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A SUBPOPULATION OF CEREBRAL B CLUSTER NEURONES OF *APLYSIA CALIFORNICA* IS INVOLVED IN DEFENSIVE HEAD WITHDRAWAL BUT NOT APPETITIVE HEAD MOVEMENTS

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Summary

The cerebral B cluster neurones of *Aplysia californica* were studied under experimental conditions designed to evoke head movements in a selective fashion: either to approach an appetitive stimulus, or to withdraw from an aversive one. Intracellular recordings indicated the presence of two types of B cluster neurones: Bn cells that had fast (narrow) spikes, and Bb cells that had slow (broad) spikes. Tactile stimulation of the tentacles, rhinophores and lips excited Bn neurones, but inhibited Bb neurones. Intracellular stimulation of Bn cells evoked contractions of body wall muscles. No contractions were observed when Bb cells were fired, indicating that it is unlikely that the Bb neurones are motor neurones. Several lines of evidence indicated that the Bn type neurones are involved in withdrawal responses but not in appetitive head turning. (1) Elimination of the descending axons of the Bn cells by lesioning the cerebropleural connectives (C-Pl connectives) did not affect the head-turning response. This lesion significantly altered the head-withdrawal response by selectively eliminating an initial fast component of the withdrawal movement. (2) In chronic recordings from the C-Pl connective, unit activity was obtained which was correlated with the presentation of an appetitive stimulus rather than with evoked or spontaneous turning movements. A substantial increase in activity also occurred during head withdrawal of the animal. On the basis of these data, we postulate that separate populations of motor neurones are responsible for the aversive withdrawal of the head, and for the directed turning response towards a stimulus.

Introduction

Much of animal behaviour can be described as either approach towards stimuli or withdrawal from them (Schneirla, 1965) and, in some cases, approach or withdrawal responses to stimuli at a given locus can involve opposite movements

Key words: *Aplysia*, head turning, head withdrawal, feeding.

of the same body parts. It is therefore of interest to compare the underlying neural controls of such contrasting responses. One example of approach and avoidance responses utilizing the same structure is provided by the head movements of *Aplysia*. These movements can serve either to withdraw the head from a noxious stimulus, or to approach appetitive stimuli such as food by means of directed turning movements (turning response). For an initial cellular analysis of the contrasting head movements, we focused on the cerebral B cluster neurones (Jahan-Parwar and Fredman, 1976). Previous evidence has suggested that B cells may be motor neurones for head movements, but it is unclear whether they are involved in withdrawal responses, appetitive movements or both types of responses (Jahan-Parwar, 1972; Fredman and Jahan-Parwar, 1977; Jahan-Parwar and Fredman, 1978*a*,*b*, 1983; Rosen *et al.* 1979). The B cells respond to tactile and food stimulation of the rhinophores, tentacles and lips (Fredman and Jahan-Parwar, 1980). Intracellular stimulation of the B neurones evokes contractions of the muscles in the tentacle, body wall and the foot (Fredman and Jahan-Parwar, 1977; Jahan-Parwar and Fredman, 1978*a*,*b*; Rosen *et al.* 1979), and backfilling the nerves which innervate the neck muscles (e.g. pleural nerve Pl 1) fills several neurones in the B cluster, together with a large number of cells in the pleural and pedal ganglia (Bablanian *et al.* 1987). The data presented in this paper show that the cerebral B cluster contains two distinct populations of neurones. A combined approach of single-cell recordings, extracellular nerve recordings and lesion studies was utilized to establish the properties of the different subsets of B cluster neurones, and to study their possible role in head movements in either turning or withdrawal responses.

Materials and methods

Experiments were performed on *Aplysia californica* (150–250 g; Marinus, CA) which were maintained in artificial sea water (Instant Ocean) at 15 °C. For dissection, the animals were immobilized by injection of an isotonic $MgCl_2$ solution of approximately 25 % of body weight.

Intracellular recordings

Preparations used were (1) isolated ganglia, (2) a reduced preparation, consisting of the head attached to the ganglia, and (3) a semi-intact preparation, in which the ganglion was pinned on a small stage inserted in a slit through the body wall of the animal. All preparations used were set up without arterial perfusion. Unless otherwise noted, the recordings were performed on six preparations each. The connective sheath covering the ganglia was removed so that the neurones were accessible. Intracellular recordings from individual neurones were performed using conventional electrophysiological techniques. The cells were impaled with double-barrelled glass electrodes. One barrel was filled with 2 mol l^{-1} potassium acetate, the other with 5(6)-carboxyfluorescein (Kodak; 5 % in 0.1 mol l^{-1} potassium acetate), and the electrode was bevelled to 10–30 MΩ.

Following recordings, the cell bodies were dye-filled by injecting negative current pulses into the dye-containing electrode, and the unfixed preparations were viewed with a Leitz fluorescent microscope.

Lesioning procedure

To cut the cerebropleural connectives (C-Pl connectives), the $MgCl_2$-immobilized animal was pinned to a slanted wax tray and opened by an incision on the dorsal surface, posterior to the rhinophores. After the connectives had been cut, the skin incision was sutured with surgical thread. Prior to lesioning, the animals were tested to ensure that they showed normal turning responses. The appetitive turning response of the animal was studied again 24 h after surgery. Turning responses were evoked in a food-aroused animal that had attached to the walls of the tank, with its head and neck fully extended and stretched out along the water surface. In this position, a small piece of seaweed was briefly touched to the lip, at approximately 5° or 10° eccentricity from the centre of the mouth. The animal was monitored with a video system from above, and the turning angle was determined every 250 ms by measuring the angle between the centre line of the animal and the starting position.

Stop–action analysis of the video recordings was also used to analyse the execution of withdrawal responses. The head withdrawal was evoked by a strong pinch of a tentacle with a forceps. In each animal, five tests were undertaken before and after surgery. The amount of head withdrawal in 250 ms was measured as a percentage of the maximal withdrawal shown by the animal. This standardization procedure minimized variability due to variations of the total length withdrawn (because of different strengths of the pinches) and differences of image scaling of the video recordings. As a control for non-specific effects of surgery, sham operations were performed, in which the C-Pl connectives were exposed and manipulated, but were not cut. In the 'blind' procedure, one experimenter performed the surgery, while the second did the behavioural testing.

Extracellular nerve recordings

Nerve recordings in freely moving animals were obtained by means of implanted suction electrodes. The surgical procedure was similar to that described above. The cut end of the cerebropleural connective was drawn into a small-diameter sylastic tubing, which fitted tightly over the partly desheathed connective. Teflon-insulated platinum-iridium wire (Medwire; 10 IR 9/47 T) was inserted into the open end of the tubing, fastened with a suture, and insulated with Vaseline. After the surgery, the animal was allowed to recover for 24 h. Video recordings of the animal were obtained simultaneously with the chronic nerve recordings, to enable precise correlations of the behaviour of the animal with the recorded nerve activity.

Results

Spike width separates the neurones of the B cluster into two populations

A survey of the neurones in the cerebral B cluster revealed that these cells can be divided into two distinct populations. The two types of neurones differed most obviously in the duration of their action potentials (Fig. 1). One type had the characteristics of the neurones previously associated with the B cluster (see e.g. Fredman and Jahan-Parwar, 1975). These neurones (Fig. 1, top traces) had narrow spikes, with a halftime of about 5 ms and amplitude of about 75 mV. There was a hyperpolarizing afterpotential that was less prominent than in the other class of B cell. This first cell type will be termed Bn (the 'n' indicating a narrow spike) to distinguish it from the second cell type, Bb ('b' indicating a broad action potential). Bn neurones received excitatory and inhibitory PSPs, with a predominance of EPSPs (see Jahan-Parwar and Fredman, 1976). The neurones of the second type (Fig. 1A, bottom trace) were characterized by a much broader spike with a halfwidth of about 13 ms and amplitude of about 65 mV. They exhibited a very prominent hyperpolarizing afterpotential. Bb neurones were occasionally silent, but most often were spontaneously active at low frequencies between 0.2 and 0.5 Hz (see lower traces in Fig. 3). The rising phase of the action potential of the Bb neurones had a prominent inflection, suggesting that the initiation of the spike occurred far from the cell body. Bn neurones were found to make excitatory connections with each other (see also Fredman and Jahan-Parwar, 1975), but no connections between the two different types of neurones or between individual Bb neurones were found.

Bn and Bb neurones have different axon distributions

Visualization of the B neurones by injecting a fluorescent dye into the cell body

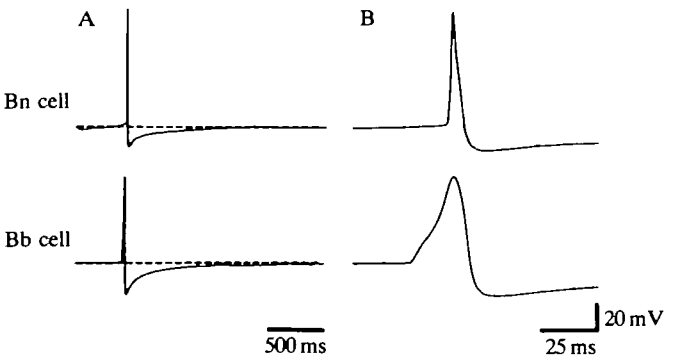

Fig. 1. Spontaneous action potentials of neurones of the two different subpopulations in the cerebral B cluster; Bn ('narrow') type neurone, top trace; Bb ('broad') type neurone, bottom trace. (A) Slow sweep shows the more prolonged afterpotential of the Bb cell compared with that of the Bn cell. (B) Faster sweep illustrates the broad action potential and inflection in the rising phase of the Bb cell spike compared with the narrow spike of the Bn cell.

revealed morphological differences between the Bn and Bb cell types (Fig. 2). Bn cells had a compact cell body with multipolar axons and usually one or several axons which projected into different cerebral nerves. In Fig. 2, the numbers next to the nerve stumps indicate the number of preparations, out of a total of 15, in which axons were observed in a particular nerve. Axons of Bn neurones were always (15/15) found in the contralateral C-Pl connective, and frequently (10/15) in the ipsilateral C-Pl connective (Fig. 2A). After entering the pleural or pedal ganglia, the axons exited through various pleural or pedal nerves that innervate the foot and the neck. No crossing of the axons in the pedal commissure was observed. Axons were also observed frequently in the ipsilateral posterior

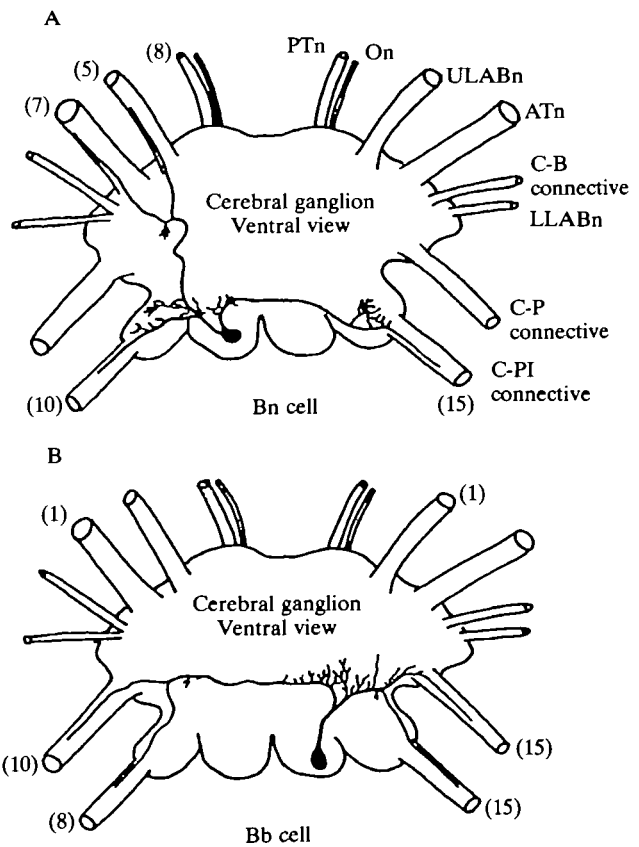

Fig. 2. Drawings illustrating the morphology of a typical Bn (A) and Bb cell (B) in a ventral view of the ganglion. Neurones were visualized by means of a fluorescent microscope after they had been injected with 5(6)-carboxyfluorescein. The numbers in brackets represent the number of preparations (out of a total of 15), in which an axon was observed in the particular nerve. Nerves and connectives are abbreviated as follows: PTn, posterior tentacular nerve; On, optic nerve; ULABn, upper labial nerve; ATn, anterior tentacular nerve; C-B connective, cerebrobuccal connective; LLABn, lower labial nerve; C-P connective, cerebropedal connective; C-Pl connective, cerebropleural connective.

tentacular (PT), anterior tentacular (AT) and upper labial (ULAB) cerebral nerves.

Bb type neurones (Fig. 2B) had an elongated, pear-shaped cell body with a long, thick axon stem. The Bb neurones always (15/15) exhibited axons in both ipsilateral pedal and pleural connectives, and frequently in the contralateral pedal (10/15) and pleural connectives (8/15). The smaller number of B cell axons observed in the contralateral connectives might be due to failure to detect the axons because of their small diameter, especially for the extremely fine axon of the Bb type neurones in the contralateral C-Pl connective. The axons of the Bb cells did not extend into the cerebral nerves, but projected into various pleural and pedal nerves.

Bb type neurones were typically encountered in the medial region of the ventral surface of the B cluster. A medial location of Bb neurones was also confirmed by nickel chloride backfills of the C-P connective which, according to the results of the fills of individual neurones, contained the axons of Bb, but not of Bn, neurones. The backfills showed approximately 10 cell bodies located near the midline of the ganglion in the B cluster of each hemiganglion. Backfilling the C-Pl connective made it possible to estimate the total number of Bn type neurones by subtracting the number of Bb neurones from the total number of cell bodies filled. These results indicated that there are about 30–35 Bn neurones in each hemiganglion, with a relatively even spatial distribution within the B cluster region.

Bn cells are excited by stimulation of the head

To gain insight into which of the B cell types might mediate withdrawal or appetitive head movements, we determined the responses of the cells to sensory stimuli applied to the head in a reduced preparation. Previous observations have indicated that head withdrawal is elicited by strong tactile stimuli or hypertonic salt solutions (Walters and Erickson, 1986). Appetitive head movements are effectively triggered by seaweed (chemo- plus tactile) stimulation (Kupfermann, 1974; Teyke *et al.* 1988). Defensive responses to noxious stimuli to the head consist of symmetrical shortening of the neck, and relatively straight-back head withdrawal (Walters and Erickson, 1986; see also Fig. 7), regardless of whether the stimulus is presented to the left or right tentacle (T. Teyke, K. R. Weiss and I. Kupfermann, unpublished observations). By contrast, the direction of appetitive head movements is dependent on the position of the stimulus on the head: stimuli to the left cause turning movements towards the left, and *vice versa*.

For these experiments we used a reduced preparation, consisting of the circumoesophageal ganglia and the head, including lips, anterior tentacles and rhinophores. This type of preparation is suited for presenting controlled tactile and chemical stimuli on the head of the animal, although it is likely that the sensory responses are depressed compared to those in an intact animal. In these preparations we observed clear responses to several different stimuli such as: (1) an aversive chemical stimulus, consisting of 1 ml of a 4 mol l^{-1} NaCl solution;

(2) food, consisting of a piece of moistened seaweed (laver) that provided both a tactile component and an appetitive chemical component; and (3) a piece of moistened paper (paper towel) that had a texture and consistency similar to that of the moistened seaweed, thus providing a tactile component in the absence of food–chemical stimulation. The paper was applied to the tentacle by means of forceps, in the same way as the seaweed. Simultaneous recordings of one Bn and one Bb neurone during tactile, seaweed and saline stimulation are shown in Fig. 3. Both tactile (Fig. 3A) and seaweed stimulation (Fig. 3B) of the tentacles evoked excitation of the Bn cell. There was no obvious difference in the magnitude of the response evoked by stimulation of the ipsilateral *versus* the contralateral side. In the Bb neurone, such stimuli evoked very weak responses, consisting of a transient inhibition, which slightly increased the interspike interval in spontaneously active cells (Fig. 3B). In general, there was no apparent difference in the response of the Bn cells to purely tactile (Fig. 3A) or seaweed stimulation (Fig. 3B). Both stimuli evoked approximately the same number of spikes and a similar prolonged depolarization of the Bn neurone. The different stimuli also did not evoke different responses in the Bb neurones. Distinctive responses of the two types of B

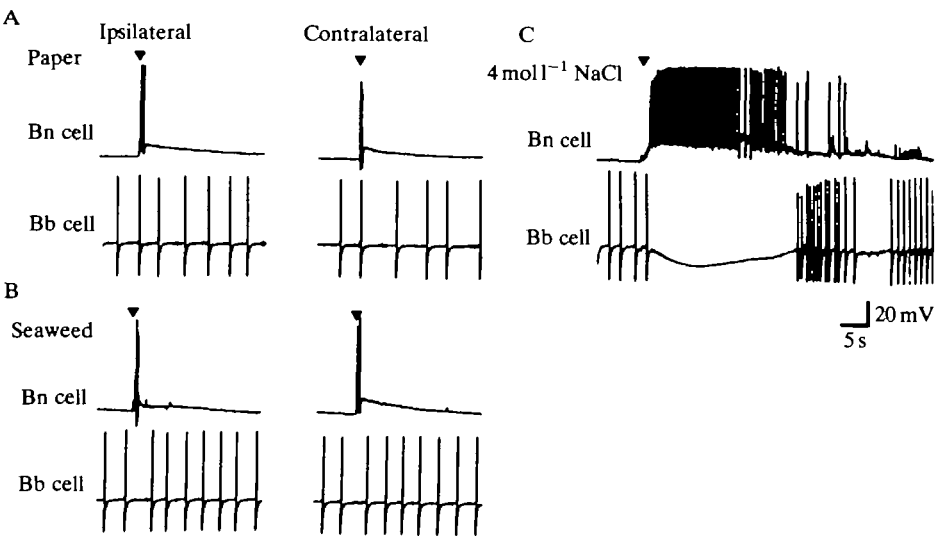

Fig. 3. Response properties of a Bn cell (top trace) and a Bb cell (bottom trace) to various stimuli applied to the tentacle in a reduced preparation. (A) Tactile (paper; see text for explanation), (B) seaweed and (C) hypertonic saline solution. Tactile and seaweed stimuli, which can evoke directed head movements, were separately presented to the tentacle ipsilateral and contralateral to the neurones. All stimuli excited the Bn cell and evoked an increase in the interspike interval in the Bb neurone (best seen in B). Note that there is no difference in the response magnitude of the cell types between tactile or food stimulation, and for stimulation of the ipsi- and contralateral tentacle. Saline solution on the tentacle (C) caused the strongest response in both types of cells. The duration of the depolarization of the Bn cell is similar to that of the hyperpolarization of the Bb neurone. The saline solution remained in the chamber following its introduction.

cluster neurones were most apparent when a hypertonic saline solution was applied to the tentacles (Fig. 3C). This stimulus triggered a prolonged spike discharge in the Bn neurone, but strongly hyperpolarized the Bb neurone. The durations of the depolarization of the Bn cell and the hyperpolarization of the Bb cell were similar. Following the initial hyperpolarization, the Bb neurone responded with an increase in firing rate, which appeared, at least in part, to reflect polysynaptic excitation.

The differential response of different types of B cells to tactile stimulation suggested that Bn cells, but not Bb cells, might receive synaptic input from the mechanoafferent neurones located in the cerebral J and K clusters (Rosen *et al.* 1979). Therefore, individual sensory neurones (79 neurones, in four preparations) were impaled and intracellularly stimulated with brief current pulses, while simultaneously recording from a Bn and a Bb neurone (eight pairs). 95 % of the sensory neurones evoked EPSPs, which were sometimes large enough to trigger action potentials in the Bn cells (Fig. 4; see also Rosen *et al.* 1979). In contrast, no synaptic input from mechanosensory neurones to Bb type neurones was observed (middle trace in Fig. 4).

The above experiments suggested that the response of the Bn neurones is similar for a purely tactile stimulus or for a food stimulus that consists of tactile and chemical components, i.e. that the neurones respond only to the tactile component of the stimulus. To test this idea further, we recorded simultaneously from Bn neurones and the metacerebral cell (MCC) in a reduced preparation (N=3). The MCC modulates feeding behaviour and has been found to be primarily excited by food stimuli on the lips (Weiss *et al.* 1978; Kupfermann and Weiss, 1982). Fig. 5 shows the responses of a Bn neurone (top trace) and the MCC (bottom trace) to a purely tactile stimulus consisting of a glass probe (Fig. 5A), and to an appetitive chemical stimulus, consisting of seaweed extract (SWE) carefully perfused near the lips at a very slow rate so as to minimize any tactile

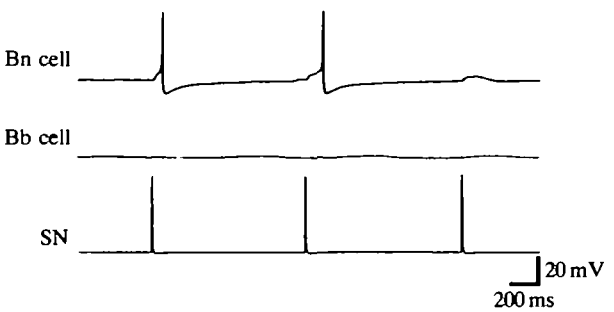

Fig. 4. Simultaneous recordings of a mechanoafferent sensory neurone (SN), a Bn neurone and a Bb neurone. Intracellular stimulation of the mechanoafferent neurone (located in the anterior part of the J cluster) with a brief current pulse evoked a short-latency EPSP in the Bn neurone, but there was no indication of a synaptic input to the Bb neurone. The third stimulation of the sensory neurone failed to trigger a spike in the Bn cell because of the decrement of the EPSP.

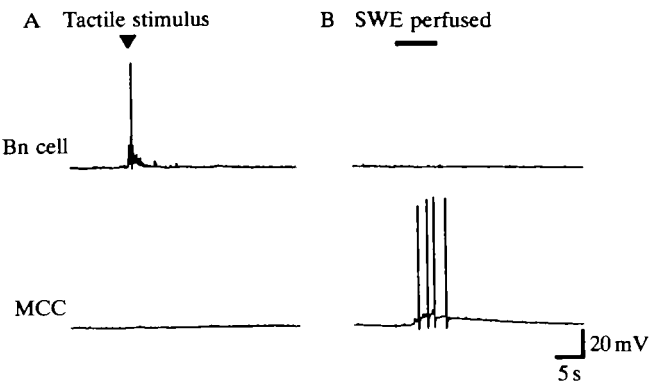

Fig. 5. Response of a Bn cell and the metacerebral cell (MCC) to different stimulus modalities, recorded simultaneously in a reduced preparation. (A) A brief tactile stimulus on the lips evoked excitation of the Bn cell, but no response in the MCC. (B) Purely chemical stimulation, produced by carefully perfusing seaweed extract (SWE) near the lips and avoiding tactile contact or water movements, did not lead to a detectable response in the Bn cell, but triggered several spikes in the MCC.

stimulation (Fig. 5B). The Bn neurone was excited by tactile stimulation, whereas little or no evoked response was observed in the MCC. The chemical stimulus, in contrast, produced little or no response in the Bn cell but evoked several spikes in the MCC. Similar results were obtained by applying moistened filter paper or a piece of seaweed to the lips while recording from Bn cells and the MCC. The Bn neurones did not respond differentially to paper or a piece of seaweed, whereas the MCC responded only to the piece of seaweed.

Firing Bn cells evokes contraction of the body wall

Jahan-Parwar and Fredman (1978*a*,*b*) and Fredman and Jahan-Parwar (1977) have reported that firing of neurones of the B cluster evokes contractions of the tentacle and neck region. We have found that firing of the Bn cells (31/36 Bn cells in seven semi-intact preparations) resulted in contractions of the tentacles, head and neck region, and more posterior parts of the body wall. Firing Bb cells at the highest possible rate did not produce any observable contractions (0/17 cells).

Lesion of the descending axons does not affect head-turning responses

Neither the results of the studies of the motor effect nor those on sensory input support a role of the Bb cells in mediating head movements. The data on the Bn cells, however, were most consistent with them having a role in producing aversive head-withdrawal responses, but did not preclude a role in appetitive directed head movements, since the sensory and motor responses in the dissected preparations may not accurately reflect the responses that occur in the intact animal. We therefore studied freely moving animals and took advantage of the fact that all descending axons of the Bn cells course through the C-Pl connective to project to the periphery. It was thus possible to eliminate the effects of the descending Bn

cell axons on head turning and withdrawal of the head by lesioning this connective. As previously reported, this lesion produced qualitatively little effect on the general behaviour of the animal (Jahan-Parwar and Fredman, 1979), and also seemed to have no effect on feeding behaviour. To determine a possible influence of the lesion on the turning response in particular, a quantitative analysis of the turning response was carried out to detect possible minor alterations in this behaviour. In initial experiments on two animals, unilateral lesions were made since it was felt that this should provide the method most sensitive to a deficit. A unilateral lesion should disrupt all connections from the Bn cells to the neck muscles on one side, allowing a comparison of the turning movements in the direction of the lesioned side with those of the intact side. In experiments similar to those of Teyke *et al.* (1988) (T. Teyke, K. R. Weiss and I. Kupfermann, in preparation), the turning angle of the animal following seaweed stimulation of different loci on the lips and the tentacles was measured in response to stimuli presented ipsilaterally or contralaterally to the lesioned connective. The averaged data of two unilaterally lesioned animals, stimulated at a spot at 10° eccentricity from the mouth, are shown in Fig. 6A. The plots give the turning angle over time (before and after the lesion) for a turning response towards the side of the lesion (ipsilateral), and towards the contralateral, intact side. In all cases, the trajectories of the turning movements are very similar, indicating that there was no asymmetry of head-turning responses. Neither the magnitude nor the time course of the movements towards the lesioned side differed from those directed towards the intact side or from the turning movements before surgery. Similar results were obtained using more medial stimuli (5°), which elicit smaller turning responses (see Teyke *et al.* 1988). Furthermore, inspection of the execution of head lifting in the lesioned animals in response to food failed to reveal any irregularities, but no exact measures of head lifting were made.

To rule out the possibility that one intact branch of the descending Bn cell axons is sufficient to mediate head turning, we studied two additional animals that had bilateral lesions of the cerebropleural connectives. In Fig. 6B, the data for the bilaterally lesioned animals were combined and compared with the combined control data established in the previous experiments. The graphs show that the head-turning responses of the bilaterally lesioned animals were virtually identical to those of the control animals. Similar results were obtained using more medial stimuli (5°). Furthermore, the responses of the lesioned animals were very similar to those of a larger group of control animals (data not shown) that had been previously tested under identical conditions for another experiment (Teyke *et al.* 1988; T. Teyke, K. R. Weiss and I. Kupfermann, in preparation). The lesion experiments suggest that the descending branches of the Bn cell axons do not contribute substantially to the performance of head-turning movements.

Lesion of descending axons slows head-withdrawal responses

Inspection of preliminary data from two animals with their C-Pl connectives lesioned revealed that the overall pattern of contraction following tentacle pinch

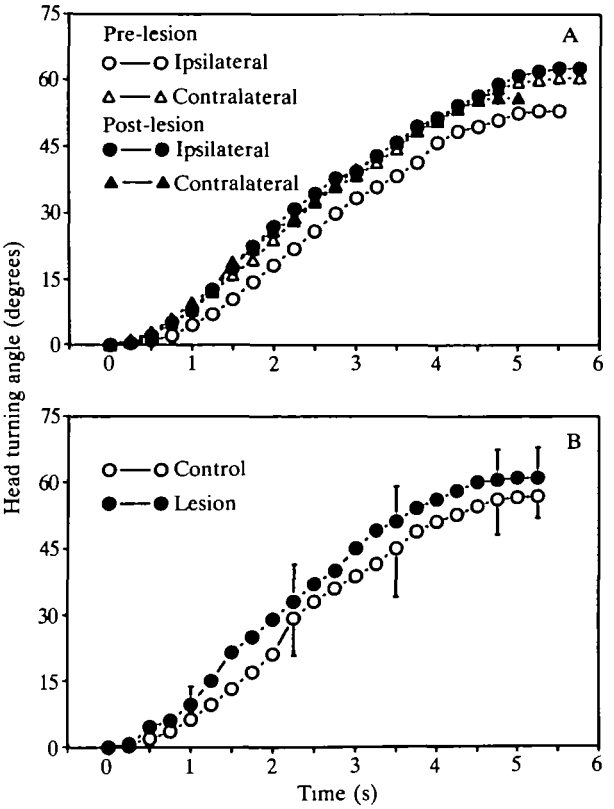

Fig. 6. (A) Effect of unilateral lesion of a cerebropleural connective on the directed head-turning response evoked by a food stimulus presented on the lips (10° from the mouth). The data show the mean head-turning responses (N=2) over time, toward (ipsi) or away from (contra) the side of the lesion (five turns in each direction) before and after the lesion. For clarity, the standard error bars have been omitted but the standard errors of each data point show considerable overlap. Note that the turning velocity (slopes of the curves) and the final turning angles are the same whether the animal turned towards or away from the side of the lesion. (B) Effect of bilateral lesions (N=2) of the C-Pl connective on the head-turning response. Control data were obtained before surgery. Data for the turning responses for stimuli 10° from the mouth in the left and the right direction have been combined. As for the unilateral lesions, there is no significant alteration of the turning responses of the bilaterally lesioned group compared with the controls.

was similar to that observed in the animals before surgery (compare Fig. 7A and B), but the velocity of the initial phase of the withdrawal response appeared to be markedly reduced (see smaller ΔL in right panel in Fig. 7). Therefore, we extended the experimental groups to include five bilaterally lesioned and five sham-operated animals, and performed the experiments in a blind fashion. For every experimental animal, a similar effect of the lesion was found. An example of the effect of the lesion on the velocity of the withdrawal response is given in

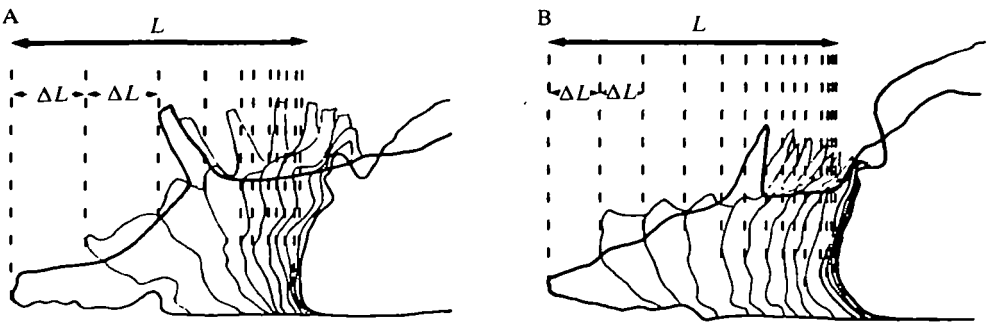

Fig. 7. Head withdrawal in response to a noxious tentacle pinch for an animal before surgery (A) and after bilateral lesion of the cerebropleural connectives (B). The head of the animal is viewed from the side and was traced every 250 ms from video recordings. Note that the overall configuration of the withdrawal response is not affected by the lesion, and neither is the total distance withdrawn (L). After the lesion, the amount withdrawn (ΔL) during the two initial 250-ms intervals is markedly reduced.

Fig. 8A. Prior to the lesion, the withdrawal response of the animal was characterized by an initial fast component of the movement, followed by a slower phase. After lesioning the connectives, the animal withdrew at almost constant speed during the total movement. The effect of the lesion was to eliminate selectively the initial higher-velocity component of the withdrawal response, and was evident only during the initial 750 ms of the withdrawal. Sham operations, as shown in Fig. 8B, did not significantly affect the time course of withdrawal velocity. Data for all experimental animals were contrasted by plotting the maximal velocity of the withdrawal response before and after surgery for the sham-operated and the lesioned groups. There was virtually no difference in the maximal velocity in the sham-operated group ($P>0.1$; Mann–Whitney U-test) before and after surgery, whereas the lesion significantly ($P<0.01$) reduced the maximal velocity of the withdrawal movements to approximately 50 % of the value before surgery (Fig. 8C). Following the lesion, the maximal head-withdrawal velocity for every lesioned animal was less than their pre-operative value or the values of the control animals before or after the sham operations.

Extracellular nerve recordings indicate that Bn cell activity is associated with the stimulus rather than with head-turning movements

To obtain additional information about the role of the B cells in normal behaviour, extracellular recordings from the C-Pl connective in freely moving animals ($N=4$) were carried out. Since lesions of the C-Pl connective did not affect the turning response of the animals (Fig. 6A,B), the cut end of the C-Pl connective could be used for nerve recordings. An increase in neural activity (relative to the level in the quiescent animal) was recorded under various circumstances, including locomotion and head lifting. In particular, a strong increase in unit activity was observed immediately following tactile stimulation of the ipsilateral or contralat-

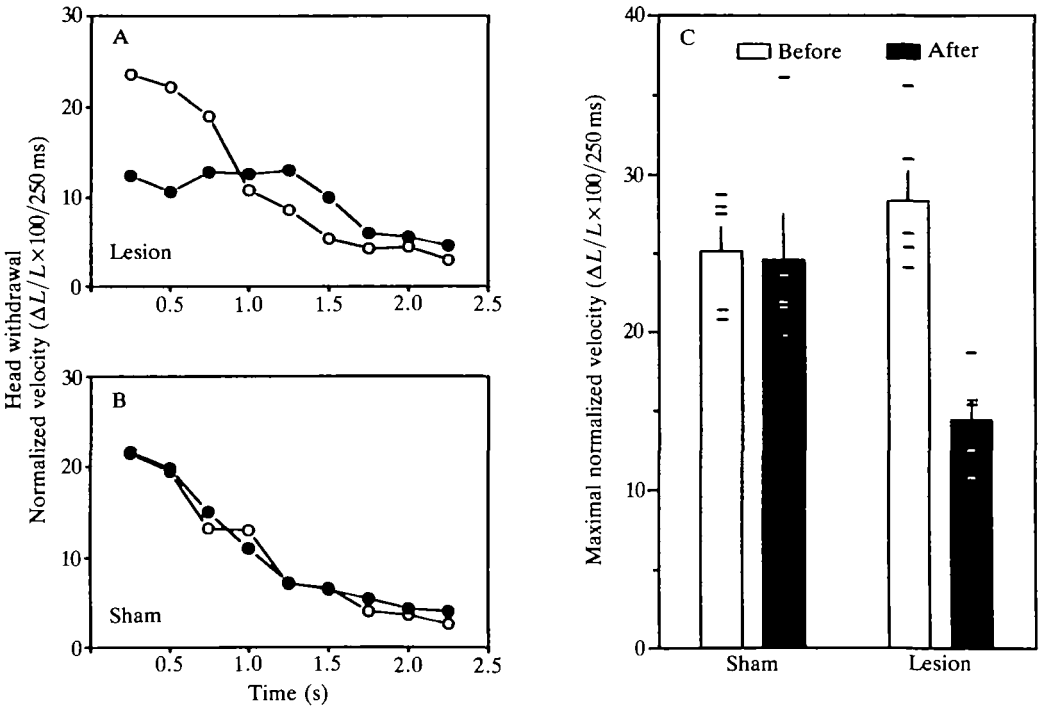

Fig. 8. Effect of bilateral lesion of the C-Pl connectives on aversive head-withdrawal responses. The graphs show examples of the velocity of head withdrawal, given as percentage of the total amount withdrawn (for explanation, see Fig. 7) per 250-ms interval over time for typical animals before (open circles) and after (filled circles) surgery. Averaged data for five trials each are compared for an animal with a C-Pl connective lesion (A) and a sham-operated animal (B). The effect of the lesion is restricted to the initial phase of the withdrawal movement. (C) Average of the maximal velocity of the head withdrawal (means±S.E.M.; individual data points are indicated) before and after surgery for the sham-operated group (N=5), and the lesioned group (N=5). The sham operations did not affect the maximal velocity of the responses (P>0.1), but in the lesioned group the maximal velocity of the withdrawal response was significantly reduced (P<0.01).

eral tentacle and rhinophore (Fig. 9). Furthermore, stimulation of the ipsilateral or the contralateral receptive surface of the animal evoked approximately the same increase in unit activity. In the cases shown in Fig. 9, the stimulus caused a slight contraction of the stimulated part of the animal. Recordings from an animal oriented in the feeding posture and performing head-turning movements (indicated above the traces of the recordings) are shown in Fig. 10. A strong increase in unit activity occurred immediately after the stimulus (arrow), but during the following evoked turning movement of the animal unit activity was not substantially increased. In general, unit activity did not increase significantly during spontaneous turning movements (see second trace), or when the animal arched its head backwards. However, a stronger increase in unit activity was recorded

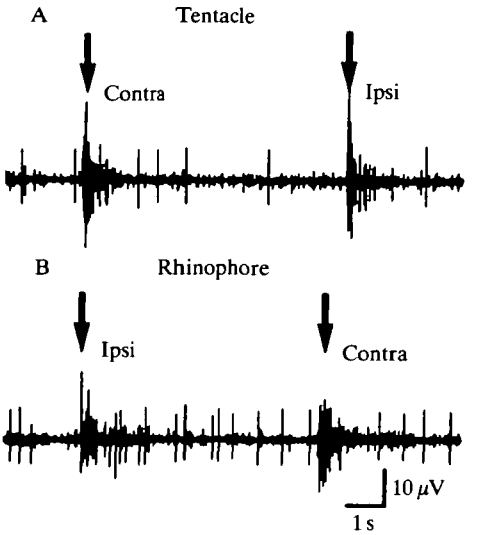

Fig. 9. Extracellular recordings from the cerebropleural connective in a freely moving animal following tactile stimulation of the contra- and ipsilateral tentacles (A) and rhinophores (B). Note that the duration and magnitude of the increase in unit activity is similar in all cases. The stimulus caused a limited regional withdrawal of the stimulated area.

following a second stimulus, which resulted in a withdrawal of the tentacles and the head (lower trace). In some instances, a slight increase in unit activity was observed at the beginning and the termination of the turning movement. In general, the responses recorded in the C-Pl connective, which were evoked by ipsi- and contralateral stimulation of the tentacles and rhinophores (see Fig. 9), resembled in their receptive field and temporal characteristics the typical Bn cell responses, recorded in response to stimuli presented to reduced preparations, as shown in Fig. 3A, B.

Following the nerve recordings, we determined whether the extracellular electrode was capable of recording Bn cell activity. The animals were killed, and the circumoesophageal ganglia with the extracellular electrode still in place were removed. The possible identity of the recorded units was determined by intracellularly stimulating various cells in different clusters in the cerebral ganglion and making simultaneous extracellular recordings from the C-Pl connective. Spikes in the connective, comparable to those recorded during the chronic recordings, were observed when Bn cells contralateral (9/10 cells) or ipsilateral (3/10 cells) to the electrode were stimulated. Besides Bn neurone activity, extracellular spikes were recorded from various other neurone types, including one of three stimulated Bb cells, and four of eight stimulated A cluster neurones. The electrode also recorded spikes of considerably smaller amplitude, probably associated with mechanoafferent neurones. It is probable that the unit activity recorded in the C-Pl connective represents activity mostly of Bn neurones, since

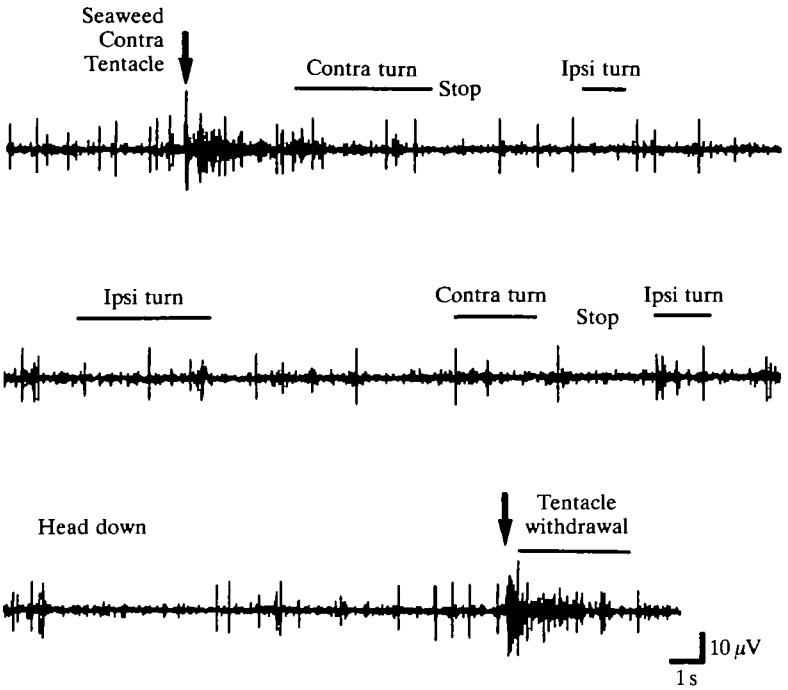

Fig. 10. Extracellular recordings from the cerebropleural connective in a food-aroused animal oriented in the feeding posture. The movements performed by the animal are indicated above the records. Increased nerve activity occurred after a seaweed stimulus was presented to the tentacle (arrow) contralateral to the side of the recording. During the evoked turning response following the stimulation, and during the spontaneous turning movements later on, the unit activity was not significantly increased. When the animal began to de-arouse and moved its head back down towards the substratum, the activity in the connective was markedly reduced. A second stimulus (arrow in bottom trace), which caused withdrawal of the head and neck of the animal, was accompanied by a strong increase in unit activity.

we demonstrated the capacity of the electrode to monitor extracellular Bn spikes in the C-Pl connective, and the receptive field and temporal response characteristics of evoked activity resembled that obtained in intracellular recordings of Bn neurones.

Discussion

This study was conducted to identify candidate cerebral motor neurones which may produce head movements in *Aplysia*. A survey of the B cluster neurones of the cerebral ganglion revealed that the cluster, which had been previously described as consisting of neurones with nearly identical properties (Fredman and Jahan-Parwar, 1975; Jahan-Parwar and Fredman, 1983), is composed of two quite

distinct populations of neurones. The two classes of neurones can be most readily distinguished on the basis of the duration of their action potential and, consequently, we have termed them Bn or Bb, referring to a narrow or a broad action potential of the cells (the previously described B neurones are the Bn type). All neurones in the B cluster surveyed in this study could be classified as belonging to either the Bn or the Bb type, but there might be additional subclasses within these populations (Jahan-Parwar and Fredman, 1983; Mackey *et al.* 1986). For example, the single serotonergic neurone within the B cluster, which modulates the synaptic efficacy of pleural sensory neurones (Mackey *et al.* 1986; S. L. Mackey, R. D. Hawkins and E. R. Kandel, personal communications), appears to meet the criteria for being a Bn type neurone.

It is probably of functional significance that stimuli which excite Bn neurones, such as tactile or food stimulation of the tentacles, rhinophores or lips of the animal, evoke hyperpolarization in the Bb cells. Tactually evoked excitation of the Bn cells appears to be mediated by excitatory connections of mechanoafferent neurones located in the J and K clusters (Rosen *et al.* 1979). Although no inhibitory connections from these sensory neurones to the Bb neurones were found (see Fig. 4), it is possible that the inhibition requires the simultaneous activity of a relatively large number of mechanoafferent neurones and is too small to be observed when individual sensory cells are stimulated.

Previous evidence (Fredman and Jahan-Parwar, 1980) has suggested that seaweed stimulation is more effective than tactile stimuli in evoking responses in the B cluster neurones (Bn cells). Therefore, we compared the responses of the Bn type neurones to a purely tactile stimulus (glass rod) and a purely chemical stimulus (seaweed extract). The results suggest that a tactile component is necessary and sufficient to evoke a response in the Bn neurones. An additional chemical component, which is present in a piece of seaweed, does not alter the response and, similarly, the chemical component alone is not sufficient to evoke a response in Bn neurones. Thus, our data do not support an integrative role of the Bn cells, of the type previously reported for multimodal neurones in *Pleurobranchaea* (Bicker *et al.* 1982) or *Tritonia* (Audesirk and Audesirk, 1980). The previously reported findings of stronger responses of the B cells to seaweed stimulation than to tactile stimulation (Jahan-Parwar, 1972; Fredman and Jahan-Parwar, 1980) may be related to the possibility that the flow of the chemical stimulus introduces a tactile component unless great care is taken to use a minimal flow rate. It is also possible that the reduced preparations were 'sensitized' and seaweed (perhaps the hypertonic component of the stimulus) produced a large contraction of the tissue, which may in turn provide excitatory proprioceptive input to the B cells. The intensity of the stimulus was found to determine the extent of the contraction of the stimulated area, and the response magnitude of the Bn neurones (Fredman and Jahan-Parwar, 1977). Thus, differences in response to chemical *versus* tactile stimulation might be due to a stronger contraction of the tentacles upon chemical stimulation, which in turn provides stronger proprioceptive stimulation of mechanosensory cells. This explanation is in agreement with

the findings of Rosen *et al.* (1979) that the mechanoafferent neurones in the cerebral J and K cluster, which respond to tactile stimulation but not to seaweed extract, make synaptic connections to the B cluster neurones (Bn type).

Taken together, the electrophysiological results are most consistent with the notion that Bn neurones are not involved in the appetitive head-turning response but have some role in defensive withdrawal responses. Similar to other withdrawal systems in *Aplysia*, such as the gill-, siphon- or tail-withdrawal reflexes (for a review, see Kandel, 1976), the strength of the excitatory connections between mechanoafferent sensory cells and the Bn neurones (Fig. 4; see also Rosen *et al.* 1979) decrements rapidly. Further support for the role of the Bn cells in aversive withdrawal reflexes is the observation that the application of a hypertonic saline solution to the tentacles evoked the strongest responses recorded in the Bn cells. Behavioural observations showed that hypertonic saline stimulation is a potent aversive stimulus, which immediately triggers withdrawal of the animal followed, after a delay, by a turning movement in the direction away from the stimulus, and then escape locomotion (Hening *et al.* 1979; Walters and Erickson, 1986). A further argument against a role of Bn cells in head turning is the large size of their receptive fields. Each Bn cell receives input from many sensory neurones (Rosen *et al.* 1979), and the receptive field can extend over the ipsi- and contralateral portions of the head of the animal (Fredman and Jahan-Parwar, 1977). Such large receptive fields would appear inappropriate for cells which mediate directed turning movements, whose response magnitude and direction is a function of the stimulus position on the receptor surface (Teyke *et al.* 1988; T. Teyke, K. R. Weiss and I. Kupfermann, in preparation). Furthermore, the results of chronic recordings from the C-Pl connective in the freely moving animal suggest that Bn cell activity is correlated with the presentation of the stimulus rather than with appetitive movements of the head and neck. The prominent increase in activity during the withdrawal of the animal supports the hypothesis that the Bn neurones are motor neurones involved in the defensive head-withdrawal reflex. We cannot completely exclude some contribution of the Bn cells to appetitive head movements in those instances in which we observed spikes in the C-Pl connective at the beginning and the termination of turning movements. This activity, however, is very brief compared with the duration of the turning movements. This neural activity may, in part, reflect self-stimulation of the tentacles, since the tentacles are bent during the acceleration and braking of the evoked movements.

Lesioning (by cutting the C-Pl connectives) the descending axons of the Bn cells, which innervate the neck region, did not affect the turning response of the animal, but did significantly alter the velocity of the head-withdrawal response upon noxious stimulation. This lesion affected the withdrawal reflex by selectively eliminating an initial high-velocity phase of the withdrawal movements. This seems to indicate a dually organized withdrawal system, similar to escape responses of other animals (e.g. crayfish: Schrameck, 1970; Krasne and Wine, 1977; and fish: DiDomenico *et al.* 1988), which consist of a short-latency fast component followed by later responses. Thus, the descending axons of the Bn cells

may contribute to head withdrawal by supplying an initial fast component to the withdrawal, lasting only for about 500–750 ms. The notion of a transient Bn cell contribution to the response is supported by the extracellular and intracellular recordings, which show that Bn cell activity is greatest during the first 0.5 s following the stimulus.

Our data do not indicate the function of the B neurones with the broad spike, the Bb neurones. These cells are unlikely to be motor neurones involved in head turning, since they do not respond to mechano- or chemosensory stimulation of the tentacles and lips, and firing the cells fails to produce muscle contractions. It is of interest that, similar to a number of peptidergic neurones in *Aplysia* (e.g. B1, B2: Lloyd *et al.* 1988; and bag cells: Kupfermann and Kandel, 1970), the Bb neurones have very broad spikes and conceivably might have peripheral modulatory effects.

Our findings are consistent with the idea that in *Aplysia* two types of movements of the same structure may be mediated by different populations of motor neurones. Head withdrawal in response to a noxious stimulus appears to be mediated, at least in part, by the Bn neurones located in the cerebral ganglion. Directed head turning in response to food does not appear to involve these neurones, and might be mediated by cells in the pedal ganglion (see e.g. the head-movement neurones described by Cook and Carew, 1988). It is possible that head withdrawal and head turning in *Aplysia* may be mediated by different muscles and consequently different motor and premotor neurones. In crayfish, two different motor programmes acting on the same group of muscles, which mediate the tonic and cyclic postural system, have been found to be governed by two largely independent subsystems of premotor interneurones (Moore and Larimer, 1988). It is also possible that the two types of head movements are mediated by the same muscles, which are activated in different sequences to execute the different responses. There are several indications in molluscs that two different motor programmes acting on a common set of muscles may be activated by different command systems (Croll and Davis, 1987; Gillette and Gillette, 1983). It has also been shown in various invertebrates that a single network acting on a common set of muscles can switch functioning from one behaviour to another (Bicker and Menzel, 1989; Getting, 1989; Kristan *et al.* 1988; Marder *et al.* 1987). In recent experiments, we have identified a cerebral neurone whose activity inhibits Bn cells while exciting a large population of neck motor neurones in the pedal ganglion (Teyke *et al.* 1989), indicating that it may inhibit the withdrawal system while activating other motor systems that appear to be involved in appetitive head movements. These findings are consistent with the notion that head and neck movements in *Aplysia*, which serve to withdraw the animal from or to approach a stimulus, may be controlled by separate motor systems.

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