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INTERSEGMENTAL TO INTRASEGMENTAL CONVERSION BY GANGLIONIC FUSION IN LATERAL GIANT INTERNEURONES OF CRAYFISH

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The central nervous system (CNS) of arthropods in general consists of a chain of ganglia in the form of a ladder. Each ganglion has a basically homologous neuronal organization and is contained in one body segment (Bullock & Horridge, 1965). This basic pattern is often modified by ganglionic fusion. What changes are caused by ganglionic fusion, particularly with regard to segmentally homologous neurones?

The lateral giant (LG) interneurones of crayfish are, because of their well known physiology and morphology, especially well suited to answer this question (Watanabe & Grundfest, 1961; Wiersma, 1947; Hama, 1961; Remler, Selverston & Kennedy, 1968; Larimer, Eggleston, Masukawa & Kennedy, 1971; Wine & Krasne, 1972). In the 6th abdominal (terminal) ganglion which has been suggested to consist of two fused embryonic ganglia (Bullock & Horridge, 1965), an 'extra LG' segment in addition to the ordinary 6th LG has been briefly reported (Johnson, 1924; Sigvardt, Hagiwara & Wine, 1982). In the present study, we reconfirmed the presence of the extra LG segment in the terminal ganglion by intracellular dye injection and recording.

The isolated abdomen of the crayfish (*Procambarus clarkii*) of either sex was pinned dorsal side up in a dissecting dish and immersed in cold van Harreveld's solution. After exposing the ventral nerve cord, the dorsal sheath of target ganglia and connectives was torn away. As the axon or main neurites of the 6th LG and extra LG segments have large diameters and run near the dorsal surface of the terminal ganglion, they could easily be seen and penetrated by microelectrodes under a binocular microscope. Glass microelectrodes filled with 3 M potassium acetate (resistance of 20–30 MΩ) were employed for intracellular recording and current injection, while for intracellular staining, electrodes filled with 5% Lucifer Yellow CH in 0·1 M-LiCl (resistance of 30–50 MΩ) were used (Stewart, 1978, 1981). To prevent the fading of fluorescence during fixation and clearance, 4% formaldehyde was added to the dye solution (M. Kanou & T. Shimozawa, personal communication).

Lucifer Yellow injection for 20–30 min into an extra LG in the terminal ganglion (Fig. 1A, asterisk) resulted in cross-migration of dye to the connected ordinary 6th LG (Fig. 1A, curved arrow). Dye injection into LGs in the terminal and in other abdominal ganglia also revealed that they are all coupled. With injection lasting less than 10 min and subsequent fixation, only the impaled extra LG segment was stained.

Key words: Crayfish, lateral giant interneurone, local neurone.

The extra LG is definitely shown to be an intraganglionic neurone (Pearson, 1979; Burrows, 1981), since all its structures – cell body, dendrites and main neurite – are confined in the terminal ganglion (Fig. 1B).

Fig. 2 shows the evidence for electrical coupling between the ordinary 6th LG and the extra LG. Such coupling was expected because of the dye coupling (Kaneko, Merickel & Kater, 1978; Spencer & Satterilie, 1980; Glantz & Kirk, 1981). Both cells, each simultaneously impaled by two intracellular microelectrodes for recording (V_1 and V_2, Fig. 2D) and for current injection (I_1 and I_2), had resting potentials of −70 to −80 mV. Injections (I_1) of constant outward and inward current produced depolarization and hyperpolarization of the extra LG segment (V_1), which spread electrotonically to the 6th LG segment (V_2, Fig. 2A). Action potentials evoked in the extra LG segment (V_1) produced an electrical coupling potential in the 6th LG segment (V_2, Fig. 2C), but did not evoke action potentials in that segment in our experiments. Transmission through this junction could be less than one to one, or transmission could have been inhibited by experimental damage. Evoked potentials in the 6th LG segment (V_2, Fig. 2B) also spread in the other way to the extra LG segment.

These results indicate that electrical coupling between the 6th and the extra LG

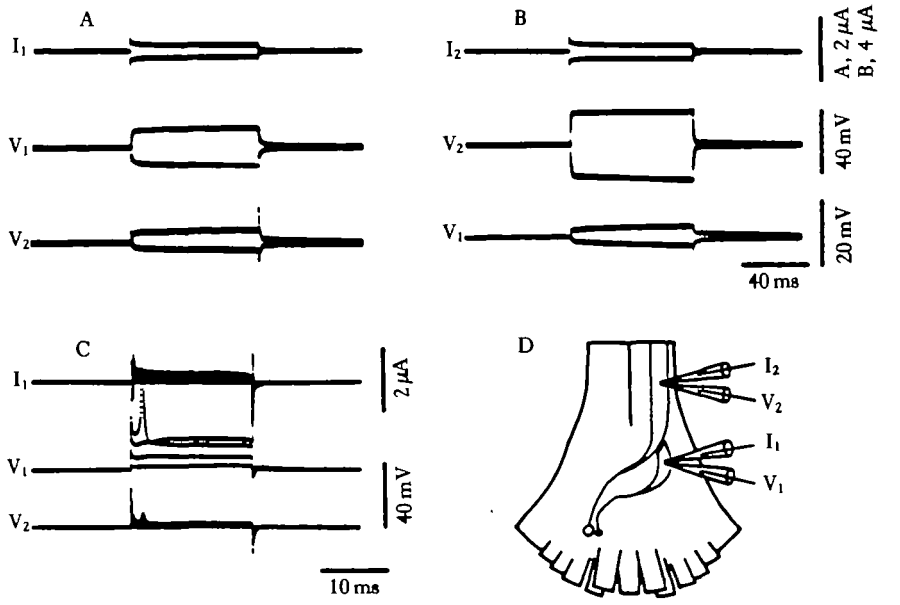

Fig. 2. Physiological evidence for electrical coupling between the extra LG and the ordinary 6th LG in the terminal ganglion. Current monitoring on top trace, upward and downward deflections denote depolarizing and hyperpolarizing currents, respectively. (A) Electrical coupling of the extra LG (I_1, V_1) to the ordinary 6th LG (V_2). (B) Electrical coupling of the ordinary 6th LG (I_2, V_2) to the extra LG (V_1). (C) Electrical excitability of the main neurite of the extra LG. Action potential evoked in the extra LG (V_1) produced the electrotonic excitatory postsynaptic potential in the ordinary 6th LG (V_2). (D) Schematic drawing of the experimental procedure: the extra LG was penetrated at the expanded neurite, while the ordinary 6th LG was penetrated at the giant axon. Each cell was impaled simultaneously with two microelectrodes for current injection (I_1, I_2) and recording (V_1, V_2).

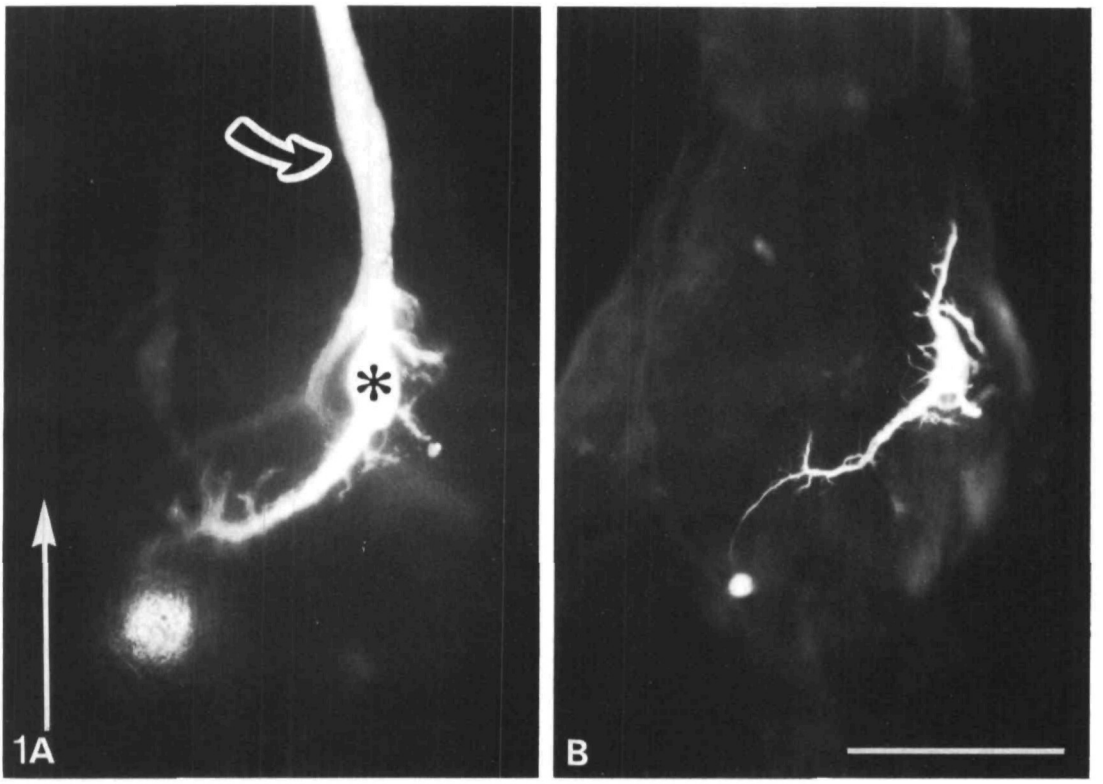

Fig. 1. Fluorescence microphotographs of Lucifer Yellow CH injected LGs in wholemounts of the terminal ganglion of the crayfish, viewed dorsally. Straight arrow indicates anterior. Scale bar: 400 μm. (A) Dye coupling between the ordinary 6th LG and the extra LG in fresh tissue. Lucifer Yellow injected iontophoretically into the extra LG (asterisk) for more than 30 min using hyperpolarizing current pulses (40–60 nA, duration of 500 ms, at 1 Hz) migrates to the 6th LG (curved arrow) across the septum. Their bilateral homologues were also weakly stained. (B) In spite of the dye coupling, only the extra LG could be stained by injection of dye for less than 10 min and subsequent fixation. Note that all its structures are restricted to the terminal ganglion.

segments is bidirectional, as it is between the 2nd and 3rd ganglia (Watanabe & Grundfest, 1961). This strongly implies that the extra LG segment is segmentally homologous to the ordinary LGs in other anterior segments.

To examine the extent of modification of this intraganglionic LG segment, we compared its morphology to that of the LGs of the anterior abdominal ganglia (Fig. 3). LG segments in the 3rd, 4th and 5th abdominal segments closely resemble each other. One of them is illustrated in Fig. 3A (see also Remler *et al.* 1968). In the terminal ganglion, the ordinary 6th LG (Fig. 3) is almost the same as the anterior ones, except for the characteristic large primary branch with relatively well-developed dendrites.

On the other hand, although its structures (i.e., contralateral cell body, expanded neurite and bilaterally arising short fine branches) are the same, the extra LG differs morphologically in many respects from other LGs (Fig. 3C). First, it has a small cell body (20–30 μm in diameter) which occurs somewhat medial to the cell body of the ordinary 6th LG. Second, in contrast with the other LGs whose giant axons extend to the next anterior ganglion, it has no axon in the connective or in any peripheral nerve. Moreover, it shows extensive variability from individual to individual. In some animals (e.g. Fig. 3D, E), a curious neurite originating from the expanded region extended anteriorly along the 6th segment LG's axon for a few hundred micrometers from the anterior edge of the terminal ganglion, but in no case ($N = 11$) did the neurite

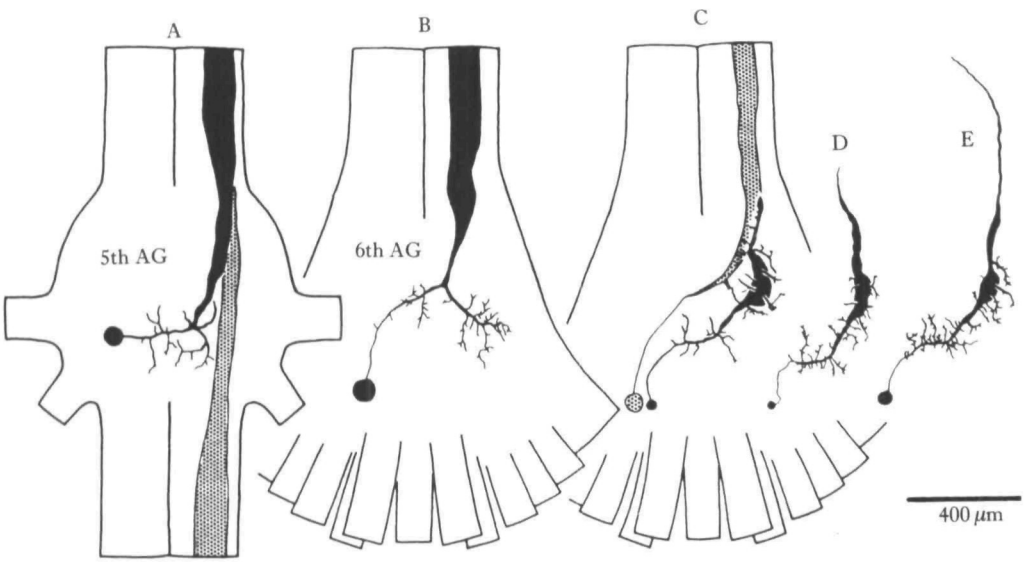

Fig. 3. Drawings of LGs viewed dorsally in the 5th and terminal ganglion of the crayfish, stained by iontophoretic injection of Lucifer Yellow CH and reconstructed from photomicrographs of dye-filled cells. Anterior is at the top. (A) The 5th LG in the 5th abdominal ganglion (5th AG), whose axon attaches to the posterior one (6th LG, dotted) to form the gap junction. (B) The ordinary 6th LG in the terminal ganglion (6th AG). (C) The extra LG in the terminal ganglion. Its anterior neurite, arising from the expanded region of the primary neurite, makes contact with the axon of the 6th LG (dotted cell). See also Fig. 1A, B. (D) and (E) Two other examples of the extra LG, showing the variability of its structure among individuals: a curious anterior neurite extensively varies in its length from animal to animal.

extend as far as the 5th abdominal ganglion. Thirdly, the expanded region of the main neurite, corresponding to the giant axon of the other LGs, has many branches extending ventrally.

The origin of local neurones has been established in DUM neurones of the locust metathoracic ganglion; the late-born progeny from the median neuroblast develop into non-spiking intraganglionic interneurones, whereas the first-born progeny develop into spiking neurones which send their axons into peripheral nerve bundles (Goodman, Pearson & Spitzer, 1980). In contrast, the extra LG appears to be converted secondarily and ontogenetically to a local type of neurone by ganglionic fusion, since its anterior homologues in unfused ganglia belong to the intersegmental type of neurone.

A number of premotor, non-spiking local interneurones involved in generating motor activity have been described in the fused terminal ganglion, as well as other unfused abdominal ganglia of crayfish (Heitler & Pearson, 1980; Takahata, Nagayama & Hisada, 1981; Reichert *et al.* 1983). These local neurones in unfused ganglia may be intrinsic. We can safely assume that in the fused ganglia there will also be some secondarily modified local neurones whose origin is the same as the extra LG.

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