Neuronal Mechanisms for the Control of Body Orientation in Clione
I. Spatial Zones of Activity of Different Neuron Groups

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Introduction

Control of body orientation and equilibrium is a vital motor function of the brain. The functional organization of nervous mechanisms responsible for the maintenance of a preferred body posture has been analyzed in considerable detail both in lower and higher vertebrates including humans (for recent reviews see Horak and Macpherson 1996; Macpherson et al. 1997; Orlovsky 1991; Platt 1993). Much less is known, however, about the neuronal organization of the postural control mechanisms. The principal difficulty with these studies is that the postural control system, especially in higher vertebrates, is extremely complex. It includes numerous sensory and motor centers located in different parts of the brain, and the integrity of these centers is necessary for normal function. This problem hinders progress in the analysis of postural mechanisms at the network and cellular level.

The invertebrates present many more opportunities for the analysis of neuronal networks controlling different motor behaviors including the maintenance of body orientation in space. The most extensive studies in this area were carried out on the crayfish (Takahata and Hisada 1982a,b; Takahata et al. 1985). It was shown that the dorsal-side-up orientation in swimming animals is controlled by input from two statocysts. Gravitational reflexes were characterized for different spatial orientations of the crayfish. However, a sufficiently complete description of the postural network in crustaceans is still lacking, primarily because of the difficulties in the identification of individual neurons involved in postural control.

In the present study, we investigated the neuronal mechanisms for postural control in the pteropod mollusk Clione limacina. The advantages of this animal model are the following: when swimming, Clione exhibits a very distinct spatial orientation behavior (Arshavsky et al. 1991b; Panchin et al. 1995a); all principal classes of neurons comprising the postural network in Clione and their connections have been identified (Panchin 1997; Panchin et al. 1995a,b); and we have developed a novel method for the in vitro study of neuronal correlates of postural activity (Deliagina et al. 1998a).

Clione is a planktonic animal. In the sea and in the aquarium at lower water temperature, Clione usually can be found oriented vertically, with its head up. Clione swims upward or maintains itself at a particular depth by continuous beating of two wings (Arshavsky et al. 1985a,b; Satterlie and Spencer 1985; Satterlie et al. 1985). Any deviation from the vertical orientation evokes a set of corrective motor responses (specific movements of the tail and wings) aimed at restoration of the initial orientation. These reflexes are driven by input from two statocysts. After removal of both statocysts, Clione is not able...
to maintain any definite orientation and continuously loops in different planes (Panchin et al. 1995a).

The activity of the gravitational postural control system of *Clione* can be subjected to modifications related to different forms of behavior (Arshavsky et al. 1991a, 1993a,b). The main mode of activity is stabilization of the head-up orientation (see preceding paragraph). The system also can switch to stabilization of the head-down orientation (Panchin et al. 1995a). This mode is observed when *Clione* leaves water layers with temperature 12–15°C and swims downward. Head-down swimming also is observed at a certain stage of hunting behavior. Horizontal swimming can also be observed under certain conditions (Deliagina, Arshavsky, and Orlovsky, unpublished observations). A different modification is a complete inhibition of postural activity during the defense reaction. In the present paper, we describe the mode of operation of the postural system when head-up orientation is stabilized. The other modes will be considered in the following papers of this series.

The main neuron groups participating in the gravitational reflexes in *Clione* and their interconnections were identified earlier by means of paired recordings (Panchin et al. 1995a,b). They are shown schematically in Fig. 1A. The statocyst internal wall is lined with 9–11 statocyst receptor cells (Tsirulis 1974). These cells are mechanoreceptors responding to the pressure exerted by the statolith (Stl). The statocysts are located on the dorsal surface of the pedal ganglia. The statocyst receptor cells send their axons to the cerebral ganglia where they affect at least two classes of cerebropedal interneurons, CPB2 and CPB3. CPB3 interneurons affect the tail motor neurons, whereas the CPB2 interneurons affect the wing motor neurons. Tail motor neurons evoke bending of the tail, whereas the wing motor neurons have double function: they evoke rhythmical locomotor oscillations of the wings and modulate these oscillations under the effect of gravitational input. CPB3a neurons send their axons to the contralateral pedal ganglion through the ipsilateral cerebropedal connective. CPB3b neurons send the axons to the contralateral pedal ganglion through the contralateral cerebropedal connective and then to the ipsilateral ganglion through the subpedal commissure. CPB3c neurons project to both pedal ganglia through the left and right cerebropedal connectives; they also have axonal branches in the subpedal commissure. CPB2 neurons initially project to the contralateral pedal ganglion and then, through the pedal commissure, to the ipsilateral ganglion. Cer. Com., cerebral commissure; Ped. Com., pedal commissure; Cer. Ped. Con., cerebropedal connective; Sub. Ped. Com., subpedal commissure; TMN, tail motor neuron; WMN, wing motor neuron.

FIG. 1. A: main types of neurons involved in the gravitational control of posture in *Clione*, and their functional interactions. Two statocysts are located on the dorsal aspect of the pedal ganglia. Statocyst receptor cells (SRCs) are excited when the statolith (Stl) exerts pressure on them. Receptor cells send their axons to the cerebral ganglia and affect 2 groups of cerebropedal interneurons, CPB2 and CPB3. CPB3 interneurons affect the tail motor neurons, whereas the CPB2 interneurons affect the wing motor neurons. Tail motor neurons evoke bending of the tail, whereas the wing motor neurons have double function: they evoke rhythmical locomotor oscillations of the wings and modulate these oscillations under the effect of gravitational input. CPB3a neurons send their axons to the ipsilateral pedal ganglion through the ipsilateral cerebropedal connective. CPB3b neurons send the axons to the contralateral pedal ganglion through the contralateral cerebropedal connective and then to the ipsilateral ganglion through the subpedal commissure. CPB3c neurons project to both pedal ganglia through the left and right cerebropedal connectives; they also have axonal branches in the subpedal commissure. CPB2 neurons initially project to the contralateral pedal ganglion and then, through the pedal commissure, to the ipsilateral ganglion. Cer. Com., cerebral commissure; Ped. Com., pedal commissure; Cer. Ped. Con., cerebropedal connective; Sub. Ped. Com., subpedal commissure; TMN, tail motor neuron; WMN, wing motor neuron.
statocyst receptor cells and the tail motor neurons, respond well to natural gravitational stimulation, i.e., a change in the spatial orientation of the statocysts (Arshavsky et al. 1991a,b; Panchin et al. 1995a). For the tail motor neurons, this was demonstrated by recording the activity of their axons in the tail nerves when the CNS-statocysts preparation was turned by forceps from the dorsal-side-up position to the ventral-side-up position. With this method, however, gravitational reflexes only could be characterized very roughly for two spatial orientations, dorsal and ventral side up. Nevertheless, these experiments led to formulation of the following hypothesis about the organization of gravitational reflexes (Panchin et al. 1995a). When the head-up orientation is stabilized, any deviation from this orientation results in excitation of the statocyst receptor cells in the lowest part of the statocyst cavity. These cells, via the CPB3 interneurons, will excite tail motor neurons projecting to the opposite side of the body wall. These motor neurons will elicit tail bending in the direction opposite to the initial body sway. The deviated tail, like the rudder of boat, will produce rotation of the animal toward the vertical until the normal, head-up orientation is restored.

In the present study, we tested the validity of this hypothesis about the organization of the postural control system in Clione. For this purpose, we characterized the spatial zones of activity of different groups of tail motor neurons and compared them with the motor effect produced by activation of each particular group. We also characterized the spatial zones of activity of some CPB3 interneurons and compared them with the zones of tail motor neurons. In addition, we investigated the role of wings in postural stabilization. We characterized the spatial zones of activity of different groups of wing motor neurons and compared them with the motor effect produced by activation or inhibition of each particular group. This study led us to a conclusion that postural reflexes in Clione, mediated by identified moto- and interneurons, are able to compensate for any possible deviation of the animal from the normal orientation.

A brief account of these studies has been published in an abstract form (Deliagina et al. 1998b).

METHODS

Experiments were carried out at the White Sea Marine Biological Station Kartesh. Mollusks were collected locally and kept in aquaria at 5–12°C. All the experiments described in the present paper were performed at temperatures in the experimental chamber of 5–12°C, when the postural network was presumably tuned at stabilization of the head-up orientation (Panchin et al. 1995a).

FIG. 2. A: drawing of the in vitro preparation used in the present study. Activity of the tail motor neurons was recorded from the tail nerve N3 and from the lateral branch of the nerve N2 [designated as N2(1)]. In the latter case, the nerve N1 was cut proximal to the site of its fusion with N2. In a few experiments, recordings also were performed from N4, from N1 and from the medial branch of N2 [designated as N2(2)]. Activity of the wing motor neurons was recorded from the wing nerve (NW). Activity of CPB3 interneurons was recorded from the transected subpedal commissure (Sub. Ped. Com.). Cer. G., cerebral ganglia; Ped. G., pedal ganglia; Pleu. G., pleural ganglia; Abd. G., abdominal ganglia. Nerves from which recordings were performed are indicated by rectangular frames. For tail nerves, the effect of stimulation is also indicated (Panchin et al. 1995a). B and C: experimental arrangement for recording activity of motor neurons and interneurons. Top view (B) and the side view (C) of the recording chamber are shown. Five pieces of filter paper soaked in the sea water (electrodes) were positioned on the bottom of the chamber and isolated from each other by the paraffin oil. CNS was positioned on the larger electrode with its dorsal side up, &&$, orientation of the rostral aspect of the CNS. Nerves (or the stumps of the transected subpedal commissure) were positioned on the smaller electrodes which were connected with inputs of the amplifiers. Chamber was filled completely with the oil and tightly closed.
Preparation

All experiments were performed on an in vitro preparation of the CNS (without buccal ganglia) isolated together with the statocysts (Fig. 2A). In Clione, tail muscles are innervated by motor axons through bilateral nerves N2(1), N2(2), N3, and N4 originating primarily from the pedal ganglia; a small number of axons in N2 come from the pleural ganglia. Retrograde staining through these nerves has shown that there are ~30 cells on each side of the CNS (presumed tail motor neurons). They are located preferentially in the pedal ganglia and, to a lesser extent, in the pleural and cerebral ganglia (Panchin et al. 1995a; Deliagina, Arshavsky, and Orlovsky, unpublished data). In preliminary experiments, we recorded gravitational responses from the axons of efferent neurons (presumed tail motor neurons) in all tail nerves. It was found that the most pronounced and persistent responses occurred in the left and right nerves N2(1) and N3. These two pairs of nerves were subjected to the most detailed analysis. Experiments with stimulation or transection of these nerves (Panchin et al. 1995a) have shown that both N3 nerves elicit ventral tail flexion, whereas each of the N2(1) nerves elicits tail flexion in the ipsilateral-dorsal direction.

Each of the wings in Clione is innervated by ~20 motor neurons, with their axons passing through the wing nerve (Arshavsky et al. 1985a,b; Satterlie 1993; Satterlie and Spencer 1985; Satterlie et al. 1985). Among these motor neurons, two cells, 1A and 2A, are very large and elicit a strong motor response. The 1A motor neuron innervates the dorsal aspect of the wing and fires in the “dorsal phase” of the locomotor cycle, whereas the 2A motor neuron innervates the ventral aspect of the wing and fires in the “ventral phase” of the cycle. When recording extracellularly from the wing nerve, the large-amplitude discharges of these two locomotor motor neurons can be recognized easily. In the present study, these discharges were used to monitor the gravitational influences on the locomotor system. In addition, we studied the gravitational effects on the motor neurons responsible for the wing retraction. These neurons can be recognized according to their tonic discharge and the absence of rhythmic input from the locomotor rhythm generator (Huang and Satterlie 1990).

The influences of the statocysts on the tail and wing motor neurons are mediated by different groups of cerebropedal interneurons. Of these groups, the CPB3b and CPB3c neurons affecting the tail motor neurons have their axons passing through the subpedal commissure (Fig. 1, C and D) (Panchin et al. 1995b). To record the gravitational responses of these neurons, the subpedal commissure (Fig. 2A) was cut, and discharges in the axons of interneurons were recorded from one or both stumps of the commissure.

Recording chamber

The method for recording activity in the nerves, used in the present study, represents an elaboration of the method described earlier (Deliagina et al. 1998a). A 35-mm polystyrene petri dish (Falkon 3801) was used as a recording chamber (Fig. 2, B and C). Five silver wires (0.5 mm diam) were inserted into the chamber through its bottom. These wires were used to make contact with the electrodes—small pieces of the filter paper positioned on the bottom of the chamber. The electrodes were soaked in and covered with a thin layer of sea water. The isolated CNS was positioned with its dorsal side up on a larger filter paper electrode, and its rostrocaudal axis was aligned with the diameter of the chamber as shown in Fig. 2B. Then the electrodes and

![Image](https://via.placeholder.com/150)

**FIG. 3.** Gravitational stimulation of statocysts. Three modes of rotation of the recording chamber are shown in A, 1–3 (arrows indicate orientation of the rostral aspect of the CNS), whereas in B, 1–3, are shown the same 3 modes if they were applied to the whole animal. A1 and B1: sagittal sway characterized by the angle $\alpha$; A2 and B2: lateral sway characterized by the angle $\beta$; A3 and B3: horizontal roll characterized by the angle $\gamma$. C and D: different patterns of gravitational stimulation. Rotation of the chamber was performed either in a stepwise manner (C) or in a trapezoid manner (D). Full clockwise turn could be followed by a counterclockwise turn (C). Shown schematically are the zones of gravitational responses of a neuron. They are characterized by the angular width ($z$) and the position of the center ($c$).
the CNS were covered with a thin layer of paraffin oil. The nerves chosen for recording were pulled carefully through the oil and placed on the corresponding electrodes. The chamber then was filled completely with paraffin oil and the lid was put on and tightly closed. No air was left in the chamber. The electrodes were connected to the inputs of the amplifiers. Up to four nerves could be recorded simultaneously. Spike discharges were recorded extracellularly in the axons; for this purpose, AC amplifiers with the bandwidth of 50–5,000 Hz were used. Usually the spike amplitude was ~1 mV.

Natural gravitational stimulation

The chamber with the preparation was mounted on a platform that allowed rotation of the chamber over a range of 0–360°. Depending on the initial orientation of the chamber in relation to the platform, three different modes of rotation could be employed. In Fig. 3, the left panels (A, 1–3) show these three modes of rotation of the chamber, whereas B, 1–3, shows a movement of Clione if these modes of rotation were applied to the whole animal. For simplicity, we shall use the terms describing orientation of the whole animal (head up, dorsal side up, etc.) when characterizing spatial orientation of the preparation. Figure 3, A1 and B1, shows rotation in the sagittal plane—the sagittal sway (pitch) characterized by the angle α. Figure 3, A2 and B2, shows rotation in the frontal plane—the lateral sway characterized by the angle β. Finally, Fig. 3, A3 and B3, shows the third mode of rotation used in this study; that is, rotation around the longitudinal axis of the animal, the axis being situated horizontally—the horizontal roll characterized by the angle γ.

Rotation of the recording chamber was performed in steps (Fig. 3C); each step was 45°, the transition from one position to another took ~1 s, and each position was held for ~4 s. As a rule, each test was repeated several times with alternating rotations in the opposite directions. Figure 3C schematically shows the test with two full turns in opposite directions. In addition to the 360° rotation, trapezoid angular movements between two positions, with an amplitude of ±90°, were employed (Fig. 3D). In this case, one of the two positions was usually chosen in the center of the angular zone of activity of the tested neuron group (see RESULTS). Rotation of the chamber was performed manually and recorded by a potentiometric transducer.

In the figures, the following designations for the spatial orientation of the preparation are used. For sagittal sway, α = 0° corresponds to the normal (vertical, head-up) orientation; positive values of α correspond to ventral (forward) sway; negative values of α correspond to dorsal (backward) sway (see also Fig. 11F). For lateral sway, β = 0° corresponds to the head-up orientation; negative values of β correspond to right sway; positive values of β correspond to left sway (see also Fig. 11E). For horizontal roll, γ = 0° corresponds to the dorsal-side-up orientation of the preparation; negative values of γ correspond to right roll; positive values of γ correspond to left roll.

To make the presentation of results more convenient and to avoid possible confusion caused by similar angular measures for the orientations in different planes of rotation, special designations were given to the six “basic” orientations. They are: head up (H), tail up (T), dorsal side up (D), ventral side up (V), left side up (L), and right side up (R). The intermediate positions were designated by indicating the closer basic orientations, like HR, HL, DR, etc.

In the present study, it was found that the most typical pattern of gravitational response in the tail and wing motor neurons, as well as in the cerebropedal interneurons, was their activation within a limited angular zone and silence outside the zone. Only in a few cases the neurons with gravitational input were not silent outside the zone. To characterize the zones, we used the following values—the width of the zone (z) and the center (c) (Fig. 3C). These values were measured for all tested neurons except for the cases when the spikes of individual neurons were difficult to distinguish because of their high-frequency. In these cases, z and c were measured for a whole group of simultaneously active neurons. The values of z and c then were averaged separately for each neuron group over all experiments. Altogether, the zones were measured in >40 experiments. Each experiment lasted for a few hours, but in some cases pronounced gravitational responses could be recorded for a much longer time, ≤2 days.

Results

Gravitational reactions of tail motor neurons

Up to 10 units of different spike amplitude and shape could be distinguished when recording from the tail nerves, N2(1) or N3. About half of them responded to gravitational stimuli. Figure 4A shows a response in LN2(1) to roll tilt (transition from the right-side-up orientation to the left-side-up orientation). With a higher time resolution (Fig. 4B), four units (1–4) could be distinguished. In the L position, the firing frequency of units 1–4 was ~5, 15, 40, and 10 Hz, respectively. The value of firing frequency of 5–15 Hz was most characteristic for the responses of tail motor neurons observed in different experiments. In the R position, these units were silent.

When stimulated by stepwise rotation, the tail motor neurons were activated in a specific zone of angles (Fig. 4C). In addition to a static response, that is, a difference in the rate of continuous firing observed in two different positions held for a long period of time, some neurons also exhibited a dynamic response, that is, a reaction to a change of orientation, which

![FIG. 4. General characterization of the gravitational responses. A: response of tail motor neurons in the LN2(1) nerve caused by transition from the right-side-up position (R) to the left-side-up position (L). Part of the recording (A) in A is presented in B with higher time resolution to show spikes of 4 different units (1–4). C: responses in the nerve RN2(1) to stepwise rotation (horizontal roll); the response to each step consists of the dynamic and static components. Width of the zone of activity is indicated (z).](http://jn.physiology.org/10.1202/33.4 on October 28, 2016)
usually lasted for 1–2 s. As shown in Fig. 4A, the static response gradually decayed with a time constant of 1–2 min.

Figure 5, A and B, shows responses in two symmetrical tail nerves, LN2(1) and RN2(1), to lateral and sagittal sway. Neurons in LN2(1) were activated with left sway, in the TL, L, and LH positions. On the contrary, RN2(1) was activated mainly with right sway, in the LH, H, HR, R, and RT positions. Activation of LN2(1) and RN2(1) with the ipsilateral-side-up orientation was also characteristic of their testing by the horizontal roll (see Figs. 4C and 5E). Sagittal sway usually evoked activation of neurons in both nerves in approximately the same zones, around the D position. Figure 5B illustrates a case when neurons responded in the H, HD, and D positions. In some experiments, however, sagittal sway elicited rather weak activation of LN2(1) and RN2(1). From Figs. 4C and 5, B and E, one also can see that, in addition to the units with a highly pronounced gravitational input, a few units are firing without any obvious relation to their angular position.

In contrast to the motor neurons from the left and right nerves LN2(1) and RN2(1), which received different gravitational inputs, neurons from another pair of nerves, LN3 and RN3, received largely identical inputs. Figure 5C shows responses in LN3 and RN3 to sagittal sway. All units in these nerves had their zones centered close to the ventral-side-up orientation (position V). The zones of smaller units occupied the DT, T, TV, and V positions; these zones were wider than the zones of larger units which occupied the TV and V positions (see also Fig. 9A). Horizontal roll (Fig. 5D) also revealed a similarity of gravitational inputs to the motor neurons from LN3 and RN3: the zones of neurons in both nerves were centered close to the V position. Lateral sway did not evoke activation of LN3 and RN3 in any zone (not illustrated).

Rotation in opposite directions did not reveal any significant directional sensitivity of the tail motor neurons. This is illustrated in Fig. 5E for the LN2(1) and RN2(1) nerves tested by horizontal roll. For example, the zones of smaller units in LN2 occupied the DL, L, and LV positions in both turns, whereas the zones of larger units occupied the L and LV positions. In some experiments, however, a difference in the zone size and position could be observed in successive tests. This most likely was caused by spontaneous changes in the excitability of neurons constituting a chain of gravitational reflexes. Sometimes spontaneous bursts of activity in the tail nerves, not caused by gravitational stimulation, were observed which considerably hampered the analysis of gravitational responses.

Three principal patterns of gravitational response have been found in tail motor neurons that allowed us to classify the neurons in three groups. A few neurons with weakly pronounced response were excluded from the analysis. The diagrams in Fig. 6 (top) show the angular zones of activity for different groups of motor neurons averaged over all experiments (n = 12–42 for different groups). Group T1 includes motor neurons from the left and right nerves N3. Group T2 includes motor neurons from the left nerve N2(1). Finally, group T3 includes motor neurons from the right nerve N2(1).

The horizontal bars indicate an average width of the zones, whereas the black circle in the center of each bar indicates the center of the zone, with the SD value shown by the horizontal lines.

The angular zones of all the three groups of tail motor neurons were ~180° in width when tested by sagittal sway, by lateral sway, or by horizontal roll. The center of the T1 zone, when tested by sagittal sway and horizontal roll, was very close to the V position. The centers of T2 and T3 zones, when tested by lateral sway and horizontal roll, were very close to the L and R positions, respectively. The centers of the T2 and T3 zones, tested by the sagittal sway, were close to the D position. On the basis of the data presented in A–C, a schematic spatial reconstruction of the zones was done (see DISCUSSION and Fig. 11, A–C).

As shown in Fig. 6, dispersion of the center of the zones, characterized by the SD value, ranged from 16 to 28° for different zones. This dispersion was most likely caused by two factors—the individual variability between the animals and the variability in positioning the CNS in the recording chamber. A dispersion of the width of zones also was measured (not illustrated in Fig. 6). Its value appeared considerably larger than the dispersion of centers (the range was 18–45°). The most likely explanation for this finding was that the width of the zone depended on the excitability in the chain of gravitational reflexes to a larger extent than the position of the center. In this relation, one should mention that in the course of
individual experiments, spontaneous variations of the width of the zones often were observed.

When recording from other tail nerves—N1, N2(2), and N4 (Fig. 2A)—a few units with position-dependent activity could be observed in some experiments. The angular zones of these units did not differ from the zones of groups T1, T2, or T3 described above. Responses in the N1, N2(2), and N4 nerves were not systematically studied, however.

Gravitational reactions of wing motor neurons

Lateral tilt of the CNS, caused by lateral sway or by horizontal roll, evoked reactions of wing motor neurons. Figure 7 shows gravitational responses in the left and right wing nerves (LNW and RNW) evoked by the horizontal roll. Two larger units in each of the nerves represent discharges of the large locomotor wing motor neurons 1A and 2A. These neurons receive alternating periodical inputs from the locomotor rhythm generator. Because of these inputs, the discharges of 1A and 2A alternate, as shown clearly in Fig. 7B at higher recording speed. The present study has shown that both rhythmic locomotor input and gravitational input converge on the 1A and 2A motor neurons. Because of the gravitational input, the 1A and 2A motor neurons exhibited their locomotor rhythmic spike activity only within a specific angular zone (Fig. 7A).

For the left wing, the zone occupied the D, DR, and R positions. For the right wing, the zone occupied the VL, L, and LD positions. Besides the locomotor motor neurons 1A and 2A, one also could see, in each of the wing nerves, the spikes of at least one nonlocomotor, tonic motor neuron. The zone of activity of this motor neuron in the LNW occupied the VL and L positions. In the RNW, the zone occupied the DR and R positions. These tonic motor neurons are most likely responsible for the wing retraction (Huang and Satterlie 1990; Delagina, Arshavsky, and Orlovsky, unpublished data). A strict reciprocity between responses of the locomotor and tonic mo-
Gravitational reactions of CPB3 interneurons

Gravitational inputs to the tail and wing motor neurons are mediated by the cerebropedal interneurons, the morphology of which is shown schematically in Fig. 1, B–E. Two types of these interneurons (CPB3b and CPB3c) have their axons in the subpedal commissure (SPC) and could be recorded from the stumps of the transected commissure (see METHODS and Fig. 2A). Units with different discharge patterns were recorded with this method—position-sensitive and tonic units. The former were considered as the discharges of the CPB3b or CPB3c interneurons according to the following criteria (Panchin et al. 1995a): they were recorded from the subpedal commissure, they transmitted gravitational signals, and they evoked gravitational responses in the tail motor neurons (see next section). The spike amplitude in these neurons was ~200 μV, which is considerably smaller than the amplitude of spikes of motor neurons in the tail and wing nerves. Unfortunately the presence of larger tonic units in the SPC in some experiments hampered detection of smaller spikes of the CPB3 interneurons.

Up to four CPB3 interneurons, with different angular zones of activity, could be identified in each (left or right) stump of the SPC. Figure 8A shows discharges of the four CPB3 interneurons recorded from the left part of the transected SPC in different positions of the preparation. The interneurons 1–4 fired in the V, L, R, and D positions, respectively. In addition, interneuron 4 fired in short bursts during transition from the R to the L position and from the L to the V position, when the preparation rapidly passed the D position. Units recorded in the right stump of the SPC also had their maximal activity in the V, L, R, and D positions, respectively (not illustrated). The firing frequency of interneurons usually was 10–15 Hz (which is similar to the frequency of motor neurons), but in some cases it was higher (~50 Hz).

The angular zones of activity of individual interneurons were difficult to measure in the cases when different neurons fired with the spikes of similar amplitude. However, in some experiments, the differences in amplitude were considerable, like in the experiment illustrated in Fig. 8, B and C. In this particular case, only two units were seen in the right SPC, the large one responding in the V position and the small one responding in the D position. When tested by the horizontal roll (0–360°), the zone of the larger unit occupied the V, VL, R, and VR positions, whereas the zone of the smaller unit occupied the L, LD, D, and DR positions (Fig. 8C). When

**Fig. 8.** Gravitational responses in the CPB3 interneurons. A: recording of responses to horizontal roll from the left stump of the subpedal commissure (LSPC). In this case, 4 different units (1–4) could be revealed by positioning the preparation in the center of their zones (positions R, L, V, and D). Discharges of unit 2 well correlated with the simultaneously recorded activity in the nerve LN2(1). B and C: recording from the right stump of the subpedal commissure (RSPC). In this case, only 2 units, with strongly differing spike amplitudes (1 and 4), were present. Gravitational stimulation was applied as trapezoid (B) or stepwise horizontal roll (C). Discharges of unit 1 well correlated with the simultaneously recorded activity in the nerve LN3. A, B, and C are from different experiments.
The diagrams (Fig. 6, bottom) show the angular zones of activity of the four groups of cerebropedal interneurons recorded in the SPC. Each group contained two cells, one recorded from the left stump of the SPC and one recorded from the right stump. The zones were averaged over all experiments \((n = 8–24\) for different groups). The IN1, IN2, IN3, and IN4 groups had their centers of zones close to the V, L, R, and D positions, respectively. All the zones were \(135^\circ–170^\circ\) in width. By comparing the diagrams of Fig. 6, top and bottom, one can conclude that the zones of activity of the CPB3 interneurons (groups IN1, IN2, and IN3) resemble the zones of activity of the tail motor neurons (groups T1, T2, and T3, respectively) except that they are narrower and IN2 and IN3 are not activated during sagittal movements. However, the zone of the IN4 group has no corresponding zone among the zones of tail motor neurons.

**Action of CPB3 interneurons on tail motor neurons**

Two types of experiments were carried out to estimate the effects produced by the CPB3b and CPB3c interneurons, through their axons in the SPC, on the tail motor neurons. First, we compared the gravitational responses in the tail nerves before and after transection of the SPC and found no marked difference (not illustrated). This finding indicates that gravitational input to tail motor neurons, mediated by the CPB3a interneurons (Fig. 1B) as well as by the axons of the CPB3b and CPB3c interneurons before they enter the SPC (Fig. 1, C and D), is considerably stronger than input mediated by the axons that crossed the SPC.

A contribution of the axons crossing the SPC, however, could be revealed in a different set of experiments illustrated in Fig. 9. After transection of all connections of the right pedal ganglion with the rest of the CNS except for the subpedal commissure (Fig. 9C), a position-dependent activity could still be observed in the right N3 nerve. As shown in Fig. 9B, the T1 group still could be activated by sagittal sway, and the activity occurred approximately in the normal zone, that is, in and around the V position, though the strength of the response was considerably reduced as compared with control (Fig. 9A). Such a response was observed in four of seven experiments. Because only one of the CPB3 interneurons, projecting through the SPC, has the zone of its activity in and around the V position (unit 1, Fig. 8) and the response disappeared after complete isolation of the pedal ganglion, this finding indicates that this particular interneuron, even when it is active alone, makes a noticeable contribution to the activation of the tail motor neurons in their normal zones.

**Equivalence of the left and right statocysts**

In behavioral experiments, it was found that *Clione* was able to stabilize its orientation close to the normal (head-up) orientation after removal of any one of the two statocysts (Panchin et al. 1995a). In the present study, we recorded gravitational responses of tail motor neurons, wing motor neurons, and CPB3 interneurons before and after removal of the left statocyst \((n = 4)\) or right statocyst \((n = 4)\). It was found that the position and width of the angular zones of activity of the inter- and motor neurons persisted after removal of any of the statocysts. The intensity of firing within the zone, however, was reduced slightly in some experiments. Figure 10 illustrates the case when removal of the left statocyst had practically no effect on the response to horizontal roll in the LN3 and LN2(1) nerves. Similar results were obtained for the wing motor neurons. In experiments with recording from the SPC \((n = 4)\) it was found that all four groups (IN1–IN4) of CPB3 interneurons retained their zones of activity after removal of one statocyst, though the frequency of discharge within the zones was somewhat reduced.

![Fig. 9. Gravitational responses in the nerve N3 could be evoked by a single interneuron. A: response to sagittal sway in the nerve RN3 before lesions. B: after the lesions (shown in C) the zones of activity of motor neurons in RN3 persisted, but the discharge frequency within the zones decreased. This response was most likely evoked by the IN1 axon passing through the SPC and firing in the V position (see Fig. 8). C: scheme of lesions. Abbreviations as in Fig. 2A.](http://jn.physiology.org/)

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These results, taken together, suggest that the equivalence of two statocysts is based on the fact that individual cerebropedal interneurons receive similar gravitational inputs from the left and the right statocysts.

**DISCUSSION**

**Spatial zones of activity of different groups of motor neurons**

In the present study, the spatial zones of activity have been characterized for different groups of motor neurons and interneurons involved in the control of body orientation in *Clione*. This was made possible by a novel method, which allows us to record gravitational reactions in an isolated CNS-statocyst preparation.

Three modes of rotation of the preparation in space were employed in the present study (Fig. 3, A and B). Sagittal sway (deviation from the vertical orientation forward or backward, Fig. 3, A1 and B1) and lateral sway (deviation to the left and to the right, Fig. 3, A2 and B2) revealed the zones of activity in two orthogonal vertical planes. Horizontal roll (rotation around the longitudinal axis positioned horizontally, Fig. 3, A3 and B3) allowed us to characterize the activity of neurons caused by a 90° deviation from the normal orientation in different directions. These tests revealed three principal groups of tail motor neurons with different spatial zones of activity (Fig. 6).

Group T1 (motor neurons in the left and right N3 nerves) responded preferentially to the backward sway with the center of the zone located close to the ventral-side-up (V) position. This group exhibited no activity with lateral sway in any direction. On the contrary, groups T2 and T3 [motor neurons in the nerves LN2(1) and RN2(1)] responded preferentially to lateral sway. Group 2 had the center of the zone located close to the left-side-up (L) position, whereas group 3, near the right-side-up (R) position. The zones of groups T2 and T3 extended also to the dorsal-side-up (D) position, but these groups exhibited no activity in the V position, in which group T1 had its zone center.

To represent the relationships between the zones of activity of different groups of tail motor neurons, we made their schematic three-dimensional reconstruction (Fig. 11, A–C) on the basis of the one-dimensional zones (Fig. 6). On these graphs (Fig. 11, A–C), the orientation of *Clione* in relation to the gravity force was represented by a radius-vector originating from the center of the sphere (shown in A). As in Fig. 6, the angles $\alpha$ and $\beta$ show deviations of *Clione* from the vertical orientation for the sagittal and lateral sway, respectively. At $\alpha = 0^\circ$, $\beta = 0^\circ$ *Clione* is oriented vertically, with its dorsal side positioned toward the reader. Positive values of $\alpha$ correspond to deviations toward the dorsal side up (forward sway); positive values of $\beta$ correspond to deviations toward the right side up (left sway). The arcs drawn on the sphere by a thick
line show the angular width and position of the one-dimen-
sional (1-d) zones revealed by sagittal sway (the arc along the
α-meridian), by lateral sway (the arc along the β-meridian),
and by horizontal roll (the equatorial arc). (For the T2 and T3
groups, the arcs along the α-meridian occurred on the opposite
side of the sphere and were not shown). The thin lines connect
the extreme points of the 1-d zones to show the presumed
borders of the three-dimensional (3-d) zones. From Fig. 11,
A–C, one can see that the T1–T3 zones are very wide, each of
them occupies approximately one-half of the sphere, and the
zones of different groups considerably overlap with each other.
The three-dimensional reconstruction of the zones for wing
motor neurons was performed in the same way and is presented
in Fig. 11D. Groups W2 and W3 of wing motor neurons had a
center of their zone close to that of the group T2 of tail motor
neurons. Similarly, groups W1 and W4 had a center of their
zone close to that of the group T3 of tail motor neurons. A size
of the zones of wing motor neurons, however, was smaller than
that of tail motor neurons.

Correlation between motor responses evoked by different
groups of motor neurons and their zones of activity

Effects on the postural orientation produced by different
groups of the position-sensitive tail and wing motor neurons
have not been measured directly. However, these effects can be
estimated on the basis of morphological data as well as on the
basis of experiments with electrical stimulation of the nerves or
their transection (Panchin et al. 1995a). The left and right
nerves N3 innervate the ventral aspect of the tail. Stimulation
of each of these nerves evokes a ventral tail flexion. After transection of these nerves, no spontaneous ventral flexion of
the tail was observed in otherwise intact Clione. One thus can
suggest that the tail motor neurons of group T1, with their
axons in the LN3 and RN3 nerves, elicit the ventral tail flexion.
The left and right nerves N2(1) innervate the left-dorsal and the
right-dorsal aspects of the tail, respectively. Stimulation of
RN2(1) evokes left-dorsal tail flexion, whereas stimulation of
RN2(1) evokes right-dorsal tail flexion. After transection of
these nerves, no spontaneous dorsal and lateral flexion of the
tail was observed. One thus can suggest that the tail motor
neurons of the group T2, with their axons in the LN2(1) nerve,
elicit the left-dorsal tail flexion. Similarly, the tail motor neu-
rons of group T3, with their axons in the RN2(1) nerve, elicit the
right-dorsal tail flexion.

The present study also has shown that gravitational input
induces left-right asymmetry in the activity of wing motor
neurons. The effect of this asymmetry on postural orientation
was not measured directly but can be estimated on the basis of
simple considerations. A unilateral activation of the locomotor
motor neurons 1A and 2A will result in an increase of the
amplitude of wing beating (Arshavsky et al. 1985a,b; Satterlie
1993; Satterlie and Spencer 1985; Satterlie et al. 1985). One
can suggest that this asymmetry in the beating of two wings
will produce a force turning Clione in the contralateral (in
relation to the “stronger” wing) direction. Simultaneous activ-
ation of the retractor motor neuron of the contralateral wing
will reduce oscillations of that wing, which will also promote
the turning of Clione toward the “weaker” wing.

Figure 11, E, 1–3, and F, 1–3, shows schematically the
postural corrective responses caused by deviation of Clione
from the vertical in the frontal and sagittal planes as well as the
groups of motor neurons eliciting these responses. With the left
sway (E2), Clione occurs in the zone of activation of the T3
group of tail motor neurons, as shown in C. This group evokes
tail flexion to the right (E2), which will elicit turning of Clione
toward the vertical orientation. With a larger left sway, Clione
is then also in the zone of activation of the W1 and W4 wing
motor neurons, as shown in D. These motor neurons evoke asymmetry in the wing beating, with the prevalence of the left
wing, which also will result in the turning of Clione toward the
vertical orientation. Thus the tail and the wing motor responses
supplement each other when restoring the normal body orienta-
tion.

With the right sway (E3), Clione occurs in the zone of
activation of the T2 tail motor neurons, as well as of the W2
and W3 wing motor neurons. These motor neurons will evoke
corrective motor responses turning Clione to the left and thus
restoring the normal body orientation. Finally, the sagittal body
sway with the dorsal (F2) or ventral (F3) side up moves Clione
into the zones of activation of the T2 and T3 groups, or T1
group, respectively (A–C). These groups evoke the dorsal (F2)
or the ventral (F3) tail flexion, respectively, which will result
in the turning of Clione toward the vertical orientation. Be-
cause the zones of tail motor neuron are very wide and
together cover the whole sphere (A–C), any deviation of Clione
from the normal orientation will result in specific group(s) of
the motor neurons being activated, and an adequate motor
response (tail flexion), aimed at restoration of the vertical
orientation, will be generated. This notion was supported in the
experiments on the isolated CNS with an artificially closed
feedback loop (Deliagina et al. 1998a). The robotics system,
driven by signals from the T2 and T3 groups of tail motor
neurons, was able to compensate for large postural distur-
bances (≤180°).

Sensorimotor transformations performed by a population of
cerebropedal interneurons

According to the anatomic data, each of the statocysts of
Clione contains 9–11 receptor cells (Tsirulis 1974). By record-
ning responses in the statocyst nerve to rotation of the statocyst
in different planes, we found that the maximal angular dimen-
sion of the zones of activity (receptive fields) in individual
receptor cells is about 135° (Deliagina, Arshavsky, and Or-
lovsky, unpublished data). In the present study, it was found
that the zones of activity of CPB3 interneurons have a similar
size (Fig. 6). The simplest explanation for this finding is that
the individual interneurons are driven by inputs from single
receptor cells of a statocyst.

The persistence of gravitational responses in the CPB3 in-
terneurons, in the tail motor neurons (Fig. 10) and in the wing
motor neurons after removal of one statocyst, found in the
present study, suggests that two homologous receptor cells,
from the left and right statocysts, converge on the same cere-
bropedal interneurons involved in the gravitational reflexes.

All three groups of tail motor neurons (T1–T3) have their
counterparts among the interneurons (groups IN1–IN3) with
approximately the same position of the zone of activity (Fig.
6). In lesion experiments, it also was found that the zone
position of tail motor neurons persisted after reduction of the
number of CPB3 interneurons projecting on them, though the
value of response within the zone reduced (Fig. 9). These findings suggest that a few interneurons with similar spatial zones converge on individual tail motor neurons.

Comparison with postural systems in other species

In terrestrial species including humans, control of body posture and equilibrium is usually based on integration of sensory inputs of three modalities—vestibular, visual, and somatosensory (for a review, see Horak and Macpherson 1995). In aquatic animals, which do not contact the substratum, somatosensory information plays a minor role. In the goldfish and some other bony fishes, vestibular input to the postural system is of primary importance. Nevertheless, deprived of this input, some fishes are able to stabilize their orientation in space relaying exclusively on visual input (Graf and Mayer 1983; von Holst 1935). In the lamprey (a lower vertebrate), vestibular input is the dominating one; when deprived of this input, the animal is not able to stabilize its orientation in space (Ullén et al. 1995a). Nevertheless, visual input can, to some extent, affect the orientation stabilized by the vestibular-driven postural system (Ullén et al. 1995b).

In Clione, gravitational input from the statocysts to the postural control network completely determines postural orientation and equilibrium (Panchin et al. 1995a). This allowed us to focus in the present study exclusively on the gravitational mechanisms for postural control and use the in vitro preparation of the CNS where the postural network is driven by only one gravitational input.

In all species that are able to stabilize their orientation in space, a deviation from the desired body orientation evokes corrective motor responses aimed at restoration of the initial orientation. These responses, as well as the underlying activity of motor neurons (usually recorded as the electromyographic responses), have been studied in many species including humans (see e.g., Amblard et al. 1988). The general conclusion from these studies is that the principal characteristics of the motor response (i.e., the content of the activated muscle groups and the temporal pattern of their activation) strongly depend on the direction and value of the deviation from the preferred orientation (see e.g., Macpherson et al. 1997). This conclusion was confirmed in the present study. It was found that the spatial pattern of the corrective motor response (a selected group of motor neurons) is determined by the direction of the deviation from the vertical orientation, whereas the value of the response may depend on the value of deviation.

In different species including humans, which stabilize their orientation in space during locomotion, postural and locomotor mechanisms strongly interact with each other (see e.g., Hirshfeld and Forssberg 1991; Nashner 1980; Nashner and Forssberg 1986). In the present study, it was found that the locomotor mechanisms in Clione are actively used by the postural system: lateral deviations from the vertical orientation induced a large asymmetry in the periodical locomotor commands sent to the muscles of the left and right wing (Fig. 7). This asymmetry was caused by gravitational input addressed primarily to the wing motor neurons but not to the rhythm-generating (CPG) interneurons because the locomotory rhythm persisted almost unchanged in different positions (Fig. 7B). A tendency to preserve the basic locomotor pattern during postural corrections is characteristic also for other species (see e.g., Nashner and Forssberg 1986; Orlovsky 1972).

An important feature of the postural control systems in different species is their remarkable flexibility, that is, an ability to stabilize different orientations (see e.g., Orlovsky 1991). The flexibility of the postural control system of Clione and the underlying neuronal mechanisms will be described in the following papers of this series.

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