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16. Because amino acid analysis of ^{3}H-labeled peptides showed a high exchange of the radioactivity with the acidic phase, we analyzed ^{14}C-labeled peptides. [^{14}C]glutamate can be metabolically formed by conversion of [^{14}C]glucose through glycolysis and the tricarboxylic acid cycle.
17. Neurons were cultured for 1 week and were then incubated for 3 hours with D-[U-^{14}C]glucose (100 μCi/ml) (Amersham; >230 mCi/mmol) and cycloheximide. Extraction of tubulin, digestion with thermolysin, and purification of the ^{14}C-labeled peptides TL.A, TL.B, and TL.C were performed as described (Fig. 1). HPLC profiles and distribution of the radioactive peaks were similar to those observed after cell incubation with [^{3}H]acetate (Fig. 1). Hydrolysis was carried out in vapor phase of *7M* HCl and 10% (v/v) TFA for 22 min at 160°C. Precolumn derivatization with phenylisothiocyanate and reversed-phase HPLC analysis of amino acid derivatives were performed essentially as described [B. A. Bidlingmeyer, S. A. Cohen, T. L. Tarvin, *J. Chromatogr.* **336**, 93 (1984)]. The eluted fractions were processed for liquid scintillation counting. In the three cases, a single radioactive fraction, associated with the Glu peak, was detected.
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15 August 1989; accepted 31 October 1989

An Identified Neuron (CPR) Evokes Neuronal Responses Reflecting Food Arousal in *Aplysia*

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Feeding behavior of *Aplysia* is associated with an arousal state characterized by a constellation of maintained behaviors and by a potentiation or depression of responses to specific stimuli. A neuron (the cerebral-pedal regulator or CPR) that has widespread actions on various systems connected with feeding has been identified. CPR excites neurons that modulate or drive (i) body posture, (ii) biting, and (iii) cardiovascular behaviors. CPR also inhibits neurons concerned with defensive responses. Food stimuli, which elicit food arousal in the animal, produce prolonged excitation of the CPR. The results suggest that the CPR may evoke a central motive state representing the neuronal correlate of feeding motivation.

MORE THAN 40 YEARS AGO IT WAS recognized that variations in behavioral responsiveness could not be adequately explained exclusively by reference to external stimuli, and it was therefore proposed that motivated behaviors are controlled by central motive states (*1*). The central motive states were postulated to evoke and maintain specific behaviors and also to modify the responses of the organism to external stimuli. The neuronal basis of central motive states has been studied in vertebrates (*2*), as well as several invertebrates (*3, 4*), including the mollusk *Aplysia*.

In *Aplysia*, food elicits behavioral changes that can be interpreted as being due to an underlying central motive state, which we have referred to as food-induced arousal (*5*). The changes include the induction of appetitive behaviors such as a characteristic feeding posture in which the head of the animal is lifted from the substratum. Furthermore, biting responses are more readily elicited by food, and once the biting responses begin there is a progressive increase in the rate and strength of the responses. Finally, defensive reflexes are inhibited, and there are modifications of cardiovascular responses (*6*). Food arousal in *Aplysia*, at least in part, is mediated by modulatory systems [for example, the serotonergic modulatory metacerebral cell (MCC) (*7*)] that are dedicated to one or another aspect of feeding behavior. In this study we sought to determine whether there are higher order neurons that modulate lower level subsystems.

We first studied isolated pedal-pleural and cerebral ganglia (*8*). We identified a single pair of bilaterally symmetrical cerebral neurons (*9*), cerebral-pedal regulators (CPRs), whose firing had excitatory or inhibitory effects on a large number of cells in the ipsilateral and contralateral pedal ganglia (Fig. 1A). A subsample of neurons studied in a solution that blocks polysynaptic transmission (*10*) indicated that most of the effects of CPR were polysynaptic (86%; $n = 32$). Of 150 pedal neurons examined (five preparations), 36% of the cells were excited and 18% were inhibited when a CPR was fired (Fig. 2). We estimate that CPR may affect the activity of as many as 2000 pedal neurons. The synaptic activity evoked by CPR in the pedal ganglion was not rhythmic, suggesting that it was not evoking a locomotor program (*11*).

Most excitatory effects exerted by the CPR were on neurons in the region where neck motor neurons have been identified [sector II (*12*) in Fig. 2]. In a reduced preparation that includes muscles that move the neck, stimulation of CPR or of pedal neurons that are excited by the CPR evoked contractions of the neck muscles. Unlike contractions evoked by presumed motor neurons, the CPR-evoked contractions were bilateral and widespread and were abolished when the ganglion was perfused with a solution that blocks polysynaptic transmission (*13*), suggesting that the CPR has no direct actions on the muscle.

To determine whether the CPR is associated with feeding circuitry we examined its actions on various neurons concerned with the consummatory aspects of feeding. Firing of the CPR evoked activity of cerebral-buccal interneuron CBI-2 (Fig. 1B), a putative command element that drives biting-like movements of the buccal mass (*14*). The resulting firing of CBI-2 was not of high enough frequency to evoke biting, but the depolarization might function to prime the neuron and enhance its responses to food stimuli. Activity of CPR also resulted in depolarization of other cerebral-buccal interneurons (*15*), including the MCC (Fig. 1B), a neuron that modulates the rate and intensity of biting (*16*).

CPR also polysynaptically modulated neurons in the abdominal ganglion and affected the cardiovascular system. Firing of CPR increased heart rate by 10 to 20%; it excited the cardiovascular command neuron L10 (*17*) and the modulatory heart motor neuron RB_{HE} (Fig. 1C). Finally, CPR firing produced a brief inhibition followed by excitation of vasoconstrictor neuron LB_{VC},

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which is excited during food arousal (*18*).

Withdrawal reflexes of *Aplysia* are strongly inhibited during the food-aroused state (*19*). We found that CPR activity inhibited several defensive systems, which are distributed throughout the central nervous system. For example, firing of the CPR polysynaptically hyperpolarized cerebral Bn neurons (Fig. 1D), which are involved in defensive head withdrawal (*20*). The hyperpolarization of the Bn neurons resulted in decreased firing of the Bn cells in response to strong tactile stimulation of the tentacles. The interneurons mediating the CPR effects on Bn cells and all other cerebral neurons appear to be located in the pedal-pleural ganglia, since sectioning of the cerebral-pedal-pleural connectives ($n = 3$) abolished all the synaptic actions of CPR.

CPR activity inhibited abdominal ganglion neuron L7, which is involved in defensive gill withdrawal (*21*), and abdominal ganglion neuron R2 and its pleural homolog PL1, which mediate mucus secretion in response to noxious stimuli (*22*). Thus CPR activity may contribute to a general inhibition of defensive responses during feeding.

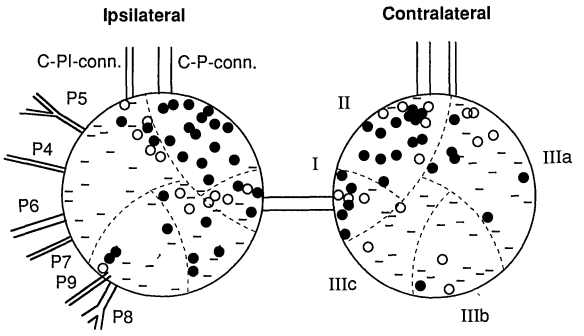
We next examined whether stimulation with seaweed, which normally induces food arousal of the animal, also excites the CPR. We recorded from a reduced preparation, in which the head of the animal remained attached to the ganglia. The CPR and the MCC were excited by seaweed applied to the tentacles, rhinophores, and lips (Fig. $3A_1$). The responses of the CPR and thus the MCC outlasted the stimulus. In four experiments, we found that if both the left and right CPRs were removed from the circuit by injecting hyperpolarizing current, the response of the MCC to seaweed stimuli was abolished (Fig. $3A_2$). In all preparations in at least some trials, a brief seaweed stimulus produced a prolonged response of the CPR (Fig. 3B), which could persist for several minutes, the typical time domain for normal head-lifting behavior. Similar long-lasting discharges of the CPR occasionally occurred "spontaneously" in the isolated ganglion preparation. We found that CPR evokes polysynaptic input to itself and the contralateral CPR, and this may contribute to the prolonged response.

We formulated a model of how the neural systems underlying the food-induced behavioral state in *Aplysia* could be organized (Fig. 4). The CPR is activated by food stimuli. The CPR then affects pedal-pleural ganglion neurons, which have widespread influences on other neurons, including motor neurons, as well as lower order modulatory neurons (for example, the MCC, and RB_{HE}). The muscles effecting the feeding posture of the animal are controlled by neurons located in the pedal ganglion, the ganglion that distributes the various effects of the CPR. Thus the postural motor system may play a pivotal role in behavioral arousal.

Since reduced preparations do not exhibit the full complement of normal manifestations of the food-aroused state, we could not directly test whether the CPR is necessary or sufficient for eliciting overall food arousal. However, our data indicate that the CPR may function in a manner analogous to that of command neurons or command elements that elicit recognizable behavioral acts, typically stereotyped rhythmic responses (*11, 14, 23*). In the case of the CPR, however, the neuron appears to elicit a central motive state rather than a specific act. Although this state is elicited by activity of a single neuron, neural representations of the state are distributed throughout the nervous system. The food-arousal state is generated by the activity of neurons that control specific behaviors such as the assumption and maintenance of a head-up posture, but in addition the state is associated with neural events that lead to the potentiation or depression of other behaviors. Neurons involved in withdrawal responses are inhibited, whereas neurons involved in consummatory feeding responses are potentiated.

The food-arousal state in *Aplysia* may require two types of systems. Specialized systems such as the MCC produce modulatory effects limited to specific aspects of behavior (*16*), whereas a more general system may coordinate the specialized lower level systems. In an arrangement of this type, coordination of the subsystems is automatically ensured, but local control at the level of the specialized systems during particular behavioral conditions can selectively accentuate one or more of the specific mani-

Fig. 2. Schematic map of a dorsal view of the pedal ganglion showing the locations of neurons in different regions of the pedal ganglion that are affected by firing of CPR; (●) excitatory; (○) inhibitory; (—) no connection.



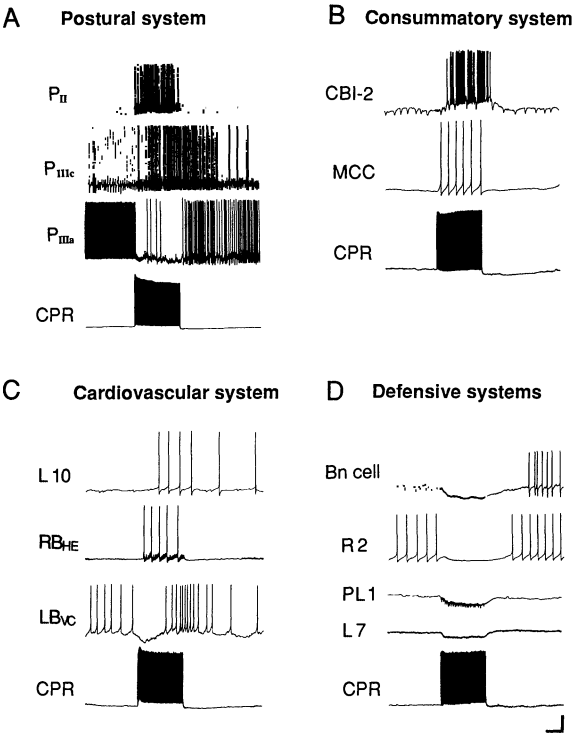


Fig. 1. Effects of firing the CPR on various systems associated with food-induced arousal. Pairs of ipsilateral cells were impaled with double-barreled microelectrodes (20 to 30 megohms) and identified by electrophysiological and morphological criteria. In each experiment, the CPR was intracellularly stimulated at 20 Hz for 5 s. For illustrative purposes, multiple follower cells of the CPR are shown in each section of the figure, but the data for each trace were obtained in separate experiments. Calibration: 2 s, 20 mV, except 5 mV for cells R2 and PL1 in (D). (**A**) Examples of the effects of firing of CPR on neurons in the pedal-pleural ganglion that are presumably part of the postural control system. The suffix in the neuron designation indicates the quadrant in which the neuron was located. Nomenclature according to (*12*). (**B**) Effects on cerebral neurons that control consummatory feeding responses (biting command element, CBI-2) and the modulatory metacerebral cell MCC. (**C**) Effects on abdominal neurons that control the cardiovascular system (command neuron L10, heart excitor RB_{HE}, and vasoconstrictor LB_{VC}. (**D**) Examples of neurons located in different ganglia that participate in defensive responses (head withdrawal motor neurons, Bn cell; gill withdrawal motor neuron, L7; defensive secretion neurons, R2 and PL1).

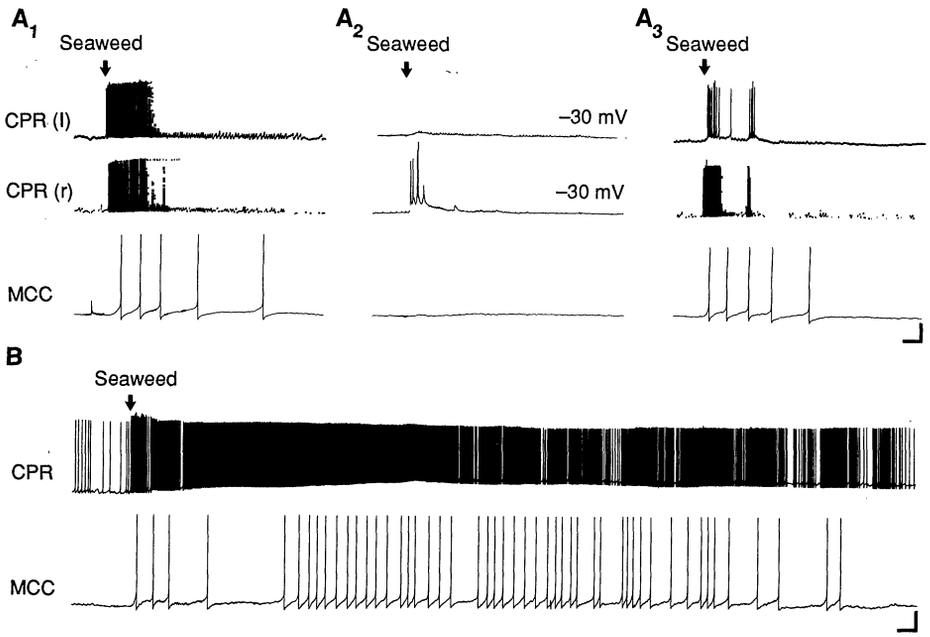


Fig. 3. Responses of the CPRs and MCC to brief seaweed stimulation on the tentacle recorded in a preparation in which parts of the head of the animal were attached to the ganglia. The ganglia were contained in a chamber separated from the chamber containing the head and tentacles. (**A_1**) Brief touch with seaweed on the left tentacle. (**A_2**) Same conditions as in (A_1), but during injection of 30-mV hyperpolarizing current into both CPR neurons. (**A_3**) Same as (A_2), but with CPR neurons returned to resting potential. Calibration: 2 s; 20 mV. (**B**) Example of a prolonged response of CPR and MCC to a brief seaweed stimulus. The preparation and stimulus was the same as in (A). Calibration: 5 s, 20 mV.

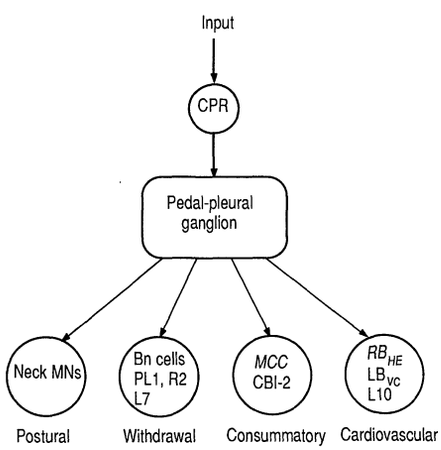


Fig. 4. Organization of postulated food-induced arousal system in *Aplysia*. Effector neurons (command elements or motor neurons) are represented in plain type, serotonergic modulatory neurons in italics. Sensory input activates the CPR and in addition has more direct access (not shown) to specific effector systems.

festations of the central motive state. Single neurons, or clusters of similar cells that exert effects on multiple systems, have been reported in other invertebrates (*4*), but the great variety of actions of the CPR distinguishes this neuron from those previously described. In higher animals, central motive states may be similarly controlled by small sets of neurons that exert widely divergent effects.

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8. One electrode was used to impale pedal ganglion neurons, and a second to stimulate cerebral neurons that project to the pedal ganglion. The position of putative cerebral-pedal interneurons was determined by $NiCl_2$ backfills of the cerebral-pedal connective.
9. The CPR is located at the ventral surface near the origin of the anterior tentacular nerve and the upper labial nerve.
10. The solution contained 160 m*M* Mg^{2+}; 30 m*M* Ca^{2+}, which raises spike thresholds and tends to block polysynaptic connections.
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24. We thank E. R. Kandel and J. Koester for comments on the paper. Supported by PHS grants MH 35564 and GM 320099 to I.K. and K.R.W. and DFG grant Te 138/ 1-1 to T.T.

24 August 1989; accepted 26 October 1989