

Morphology and physiology of giant fibres in the seventh abdominal ganglion of the Scorpion *Heterometrus fulvipes*

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Abstract. The morphology of giant fibres in the last metasomatic ganglion of the scorpion, *Heterometrus fulvipes*, was studied by anatomical methods, making use of cobalt chloride back-filling of the 5th metasomatic segmental nerve. The pattern of activation of these giant fibres was examined by electrophysiological methods. The functional organisation of these giant fibres is supposed to bear significance with reference to the neural and muscular activity of the last metasomatic segment and the consequent movement of the stinger, and is thus of survival value to the animal.

Keywords. *Heterometrus fulvipes* ; cobalt chloride technique ; abdominal ganglion ; giant motor neurons ; synaptic properties ; stinger.

1. Introduction

Advances in histological and electrophysiological techniques have permitted detailed analysis of anatomical and functional organisation of the nervous system of many groups of invertebrates. Eventhough arachnids are an important group of terrestrial arthropods, they have received a cursory attention compared to insects and crustaceans.

The earliest studies on the nervous system of scorpions (Newport 1843, Police 1902; Hanstrom 1923) gave descriptions of the internal anatomy of the brain and the abdominal ganglia. The studies of Babu (1965) and Babu and Venkatachari (1966) revealed the gross morphological and histological aspects of the central nervous system of the scorpion. They also reported that large cells of the seventh abdominal ganglion give rise to giant axons that control the movement of the stinger. However, detailed aspects concerning the cytomorphology of these neurons and their synaptic links have not been studied so far. The present investigation on these giant neurons is an attempt to elucidate the morphology by cobalt chloride technique, and the functional properties by electro-physiological methods.

2. Materials and Methods

Locally available scorpions, *Heterometrus fulvipes*, were used in the present study. The origin and distribution of nerves that innervate the 5th segmental muscles were carefully studied by dissections under binocular microscope and by methylene blue staining. The method described by Babu and Subhashini (1979) was adapted for back-filling of 5th segmental motor neurons by cobalt chloride (CoCl₂). The scorpion Ringer (Padmanabha Naidu 1967) was used for preparation of various solutions and during the study of electrical activity of the motor neurons.

Electrical stimulation and recording were done with paired platinum electrodes. Square-wave pulses were obtained from Grass S44 stimulator. The nerve potentials were fed through a Grass P5 preamplifier and displayed on a Tektronix 502A oscilloscope. Recordings were made by a Philips type PP1011 oscilloscope camera.

3. Results

The last metasomatic segment is large and differs from the other metasomatic segments in its anatomical organisation and innervation pattern, as it bears a stinger and two pairs of large muscles that control its movement (figure 1 : Dm; Vlm). The 5th metasomatic segmental nerve (figure 1 : 5n) which contains the giant fibres (figure 4 : Gf) innervates the 5th metasomatic segmental muscles. Stimulation of the 5th metasomatic segmental nerve or the large mass of musculature leads to the swinging action of the stinger.

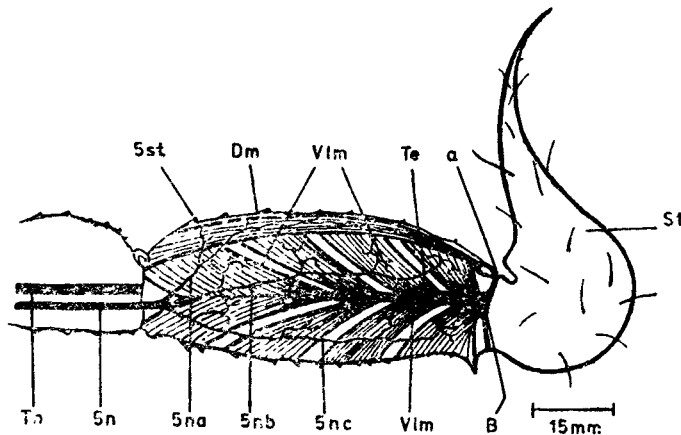


Figure 1. Schematic representation of the 5th metasomatic segment (5 St) of the scorpion showing the distribution of muscles and their innervation. The paired ventro-lateral muscles (Vlm) constitute the bulk of the 5th metasomatic segment and these are attached by a large tendon (Te) at point-B of the stinger (St). The contractions of these large muscles lead to an up- and downward movement of the stinger. The paired small dorsal muscles (Dm) are attached at point-a and the contractions of these muscles produce sideward movements of the stinger. 5n = Fifth metasomatic nerve and its branches (5na, 5nb, and 5nc). Tn = Telsonic nerve.

3.1. Giant motor neurons

Of the 150 ganglia that were attempted with CoCl_2 , 20% were successfully filled and these provided adequate information about the organisation of the giant motor neurons innervating the 5th metasomatic segment. In good CoCl_2 preparations the neurons appeared black in whole mounts with the surrounding nervous tissue transparent and pale yellow. When the 5th nerve was filled with CoCl_2 , two ipsilateral (figure 2 : G1 and G2) and two contralateral (figure 2 : G3 and G4) groups of motor neurons were located.

Among these four, the G1 group was the largest, consisting of 8–12 large cells that lie in the cellular rind extending ventrolaterally proximal to the 5th nerve (figures 2 and 5 : G1). The soma is ovoid to spherical ($50\text{--}62\ \mu\text{m}$) with a nucleus of $10\text{--}20\ \mu\text{m}$ in diameter. The soma gives off a short stem process ($13\ \mu\text{m}$) which ascends up mid-dorsally into the neuropile forming a neuropilar segment that gives off 10–16 primary dendritic branches (figure 5 : Dn) in the posterior part of the ganglion. Such a large number of primary dendritic branches in the neuropilar segment were also reported in other arthropods (Kennedy *et al* 1969; Davis

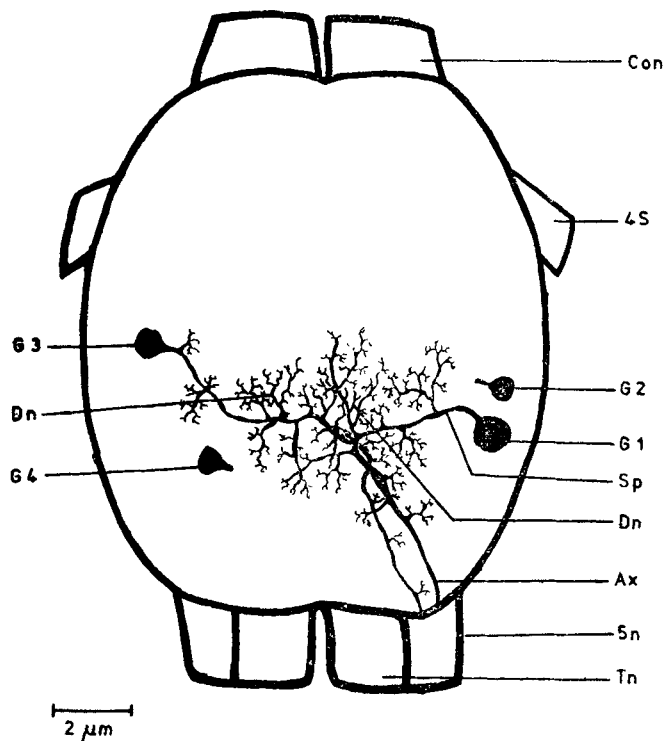


Figure 2. Camera lucida drawing as viewed from the dorsal side of the seventh abdominal ganglion of the scorpion showing the motor neurons. The back-filling was made through the 5th segmental nerve (5n). Two ipsilateral (G_1 and G_2) and two contralateral motor neurons (G_3 and G_4) enter the 5th segmental nerve as motor fibres. The axons arising from the G_1 group constitute the giant fibres. Ax = Axon; Con = Connective; Dn = Dendrites; Sp = Stem process; Tn = Telsonic nerve; 4s = Fourth segmental nerve.

1970; Tyrer and Altman 1974; Truman and Reiss 1976) by CoCl_2 or fluorescent dye technique. These primary dendrites in scorpion are thick and divide into several secondary, tertiary and even quaternary branches (figures 2 and 5 : Dn). The neuropilar segment takes a V turn in the neuropile and enters the ipsilateral 5th nerve as a giant motor fibre (figures 2 and 5 : Ax). As they emerge the fibres measure only $8\ \mu\text{m}$, but one mm away from the ganglion their size increases to $40\ \mu\text{m}$ in diameter (figure 4 : Gf). Thus these motor neurons are ipsilateral, since their soma, dendrites and axon are in the same half of the ganglion.

3.2. Sensory fibres

Figure 3 shows four different categories of ipsilateral sensory neuron projections in the 7th ganglion. The sensory fibres from the 5th nerve enter the neuropile and divide into a number of branches in areas of the dendritic neuropile of giant motor neurons (figures 3 and 6 : 5 Sn). The branching of these sensory neurons is extensive in the posterior region of the ganglion where small blebs of $1\text{--}3\ \mu\text{m}$ are seen.

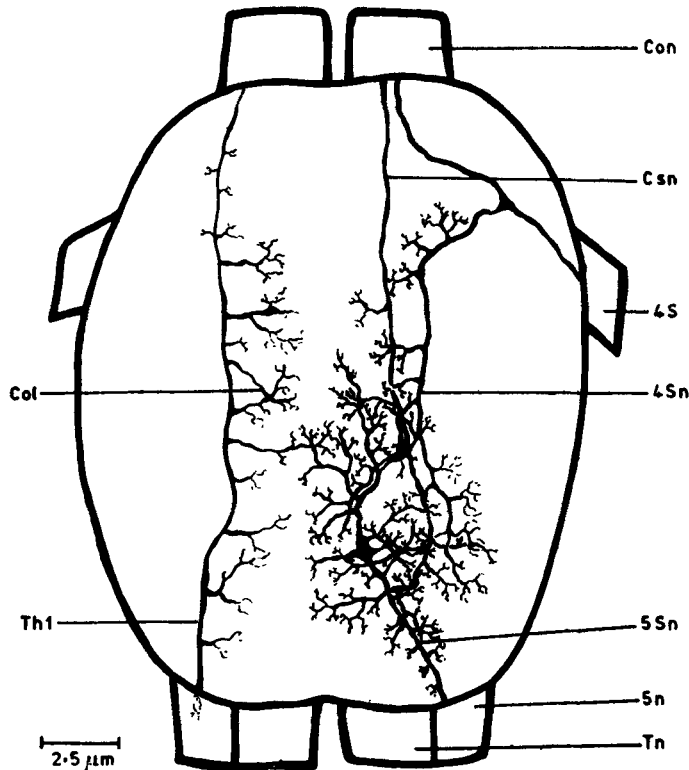


Figure 3. Camera lucida drawing as viewed from the dorsal side of the seventh ganglion of the scorpion showing the presynaptic terminations. This composite drawing was made from separate whole mount preparations where in each preparation the telsonic (*Tn*) or 5th (*5n*) or 4th (*4s*) or connective (*Con.*) was used for back-filling. Note the ipsilateral terminations of these fibres. Except the through fibre the rest of them terminate predominantly in the dendritic neuropile of the giant motor neurons. Note the sensory neurons from 5th nerve (*5sn*), 4th nerve (*4Sn*) and terminations from connective (*Csn*). *Th1* = Through fibre. *Col.* = Collateral.

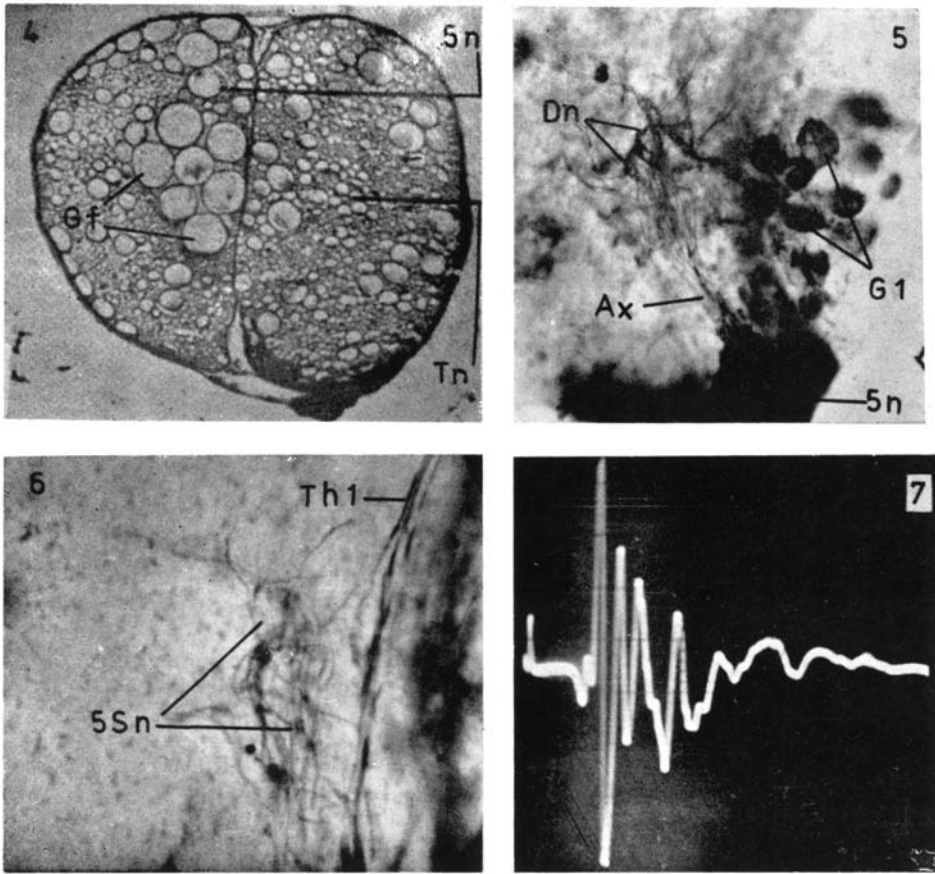


Figure 4. Transverse section of the 5th metasomatic segmental (*5n*) and telsonic (*Tn*) nerves showing the giant axons. $\times 400$.

Figure 5. G1 group of motor neurons showing the cell bodies, dendritic branches and axons entering the 5th nerve. Back-filling was done through the 5th nerve. $\times 128$.

Figure 6. Sensory terminations in the seventh ganglion arising from the 5th nerve (*5Sn*). *Th1* = Through fibres. $\times 128$.

Figure 7. Electrical activity recorded from 5th nerve branch 'b' while stimulating the ipsilateral connective of the ventral nerve cord. The first, large compound action potential represents the giant fibre activity while the subsequent three peaks represent the activity of slower fibres. The stimulus artifact is seen at the beginning of the sweep. Calibration : 2 msec/cm.

Sensory neurons from the 4th metasomatic segmental nerve also enter the 7th ganglion. The primary axon of the sensory neuron enters the ipsilateral connective, while it also gives off branches (4–6 μm) which extend posteriorly and terminate near the dendritic neuropile of the final motor neurons (figure 3 : 4Sn).

Descending ipsilateral presynaptic terminations are also observed from the connective (figure 3 : Csn). These axons in the 7th ganglion move posteriorly and mid-dorsally where they branch extensively near the region of the dendritic neuropile of the giant motor neurons. Along their length these axons give off branches at various regions of the ganglion. Besides these, there are also sensory projections from the telsonic nerve which branch extensively and terminate in the vicinity of dendritic neuropile of giant motor neurons.

Another category of sensory projections, which are primarily through fibres, was also noticed (figures 3 and 6 : Th1). As these fibres pass through the ganglion, they give off collaterals (figure 3 : Col) towards the central axis of the ganglion along their entire course.

3.3. *Transmission through the seventh ganglion*

Recordings were made from 5th nerve while stimulating the connectives, 4th segmental and telsonic nerves ipsilaterally and contralaterally.

Ipsilateral stimulation of the connective elicited several peaks of large action potentials (figure 7) in 5na, 5nb and 5nc branches of the 5th nerve. The thresholds, ganglionic delays and conduction velocities for the fastest peak were almost equal in all these three branches. The fastest spike conducts at 6 to 7.0 m/sec and has the lowest threshold (2.2 V). The ganglionic delay ranged between 0.5 to 1.0 msec. Critical alteration of thresholds has shown that the fastest spike of 5na-branch consists of 3–4 giant fibres, 5nb-branch 4–5 fibres and 5nc-branch 3–4 fibres. Thus the fast giant axons are distributed on the large musculature of the 5th metasomatic segment.

Ipsilateral stimulation of 4th segmental and telsonic nerves also evoked similar compound action potentials in the 5th nerve. The conduction velocities of the fastest spikes varied between 6–7.0 m/s. These spikes can be elicited at a threshold of 2 V and the ganglionic delays were of 0.5–0.8 msec. These results show that ipsilateral stimulation of connective, or 4th segmental or telsonic nerves activates the giant fibres of the 5th segmental nerve, apart from slow conducting fibres. These synaptic junctions appear to be quite labile since transmission fails quickly even at low frequency presynaptic stimulation.

Contralateral stimulation of preganglionic fibres and recordings from 5th nerve has shown a different pattern of activity. Stimulation of either a connective or 4th or telsonic nerve and recording from any branch of 5th nerve showed only small compound action potentials. The velocity of the fastest spike ranged from 3 to 4 m/s and the threshold values varied from 6 to 10 volts. The ganglionic delays were also much longer and these varied from 3 to 5 msec.

4. Discussion

The foregoing results demonstrate several characteristic features in the functional organisation of the central nervous system of the scorpion, *H. fulvipes*.

Both anatomical and physiological studies show monosynaptic junctions between the giant motor fibres and ipsilateral presynaptic terminations from connective, 4th and telsonic nerves of the seventh abdominal ganglion. These sensory fibres, after extensive branching in the ganglion, terminate predominantly in the dendritic neuropile of the giant motor fibres. The small blebs found in these regions of the neuropile were also reported in several insects (Strausfeld 1971; Pitman *et al* 1973; Tyrer and Altman 1974) and these were considered to be the synaptic regions. These anatomical evidences suggest a direct link between these sensory and motor fibres of the 5th segmental nerve and can be considered as axo-dendritic type of synapses. Thus the sensory input from different sources converges mostly on the dendritic neuropile of G1 and G2 groups of neurons and to some extent on the G3 and G4 groups also.

The central representation of sensory input is of fundamental importance since it determines the response pattern of motor and interneurons. In the present CoCl_2 preparations, two types of sensory terminations were identified; (1) those that terminate ipsilaterally in the 7th ganglion, (2) those that are through fibres but give off collaterals in the ganglion.

The former type of primary sensory neurons is common in insects (Fielden and Hughes 1962; Mill 1963; Murphey *et al* 1974; Tyrer and Altman 1974; Babu and Subhashini 1979) and crustaceans (Wiersma *et al* 1955; Wiersma 1958; Wiersma and Hughes 1961; Wiersma and Bush 1963). The electrophysiological studies of Sanjeeva Reddy and Rao (1970) on the scorpion had shown that primary sensory neurons give off collaterals as they pass through the 7th ganglion. They demonstrated that these collaterals activate contralateral interneurons. Thus these fibres correspond to the latter type of sensory fibres mentioned above. It is apparent that activation of motor neurons takes place by sensory neurons terminating ipsilaterally in the ganglion, while the contralateral neurons are activated by the collaterals of the through sensory fibres.

The electrophysiological evidences confirm the existence of monosynaptic junctions between the giant motor fibres and ipsilateral sensory terminations. The lability, short delays and low thresholds of giant fibres elicited on ipsilateral stimulation are properties of monosynaptic pathways. Thus these neurons act as final motor neurons ensuring fast and quick relay of responses to the target organs. Similar monosynaptic pathways are characteristic of other giant fibre systems (Roeder 1948; Bryant 1959; Kennedy *et al* 1969).

Another significant feature is the interrelationship of giant fibres with sensory input from contralateral region. The cobalt fillings clearly demonstrate only ipsilateral sensory projections implying the absence of any direct sensory contact with motor fibres in the other half of the ganglion. The high lability of the electrical response, high thresholds, long delays and slow conducting responses indicate absence of direct contact between motor fibres of one side and sensory terminations of the other half of the ganglion. These properties imply the occurrence of one or more interneurons between the sensory and motor fibres. Further, the contralateral sensory input activates only the slow conducting neurons but not the fast giant fibre system. Sanjeeva Reddy and Rao (1970) had made similar suggestions and demonstrated through electrophysiological studies that the collaterals of through fibres mediate such responses,

The giant motor neurons of the scorpion are typically monopolar and these are characteristic of invertebrate nervous systems (Bullock and Horridge 1965). Different categories of giant neurons were reported in both invertebrates and vertebrates. Unicellular giant interneurons were demonstrated for locusts (Cook 1951), crickets (Murphey 1973), cockroaches (Pumphery and Rawdon-Smith 1937; Roeder 1948; Babu and Subhashini 1979), crayfish (Johnson 1924), loligo (Young 1939) and bony fishes (Stifanelli 1951). A somewhat different category of unicellular giants which are segmental in origin and whose axons fuse to form the lateral and median giants of earthworm (Mullony 1970) and lateral giants of crayfish (Remlar *et al* 1968) was reported. In contrast to these, certain giant fibres of cephalopods and polychaetes are multicellular in origin (Young 1936; Nicol 1948).

The high conduction velocities (6–7 m/s) of the giant fibres in the scorpion are comparable to giant fibres found in other invertebrates. Among annelids, *Neanthes* giant fibres of 15–37 μm diameter conduct at 4 to 5 m/s; *Myxicola* giant fibres of 100–1000 μm conduct at 6–20 m/s. In arthropods 70 μm large fibres in the cord of *Homarus* conduct at 7 m/s; *Callianassa* 35–40 μm giant fibres conduct at 6.0 to 7.5 m/s; *Periplaneta* giant fibres of 10–40 μm conduct at 6–9 m/s. In molluscs, the *Sepia* giant fibres of 35 to 200 μm conduct at 3–8 m/s. A more comprehensive data on the size and conduction velocities in different groups of animals was given in Bullock and Horridge (1965).

Thus the giant motor fibres in the 7th ganglion integrate sensory input impinging on them monosynaptically from intra- and intersegmental body regions of the scorpion. The activated giant fibres, because of their high conduction velocities and large spike potentials, bring about quick and synchronous contraction of the large mass of musculature in the 7th metasomatic segment, resulting in movement of the stinger, whose actions have survival significance to the animal.

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