lyzed as described (*7*). We constructed the pSP*gsc* expression vector by creating an Nco I site at the initial methionine and a Sal I site after the termination codon of the *gsc* cDNA type B form [nucleotides 115 to 847 of the sequence reported in (*6*)] by means of PCR add-on mutagenesis. This fragment was then used to replace a bovine preprolactin fragment in pSP35T (*27*) after excision with Nco I and Sal I. The parent vector of pSP35T is a modified form of pSP64 [P. A. Krieg and D. A. Melton, *Nucleic Acids Res.* **12**, 7057 (1984)]. The resulting pSP*gsc* construct contains 5′ and 3′ untranslated sequences of β-globin, as well as a polyadenylate tail. Capped synthetic mRNA was transcribed from pSP*gsc* in vitro with SP6 RNA polymerase (*6*). This mRNA could be diluted 20 times to yield results similar to those obtained in our previous studies with the vector p*gsc* (*6*), which lacks β-globin sequences.

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21. Whole mount in situ hybridizations were carried out as described, with a full-length *gsc* clone as probe (*6*). Embryos were developed in the staining solution for 6 hours to avoid reaching reaction saturation. Slides of intact whole mounts or histological sections were scanned and analyzed digitally at the computing facility of the CNRS Strasbourg, directed by J.-L. Vonesch.
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Neuronal Activity During Different Behaviors in *Aplysia*: A Distributed Organization?

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The active neuronal populations in the *Aplysia* abdominal ganglion during spontaneous and evoked behaviors were compared with the use of multineuronal optical measurements. In some preparations, more than 90 percent of the neurons activated during the reflex withdrawal of the gill also were activated during respiratory pumping and during small spontaneous gill contractions. Although the same neurons made action potentials in all three behaviors, the activity patterns were different. There was a substantial interaction between the neural substrates underlying evoked and spontaneous behaviors when they were made to occur together. If a gill withdrawal reflex was elicited a few seconds after a respiratory pumping episode, the evoked neuronal activity in most neurons was clearly altered. These results suggest that a distributed organization involving a large number of neurons may be responsible for generating the two behaviors. Different behaviors appear to be generated by altered activities of a single, large distributed network rather than by small dedicated circuits.

Behaviors can be generated either by dedicated neuronal circuits or by a distributed neuronal network (Fig. 1) that can be reconfigured to generate several behaviors. It may be difficult to distinguish a distributed neuronal organization from dedicated circuits by direct observations. For example, earlier voltage-sensitive dye recordings show that between 200 and 300 neurons in the abdominal ganglion of *Aplysia* are activated during the gill withdrawal reflex (*1*, *2*). However, as shown in Fig. 1 both kinds of organization can be constructed with similar numbers of neurons and both may share sensory and motor neuron pools among several behaviors. A straightforward way to distinguish the two organizations is to examine all the possible synaptic connections in the system. Unfortunately, this is often impractical because the number of possible connections can be very large (increasing approximately as the square of the number of neurons).

Some researchers have suggested that dedicated circuits may generate some simple behaviors (*3*, *4*). On the other hand, there is evidence of distributed neuronal organizations in both invertebrate and vertebrate systems (*5*). However, in these studies only a small fraction of the active neurons was examined. Thus, it was impossible to determine how the whole nervous system functioned during different behaviors.

We attempted to distinguish the two possible organizations by comparing neuronal activity in *Aplysia* during spontaneous and evoked gill withdrawal behaviors. In the dedicated model (Fig. 1), sensory neurons activate many distinct circuits and interneurons. However, during spontaneous behaviors sensory neurons are not involved (*6*). This dedicated model would predict that only one circuit should be activated by the intrinsic generator, and thus we would expect a much smaller number of active inter-

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neurons. In contrast, distributed models predict that the same large population of neurons would be activated during both spontaneous and evoked behaviors (Fig. 1).

Voltage-sensitive changes in the absorption of stained neurons were detected (*7*) with a 124-element photodiode array; up to 30% of the 900 neurons (*8*) in the abdominal ganglion can be simultaneously recorded (*1*). We compared the neuronal activity during three different behaviors—the gill withdrawal reflex, a small spontaneous gill contraction, and respiratory pumping—in six recordings from the same ganglion (Fig. 2). The active neuronal populations are similar during the three different forms of gill contractions. In two recordings of the gill withdrawal reflex, we found 62 and 69 active neurons, respectively. Among these neurons, only two are not activated during small spontaneous gill contractions. Only 5 out of 72 neurons are active during respiratory pumping but not during gill withdrawal. More than 90% of the neurons active in one behavior were active in the other two. The variation in the percentage overlap of neurons between two different behaviors was similar to the trial-to-trial variations seen with one behavior (Fig. 2) (*9*). With correction for the 30% completeness of the voltage-sensitive dye recording (*1*), the actual number of activated neurons in this experiment would be about 200.

The data from Fig. 2 are displayed in histogram form in Fig. 3. The two middle sections are histograms of the total action potential count and the number of active neurons. Just before the onset of a behavioral event, there is a peak in action potential activity, which involves a large number of active neurons. Between 41 and 48 neurons made at least one action potential during a 512-ms time interval at the onset of the gill withdrawal reflex and respiratory pumping. In the histogram at the bottom, the neurons activated during one of the small spontaneous gill contractions (event B) were removed, so that the neurons shown are those that were activated only during the gill withdrawal reflex or respiratory pumping. During the three behaviors the same large population of neurons was activated. The overlap in neurons used in the gill withdrawal reflex and respiratory pumping was examined in four additional preparations. Seventy to 90% of the neurons activated in one behavior were also active during the second.

Many neurons showed significantly different firing patterns and total numbers of action

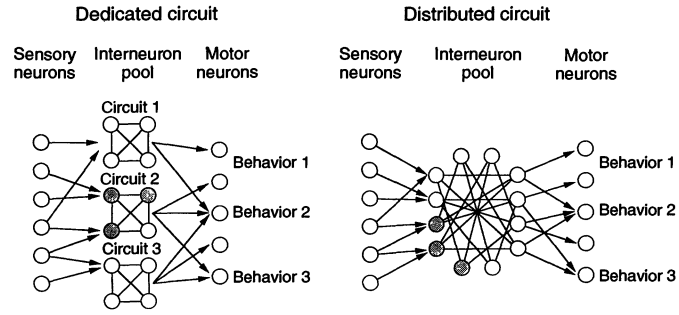

Fig. 1. Distributed and dedicated neuronal organizations, which differ in the connectivity of the neurons. In a dedicated organization, the connections among neurons form clusters and each cluster (a circuit) is dedicated (necessary and sufficient) to generate one behavior. In distributed organizations, specific functions are generated by different configurations of the network; there are no fixed circuits, and neuronal resources are shared by all the functions of the system. In a dedicated system, the activity resulting from spontaneous intrinsic rhythms (shaded neurons) is limited to one circuit. In a distributed system, however, intrinsic activities in interneurons can spread to the whole system to generate a spontaneous behavior.

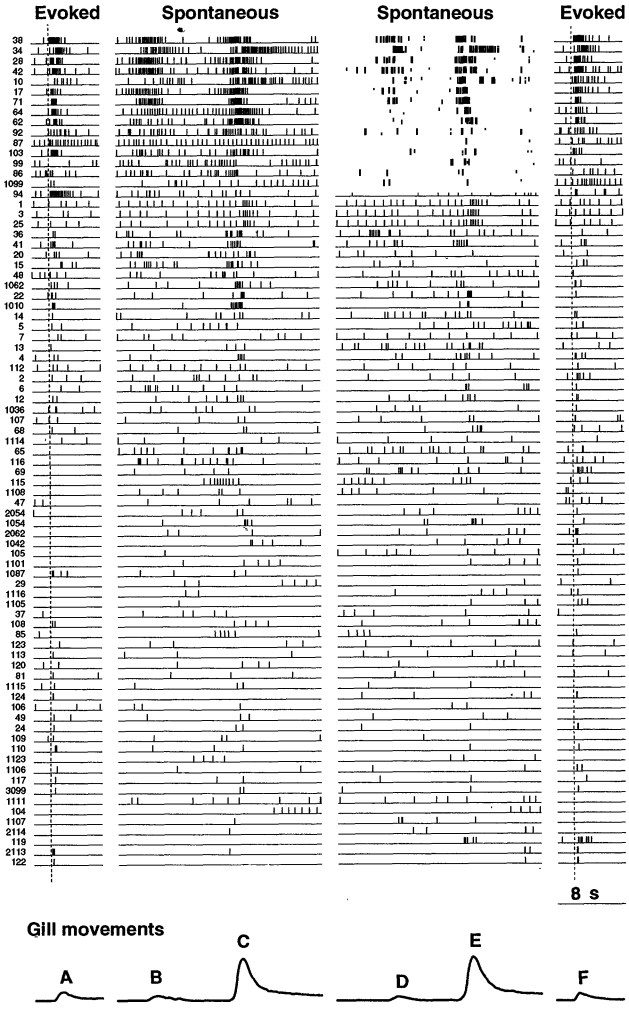

Fig. 2. Comparison of the neuronal activity during spontaneous and evoked gill contractions. Each line represents the activity of one neuron during the gill withdrawal reflex (**A** and **F**), respiratory pumping episodes (**C** and **E**), and small spontaneous gill contractions (**B** and **D**). Each vertical tick on the line indicates the timing of an action potential in the neuron. The activities of all of the neurons whose spike signals were detected in the optical recording are shown in a descending sequence according to the total number of action potentials in the four recordings. The gill withdrawal reflex was elicited by water jet (0.2 ml and 6 to 7 mmHg) at the time indicated by the dashed line.

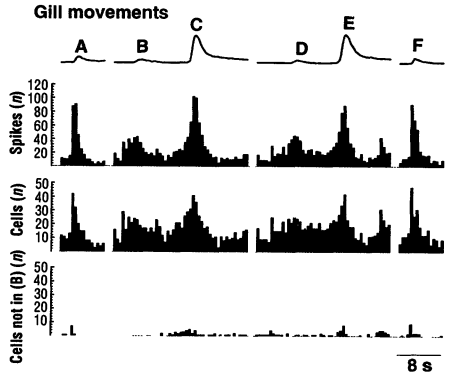

Fig. 3. Overlap of the active neuronal population. Data from the experiment shown in Fig. 2 are presented here in histograms. The height of each bar represents the numbers of action potentials (top) or active neurons (middle) recorded during a 512-ms time interval. In the bottom histogram, neurons activated during a small spontaneous gill contraction (B) were removed. The top section shows the amplitude of the gill contractions. (**A** and **F**) Gill withdrawal reflex; (**B** and **D**) small spontaneous gill contraction; (**C** and **E**) respiratory pumping episode.

potentials during the different behaviors. During the gill withdrawal reflex, most of the neurons fired a brief burst in response to the sensory input. On the other hand, during the respiratory pumping episodes and the small spontaneous gill contractions many neurons fired in a long train before the onset of the gill movement (Fig. 2). There were twice as many action potentials during respiratory pumping episodes than during the gill withdrawal reflex.

The results shown in Figs. 2 and 3 suggest a distributed neuronal organization. The activity of this large population of neurons may be necessary for all behavioral events related to the gill withdrawal, as predicted by distributed models. During different behavioral events, most neurons are activated differently; this is consistent with a distributed mechanism, where different behaviors are generated by altered configurations of the same circuit. Our results, however, do not support a dedicated model. We find few neurons that are exclusively activated during one behavior.

If two behaviors are generated at the same time, there should be a competition for common neuronal resources. In a distributed system, this interaction is expected to be extensive and to affect many neurons. We compared the neuron activity in a gill withdrawal initiated in the middle of the interval between two respiratory pumping episodes with the activity in a reflex elicited during the falling phase of a respiratory pumping episode. Substantial modification can be seen in the spiking activity of many neurons. In the preparation used for the first and second panels of Fig. 4, the overall response to the touch was significantly prolonged, and the gill contraction was also prolonged. On the other hand, in the preparation shown in the third and fourth panels of Fig. 4, the preceding respiratory pumping reduced the response to the touch; most of the neurons fired fewer action potentials. The modification caused by a preceding respiratory pumping was consistent in repeated trials in one animal ($n = 4$). But in experiments on different animals the overall neuronal activity could be increased, decreased, or even unaffected. The existence of a modulatory effect on the activity of a large fraction of the neurons is consistent with a distributed circuit.

A large number of neurons respond to a light touch to the siphon. In addition to the 200 to 300 abdominal ganglion neurons that respond (*1*), there are another 100 neurons in the pleural ganglion and 600 neurons in the pedal ganglion that are activated (*10*). Approximately 15% of the central neurons of *Aplysia* are affected by this very mild and localized stimulus. It is unlikely that this large population of neurons is dedicated to one simple behavior. It would be more reasonable to think that these neurons are used in a distributed way to carry out several behaviors and their coordination. It had been thought that the monosynaptic connection made by LE sensory neurons accounted for 58% of the excitatory input to the gill motor neurons (*4*). If true, this result would argue strongly for an important dedicated component with a simple architecture. However, it is clear that the LE contribution is much smaller, probably between 0 and 10% (*11*).

There is a possible weakness in our argument in favor of a distributed organization. Both of the behaviors we have examined include gill contractions. Because we do not know the proportion of motor neurons and interneurons in the optical recordings, there is the possibility (albeit unlikely) that the majority of neurons that we detected optically are motor neurons. Because the behaviors had similarities, it would be expected that the active neurons would also be similar; experiments examining additional behaviors are required to rule out this possibility.

In a distributed system, temporary circuitry emerges from the anatomical connections only when a particular form of sensory input or intrinsic activity occurs. Getting (*12*) introduced the concept of dynamic circuitry from the study of the neuronal organization that generates *Tritonia* swimming and withdrawal behaviors. Our results suggest that in *Aplysia* similar mechanisms may occur on a much larger scale and may mediate the gill withdrawal reflex, respiratory pumping, and other behaviors. Electrophysiological measurements on neurons in the abdominal ganglion have suggested the existence of more than 20 groups of interneurons involved in the defensive withdrawal circuit (*13*). These interneurons might have distinct follower cells and be dedicated to specific functions. Thus, there may be clusters of neurons behaving as dedicated functional units that contribute to a distributed network generating different behaviors. Additional experiments are required to determine whether such functional clusters exist and behave as a unit during all behaviors. Although a distributed neuronal organization appears to be more difficult to understand than one consisting of dedicated circuits, it is similar to some models in which nonspecific connections can be "trained" into configurations with specific function (*3*, *14*).

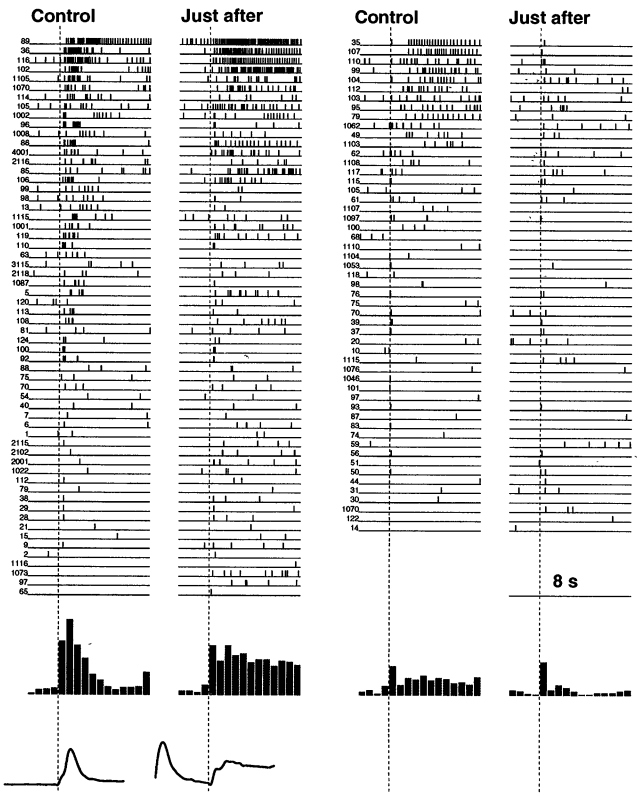

Fig. 4. Interaction between spontaneous and evoked gill withdrawals in two preparations. The dotted line marks the time of the mechanical stimuli to the siphon. In the second and fourth panels, the gill withdrawal reflex was evoked at 6 and 8 s after the onset of a respiratory pumping episode. The first and third panels are control trials; the withdrawal was elicited between two respiratory pumping episodes. The middle section shows histograms that plot the total number of action potentials. The bottom shows the change in the gill area; shown in the second panel is a large spontaneous gill contraction before the optical recording. There was no video recording of the gill contraction for the animal shown in the right panels. The large modulatory effect of a preceding respiratory pumping episode was not apparent when a delay of 30 s was used.

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In Vivo Ca^{2+} Dynamics in a Cricket Auditory Neuron: An Example of Chemical Computation

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Fura-2 calcium imaging in the cricket omega neuron revealed increased intracellular free calcium ion concentration in response to simulated cricket calling songs and other sound stimuli. The time course of the increase and decrease in intracellular calcium coincided with the time course of forward masking, a time-dependent modulation of auditory sensitivity. The buffering of calcium transients with high concentrations of a kinetically fast calcium buffer eliminated the post-stimulus hyperpolarization associated with forward masking, whereas the uncaging of calcium inside the neuron produced a hyperpolarization. The results suggest that sound-stimulated intracellular calcium accumulation acts by means of a calcium-activated hyperpolarizing current to produce forward masking. These findings underscore the importance of chemical dynamics in neural computation by demonstrating a behaviorally relevant role of calcium dynamics in vivo.

With the advent of techniques for measuring the spatial and temporal dynamics of chemical species in neurons (*1*), it is possible to ask how the dynamics of chemical activity (such as ion concentration) contribute to neural computation. Here we report how one form of chemical activity, the temporal dynamics of intracellular free calcium ion concentration, $[Ca^{2+}]_i$, in a cricket auditory interneuron, underlies forward masking, a psychophysical and electrophysiological phenomenon in cricket hearing. Forward masking is a form of temporal inhibition in which a loud sound suppresses the response to subsequent sounds (a temporal analog of lateral inhibition). This temporal inhibition may be used for automatic gain control or background subtraction, allowing female crickets to "focus" on the loudest caller in the presence of multiple calling males and background noise. Because this ability would enable the cricket to home in on an individual chirping male, it has been referred to as a form of selective attention (*2*) in analogy to the "cocktail party phenomenon" observed in human auditory psychophysics (*3*). An electrophysiological correlate of forward masking is observed in the omega neuron, one of the first interneurons in the cricket auditory pathway. This correlate appears as a long-lasting, post-stimulus hyperpolarization and concomitant reduction in action potentials after sound stimuli (*2*).

The response of an omega neuron to a simulated calling song and the forward masking effect are shown in Fig. 1 (*4*). The response to chirps of a single calling song presented at 60-dB sound pressure level (SPL) is shown in Fig. 1A and Fig. 1B shows the response to two simultaneous calling songs. The chirps of the 90-dB song precede the chirps of the 60-dB song and suppress the number of action potentials elicited by the 60-dB song, compared with the number of action potentials evoked by the 60-dB song by itself. This effect is accompanied by a small hyperpolarization that occurs between chirps. The masking increases from the first to the fourth chirp in the sequence, at which point the action potentials evoked by the quieter calling song are completely eliminated. The hyperpolarization that follows each chirp also accumulates with each successive chirp. Figure 1C summarizes this forward masking effect, showing the number of spikes per chirp in the absence of masking and with two different masking intensities. As the masking intensity is increased the curves shift to the right, showing that the cell's auditory threshold is temporarily raised by the masking stimuli. The amount of masking produced by the 90-dB song on the 60-dB song increases with each subsequent chirp (Fig. 1B), suggesting that the masking effect takes longer to decay than the inter-chirp interval. As a result, the response to later chirps is affected by the residual masking from earlier chirps. This observation and the data in Fig. 1C suggest that the physiological variable that controls masking increases with sound intensity and decays slowly (several seconds), compared to the inter-chirp interval. Forward masking cannot be explained by inhibition from the known connections onto the omega neuron (*5*). Therefore, we hypothesized that a calcium-activated hyperpolarizing current produces forward masking and, thus, that the dynamics of forward masking are determined by the dynamics of $[Ca^{2+}]_i$.

To investigate this hypothesis, we mea-

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