To examine mammastatin production by normal and transformed human cell lines, we performed immunoperoxidase staining of detergent-permeabilized normal and transformed cells using the 3C6 monoclonal antibody (Fig. 3). As a control, irrelevant IgM antibodies were used and showed no staining (*20*). Normal human mammary cells contain immunoreactive mammastatin, which is present in greater than 90% of the cells (Fig. 3A). In contrast, only 10 to 15% of MCF-7 cells showed even weak staining. Similarly, immunoperoxidase staining of the other transformed cell lines (Table 2) showed inconsistent low levels of staining (*21*).

These studies show that normal human mammary cells produce proteins of approximately 47 and 65 kD that inhibit the growth of transformed mammary cell lines in culture. These include both estrogen receptor–positive, as well as estrogen receptor–negative, cell lines. This may indicate that mammastatin does not require estrogen receptor for its action. However, it remains to be determined whether estrogen or other steroid hormones regulate the production of mammastatin by human mammary cells.

Decreased immunoperoxidase staining of transformed mammary cell lines compared to normal mammary cells may indicate decreased production of inhibitor by these cells. Indeed, we have been unable to detect immunoreactive or biologically active mammastatin in conditioned medium of transformed mammary cell lines (*22*). Decreased production of mammastatin by transformed mammary cells might contribute to the loss of normal growth control in these cells. If this were the case, then new therapeutic strategies might be developed that use mammastatin to inhibit the growth of human breast cancer.

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11. MCF-7 cells were plated at 10^4 cells per milliliter and at day 1, 10% NHMC-conditioned media or purified mammastatin (10 or 20 ng/ml) was added with or without monoclonal antibody 3C6 (5 μg/ml). Cell number was determined at day 7.
12. Affinity-purified mammastatin was dialyzed into 1/10 PBS and then concentrated by lyophilization (10×). Concentrated sample was loaded onto a 75-cm S-200 sephacryl column and eluted with PBS. Fractions were analyzed for MCF-7 growth inhibitory activity as in Fig. 2.
13. Purified mammastatin (100 ng/ml) was heated to 37°, 55°, 70°, or 100°C for 60 min. Inhibitory activity of these samples was then determined as in Fig. 2.
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20. Immunoperoxidase staining of cell cultures was performed with 3C6 and an irrelevant IgM at equal concentrations. Samples that stained with 3C6 were negative with irrelevant antibody.
21. Immunoperoxidase staining was performed on the cell lines listed in Table 2 as described in Fig. 3. BT-20, MDA-MB-231, ZR-75-1, evejos, and HBL-100 showed less than 10% weakly staining cells. Non-mammary cell lines were negative.
22. Conditioned medium was obtained from MCF-7, BT-20, and MDA-MB-231 cell lines as described for NHMC and tested for MCF-7 growth inhibitory activity. These media were also analyzed by immunoblot. Neither inhibitory activity or immunoreactive mammastatin was detected.

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Switching of a Neuron from One Network to Another by Sensory-Induced Changes in Membrane Properties

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A neuron that is an integral member of the pyloric neural network of the lobster stomatogastric nervous system leaves this network and instead fires exclusively with another stomatogastric nervous system network, the cardiac sac network, whenever the cardiac sac network is active. This switch is associated with the neuron losing, in a long-lasting fashion, regenerative oscillatory membrane properties that underlie its participation in the pyloric network. Functional membership of neurons in central networks is thus not fixed, and long-lasting neuromodulatory influences, controlled at least in part by sensory inputs, can switch neurons from one network to another.

NERVOUS SYSTEMS GENERATE behaviors and modify those behaviors in response to sensory stimuli. Many behaviors involve the coordination of several different groups of neurons, known as neural networks, with each network generating specific aspects of the total behavior. An understanding of the mechanisms underlying this coordination is therefore of fundamental importance in order to explain nervous system function. Work in vertebrate and invertebrate preparations suggests that considerable flexibility exists within individual networks and that a single anatomically defined network can assume several different functional configurations, each of which produces a different output (*1, 2*). Although these results suggest that neurons can belong to different configurations of a single network, they do not address whether a neuron can belong to two or more different networks otherwise composed of different neurons. We describe here a neuron that switches between two different networks depending on whether one or both networks are active and show that this switch is associated with changes in the intrinsic membrane properties of the cell.

We used in vitro preparations (*3*) of the stomatogastric nervous system (STS) of the lobster, *Palinurus vulgaris*. The crustacean STS contains four neural networks that generate the motor patterns of the four different regions of the foregut (*4*). The pyloric network generates the motor patterns of the

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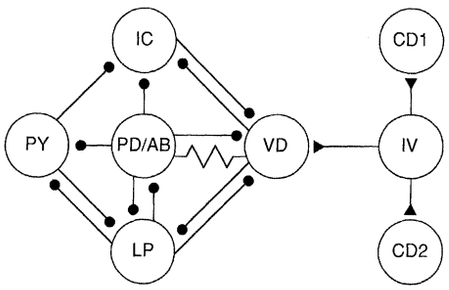

Fig. 1. The VD neuron is synaptically connected to both the pyloric (left) and CS (right) networks. The electrically coupled PD and AB neurons of the pyloric network have been grouped together. Pyloric network neurons: AB, anterior burster; IC, inferior cardiac; LP, lateral pyloric; PD, pyloric dilator; PY, pyloric; and VD, ventricular dilator. CS network neurons: CD1 and CD2, cardiac sac dilators 1 and 2; and IV, inferior ventricular. Filled circles represent inhibitory chemical synapses; triangles, excitatory chemical synapses; and the resistor, electrical coupling.

pylorus (the most posterior foregut region), and the cardiac sac (CS) network generates rhythmic dilations of an anterior foregut region. These two networks are composed of different neuronal populations (Fig. 1) and can be simultaneously active. However, two considerations argue that the ventricular dilator (VD) neuron, a well-described, integral member of the pyloric network (*5*), might also participate in CS network activity. First, in addition to the synaptic input it receives from pyloric network neurons, the VD neuron is strongly excited by the inferior ventricular (IV) neurons of the CS network (Fig. 1) (*6*). Second, the VD neuron innervates a muscle that is also innervated by a CS network neuron, cardiac sac dilator 2 (CD2) (*4*, *7*).

The pyloric network is generally continuously active in vitro, whereas the CS network is only occasionally active. Recordings of spontaneous VD neuron activity showed that it displays two different firing patterns depending on whether the pyloric network alone is active or the two networks are active simultaneously. When only the pyloric network is active (Fig. 2A), the VD neuron depolarizes and fires short (about 250 ms) spike bursts with each pyloric cycle. The pyloric cycle is indicated in Fig. 2A by the activity of the pyloric dilator (PD) neuron (*5*). When both networks are active (Fig. 2B), the VD neuron depolarizes and fires exclusively with the CS network activity, exhibiting long (2 to 10 s) spike bursts every 10 to 30 s. CS network activity is indicated in Fig. 2B by the recording of the CD2 neuron (*7*).

In preparations in which the CS network is originally silent, CS network activity can be gated (*8*) by stimulation of a mechanosensory input associated with the stomach wall or electrical stimulation of the corresponding sensory nerve [the lateral posterolateral nerve (lpln) (*9*)]. During our recordings, this activation of a quiescent CS network was always associated with the VD neuron switching from a pyloric to a CS network activity pattern (Fig. 2C). Before stimulation, the CS network [monitored by recording from the cardiac sac dilator 1 (CD1) neuron] was silent, and the VD neuron fired with the pyloric network. Shortly after tonic 2-Hz lpln stimulation began, the CS network became rhythmically active, and the VD neuron switched from a pyloric firing pattern to fire exclusively with the CS network, although the rest of the pyloric network continued to cycle [the rhythmic inhibitions of the VD neuron are due to pyloric network input (*4*)]. The two firing patterns of the VD neuron thus appear to be specifically associated with CS network activity. The VD neuron fires with the pyloric network if the CS network is silent, but always switches to the CS network when this network is active, independent of whether the CS network activity is spontaneous or is gated by sensory input.

We next attempted to determine the basis of the VD neuron switch. The VD neuron is strongly excited by the IV neurons of the CS network (*6*), and we show elsewhere (*10*) that it is this input that causes the VD neuron to fire during CS network bursts when the CS network is active. What is more difficult to explain is why the VD neuron does not continue to fire with the pyloric network between CS network bursts, particularly since the rest of the pyloric network continues to be rhythmically active (Fig. 2B, PD trace). We stimulated the lpln with brief high-frequency pulse trains. This stimulation induces a single CS network cycle (*9*) and results in a long-lasting (tens of seconds) VD neuron inactivation similar to that seen during rhythmic CS network activity (Fig. 3A).

One source of this inactivation could be an inhibition of the VD neuron (either by a neuron that fires continuously during the VD neuron inactivation or by a neuron that induces a long-lasting inhibitory postsynaptic potential in the VD neuron). Although we cannot totally reject this hypothesis, two observations argue against it. First, examination of the spiking activity in the only nerve (the stomatogastric nerve) that could carry an inhibitory input that is active during the VD neuron inactivation, and examination of the VD neuron itself (at several membrane potentials) failed to reveal any continuing inhibitory input. Second, VD neuron inactivation often occurred without any long-lasting change in the membrane potential of the VD neuron (Fig. 2C), and the steady-state current-voltage relations of active and inactivated VD neurons were identical (*9*).

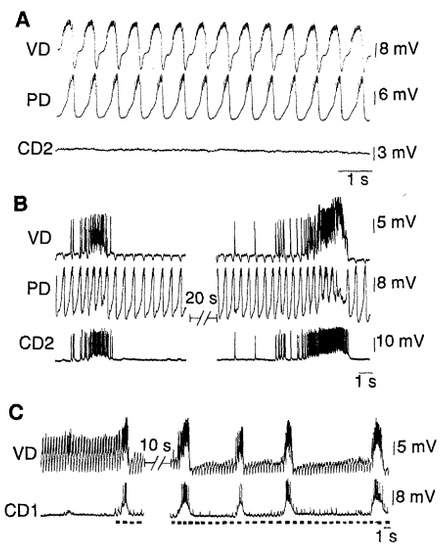

Fig. 2. (**A and B**) The VD neuron can fire either with the pyloric network (PD neuron trace) (A) or the CS network (CD2 neuron trace) (B). (**C**) Tonic stimulation (dotted line) of a sensory nerve, the lpln, activates the CS network (CD1 neuron trace), and the VD neuron switches from the pyloric network pattern (left) to the CS network pattern (right).

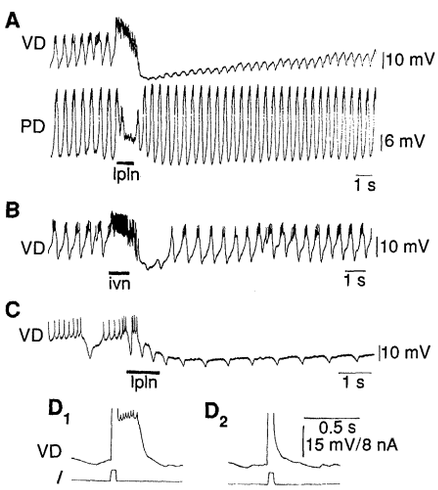

Fig. 3. The VD neuron inactivation is associated with loss of its regenerative membrane properties. (**A**) After brief lpln stimulation (lpln), which induced a single CS network cycle and high-frequency VD neuron spike burst, the VD neuron was inactivated and no longer fired with the pyloric network (PD trace). (**B**) A similar high-frequency VD neuron spike burst obtained by stimulating the axons of the IV neurons (ivn) did not inactivate the VD neuron. (**C**) VD neuron inactivation not preceded by a high-frequency spike burst was obtained by brief lpln stimulation (lpln) in the presence of curare. (**D_1** and **D_2**) A brief pulse of depolarizing current (*I*) injected into the VD neuron soma induced a regenerative depolarization when the VD neuron was firing with the pyloric network (D_1), but induced only a passive response when the VD neuron was firing with the CS network (D_2). All lpln stimulations were 40 Hz for 1 s.

We also tested whether the VD neuron inactivation might be due to a reduction in the electrical coupling between the VD neuron and the PD and anterior burster (AB) neurons of the pyloric network (Fig. 1), as this coupling is the only excitatory input the VD neuron receives from the pyloric network. The strength of this connection was identical for active and inactivated VD neurons.

Another reason for the VD neuron inactivation could be that the high-frequency VD neuron firing during CS network bursts induces a post-tetanic depression of the neuron. This hypothesis can be rejected because the VD neuron spike burst and inactivation can be experimentally dissociated. First, high-frequency VD neuron spike bursts induced either by injecting depolarizing current into the VD neuron or stimulating the IV neurons (Fig. 3B) do not inactivate the VD neuron. Second, curare blocks the VD neuron firing induced by lpln stimulation [by blocking the excitatory IV to VD neuron synapses (*6*)], but has no effect on VD neuron inactivation (Fig. 3C).

Finally, the VD neuron inactivation could be due to changes in the expression of regenerative membrane properties in the neuron itself. The rhythmic depolarization and firing of the pyloric network neurons depend on "plateau" or "oscillatory" membrane properties that are intrinsic to the neurons (*11*) but can be modified by extrinsic inputs (*11, 12*). A neuron expressing these properties responds to a brief depolarizing current injection sufficient to bring the membrane potential of the neuron beyond a certain threshold with a sustained depolarization (plateau) that long outlasts the current pulse (*11*). When the VD neuron fires with the pyloric network it always expresses these properties (Fig. $3D_1$), but whenever it is inactivated [during CS network activity induced by tonic lpln stimulation (Fig. $3D_2$), during spontaneous CS network activity, or after brief lpln stimulation (*10*)], the neuron shows only passive responses.

We have not identified the input that induces the VD neuron inactivation, but it is not the IV neurons, as their direct stimulation (Fig. 3B) does not cause VD neuron inactivation. This input is presumably neuromodulatory in nature, because brief lpln stimulation leading to a single CS network cycle inactivates the VD neuron for tens of seconds (Fig. 3A), and 10 to 20 min of continuous CS network activity (either spontaneous or induced by lpln stimulation) inactivates the VD neuron for several hours.

In summary, when the CS network is active, it always appropriates an otherwise integral member (the VD neuron) of the pyloric network. This work thus argues that individual neurons can belong to more than one neural network. Switching of the VD neuron occurs when the CS network is activated by electrical stimulation of a sensory input or by mechanical stimulation of the stomach wall in semi-intact preparations (*9*); this switching may therefore be physiologically relevant. Our results are consistent with the VD neuron switch arising from a long-lasting neuromodulatory suppression of the intrinsic regenerative membrane properties of the neuron; changes in these properties may thus not only cause a single network to assume different configurations (*2*), but also switch neurons between different networks.

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Taxonomic Differences in the Scaling of Brain on Body Weight Among Mammals

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Theories for the evolution of brain weight in mammals suggest that closely related species have diverged largely as a result of selection for differences in body weight, but that differences among more distantly related species have arisen due to greater net directional selection on brain weight. This pattern of changing selection causes brain weight to evolve more slowly than body weight among closely related species, such as those in the same genus, than among more distantly related species, such as those from different families or orders; a phenomenon known as the "taxon-level effect." Thus, brain weight differs more for a given difference in body weight as the species compared are more distantly related. An alternative explanation for the taxon-level effect is proposed. Distantly related species are more likely to inhabit different ecological conditions than are more closely related species. Where the taxon-level effect occurs, brain weight appears to have evolved in response to the demands of these different ecological conditions. As a consequence, brain weight differs more among distantly related species, for any given difference in body weight, than among closely related species. This effect, rather than a progressive pattern of changing selection pressures, may account for the taxon-level effect in mammals.

THE RELATIONSHIP OF BRAIN weight to body weight in mammals can be described by an allometric power formula, brain weight $\approx a(\text{body weight})^b$, which becomes linear when both variables are expressed in logarithmic form: $\log(\text{brain weight}) \approx \log(a) + b\log(\text{body weight})$. The taxon-level problem in the evolution of mammalian brain weight refers to the finding that the slope b of the logarithmic relation depends upon the taxonomic level within which it is estimated (*1–4*). Slopes fitted to species of the same genus are reported to be typically about 0.2 to 0.4, but if a slope is fitted across species from different orders it ranges up to around 0.75 (*1–4*). This means that brain weight differs more for a given difference in body weight among more distantly related taxa (Fig. 1).

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