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SHORT COMMUNICATION

INTERNEURONES MEDIATING THE ESCAPE REACTION OF THE MARINE MOLLUSC *CLIONE LIMACINA*

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Neural mechanisms underlying the escape reaction have been extensively studied for over 20 years in many species of animals belonging to several different phyla (for a review, see Eaton, 1984). Among molluscs, the escape reaction has been most thoroughly studied in *Tritonia*. In this animal a strong mechanical or chemical stimulus applied to the posterior part of the body evokes swimming (for a review, see Getting, 1983; Getting and Dekin, 1985). Electrophysiological experiments on the isolated nervous system revealed that neurones constituting the central pattern generator (CPG) for swimming were strongly depolarized in response to sensory stimulation and that this depolarization caused rhythmic activity in the CPG. However, attempts to identify command neurones mediating the escape reaction in *Tritonia* have failed (Getting, 1983).

This paper deals with the escape reaction in another gastropod mollusc, *Clione limacina* (subclass Opistobranchia, order Pterapoda). We have been able to identify some of the interneurones involved in this animal's escape reaction. *Clione* swims using rhythmic movements of two wings, which are controlled by the locomotor CPG located in the pedal ganglia. The CPG can generate the locomotor rhythm (fictive swimming) *in vitro*, i.e. after isolation of the pedal ganglia from the rest of the nervous system, and the neuronal organization of the CPG has been analyzed (Arshavsky *et al.* 1985*a–c*; Satterlie, 1985, 1989). The CPG consists of two groups of interneurones (7 and 8) controlling various groups of wing motoneurones, including two large cells, 1A and 2A, which innervate the dorsal and ventral wing muscles, respectively (Arshavsky *et al.* 1985*a*,*b*).

Locomotor activity is not continuous in freely swimming *Clione*: periods of locomotor activity (lasting 1–3 min) usually alternate with periods of inactivity during which the animal sinks passively; this results in 'vertical migrations' (Litvinova and Orlovsky, 1985). The effects of tactile stimulation depend on the phase of vertical migration in which the stimulus is applied. In a passive animal,

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touching the tail triggers the locomotor system and the animal swims away from the irritation (escape reaction). In an active animal, tail stimulation results in acceleration of wing oscillations. Touching the head results in termination of wing oscillations (Arshavsky *et al.* 1990; Satterlie *et al.* 1985). Interneurones mediating the first type of response (escape reaction) will be described below.

The study was carried out at the White Sea Marine Biological Station, *Kartesh*. Experiments were performed on a preparation consisting of the central nervous system (CNS) (see Fig. 2A) with intact connections to the posterior part of the body. A few experiments were carried out on the isolated CNS. Surgical operations were performed at low temperature (on ice) to prevent excitation of nociceptive inputs. The preparation was fixed to the bottom of a Sylgard-lined chamber filled with sea water. Glass microelectrodes filled with 3 mol l^{-1} KCl (tip resistance, 20–60 MΩ) were used for intracellular recordings. The same electrode was used for both recording and current injection. The artefact caused by a polarizing current was partly compensated for by means of a bridge circuit. For intracellular staining, the microelectrodes were filled with a solution of Lucifer Yellow; the dye was injected into a cell by passing a hyperpolarizing current (Stewart, 1978). To facilitate the insertion of the microelectrode, the epineural sheath was softened with a 1% solution of Pronase E (Sigma) for 5–10 min. Neuronal activity was recorded with a pen recorder that was not rectilinear and had a frequency range of 0–200 Hz, which somewhat attenuated the amplitude of recorded spikes.

Fig. 1 shows the responses of two neurones of the locomotor CPG (the group 7 interneurone and the 1A motoneurone) to tactile stimulation of the tail. The first stimulus in Fig. 1A evoked a short-term depolarization of the neurone and a series of locomotor cycles. The second stimulus in Fig. 1A and the stimulus in Fig. 1B

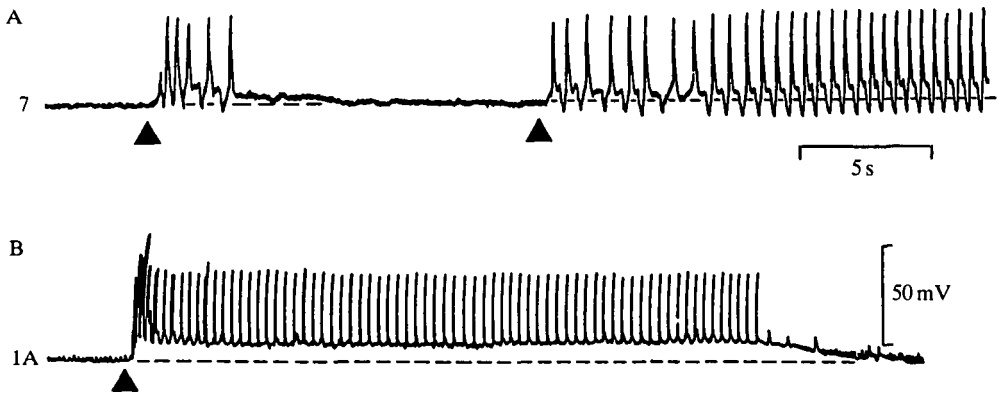

Fig. 1. Intracellularly recorded response of a type 7 locomotor interneurone (A) and of the 1A motoneurone (B) to tactile stimulation of the tail (marked by arrowheads). The interrupted line shows the resting membrane potential.

evoked long-lasting depolarizations of the neurones and episodes of fictive locomotion lasting for many seconds. A stimulus applied during ongoing locomotor activity resulted in acceleration of the rhythm (not illustrated).

In each cerebral ganglion we found at least 20 neurones that sent axons to the pedal ganglia. When stimulated intracellularly, these neurones affected the locomotor CPG in the pedal ganglia. The majority of these neurones exerted an excitatory effect ('cerebral locomotion excitors', CLEs), but some exerted an inhibitory effect ('cerebral locomotion inhibitors', CLIs). One type of CLE was identified (CLE1) which mediated the escape reaction. Its morphology is shown in Fig. 2A. The axon of CLE1 projects through the cerebral commissure, contralateral cerebral ganglion, cerebro-pedal connective, contralateral pedal ganglion and pedal commissure and terminates in the ipsilateral pedal ganglion. The axon forms short neuropilar branches in all the ganglia. Activity of CLE1 is shown in Fig. 2B,C. Excitation of CLE1 by current injection resulted in activation of the locomotor CPG, which was evident from a series of rhythmic IPSPs and EPSPs in the 2A locomotor motoneurone (Fig. 2B). Tactile stimulation of the tail (arrowhead in Fig. 2C) excited CLE1 and activated the locomotor CPG. In another experiment, excitation of CLE1 by current injection resulted in acceleration of the existing locomotor rhythm (monitored by discharges in the type 7 locomotor interneurone, Fig. 2D). This CLE1 also responded to tactile tail stimulation (Fig. 2E).

When CLE1 activity was suppressed by injection of hyperpolarizing current, stimulation of the tail could still activate the locomotor CPG (not illustrated), suggesting that the escape response is mediated by a group of excitatory interneurones. An attempt to evaluate the contribution of a single CLE1 to the response (by measuring the decrease in response caused by suppression of the neurone) failed because of the large variability of the response (as illustrated in Fig. 1A).

As shown previously, the locomotor activity and the heart rate of *Clione* are strongly correlated: any spontaneous or reflex change of activity of the locomotor CPG is always accompanied by a corresponding change in the heart rhythm (Arshavsky *et al.* 1990). This correlation is partly due to the direct action of the locomotor CPG upon the heart excitatory motoneurone (HE) located in the left pedal ganglion (see also Fig. 9 in Arshavsky *et al.* 1990). In the present study we have revealed one more mechanism linking the locomotory and circulatory systems. In the experiment illustrated in Fig. 2D, activity in CLE1 was recorded together with both the locomotor cell (interneurone 7) and the heart excitatory motoneurone HE. When CLE1 activity caused an increase in the frequency of the locomotor CPG, there was a concomitant burst of action potentials in HE. Thus, the interneurone CLE1 plays an important integrative role in the organization of motor behaviour in *Clione*.

The activity of a cerebral inhibitory neurone, CLI1, and its influence upon the locomotor CPG are illustrated in Fig. 3. This cell exerted a strong inhibitory action upon the locomotor CPG (Fig. 3A). In turn, CLI1 was inhibited during periods of

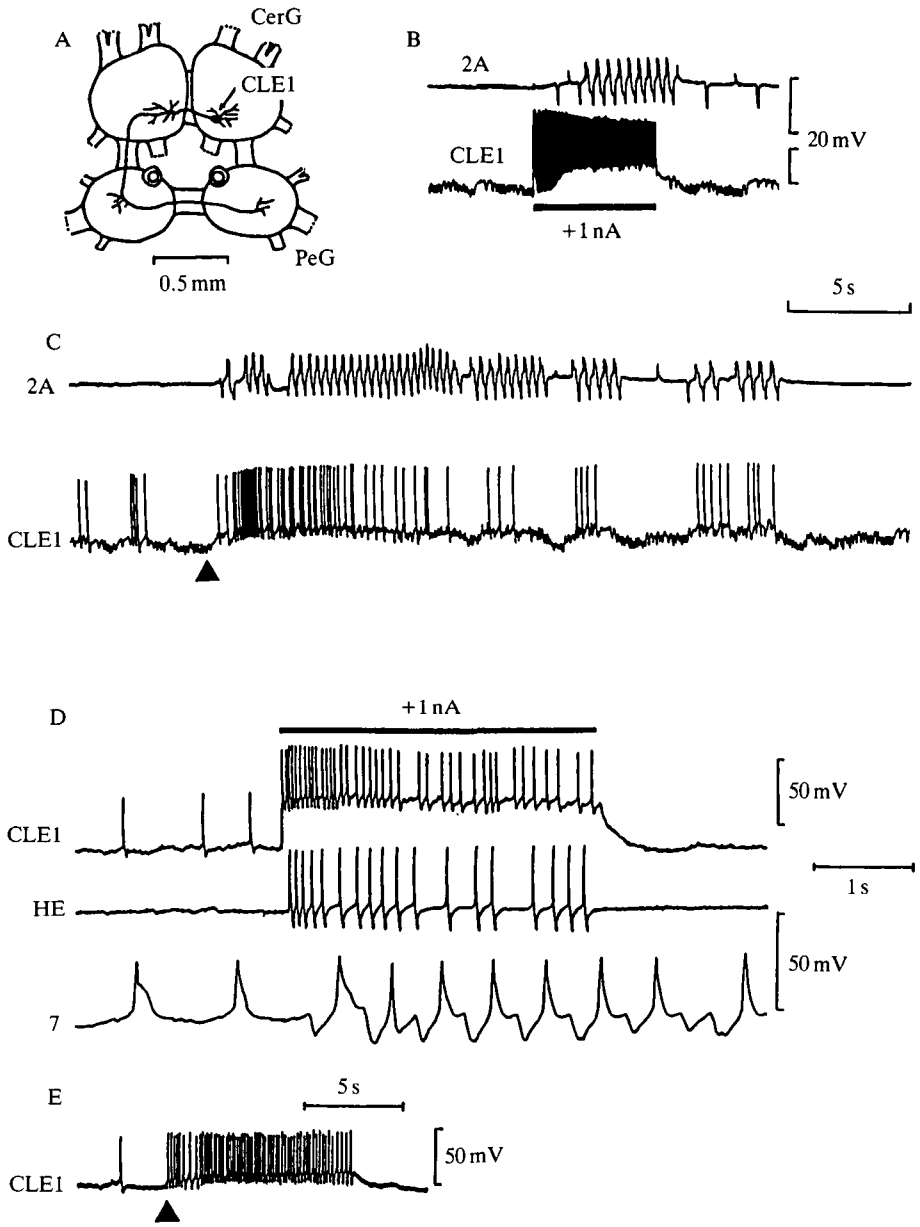

Fig. 2. Characterization of CLE1. (A) Morphology of CLE1 (revealed by staining with Lucifer Yellow); CerG, cerebral ganglia; PeG, pedal ganglia (dorsal view). (B) Excitation of CLE1 by current injection (bar) evoked the locomotory rhythm, monitored in the 2A motoneurone. (C) Tactile stimulation of the tail (arrowhead) resulted in activation of both CLE1 and 2A. (D) Excitation of CLE1 by current injection (bar) resulted in activation of the heart excitatory neurone (HE) and acceleration of the locomotory rhythm, monitored in the group 7 interneurone. (E) Response of the same CLE1 to tactile stimulation of the tail. Recordings B,C and D,E are from different experiments.

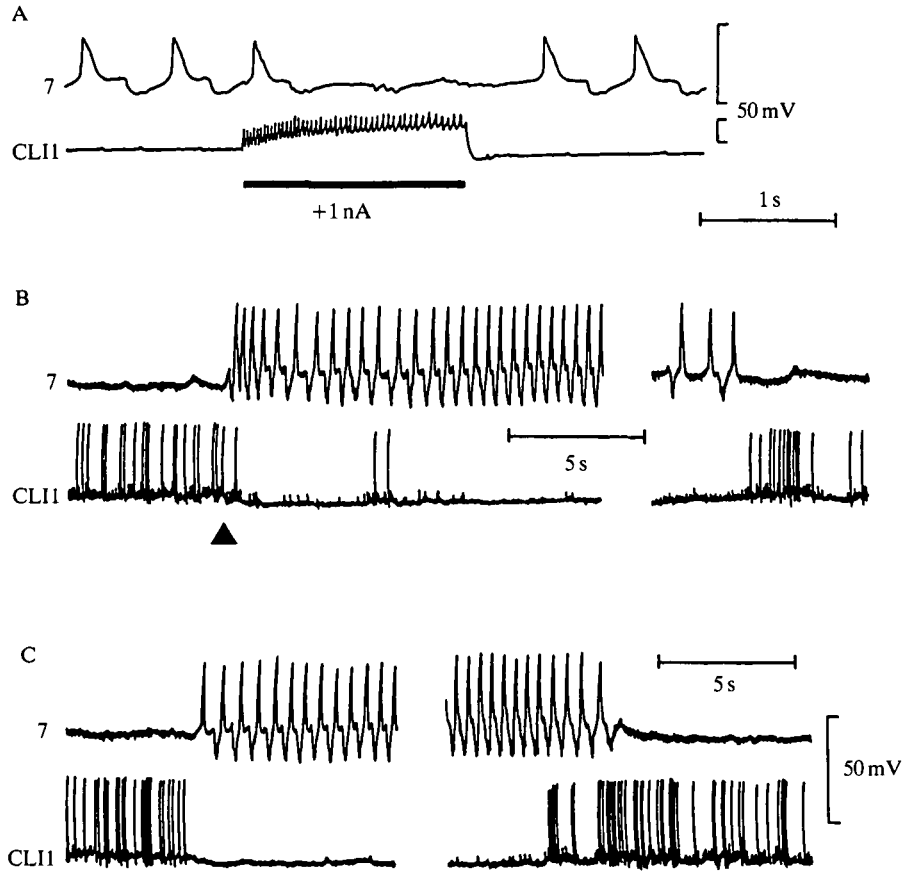

Fig. 3. Characterization of CLI1. (A) Excitation of CLI1 by current injection (bar) inhibited the locomotory rhythm, monitored in the type 7 interneurone. (B) Tactile stimulation of the tail (arrowhead) resulted in inhibition of CLI1 and activation of the locomotor central pattern generator. (C) CLI1 was inhibited during a spontaneous burst of locomotor activity.

locomotor activity evoked by tail stimulation (Fig. 3B) or arising spontaneously (Fig. 3C). We suggest that activation of the locomotor CPG during the escape reaction is due to excitation of excitatory interneurones (CLEs) and inhibition of inhibitory interneurones (CLIs).

As shown in Fig. 1, tail stimulation could evoke either a short-term activation of the locomotor CPG (the first stimulus in Fig. 1A) or a long-term activation lasting up to several minutes, considerably longer than the duration of the stimulus. In our opinion, these long-lasting effects are due to activity in an interneurone PeLE1, located in the pedal ganglia, or in a similar group of neurones. In the isolated CNS preparation we managed to record PeLE1 four times; its soma was

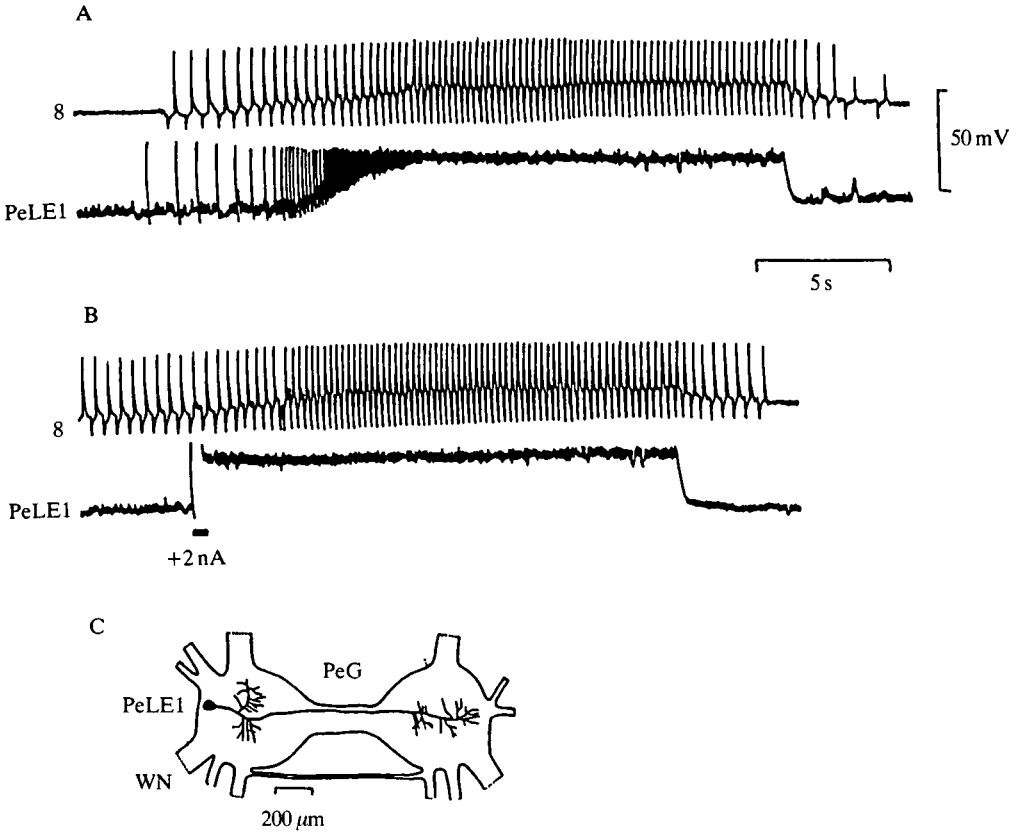

Fig. 4. Characterization of PeLE1. (A) Spontaneous activity in PeLE1 resulted in an episode of locomotor activity monitored in the group 8 interneurone. (B) A brief pulse of depolarizing current (bar), injected into PeLE1 resulted in a depolarizing plateau and in acceleration of the locomotory rhythm. (C) Morphology of PeLE1 (revealed by staining with Lucifer Yellow). PeG, pedal ganglia (ventral view); WN, wing nerve.

located in the ventro-lateral region of the pedal ganglion with its axon crossing into the contralateral ganglion (Fig. 4C). PeLE1 is capable of repeatedly generating (with a period of about 1 min) a long-lasting 'plateau potential' differing from the resting potential by +25 to +35 mV. Fig. 4A shows one such wave of depolarization that arose spontaneously and Fig. 4B shows a similar wave triggered by intracellular injection of a short pulse of depolarizing current. In both cases the 'plateau potential' generated in PeLE1 was accompanied by a clear depolarization of locomotor interneurone 8 and by activation of the locomotor CPG (monitored by rhythmic discharges in interneurone 8).

It seems very likely that the alternating episodes of locomotor activity and passive sinking observed in freely swimming *Clione* are due to a specific pattern of activity of the PeLE1 interneurone(s). We also suggest that PeLE1 is involved in the escape reaction and is triggered by tail stimulation either directly or through

CLE1. It seems likely that it is the involvement of PeLE1 that determines the long-lasting response to a brief tactile stimulation (Fig. 1). Unfortunately, we succeeded neither in making paired recording from CLE1 and PeLE1 nor in recording from PeLE1 in the 'tail plus CNS' preparation, mainly because PeLE1 is located on the ventral surface of pedal ganglia where it is difficult to penetrate.

The PeLE1 neurone is the second cell, identified in the *Clione* CNS, that is capable of generating plateau potentials. Previously we described interneurone 12 in the locomotor CPG which possesses membrane properties similar to those of PeLE1 (Arshavsky *et al.* 1985*d*, 1989). These two types of interneurones play very different roles in locomotor control: interneurone 12 regulates activity of the locomotor CPG within a definite phase of the locomotor cycle, while PeLE1 is responsible for long-term activation of the CPG. The ionic mechanisms responsible for generating the plateau potential in these cells have not yet been investigated.

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