between colonies within rivers (D_{cr}), and 21.3 percent within colonies (H_c). Differences between river systems within the major groups (D_{rs}) are negligible (0.4 percent).

Management of the endangered Arizona populations is proceeding at several levels. Remaining natural habitats in Arizona are protected from future development, and efforts to control the predaceous and exotic *G. affinis* are under way (*18*). Several springs and marshes within the Gila drainage are being restocked with *P. o. occidentalis* derived from a hatchery population originating at Monkey Spring (location C) (*19*). Although restocking is a logical step toward maintaining this species in Arizona, the current plan has a flaw. Monkey Spring is a thermally stable, isolated springhead, and the resident fish are genetically invariant by our criteria (Table 1). In addition, topminnows from Monkey Spring display low fecundity (*20*). A better choice for restocking might be wild or first generation fish from a thermally fluctuating natural environment such as Sharp Spring (location D). These topminnows are genetically the most variable of the Arizona populations (Table 1), and females show high fecundity (*6*).

It is critical that the three major groups of *P. occidentalis* remain discrete in nature since 53 percent of the genetic diversity in this species results from intergroup differences. Hence, repopulation efforts in extreme southeastern Arizona should employ stocks from San Bernardino Ranch, Arizona (location E), or other localities within the upper Yaqui basin of Mexico (group 2). However, experimental mixing of stocks within each of the groups could increase local genetic diversity. Intragroup hybrids might prove to be more successful colonists than any stock deriving from a single population. Localized intragroup mixing could reverse effects of population subdivision caused by recent habitat destruction. Time for experimental studies of hybridization, fitness, and adaptive plasticity with most endangered species is limited. Yet such studies are feasible with this small, short-lived, viviparous fish, and conservation efforts based on genetic knowledge of remnant populations are possible.

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Reciprocal Inhibition and Postinhibitory Rebound Produce Reverberation in a Locomotor Pattern Generator

Abstract. *The central pattern generator for swimming in the pteropod mollusk* Clione limacina *consists of at least four pedal interneurons, two each controlling parapodial upstroke and downstroke. The two sets of antagonistic interneurons are linked by reciprocal monosynaptic inhibitory synapses, and all exhibit apparently strong postinhibitory rebound. This simple neuronal network produces reverberating alternate cyclic activity in the absence of tonic drive or apparent feedback modulation.*

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Patterned rhythmic activity can be generated in neuronal circuits in two ways, by including endogenously active neurons in the circuit (endogenous oscillators) or by making specific synaptic connections between neurons that are not spontaneously active (network oscillators) (*1*). In the latter case patterned activity is shaped not only by network synaptic interconnections, but also by intrinsic properties of central pattern generator neurons and, in many cases, by tonic or phasic drive from outside the pattern generator. In perhaps the simplest circuit capable of generating an alternating two-phase activity cycle [two antagonistic neurons connected by reciprocal inhibitory connections (*2*)], both intrinsic burst-terminating properties of component cells and an overall tonic drive to the network are necessary for the production of continuous alternating activity (*1*). The addition of another intrinsic membrane property (postinhibitory rebound) to a similar two-phase circuit generates stable cyclic activity without tonic drive in a computer-modeled network (*3*). Postinhibitory rebound contributes to the generation of rhythmic activity in a number of central pattern generator circuits (*4*). Here I report on a central pattern generator network that includes reciprocal inhibitory connections between antagonistic pattern generator interneurons, each of which exhibits strong postinhibitory rebound. This network produces continuous, stable oscillations in the apparent absence of (i) tonic drive to the network and (ii) intrinsic oscillating properties of member neurons. The network directly controls swimming in the pteropod mollusk *Clione limacina*.

Forward or hovering swimming in *Clione* is accomplished by alternate dorsal and ventral flexions of a pair of laterally projecting winglike parapodia. Swimming is a continuous, spontaneous activity. High-speed film analyses indicate that the two wings move in synchrony with virtually symmetrical upstroke and downstroke movements (*5*). The pattern generator for flapping wing move-

ments is restricted to the pedal ganglia, and it operates in the absence of peripheral sensory feedback (6).

Premotor swim interneurons were identified electrophysiologically and morphologically (7). Two classes of swim interneurons were found, one with phasic spike activity associated with wing upswing (upswing-interneurons) and the other similarly associated with wing downswing (downswing-interneurons). In both groups, a single overshooting spike (up to 70-mV amplitude and 40- to 100-msec duration) alternates with a single inhibitory postsynaptic potential (IPSP) (up to 20-mV and 100-msec duration) (Fig. 1A). The two groups of interneurons fire in exact antiphase so that a spike in one immediately precedes the IPSP of the other (maximum delay, 4 msec) (Fig. 1A). The swimming rhythm can be altered by intracellularly stimulating with depolarizing or hyperpolarizing currents into individual upswing or downswing interneurons (Fig. 1A).

Only four swim interneurons have been found, including one upswing and one downswing interneuron in each pedal ganglion (8). All four cells are similar in morphology. Interneuron cell bodies are 20 to 35 μm in diameter and have a single axon that bifurcates in the ipsilateral pedal ganglion (Fig. 1B). One branch provides arborizations in the lateral neuropil, near the emergence of the wing nerve (area of motorneuron branching). The other main branch runs to the contralateral pedal ganglion via the pedal commissure and repeatedly branches, again in the lateral neuropil (Fig. 1B). Fine processes of ipsilateral interneurons interdigitate in two areas—in the lateral neuropil near the upswing-interneuron cell body and in the medial neuropil. Processes of contralateral interneurons also extend to these areas. Upswing-interneuron cell bodies are located in the lateral portion of each pedal ganglion, whereas downswing interneuron cell bodies are found near the origin of the cerebropedal connective (Fig. 1C).

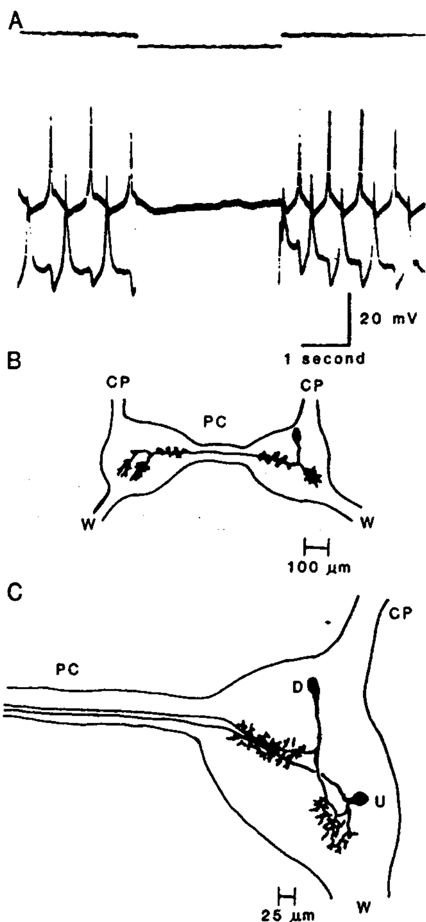

Fig. 1. (A) Simultaneous intracellular recording from a pair of antagonistic swim interneurons (middle trace, upswing interneuron; bottom trace, downswing interneuron). The top trace is a current monitor. The cyclic activity is exactly antiphasic. Stimulating the downswing interneuron with a long hyperpolarizing current (3 nA) totally stopped swimming for the duration of the current pulse; swimming immediately resumed at the termination of the pulse. (B) Tracing of a dye-filled downswing interneuron showing the main branching pattern in the pedal ganglia. (C) Similar tracing of an antagonistic pair of swim interneurons. Only one pedal ganglion is shown. Axon processes of the two cells interdigitate in the lateral (lower) and medial (upper) neuropil. Abbreviations: CP, cerebro-pedal connective; D, downswing interneuron; PC, pedal commissure; U, upswing interneuron; W, wing nerve.

Bilateral coordination of activity in the contralateral pair of downswing interneurons is accomplished through strong electrical coupling so that stimulation of spiking in one cell always induces simultaneous firing in the contralateral homologue (Fig. 2A). Similar coupling has not been demonstrated between contralateral upswing-interneurons since the lateral position of the cell bodies makes simultaneous penetration difficult.

Synaptic transmission was stopped by bathing the preparation in high Mg^{2+} saline to test the possibility of endogenous production of cyclic electrical activity in swim interneurons (9). All swimming activity and all firing activity in interneurons disappeared, suggesting that the interneurons are not spontaneously active. With this treatment, interneurons could be induced to spike by depolarizing the cell soma. Similarly, interneurons would spike at the termination of injected hyperpolarizing currents, suggesting the presence of postinhibitory rebound in these cells (Fig. 2A). Further evidence for a rebound effect was obtained by stimulating an interneuron with a "conditioning" hyperpolarizing current pulse followed by a normally subthreshold depolarizing pulse. The depolarizing pulse would frequently induce firing even though neither hyperpolarizing nor depolarizing pulses alone would stimulate a spike (Fig. 2B). The relatively small hyperpolarizing currents needed to induce rebound firing in swim interneurons (as little as 1 nA in some cells) suggests that the rebound effect is relatively strong in these cells.

The short (under 5 msec), constant latency between upswing-interneuron spikes

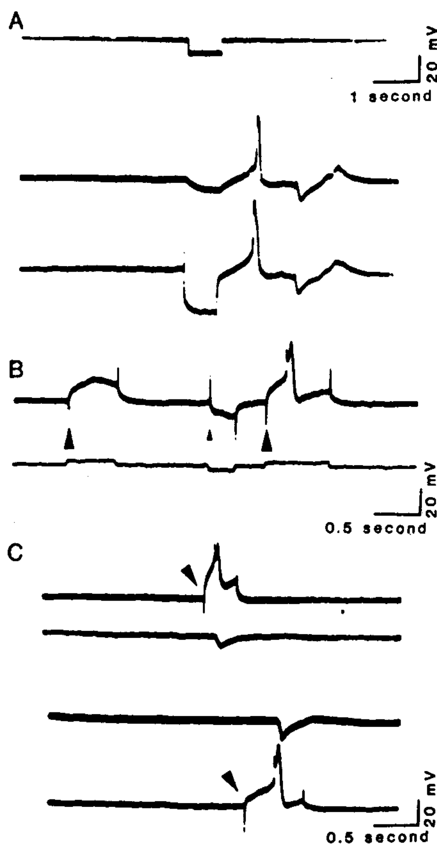

Fig. 2. (A) Intracellular recording from a pair of downswing interneurons bathed in high Mg^{2+} seawater. Stimulating one interneuron with a hyperpolarizing current pulse (bottom trace) hyperpolarized the contralateral homologue (middle trace). Both cells fired a single simultaneous spike at the termination of the stimulus. The top trace is a current monitor. (B) Similar recording from a downswing interneuron. A subthreshold depolarizing current (large arrow) triggered a spike only if preceded by a "conditioning" hyperpolarization (small arrow). The bottom trace is a current monitor. (C) Dual recordings from an antagonistic pair of swim interneurons in high Mg^{2+}–high Ca^{2+} seawater. Both paired traces represent recordings from the same set of interneurons. Induced spikes, through intracellular depolarizations (arrows) triggered a short-latency IPSP in the antagonistic interneuron, indicating that the two are linked by reciprocal monosynaptic inhibitory synapses. Stimulating injected currents: (A) 3.5 nA, (B) 1.5 nA, and (C) 3 nA.

and downswing-interneuron IPSP's, and between downswing-interneuron spikes and upswing-interneuron IPSP's, suggests that monosynaptic, reciprocal inhibitory connections exist between the two interneuron groups. This possibility was tested by exposing the preparation to saline in which both [Mg^{2+}] and [Ca^{2+}] were proportionately increased by 2.5 times (*9*). All spontaneous cyclic activity disappeared in interneurons subjected to this treatment. Initiated spikes in one interneuron of an antagonistic pair always produced IPSP's in the other cell, with a synaptic delay similar to that in untreated preparations (1 to 4 msec) (Fig. 2C). Furthermore, the amplitude and duration of the IPSP's in the treated ganglia fell within the normal range of those measured in untreated preparations (up to 20 mV), thus suggesting that the reciprocally inhibitory connections may be solely responsible for the hyperpolarizing phases of the cyclic interneuron electrical activity in both treated and untreated preparations.

During replacement of the high Mg^{2+}–high Ca^{2+} saline with seawater, a dilution of the former was reached in which repetitive firing was recorded in antagonistic pairs of swim interneurons after a short current pulse. At this point, about half of the test saline had been replaced by natural seawater. Repetitive cycling, including up to 15 complete cycles, could not only be initiated by injecting a depolarizing current into one cell of the pair (Fig. 3A), but could similarly be induced by applying a short hyperpolarizing pulse (Fig. 3B). Tonic depolarization of swim interneurons was not evident during these "swimming" bouts as the membrane potential returned to the resting level between spikes and IPSP's (Fig. 3), thus arguing against the presence of tonic excitatory input to the network during reverberating activity (*10, 11*). It is important to note that the spike threshold of swim neurons was still sufficiently elevated that swimming activity was absent even during interneuron firing. This result suggests that the cyclic firing is due to a true reverberating circuit operating in the absence of polysynaptic feedback connections and apparent tonic excitation. With further washing, the duration of similarly induced cyclic activity increased until continuous, spontaneous activity was evident and wing movements resumed.

Cycle frequency during reverberating interneuron activity in 50 percent high Mg^{2+}–high Ca^{2+} seawater was 1 to 2 Hz. This is similar to the wing beat frequency during hovering swimming in the intact

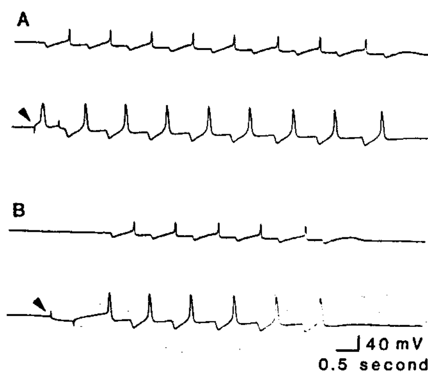

Fig. 3. Intracellular recordings from antagonistic interneurons during recovery from high Mg^{2+}–high Ca^{2+} saline. The interneurons were electrically silent in the absence of stimulation. In (A), a single depolarizing current was used to stimulate the upswing interneuron (bottom trace, arrow), resulting in repetitive cycling. In (B), similar repetitive cycling was induced after a single hyperpolarizing current (arrow). Stimulating currents were 2.5 nA.

animal and in untreated pedal ganglion preparations after removal of the cerebral ganglia (1 to 3 Hz). The basic pedal ganglion rhythm thus seems determined largely by the properties of reciprocal inhibitory connections between swim interneurons and of postinhibitory rebound in these cells, and the major modulatory inputs to this network, at least swim-accelerating inputs, seem to originate in the cerebral ganglia. Hovering swimming, which *Clione* does much of the time, would then represent the unmodulated or minimally modulated output of the pedal pattern generator.

The data suggest that the basic pattern generator for swimming locomotion in *Clione* consists of two groups of antagonistic interneurons that display strong postinhibitory rebound and that are linked by reciprocal inhibitory connections (monosynaptic). This network is capable of stable oscillatory activity driven by auto-excitation (rebound firing), thus providing a clear biological example of a neural network previously proposed based on a computer model (*3*). This simple circuit controls a continuous, spontaneous behavior that consists of two relatively symmetrical phases of activity.

The swimming system of *Clione* exhibits many of the intricacies of locomotory control of higher animals (*12*), including premotor central pattern generation, descending control and peripheral sensory modulation of the pattern generator, and motoneuron recruitment. On the basis of the simplicity of the pattern generator described here and the simultaneous microelectrode accessibility of a variety of neuronal cell types in this preparation, the *Clione* swimming system seems to be an excellent model system for the study of central and peripheral control of a two-phase locomotory behavior.

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6. The swim pattern generator was isolated in cutting experiments. Also see Y. I. Arshavskii *et al.* [*Neirofiziologiya* **14**, 102 (1982)]. Removal of all but the cerebral and pedal ganglia had no effect on alternate wing movements. Removal of the cerebral ganglia did not stop wing movements, but reduced swim frequency (R. A. Satterlie and A. N. Spencer, *J. Exp. Biol.*, in press). Maximum swim frequency in intact animals is 10 Hz.
7. Adult animals (1 to 2 cm long) were dipped from surface waters off the breakwater of Friday Harbor Laboratories, Friday Harbor, Wash. The animals were opened dorsally and the entire ring of ganglia was removed with wings attached. Intracellular recordings from pedal neurons were made with standard direct-current electronics and microelectrodes (10 to 50 megohms) filled with 3*M* KCl or 2.5*M* $KC_2H_3O_2$. Lucifer yellow CH (4 percent) was iontophoretically injected into interneuron somata with 1- to 10-nA negative current pulses (500-msec duration at 1 Hz). Filled cells were observed and photographed live and after being fixed in 10 percent Formalin in seawater, dehydrated and cleared in methyl salicylate.
8. Despite extensive probing of the pedal ganglia, only four swim interneurons have been found. This does not rule out the possibility that others exist, or that other cells have an influence on pattern generation. The absolute position of swim interneuron cell bodies cannot be visually recognized because of their small size and because they lie among other somata of similar size.
9. High Mg^{2+} saline consisted of a 1:5 mixture of 0.33*M* $MgCl_2$ in seawater. High Mg^{2+}–high Ca^{2+} saline consisted of 2.5*N* concentration of Ca^{2+} and Mg^{2+} and was made by adding 9.4 ml of 1*M* $MgCl_2$, 0.4 ml of 1*M* KCl, and 25.8 ml of distilled water to 62.5 ml of seawater [P. A. Getting, *J. Neurophysiol.* **46**, 65 (1981)]. High Mg^{2+}–high Ca^{2+} saline effectively blocks polysynaptic transmission by raising neuronal firing threshold [J. L. Cohen, K. R. Weiss, I. Kupfermann, *J. Neurophysiol.* **41**, 157 (1978)].
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