz) before (pre) and in separate trials at 3, 5, 7 and 9 s after cessation of a DSI spike train (5 Hz, 10 s). The direct excitatory DSI-DFN connection can be seen during the DSI train. The amplitude of the C2-evoked e.p.s.p. was enhanced for more than 9 s after cessation of DSI activity. **D**, Average decay of the modulation under conditions identical to those in **C** ($n=3$). The modulation was maximum at the first point tested (2 s following DSI stimulation). Insets in **A**, **B** and **C** show the cells involved in each experiment. Arrows indicate synapses being modulated by DSI. In all cases, recordings were from isolated brain preparations superfused with saline



at 10 °C and containing increased divalent ion concentrations to limit polysynaptic contributions to the responses. High-divalent-ion saline consisted of (in mM): 285 NaCl, 10 KCl, 25 CaCl₂, 125 MgCl₂, 10 HEPES (pH 7.6), 11 D-glucose. C2 and DSI were stimulated with short current pulses, evoking a single action potential per pulse. DSI modulation of the C2-DFN connection has also been observed in sea water by us (data not shown) and others²⁸.

