ing an intravenous injection of [^{3}H]-tryptophan, as described by Costa and his co-workers (*9*). Table 1 shows a comparison of the specific radioactivity of tryptophan (Trp*) and serotonin (S*) in tolerant-dependent (3.7-day morphine pellet implants) and sham-implanted control mice at various times after the intravenous injection of [^{3}H]tryptophan into the tail vein. The specific radioactivities of both tryptophan and serotonin are consistently somewhat higher in the tolerant-dependent mice than in the control mice. This result implies a more rapid transport of the amino acid from the blood into the brain, but it has no bearing on the question of serotonin turnover. The conversion index, CI (*10*), was calculated from

$$CI = \frac{S^* \times S}{Trp^* \times t} \quad (1)$$

where S is the brain serotonin content and t is the time interval (in minutes) from the injection of labeled tryptophan. No significant difference in the conversion index between tolerant-dependent mice and sham-implanted control mice was found at any time. Since Eq. 1 does not correct for serotonin efflux, the values obtained tend to decrease during the later time periods.

The fractional rate constant (k_S) for the turnover of brain serotonin was calculated (*9*) from a smoothed curve of the data in Table 1 for morphine-implanted mice (2.04 hr^{-1}) and sham-implanted mice (2.28 hr^{-1}) from the specific radioactivities of tryptophan and serotonin between 30 and 50 minutes after the injection of the labeled amino acid.

$$k_S = \frac{(S^*_{t_2} - S_{t_1})/(t_2 - t_1)}{[Trp^* - S^*)_{t_1} + (Trp^* - S^*)_{t_2}]/2}$$

where t_1 and t_2 equal 30 and 50 minutes, respectively, after the injection of the labeled amino acid. The rates of serotonin turnover calculated from the k_S for morphine-implanted and sham-implanted mice were 6.18 and 6.48 nmole g^{-1} hr^{-1}, respectively.

These studies demonstrate by direct measurements of serotonin turnover that the rate of serotonin synthesis and degradation remains unchanged in mice that have a high degree of tolerance to and physical dependence on morphine. However, Way and his co-workers (*2*) reported that, after treatment with the monoamine oxidase inhibitor pargyline, mice with morphine tolerance and physical dependence showed a much greater increase in serotonin accumulation than mice receiving only pargyline. The fact that there is a discrepancy in the results obtained by the two methods suggests that under some conditions the indirect pargyline method does not serve as a valid measure of serotonin turnover.

Findings by Way and his co-workers (*2*) suggested that PCPA inhibited the development of tolerance and physical dependence in mice. In extensive additional studies involving PCPA, details of which are being published elsewhere (*11*), we have been unable to confirm these findings. In our experience PCPA, in doses sufficient to reduce brain serotonin to one-third of its normal value, has no effect whatsoever upon the development of tolerance or physical dependence produced by opiate narcotics in mice.

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Oscillator Neurons in Crustacean Ganglia

Abstract. *The motor rhythm of ventilation in hermit crabs and lobsters appears to be controlled by a pair of neurons, one in each half of the subesophageal ganglion. Their membrane potentials oscillate and upon depolarization and hyperpolarization elicit spiking in two pools of motor neurons on each side, without spikes in the oscillator neurons themselves. The fact that higher order (command) interneurons can control the rate of the oscillator by means of a smoothly graded input lends support to the idea that oscillator neurons respond periodically to a constant ionic stimulus.*

A local, rhythmically active system in the subesophageal ganglion of decapod crustacea innervates the muscles of the coxal and basal segments of the scaphognathite by means of 11 motor neurons divided into levator and depressor pools and distributed between two branches of the second maxilla root. Alternating bursts of motor action potentials produce an up-and-down sculling motion that drives a stream of water through the gill chambers (*1*). The motor axon discharge pattern from the two branches on one side of a totally isolated ganglion of the hermit crab, *Pagurus pollicarus,* is shown in Fig. 1A. Such rhythmic, well-organized activity may persist in vitro for up to 48 hours when ganglia are superfused with cold saline containing hemoglobin (*2*). Although none of the visible cell bodies penetrated on the surface of the desheathed ganglion display electrical activity, it is possible, with fine-tipped (80 to 150 megohm) microelectrodes, to record within the neuropil from motor neurons and interneurons of the ventilation control system. The membrane potentials of the cells may be altered by current passed through either the recording electrode (by means of a bridge circuit) or the second barrel of a double-barreled electrode.

Oscillator interneurons are very hard

to find and harder to hold once penetrated—doubtless because of the thinness of the penetrated processes. Figure 1B shows a record from a lobster (*Homarus*) oscillator along with ongoing spike activity in the two groups of motor axons. (This preparation was left in situ with all subesophageal roots cut on both sides and the circumesophageal connectives severed.) The membrane potential of the oscillator moves up and down in exact correspondence with the timing of the axon bursts, yet there is no sign of any action potential in the oscillator itself—nor does any oscillator interneuron show spike activity. The potential goes from about − 15 mv to − 25 mv, oscillating around a mean of − 20 mv. (It is impossible to attach greater significance to these voltages than that the electrode was inside the cell originating the potentials.) To test whether this cell can indeed control activity in the motor neurons (as opposed to merely reflecting activity in some other controllers) current was passed through the recording electrode, as indicated by the arrows, to depolarize or hyperpolarize the membrane. In this case a single electrode was used with a bridge circuit, which resulted in a noisy and erratic intracellular voltage record during the periods of current flow; nonetheless, the resultant effect on the motor activity is clearly consistent with the observation on the free-running system. Depolarization of the oscillator silences or greatly slows activity in all the motor neurons of the upper trace (except two that normally fire well into the period of spontaneous depolarization of the oscillator) and at the same time elicits prolonged firing from the lower group. Imposed hyperpolarization produces continued discharges from the motor neurons of the upper group while silencing the ones previously activated by depolarization. If brief depolarizing or hyperpolarizing pulses are interpolated into the free-running rhythm they reset the rhythm, turning on the appropriate group of motor neurons and silencing the other group if it is then active. During periods of polarization by applied current flow there is no sign of membrane potential oscillation at the free-running rhythm (for example, Fig. 1C). This fact, along with the observation of resetting by pulses of both polarities militates strongly against the possibility that the penetrated cell is itself being driven at the free-running rate by some other neuron (or neurons) that is the real oscillator. Withdrawal of the microelectrode just far enough to lose the resting potential of the cell renders imposed current ineffectual in changing the activity of the motor neurons.

Results nearly identical to those from the lobster can be obtained from the hermit crab ganglion (Fig. 1, E–G). These results were obtained with a double-barreled microelectrode; the records of current passage, taken during a period of spontaneous quiescence, show far more accurately the actual potential change imposed on the oscillator. The membrane potential during free-running activity was between − 26 and − 31 mv, with a mean of approximately − 28 mv. The resting potential in the quies-

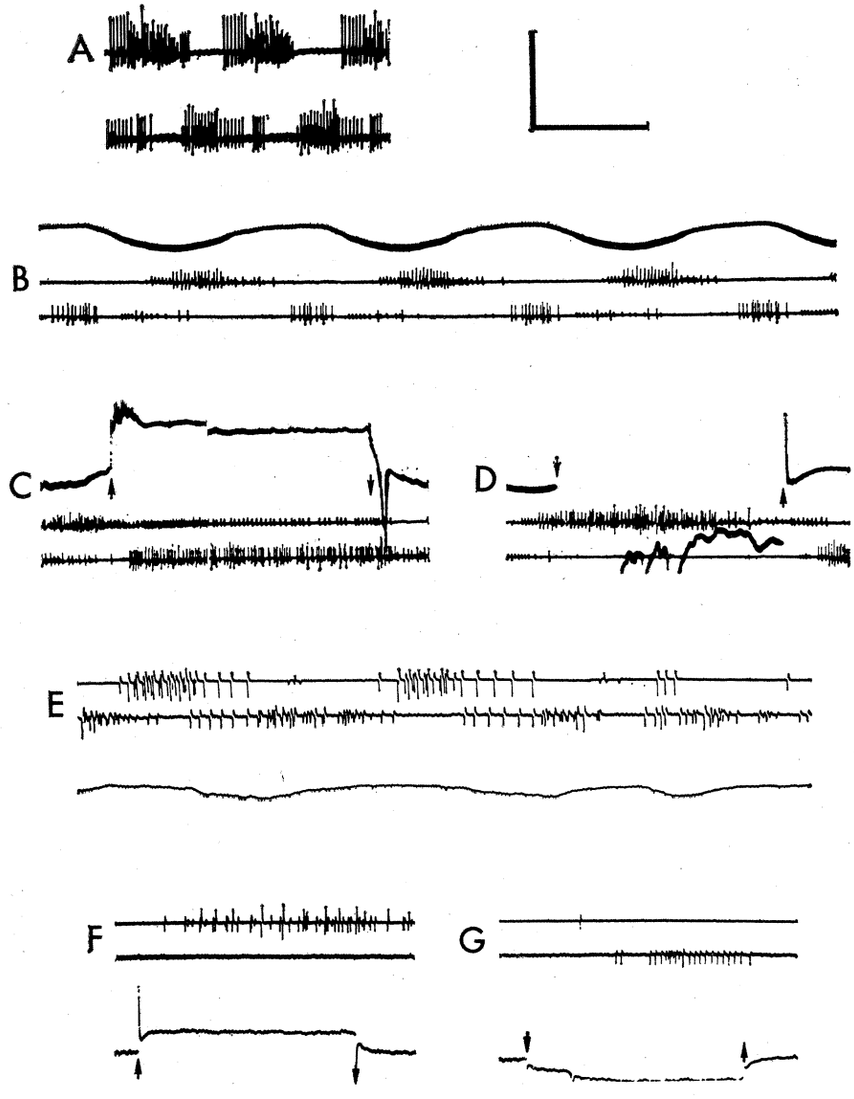

Fig. 1. (A) Motor axon activity in the two branches of the second maxilla root of a hermit crab subesophageal ganglion in vitro, recorded with suction electrodes. The upper trace is from the proximal (levator) branch, the lower trace is from the distal (depressor) branch. The patterns are usually quite uniform, yet variations in the detail of the burst appear constantly. (B) Record from a lobster; the top trace is a microelectrode in an oscillator neuron; the middle and lower traces are from motor root branches—free-running rhythm (the thickening of the intracellular trace on the repolarizing phase is an artifact; it is absent on a simultaneously registered high-gain a-c record). (C) Effect of depolarization of the oscillator; a segment of the record has been cut out of the middle. (D) Effect of hyperpolarization of the oscillator. (E–G) The same sequence from a hermit crab ganglion; the top two traces are the motor branch records; the bottom trace is the intracellular record from the oscillator. (E) Free-running rhythm; note how an irregular oscillator rhythm in the last cycle is matched by irregular bursting. (F and G) Depolarization and hyperpolarization of the oscillator (spontaneous cycling had stopped). Calibrations: intracellular records B, C, and D, 34 mv; E, F, and G, 40 mv. (Extracellular voltages are not calibrated). Time: A, 250 msec; B, C, and D, 310 msec; E, F, and G, 320 msec.

cent period was about − 36 mv; the current pulses produced voltage shifts of ±8 mv. Uncertainties concerning the location of the electrode tip in relation to the terminals make it impossible to attach significance to the absolute magnitudes of these potentials; however, the 16-mv difference between peak levels during imposed polarization is greater than the 5-mv oscillation magnitude, indicating that the site of potential oscillation is probably closer to the terminals than the electrode tip is.

On one occasion repenetration of an oscillator proved possible after the electrode had just been pulled out. After several repetitions of this maneuver the oscillator depolarized to nearly 0 mv, oscillation of measurable size ceased, and so, too, did the activity in the motor branches cease; it did not resume in that hemiganglion although the ventilation output of the contralateral side continued for some time afterward. These data show that change in the membrane potential of a single neuron is sufficient to determine the entire rhythm of the local neural system and that the observed oscillations of membrane potential are the causative agent for the motor neuron activity, not caused by it. They are consistent with the hypothesis that a single oscillator neuron in each hemiganglion controls the scaphognathite beat pattern.

The mechanism responsible for the cyclic variation of membrane potential in the oscillator is not known. The Q_{10} of the cycle rate is 2.9 from 9.5° to 15°C, and rises to nearly 10 between 4.5° and 9°C. This is consistent with a role for an electrogenic ion pump in the generation of the oscillation. Such a system could pump Na^+ periodically as its internal concentration builds up, and then stop until more Na^+ has accumulated, thus generating the spikeless oscillation of the membrane potential (*3*). The fact that the oscillator rate is highly sensitive to the amount of oxygen also fits the model of a pump-driven rhythm. If we assume that the pump can achieve a maximum rate greater than the normal Na^+ influx, and is thus shut down over much of the cycle while the internal concentration of Na^+ builds up, then obviously the cycle rate can be modulated by increasing Na^+ influx; the period between pump runs would be shortened as Na^+ accumulated more rapidly. This implies that the oscillator's cycle rate ought to be subject to proportional control, and finding such a control would further strengthen the model.

The local oscillators are indeed subject to control from the rest of the central nervous system through the action of command interneurons (*1, 4*), but to demonstrate smooth, proportional control of the oscillator is not simple. Repetitive stimulation of the circumesophageal connectives of the preparation in vitro can initiate the cycle in a quiet ganglion or accelerate or inhibit the cycle in a bursting ganglion. The sign and intensity of the effect vary unpredictably as a function of the stimulus intensity and frequency, indicating the presence of several command interneurons, both excitatory and inhibitory. Fortunately, on two occasions, the probing microelectrode entered units whose properties fit the pattern to be expected of the terminals of excitatory command interneurons, and these cells do indeed exert smoothly graded control of the oscillator function. Figure 2 shows records from one of these cells penetrated by a double-barreled electrode. The intracellular potential is shown on the lower trace, the motor activity from one of the root branches on the upper trace in Fig. 2, A–C. Figure 2A shows the situation when no extrinsic current was passed into the cell (the resting potential was − 65 mv; the calibration pulse at the start of the trace is 10 mv in amplitude); one or two small units fired slowly. Figure 2B shows the effect of passing an outward current through the second barrel of the microelectrode. The resulting depolarization triggered a small blocked spike at its start; as the current was maintained, rhythmic bursts of activity appeared in the motor axons and continued as long as the depolarization. Figure 2C shows that stronger outward current elicited a full-blown action potential in the interneuron (note the deeper repolarization in its wake at the start of the plateau) and faster cycling of the oscillator although no further action potentials appear in the command neuron. Indeed if only a single action potential was produced in the command interneuron by a brief depolarizing pulse, there was no motor output.

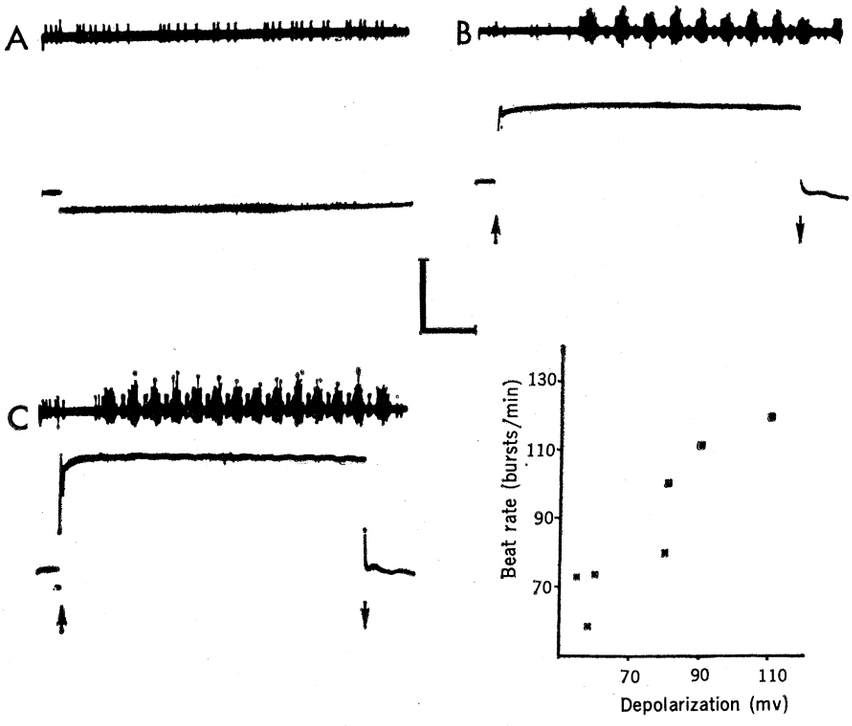

Fig. 2. Control of the local oscillator by an interneuron. (A–C) The upper trace in each shows the motor activity in the proximal branch of the second maxilla root. (The record for the distal branch has been removed because it was very noisy and provided no further information). The lower trace shows the transmembrane potential of an interneuron penetrated with a double-barreled electrode. In (A) no extrinsic current was passed; the brief pulse at the start is a calibration signal, and the spontaneous activity consists of slow spiking in one or two small units. (B) and (C) show the effect of passing two intensities of depolarizing current across the membrane; the slow time base causes spikes within bursts to apparently merge together. (D) The rate of generation of bursts is plotted as a function of current-induced depolarization measured from the resting potential. Despite a considerable scattering of the data, it is clear that a continuous relation exists between the two. Calibrations: 40 mv., 300 msec.

The cycle rate of the oscillator was a continuous function of the depolarization of the command neuron: this relation is plotted for several current strengths in Fig. 2D. Hyperpolarization of the command neuron was without effect on the motor output. Action potentials were effective in activating the oscillator only if delivered in trains with a frequency high enough to build up a sufficient average depolarization. Again currents of far greater amplitude than those shown did not produce cycling once the electrode was withdrawn just far enough to lose the resting potential. On the late part of the depolarization plateau of Fig. 2C, as well as after termination of the current pulses in Fig. 2, B and C, there is a variation of the membrane potential in time with the motor rhythm. This oscillation within the command neuron may be due to feedback, from a later stage, that serves to entrain the command neuron and assure rhythmicity; yet it is clearly not a necessary part of the oscillatory mechanism (the early part of the record in Fig. 2C shows no oscillation on the plateau, and in Fig. 2B there is no sign at all of oscillation in the command neuron). Although the local oscillator may have the ability to entrain the command unit's transmitter output to its rhythm, oscillation does not require periodic input from the command element; rather, its rate appears to be a direct function of the steady-state input. (Since I could not record simultaneously from command and oscillator neurons, there is no way to be certain that the command neuron makes a direct connection to the oscillator. Yet if any stages intervene between the two they must be nonperiodic as well, since, as previously indicated, the oscillator displays no sign of rhythmic input. Thus the conclusion that the oscillator can convert various levels of steady drive into different frequencies is still valid.)

The complete absence of action potentials in the oscillators is not a surprising finding. It is becoming increasingly clear that many short neurons, with total lengths comparable to their space constants, do not employ action potentials since electronic propagation of signals is sufficient to convey information with acceptably small loss (*5*). In one case a coxal receptor located outside but close to the thoracic ganglion of a crab was shown to convey excitation into the ganglion and elicit a complete reflex entirely without spikes (*6*). Because the oscillators have not been visually identified and measured, their size is not known; but since the ganglion itself is not over 200 to 300 μm in half-width their processes cannot be any longer than those of the coxal receptors, and a similar mode of operation is entirely possible.

It is more difficult to account for the complementary actions of depolarization and hyperpolarization of the oscillator on the motor neurons. No interneurons were encountered with properties that would fit them to be intermediates between oscillators and motor neurons. It is worth speculating that this is another case of an interneuron with a dual action whose followers are either excited or inhibited according to specific postsynaptic responsiveness to the driver neuron's transmitter (*7*). In such a system one group of motor neurons would be depolarized and excited and the other group inhibited by increased transmitter output from the oscillator on its depolarization phase. Then as the oscillator repolarized, its transmitter release would decrease, the excited cells would become silent while the inhibited cells fired on rebound depolarization; the relation of a particular motor neuron's spike pattern to the input drive would be set by its own characteristics of refractoriness and accommodation (*8*). Thus command interneurons and oscillator interneurons both appear to be able to control periodic activity of their follower cells with a smooth, graded release of transmitter whose duration greatly exceeds the cycle period of the controlled output. Others have reported unsuccessful attempts to detect local oscillator cells, which has led to the proposal that all oscillator systems consist of diffuse networks of neurons operating in concert (*9*). Finding discrete oscillator neurons in lobsters and hermit crabs encourages the belief that such neurons are present elsewhere and again demonstrates the parsimony of the crustacean nervous system in utilizing the available neurons.

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Developmental Behaviors: Delayed Appearance in Monkeys Asphyxiated at Birth

Abstract. *Developmental behaviors were studied in monkeys subjected to asphyxia at birth. Visual depth perception, visual placing, and locomotion appeared significantly later than in nonasphyxiated monkeys. After these behaviors had been established in asphyxiates, however, there was little difference from those observed in normal monkeys. These results were compared with reports of permanent learning deficits that occur in monkeys asphyxiated at birth for similar periods of time. Such comparison suggests that the neural structures responsible for the developmental behaviors studied are not damaged by asphyxia to the same extent as those for acquisition. Delay in development may be an early indication of brain damage with subsequent mental retardation.*

A regularly occurring pattern of structural brain damage by birth asphyxia of 10 to 17 minutes duration in rhesus monkeys has been established by Windle and his associates (*1*). Similar lesions have been seen in human infants after birth asphyxia (*2*). In this respect the infant monkey appears