rocal feedback connections between command neurons and other members of a motor network provide a possible means for self-supporting oscillation of the network (*14*). Such self-reinforcement by positive feedback can, in principle, help sustain a motor pattern beyond the initiating stimulus, providing one plausible neurophysiological explanation for the ethological concept of "triggering" (*15*), whereby a behavioral sequence long outlasts the initiating stimulus. Our data also imply that the activity of a central motor network can, in principle, be initiated at many loci. Direct excitation of the network mediating feeding in *Pleurobranchaea* can occur by stimulation of other neurons that are reciprocally connected to the command neurons (*16*). Owing to such reciprocal interconnections within a motor network, specialization of a given population of neurons for the command role may derive at least in part from privileged access to the central or sensory inputs that control the corresponding behavior. That is, command neurons may be differentiated from other members of a motor network not only on the basis of output effects, but also on the basis of input organization.

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13. The CD neurons send ascending axons to the brain through the cerebrobuccal connective (*8, 10*), and are necessary for the proper coordination of the brain and buccal ganglion (*8*). The AV neurons are associated with feeding (*12*) and have strong, widespread chemical and electrical synapses throughout the buccal ganglion. Single AV action potentials trigger repetitive discharges in many buccal ganglion neurons (Fig. 2), and thus resemble the "trigger" neurons in the buccal ganglion of *Planorbis* [M. S. Berry, *J. Exp. Biol.* **57**, 173 (1973)] and the "cyberchrons" of *Helisoma* [S. B. Kater, *Am. Zool* **14**, 1017 (1974)].
14. Reciprocal connections among small populations of motor neurons have been demonstrated in invertebrate preparations [for example, A. I. Selverston, *Am. Zool.* **14**, 957 (1974)] and shown by computer modeling studies to be capable of generating rhythmic motor output in response to tonic input [D. H. Perkel and B. Mulloney, *Science* **185**, 181 (1974); D. Dagan, L. H. Vernon, G. Hoyle, *ibid.* **188**, 1035 (1975); H. S. Warshaw and D. K. Hartline, *Brain Res.* **110**, 259 (1976)].
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17. This work was supported by NIH postdoctoral fellowships to R.G. and M.P.K., and by NIH research grants NS 09050 and MH 23254 to W.J.D. Some of the experiments on whole-animal preparations were performed at Friday Harbor Laboratories, Friday Harbor, Wash. We thank P. A. Getting and A. O. D. Willows for discussion and criticism of an earlier draft of this report.

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## Neural Correlate of Behavioral Plasticity in Command Neurons of *Pleurobranchaea*

Abstract. *Food stimuli normally excite the command neurons of* Pleurobranchaea *that cause feeding. In contrast, the same food stimuli selectively inhibit these neurons in specimens that have been trained to suppress feeding and withdraw from food by means of an avoidance conditioning paradigm consisting of paired food and conditional shock. Food stimuli excite the feeding command neurons of yoked control specimens exposed to unpaired food and shock, but inhibit the feeding command neurons of untrained specimens that have been satiated with food. These results suggest that the command neurons serve as a neural locus at which an animal's behavior is modulated by past experiences. These results also establish a neural correlate of behavioral plasticity, in the form of synaptic inhibition of the command neurons.*

Behavioral plasticity may be defined broadly as a modification of behavior that is acquired because of experience (*1*) and includes such phenomena as habituation, sensitization, and learning. One approach to understanding the neurophysiological mechanisms of behavioral plasticity is to analyze the neuronal circuitry that mediates a modifiable behavioral act, and then to examine this circuitry for physiological changes that accompany the behavioral modification (*2*). The feeding behavior of the mollusc *Pleurobranchaea californica* is amenable to this approach; the behavior (*3*) can be modified by classical (*4*) and avoidance (*5*) conditioning, as well as food satiation (*6*), and the neuronal circuitry controlling feeding is known in some detail (*7–9*). In particular, command neurons for feeding, termed paracerebral neurons (*8*), have been identified in two bilaterally symmetrical groups in the cerebropleural ganglion (brain). Intracellular stimulation of single command neurons in whole-animal preparations causes rhythmic, coordinated biting and swallowing that is indistinguishable from normal feeding (*8*).

As reported (*8*), paracerebral command neurons in hungry, untrained specimens are excited by application of food stimuli to the chemosensory oral veil, and such natural excitation of the command neurons is usually followed immediately by overt feeding. We report here that when *Pleurobranchaea* is trained in a conventional avoidance conditioning paradigm (*5*) to suppress feeding (passive avoidance conditioning) and withdraw from a normally palatable food substance (active avoidance conditioning), application of this food to the oral veil inhibits rather than excites the feeding command neurons. Likewise, when specimens are fed to satiety, the feeding command neurons are inhibited rather than excited by food stimuli. Our study therefore shows that in *Pleurobranchaea*, modulation of behavior by experience is accompanied and presumably caused by a corresponding modulation of the activity of command neurons that control the behavior.

Specimens of *P. californica* (volume, 50 to 350 ml) were purchased from Pacific Bio Marine (Venice, California), matched in pairs on the basis of size (± 10 percent), and divided into experimental and control groups by a random procedure (coin flip). Experimental animals were conditioned in seawater (12° to 15°C) in 10 to 18 hourly trials as described (*5*). In each trial, a liquid homog-


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enate of raw squid mixed with an equal volume of seawater (designated 1 × squid homogenate) was applied evenly to the oral veil with a disposable syringe at an approximate flow rate of 0.2 ml/sec. If a specimen either exhibited a component of the feeding behavior [the proboscis extension or bite-strike response (*3*)] or failed to withdraw from the food stimulus during 90 seconds (criterion time) of continuous application, it received an aversive electric shock (*10*). Each control animal was yoked to its matched experimental animal and received unpaired food (on the hour) and shock (on the half hour), delivered in the same way and in the same quantities as received by the matched experimental animal in the corresponding trial.

Experimental and control animals were tested together 12 to 36 hours after conditioning with squid homogenate from the same batch as that used in conditioning. The identity of the animals was concealed during testing. Behavioral parameters measured during testing included: (i) the thresholds of the proboscis extension and bite-strike feeding responses, defined as the minimum concentration of squid homogenate necessary to elicit the response (*6*); (ii) the latencies of the same responses following the onset of application of 1 × squid homogenate (to a maximum, or criterion, time of 90 seconds); and (iii) the pres-

Table 1. Summary of the responses of 52 feeding command neurons from 22 whole-animal preparations to the application of 1 × squid homogenate to the oral veil. Responses were scored blind as E (excitatory), 0 (no response), A (ambiguous), and I (inhibitory) (see text). Command neuron responses are expressed as the fraction of the total number of cells sampled in each category. Variability between animals was assessed by determining the fraction of preparations in which 50 percent or more of the command neurons sampled responded in the same way as the majority of cells in the corresponding category. These data are: naive, 4/7 [see (*15*)]; conditioned, 5/7; control, 4/4; and satiated, 4/4.

| Type of preparation | *N* | Response of command neuron | | | |
|---|---|---|---|---|---|
| | | E | 0 | A | I |
| Naive | 7 | 7/16 | 0/16 | 3/16 | 6/16* |
| Conditioned | 7 | 2/14 | 1/14 | 2/14 | 9/14 |
| Control | 4 | 10/10 | 0/10 | 0/10 | 0/10 |
| Satiated | 4 | 1/12 | 1/12 | 0/12 | 10/12 |

*See (*15*).

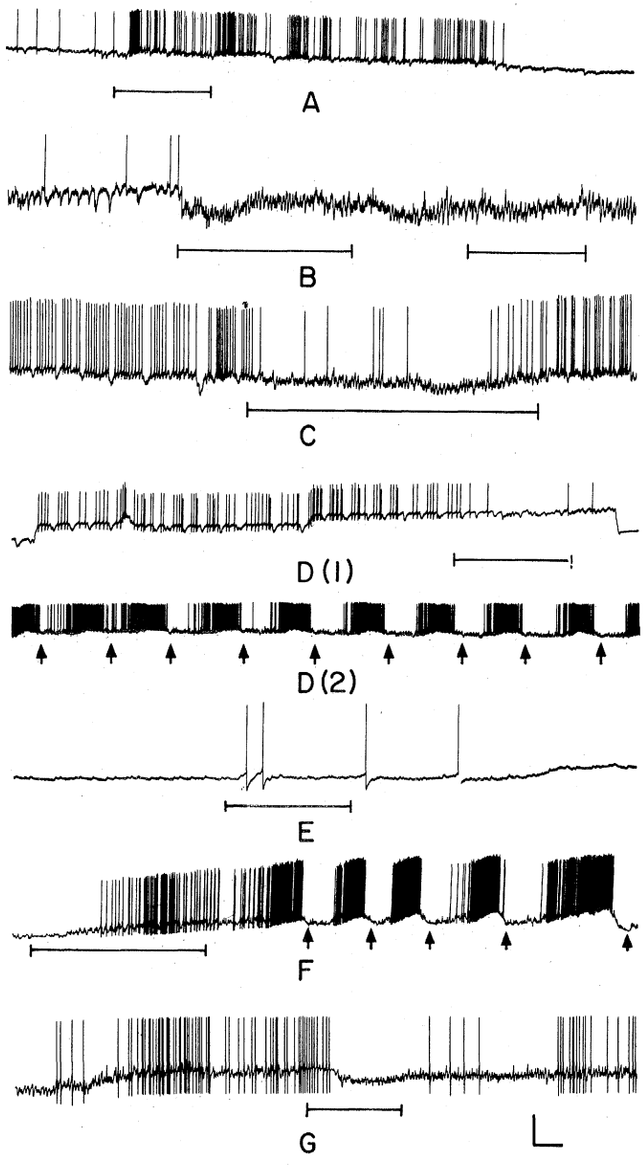


Fig. 1. Responses of feeding command neurons in whole-animal preparations of *Pleurobranchaea* recorded intracellularly during the application of food stimuli (1 × squid homogenate) to the chemosensory oral veil (bars beneath each record). (A) Typical excitatory response of a paracerebral command neuron in an untrained, hungry specimen. Visible feeding movements were in this case weak. (B to D) Inhibitory responses of the paracerebral command neurons of three different animals trained in an avoidance conditioning paradigm to suppress feeding and withdraw from 1 × squid homogenate. (B) Inhibition of a "resting" command neuron. (C) Inhibition of a command neuron that was tonically depolarized by injection of current through the recording electrode. (D) Trace 1, inhibition of a command neuron by food stimuli following stepwise depolarization (at the beginning and middle of the record); trace 2, strong depolarization of the same neuron immediately after trace 1 was obtained caused normal, cyclic bursting in the command neuron. Each burst was accompanied by the animal's making visible bites (upward arrows). (E) A metacerebral giant neuron of a conditioned specimen, showing excitation by food stimuli. (F) Excitation of a paracerebral command neuron of a control animal. Upward arrows correspond to the animal making visible bites. (G) Inhibition of a tonically depolarized paracerebral command neuron of a satiated animal. Calibration (lower right): (A) 25 mV (vertical), 2 seconds (horizontal); (B) 4 mV, 1 second; (C) 10 mV, 1 second; (D to F) 25 mV, 2 seconds; (G) 12.5 mV, 2 seconds.

ence or absence of active withdrawal from 1 × squid homogenate (*11*). Only well-conditioned animals (that is, animals having maximum or near maximum thresholds and latencies, and strong aversion to food stimuli) that were matched with unchanged controls were accepted for neurophysiological experiments. Likewise, only unchanged control animals matched with well-conditioned experimentals were studied neurophysiologically. To satiate specimens, hungry animals were fed raw squid until they refused to eat for a period of at least 1 minute.

Individual feeding command neurons were studied neurophysiologically approximately 0.5 to 2 days after the conditioning and control procedures, and 2 to 6 hours after the animals were satiated. Whole-animal preparations were made as described (*8*), and the somata of individual command neurons were impaled under visual control with a glass capillary microelectrode filled with 3*M* KCl (tip resistance, ~10 megohms). The microelectrode was attached to an electrometer having a bridge circuit for current passage, which in turn led to a Tektronix oscilloscope in parallel with a Brush-Gould pen recorder for making permanent records. After a command cell was impaled and identified (*12*), the animal was subjected to a prearranged sequence of stimuli, including (i) a variety of types and concentrations of liquefied food stimuli (*13*), (ii) tactile stimulation of various external body parts, and (iii) electrical stimulation of brain nerves (*14*). About 63 percent of the command cells were studied in blind neurophysiological experiments, that is, without the investigators knowing the identity of the animal until after the experiment was completed. The results of these experiments were the same as those obtained in the remaining experiments that were not conducted blind. Recorded data were analyzed later by investigators who did not know the identity of specimens from which the recordings were obtained. Intracellular responses to stimuli were scored as excitatory (E), no response (0), ambiguous (A), or inhibitory (I). Ambiguous responses (9.6 percent of the total) were variable ones that contained more than one of the other three response categories. Useful data were obtained from 52 feeding command neurons in 22 specimens. In this report we discuss only the responses to 1 × squid homogenate.

In naive, hungry specimens, application of 1 × squid homogenate to the oral veil excited the command neurons (Fig. 1A) and frequently elicited rhythmic biting from the whole-animal preparations (*8*). In contrast, the command neurons of animals trained in the avoidance conditioning paradigm responded with a barrage of hyperpolarizing potentials when food stimuli were applied to the oral veil (Fig. 1B); typically, these potentials were accompanied by the animal's making an active withdrawal response. This hyperpolarizing response had a reversal potential below the typical rest potential of 50 to 60 mV, and was capable of suppressing high-frequency action potential discharge induced by injected current (Fig. 1C). Therefore, the response was inhibitory. Feeding behavior could nonetheless be driven in such conditioned preparations by imposed depolarization of the command neuron (Fig. 1D). Therefore, the remainder of the feeding network was not incapacitated by avoidance conditioning. The metacerebral giant neuron, which normally has a weak excitatory effect on feeding behavior and is activated by food stimuli (*9*), was excited rather than inhibited by 1 × squid homogenate in conditioned preparations (Fig. 1E), as were other brain neurons that burst cyclically and in phase with the feeding rhythm. Therefore, the inhibitory effect in conditioned specimens is at least partially selective to the paracerebral command neurons. The responses of feeding command neurons of control animals were indistinguishable from those of hungry untrained animals; that is, 1 × squid homogenate was excitatory (Fig. 1F). Therefore, the inhibitory response in conditioned specimens was not caused simply by repeatedly shocking the oral veil. The responses of command neurons of satiated animals were indistinguishable from those of conditioned animals; that is, food stimuli were inhibitory (Fig. 1G). Satiated specimens seldom withdrew from the food stimulus, however; therefore, the inhibition of paracerebral command neurons is presumably not caused by neurons that mediate withdrawal. The responses to 1 × squid homogenate of all 52 paracerebral feeding command cells included in this study are summarized in Table 1.

These data establish a neurophysiological correlate to behavioral plasticity in *Pleurobranchaea*. Different experiences that reduce the animals' propensity to eat (that is, reduce the level of feeding motivation) are manifest similarly as synaptic inhibition at the level of the feeding command neurons. Thus, intracellular recordings from the command neurons provide a neurophysiological reflection of motivation (*15*). We presume that avoidance learning and satiation involve distinct neuronal mechanisms and pathways; the finding that these different experiences cause a similar change in the responses of the command neurons to food stimuli suggests that the respective pathways converge presynaptically to the command neurons, perhaps on the unidentified neurons that inhibit the command neurons. Our study suggests that the command neurons represent a neurophysiological locus at which the activity of the feeding network is modulated, or "gated," by the organism's behavioral experience. The command neurons now also provide an identified reference point in the feeding circuitry, from which the physiological mechanisms underlying avoidance learning may be sought.

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10. Shock was applied under seawater to the animals' anterior end, including the oral veil, through 12-gauge tinned copper bus wire electrodes separated by 2 cm and insulated except at the exposed tips with epoxy glue. A shock consisted of rectangular pulses of 20-msec duration and 70-volts amplitude, repeated at 20 hertz for 60 seconds.
11. About 80 percent of conditioned specimens showed elevated feeding thresholds (usually by a factor of 100) and increased latencies (usually from a few seconds before conditioning to a criterion time of 90 seconds), as well as an acquired aversion to 1 × squid homogenate. About 75 percent of control animals showed normal (unchanged) feeding thresholds and latencies, and no withdrawal from 1 × squid homogenate. Our results are thus in general agreement with those of Mpitsos and Collins (*5*).
12. Every command neuron studied here was identified by at least three and often four of the following criteria: (i) soma position [see (*8*)]; (ii) the presence of a descending axon in the ipsilateral cerebrobuccal connective, demonstrated by antidromic or orthodromic stimulation, or both; (iii) the presence at rest of cyclic hyperpolarization that was 1.0 to 5.0 mV in amplitude, 0.2 to 1.0 second in duration, and repeated at a period of 0.5 to 3.0 seconds. This hyperpolarization was recorded almost invariably from somata of the paracerebral neurons but never from nearby somata of other neurons; and (iv) the capacity to drive cyclic feeding behavior when depolarized by injected current.
13. Food stimuli were maintained within 1°C of the

seawater bath temperature and applied to the oral veil with a Pasteur pipette. Squid homogenate was taken from the same batch used in conditioning.

14. Most of the command cell penetrations were held long enough (~ ½ hour) to enable us to apply a complete sequence of stimuli. Several penetrations were held long enough (about 1 hour) to enable us to fully replicate the stimulus sequence in order to test the stability of the responses. The responses to the second sequence were generally identical to the first.
15. Of the six command neurons in naive animals that were inhibited by food stimuli, four were from two animals that refused to feed during the experiment, and two were from the last half of an experiment in which the animal had ingested large quantities of seawater during analysis of the first two command neurons, visibly distending the gut and inducing satiety-like effects. Apparently, when specimens are unwilling to eat for any reason, command neurons are inhibited rather than excited by food stimuli.
16. This work was supported by NIH research grants NS 09050 and MH 23254 to W.J.D. and by an NIH postdoctoral fellowship to R.G. Pilot experiments were conducted at Friday Harbor Laboratories, Friday Harbor, Wash. We thank the director, A. O. D. Willows, for making facilities available to us, J. Villet for technical assistance, and M. Kovac for criticism of the manuscript.

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## Biologically Active Pituitary Hormones in the Rat Brain Amygdaloid Nucleus

Abstract. *While an attempt was being made to identify the source of the growth hormone releasing factor present in cerebral spinal fluid of man, it was discovered that cells of the rat amygdaloid nucleus, grown in tissue culture, produce a material that is immunologically and chromatographically identical to growth hormone found in the pituitary. Immunoperoxidase staining revealed dense accumulation of the peroxidase-antibody to growth hormone complex in amygdala cells. Significant amounts of growth hormone and adrenocorticotropin could be extracted from this limbic structure. Extracts containing immunoequivalent amounts of growth hormone were measured by bioassay in hypophysectomized rats. Stimulation of the growth of epiphyseal cartilage by extracts of the amygdala was comparable to the stimulation by extracts of anterior pituitary glands. The stimulatory effect of amygdala extracts on adrenal and gonadal size and weight and on growth of thyroid follicular epithelium was also comparable to that of pituitary extracts.*

While investigating the source of a growth hormone releasing factor present in human cerebral spinal fluid (*1*), we first studied the amygdaloid nucleus of the limbic system because of the many reports linking that brain structure to endocrine function of the anterior pituitary gland (*2*). Unexpectedly, we have discovered significant amounts of immunoreactive growth hormone in this structure and in media harvested from these cells grown in tissue culture. Significant amounts of immunoreactive adrenocorticotropin (ACTH) are also present in the amygdaloid nucleus of the rat brain, confirming a similar report of the extrapituitary presence of ACTH in discrete regions of the central nervous system (*3, 4*). Likewise, bioassay of rat amygdaloid nucleus extracts in hypophysectomized animals reveals significant bioactivity similar to the bioactivity of growth hormone, ACTH, gonadotropin, and thyroid stimulating hormone, as well.

Adult male and female Sprague-Dawley rats (approximately 200 g) were quickly decapitated and the brain was removed. A coronal slice was made at the level of the chiasmal crossing, and the amygdaloid nucleus quickly removed. This tissue was extracted and frozen for subsequent studies or immediately prepared for tissue culture by enzyme dispersal of single cells by means of short-term incubation with Pronase. Rat growth hormone was extracted by homogenization of the tissue in 0.01*N* sodium hydroxide and phosphate-saline buffer (*5*). Rat growth hormone, either from the anterior pituitary or from portions of the rat brain, was measured by solid phase radioimmunoassay (*6*) adapted in our laboratory for rat growth hormone. Rat ACTH was immunoassayed with an antiserum to 1-24 ACTH, which has a molecular weight of 2800; but we based our calculations on 1-39 ACTH which has a molecular weight of 4500. With these approaches, rat amygdaloid nucleus contained approximately 250 ng of growth hormone per gram of wet tissue. The ACTH content was approximately 4.1 ng per milligram of tissue or roughly 5 percent of pituitary content. Chromatography of this growth hormone-like material on Sephadex G-100, cycled with a Veronal buffer at *p*H 8.4, revealed a profile identical to that of $^{125}$I-labeled rat growth hormone. Sections stained immunohistochemically with conjugated peroxidase–antibody to growth hormone complex revealed numerous cells of the amygdala containing a dense accumulation of the granular precipitate (*7*). Preliminary examination of other brain areas revealed the presence of a material similar to the immunoreactive growth hormone in the cortex, hippocampus, and hypothalamus, but none was present in the caudate nucleus or in the cerebellum. On the basis of wet weight of tissue, the amygdaloid nucleus contained the largest amount of this material.

Because of the possibility that this growth hormone might be of pituitary rather than of brain origin, we examined the effects of injecting radioactively labeled growth hormone into the systemic circulation. The $^{125}$I-labeled growth hormone, $11 \times 10^6$ count/min, was injected into the tail vein of lightly anesthetized animals, and portions of muscle, kidney, fat, and brain were examined for the presence of radioactivity. No radioactivity was found in any part of the brain examined, whereas kidney and liver contained large amounts of radioactivity.

To provide further evidence that amygdaloid growth hormone was derived from this extrapituitary source, we also examined rats subjected to hy-

Table 1. Mean and standard deviations derived from bioassay of extracts of anterior pituitary (RAP) and amygdaloid nucleus (RAM) from adult rats 14 days after hypophysectomy. Four to five animals were included in each group. Experimental animals received comparable amounts of immunoreactive growth hormone (IRGH) in four daily divided doses administered intraperitoneally. A minimum of ten width measurements were made of individual silver nitrate–stained tibial epiphyses by means of an ocular micrometer, where 1 micrometer unit (m.u.) is equivalent to 0.005 mm.

| Extract and dose ($\mu$g IRGH/ per rat) | Sex | Tibia epiphyseal width (m.u.) | Testis (mg) | Ovaries (mg) | Adrenals (mg) |
|---|---|---|---|---|---|
| Saline-0 | M | 8.3 ± 0.5 | 154 ± 0.7 | | 7.6 ± 0.4 |
| RAP-113 | M | 10.7 ± 0.4* | 193 ± 4.0* | | 11.3 ± 0.9† |
| RAM-73 | M | 10.3 ± 0.2* | 204 ± 6.2* | | 10.5 ± 2.8† |
| Saline-0 | F | 11.1 ± 2.0 | | 15.2 ± 3.2 | 11.2 ± 1.6 |
| RAP-95 | F | 13.6 ± 3.2† | | 19.1 ± 4.9† | 13.2 ± 1.9† |
| RAM-90 | F | 15.1 ± 3.2* | | 29.7 ± 4.5* | 16.7 ± 2.7* |

*$P < .01$. †$P < .05$.


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