J. exp. Biol. 148, 477–482 (1990)
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SHORT COMMUNICATION

AN IDENTIFIED SET OF LOCAL NONSPIKING INTERNEURONES WHICH CONTROL THE ACTIVITY OF ABDOMINAL POSTURAL MOTONEURONES IN CRAYFISH

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Accepted 12 August 1989

Several interneurones have been reported to be involved in the control of activity of abdominal postural motoneurones in crayfish (Miall and Larimer, 1982*a*; Larimer and Jellies, 1983). These interneurones are mostly interganglionic, except for two (each encountered only once) which are local (Jellies and Larimer, 1985). The two local interneurones were found to be nonspiking, as reported in a variety of motor control systems of arthropods (Siegler, 1985). In the abdominal posture system, however, it is still unknown to what extent this type of neurone participates in the control of motoneurone activity. In this study, we repeatedly encountered and identified a set of local nonspiking interneurones which could control the postural motoneurone activity in the same fashion in different preparations.

The interneurones were penetrated with microelectrodes filled with Lucifer Yellow (5 % in 1 mol l^{-1} $LiCl_2$ or 3 % in 0.1 mol l^{-1} $LiCl_2$; Stewart, 1978). The abdominal nerve cord of the crayfish *Procambarus clarkii* Girard was isolated and pinned dorsal side up on a Sylgard-lined dish filled with crayfish saline (van Harreveld, 1936). We stained 42 examples of local interneurones in the fourth abdominal ganglion of 40 different preparations. They all showed a bilateral structure, extending dendritic branches on both sides. These bilateral branches were connected by a thick dorsal process running transversely. No axonal structure was observed. The cell body was always located in the ventrolateral portion of the ganglion and was connected to the neuropilar processes *via* a thin neurite. These local bilateral interneurones are called LB cells in this paper.

The LB neurones could be classified into two morphological types. The LB_1 cells (N=29) extended their branches on both the ipsilateral and contralateral sides of the ganglion to their somata, with a few dendrites near the midline

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Key words: crayfish, abdominal posture system, nonspiking interneurone.

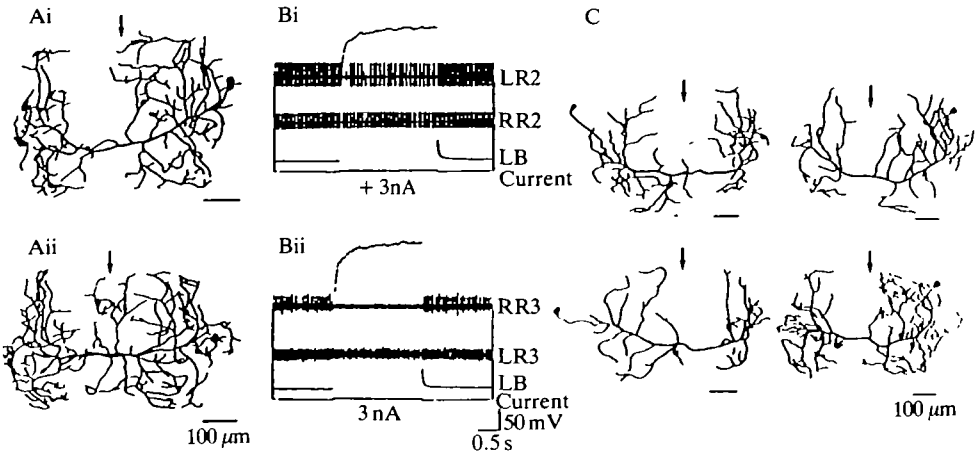

Fig. 1. Structure of LB_1 cells and the effect of current injection on spike activity of the abdominal postural motoneurones. (Ai) Structure of an LB_1 cell stained with Lucifer Yellow. (Aii) Two LB_1 cells stained simultaneously. Anterior is at the top. Arrows indicate the midline. (Bi,ii) Effect of current injection into the cell shown in Ai. (Bi) Intracellular injection of 3 nA depolarizing current inhibited spontaneous spike discharges of the second roots on both sides. (Bii) 3 nA depolarizing current also decreased the spike discharge rate of the third roots. LR2, motor activity in the left second root; RR2, the right second root; LB, intracellular recording from the LB cell; Current, monitor of intracellular current injection. (C) Variability of morphology among LB_1 cells believed to be homologues.

(Fig. 1Ai). On two occasions, two LB_1 cells whose somata were in the same hemiganglion were stained in the same preparation (Fig. 1Aii). The LB_1 cells showed morphological variability from animal to animal (Fig. 1C). The LB_2 cells (N=13) had an asymmetrical distribution of dendrites, mainly on the contralateral side to the soma, near the midline (Fig. 2A). This type of cell showed little variability among animals. Simultaneous staining of LB_1 cells indicated that there are more than one LB_1 cell in each hemiganglion. LB cells might form their cluster in the same way as the unilateral nonspiking interneurones in the sixth abdominal ganglion (Hisada *et al.* 1984). The exact number of LB_1 cells in the fourth ganglion is unknown.

The two types of LB cell showed no discernible difference in physiological characteristics, despite their morphological differences. Depolarizing current injection into an LB cell affected the motoneurone spike activity in both the second and the third superficial roots of the fourth ganglion on both sides (Figs 1B, 2B). In the second root, which contained the slow extensor motoneurone axons (Kennedy *et al.* 1966), only a unit with the largest extracellular spike amplitude was depressed by injection of depolarizing current into the LB cell. Hyperpolarizing current injection had no visible effect. This motoneurone usually showed spontaneous spike discharges (2–9 spikes s^{-1}), and was identified as the peripheral inhibitor innervating the slow extensor muscle on the basis of intracellular staining

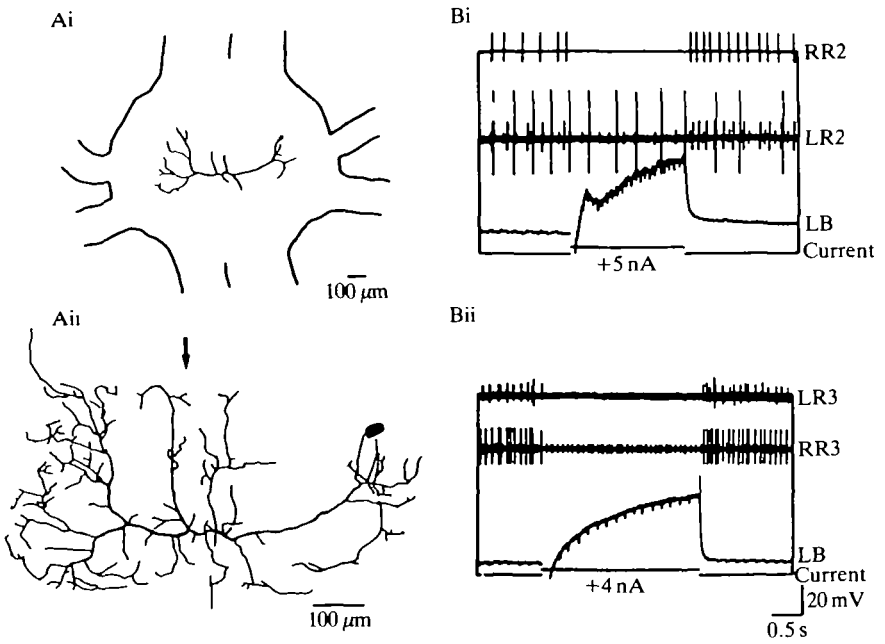

Fig. 2. Structure of an LB_2 cell and the effect of current injection. (Ai) Location of an LB_2 cell stained with Lucifer Yellow in the fourth abdominal ganglion. (Aii) Reconstructed structure of the cell shown in Ai. Anterior is at the top. The arrow indicates the midline. (Bi) Intracellular injection of 5 nA depolarizing current into the cell shown in A inhibited spontaneous spike discharge of the slow extensor inhibitors in the second roots. Second largest unit in the LR2 (second trace) is the slow extensor inhibitor. (Bii) 4 nA depolarizing current injection in the other LB_2 cell inhibited spike discharges of the third superficial roots on both sides.

(N=12). The staining revealed the soma contralateral to the axon's exit, and bilateral dendritic branches (Wine and Hagiwara, 1977; Miall and Larimer, 1982*b*).

The units which fired spontaneously in the third superficial root were unidentified. This root contains six motoneurones (five excitors and one inhibitor) innervating the slow flexor muscle (Kennedy and Takeda, 1965; Kennedy *et al.* 1966). Since the slow flexor inhibitor has been reported to be usually silent and to operate in reciprocity with the excitors (Kennedy and Takeda, 1965; Kennedy *et al.* 1966), we assumed in this study that those spontaneously active units were the slow flexor excitors.

The LB cells could simultaneously control the activity of several presumed slow flexor excitors. These motoneurones decreased their spike discharge rate when depolarizing current was injected into the LB cell (Figs 1Bii, 2Bii). The slow flexor inhibitor was not excited. Hyperpolarizing current injection usually had no noticeable effect, except in a very few cases where weak excitation was observed in the discharging motoneurone. Neither type of LB cell had any visible effect on the

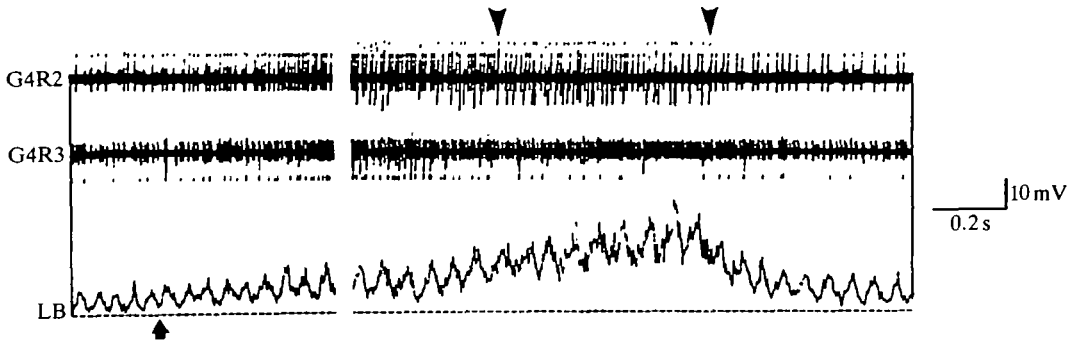

Fig. 3. Activity of an LB cell during fictive abdominal movement. The animal first showed an increase in the extensor inhibitor (second largest unit in G4R2) and in the flexor excitor activity (G4R3). The LB cell was slightly depolarized when the flexion pattern of motoneurone activity began (indicated by an arrow). A few seconds later, the activity pattern of motoneurones changed so that the spike discharge rate of the flexor excitors and the extensor inhibitor now showed a decrease while the largest unit of the extensor excitor in G4R2 showed an increase (arrowheads). This reciprocal pattern of postural motoneurone activities indicated that the network for abdominal extension movement was activated during that period. Correspondingly, the LB cell showed maximal depolarization during the period. After the termination of this fictive movement, the cell repolarized to the previous potential level (as indicated by the broken line). The oscillation in the LB cell's membrane potential (15–20 cycles s^{-1}) seen in this figure was not observed in other preparations. The functional meaning of this oscillation is not known.

fast motoneurones in either the second or the third roots, on the swimmeret motoneurones in the first root, or on the interganglionic interneurones in both the 3–4 and 4–5 connectives.

The membrane potential change of LB cells during the 'fictive' abdominal posture movement was studied using the whole-animal preparation that had been developed in a previous study (Takahata and Hisada, 1986). LB cells were penetrated in three different animals. The fictive abdominal posture movement was induced by forcibly moving the walking legs that were left free in the air (Page, 1981). During the fictive abdominal extension, as judged by an increase in the extensor excitor and a decrease in the flexor excitor activities (Fig. 3 arrowheads), the LB cell showed a sustained depolarization as long as the extension pattern of motor activity continued (Fig. 3). The maximal amplitude of this depolarization was about 20 mV, which was sufficient to suppress the postural motoneurones in the current-injection experiment (Figs 1B, 2B). We concluded that the LB cells were at least partly involved in the control of postural motoneurone activity during the abdominal movement. We could not determine whether the depolarization was the exclusive cause of the suppression of the flexor excitor activity.

Two examples of local interneurones (LIs) have been reported previously in the third and fifth abdominal ganglia (Jellies and Larimer, 1985), with a bilateral morphology similar to that of LB_1 cells and quite different from that of LB_2 cells.

Although this suggests the possibility that the LB_1 cells are serially homologous with the LI cells, the difference in motor effect between them makes this possibility less likely: depolarization of LB_1 cells decreased the spike activities of both the slow extensor inhibitor and slow flexor excitors, whereas depolarization of the LI cells caused activity to increase in the flexor inhibitors and decrease in the flexor excitors. Since no increase in the activity of the flexor inhibitor was observed in any of our preparations, we concluded that the LB_1 cells were not identical with the LI cells. The present study thus shows that there are several types of local nonspiking interneurones involved in abdominal posture control besides the LI cells.

The functional significance of nonspiking interneurones in abdominal posture control is yet to be shown. The bilaterally coordinated output of LB cells, which is also well coordinated in terms of the antagonism between flexor and extensor activities, seems to be consistent with the idea that local interneurones act as the driver interneurones interposed between the interganglionic command neurones and the motoneurones (Evoy, 1967; Evoy and Kennedy, 1967). However, the observation reported here and by Jellies and Larimer (1985), that the local nonspiking interneurones operate not by exciting the extension circuit but by inhibiting the flexion circuit, might suggest that the interneurones act as part of an auxiliary or modulatory circuit which facilitates the extension movement by inhibiting the neural activity operating antagonistically to that movement. The neural circuit which actively drives the extensor motoneurones during the extension movement remains to be described.

We would like to thank Dr T. Nagayama for his helpful suggestion on the study. This work was supported by Grants-in-Aid from the Ministry of Education, Science and Culture to MH (nos 61480016 and 61840023) and to MT (no. 63304005).

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