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NERVE PATTERN OF EXTENSOR TIBIAE MUSCLE OF HIND LEG OF AK GRASSHOPPER, *POEKILOCERUS PICTUS* FABRICIUS (ORTHOPTERA: PYRGOMORPHIDAE)

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ABSTRACT

Insect nervous system has been used as a model to study the neural basis of behavior. However, this study is still concentrated on a very few species of locust. The present work was planned to explore the phenomenon in Ak Grasshopper (*Poekilocerus pictus* Fab.) to answer that how many muscles are located in its hind femur and what is their innervation pattern. For that, hind legs of adult Ak grasshopper were dissected. The innervation pattern of extensor tibiae muscle was investigated by the anterograde filling of main leg nerve with 2% Nickel Chloride. Results showed a similar pattern to *Locusta migratoria* as hind femur in Ak grasshopper has extensor and flexor tibiae muscles which are innervated by branches of N5. It passes into the gap between flexor and extensor tibiae and follows the line of the extensor apodeme. After entry into the femur, it gives off branches which enter the large fan shaped outer bundle and dorsally to supply proximal bundles. This type of study could be helpful in devising a controlling strategy for jumping insects.

Keywords: Anterograde, Ak Grasshopper, Extensor tibiae, Hind leg, Nerve

INTRODUCTION

Insects are major element in the biotype and they impinge upon almost all aspects of our lives, often with drastic effects. The insect nervous system has been widely and successfully used as a model system in the study of the neural basis of behavior. Understanding how they work is an essential step to our living in harmony with them (Holye, 1975; Burrows, 1996). Particularly the neuromuscular system of locust; controlling walking and jumping has received considerable attention. For that the locust metathoracic extensor tibiae muscle was one of the most closely studied insect muscle for innervation, physiology and neural control (Hoyle, 1955a, 1978; Hoyle and Burrows, 1973; Burrows and Hoyle, 1973; Heitler and Burrows, 1977a,b; Durr and Matheson, 2003).
The basic innervation and neuromuscular physiology of the acridid extensor tibiae (ETi), which is a jumping muscle, was first described by Hoyle (1955a,b) and continued to be a major target for the study of insect neuromuscular physiology (Usherwood, 1967, 1977; Hoyle, 1975; Zakotnik, 2006). Locust has six legs and each consists of five parts; a short proximal coxa, a small trochanter that in the front legs can move relative to the femur, a tibiae with the same length as the femur, and a distal tarsus that is divided into a series of moveable parts, with a terminal claw or unguis. The disparity in the sizes of the legs is such that the mass of the femur of a hind leg is about ten times that of a femur of front leg; this is associated with the specialization of the hind legs for jumping. Each of leg muscle in the body is innervated by only a small number of neurons ranging from one to about nine. Despite the disparity in mass, the flexor tibiae are innervated by nine excitors and two inhibitors while the extensor tibiae are innervated by only two excitors and one inhibitor (Burns and Usherwood, 1978; Sasaki and Burrows, 1998).
However, ongoing study of insect neuromuscular physiology is still concentrated on a very few species. Our aim was to extend this work for other insect species especially grasshoppers which are solitary phases of locusts to find out more general issues about their control strategies. The present work planned to answer the following questions in Ak Grasshopper: How many muscles are located in the hind femur of Ak Grasshopper and what is their innervation

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pattern? To which extent the structure and innervation patterns are correlated to other species?

MATERIALS AND METHODS

Ak Grasshopper, *Poekilocerus pictus* were collected from the field and brought to the laboratory at Govt. College University, Faisalabad, Pakistan. Animals were acclimatized before dissection and kept in the refrigerator for 20 minutes to anesthetize by cooling at 0°C (Personiusa and Chapman, 2002). Locust ringer solution was prepared by following the recipe of Clements and May (1974). Insects were mounted in wax filled petri dishes. Extensor tibiae muscles and nerves were made visible by removing the cuticle and subsequently clearing the underlying tracheae and fatty tissues. Locust ringer solution was poured into dishes and frequently changed during dissection.

Nickle Chloride staining

The innervation pattern of extensor tibiae muscle was studied by filling it in anterograde fashion with 2% $NiCl_2$ solution. The exposed nerve was cut at suitable length and a Vaseline® chamber was made near the cut ends using Vaseline® filled syringe and was exposed to distilled water for 1-2 minutes to open the cut ends of nerves. After that chamber was filled with few drops of 2% $NiCl_2$ solution and was sealed with Vaseline®. Preparations were incubated over nightly at 4°C in a refrigerator. After incubation, Vaseline® chamber was removed and preparations were rinsed for 2-3 times with ringer solution and few drops of rubeanic acid was poured into Petri dishes. A bluish color precipitation was developed after 10-15 minutes (Sakai and Yamaguchi, 1983). Now preparations were fixed for 2 hours in formaldehyde (4% in distilled water) at 4°C in a refrigerator. After fixation, extensor tibiae muscle and metathoracic ganglion were carefully removed. Now tissues were dehydrated for 5 minutes in each ascending series of 30, 50, 70, 80, 90 and 100% of Isopropanol. Photographs of freshly dissected preparations and Nickel chloride stained preparations were taken by a high resolution digital camera attached to a stereomicroscope. Further, graphics editing software (Adobe Photoshop CS3, Adobe system, San Jose, California, USA) was used to adjust brightness, contrast and to convert false colors of images. Line drawings were also made.

RESULTS AND DISCUSSION

Morphology of hind leg

Hind legs of grasshopper are different from the meso- and prolegs in design as the metalegs are directly involved in jumping and their design is exactly according to their function. Femur of the hind leg is greatly enlarged with herring-bone like markings which show the insertion points of extensor muscle (Fig. 1a). It is dorso-ventrally expanded to accommodate large extensor tibiae muscle and has more mass of the femora as compared to the front or middle legs. The ability of femur to with stand longitudinal loads is directly related to series of longitudinal ridges in femur as seen in the hexagonal cross section view (Fig. 1). Tibiae of the hind leg are long and thin tubular construction with the twice length of the tibia of middle leg but have the same diameter. On the dorsal side of the tibia, a double row of spines is present which increase its stiffness to a certain point (Fig.1b, c). Internally, it contains only trachea, nerves and the small muscles that facilitate the tarsus to move.

Musculature of Femur

The two big muscles of the hind leg are extensor and flexor muscles. We have reinvestigated the morphology and innervations of extensor tibiae muscle in *Poekilocerus pictus* and described their origin, size, and shape of the muscle. The muscle fibers are arranged pinnately and their length is variable depending on the shape of the femur. They are attached to the long, deep, and spatulate extensor apodemes.

Extensor Tibiae muscle of hind leg

The extensor tibiae muscle is dorsally placed and occupies the greater part of the femur.

The point of attachment of muscle fibers with apodemes is externally visible in the form of patches on the cuticle (Fig. 1a). Each corresponding patch is named muscle unit which is a group of the muscle fibers that receive a single, discrete branch of a nerve supply and a similar discrete branch from the tracheal system (Fig. 2). Two rows of muscle units are present placed one above the other. The apodeme narrows sharply in the distal part of the femur to which this muscle attaches (Fig. 2).

Innervations of extensor tibiae muscle of hind leg

Insect nervous system consists of central, peripheral and stomatogastric nervous system. Central nervous system consists of the brain and ventral nerve cord. The brain receives the sensory input from the sense organs of the head and from more posterior ganglia via ascending interneuron. Ventral nerve cord consists of a chain of bilaterally symmetrical segmental ganglia involved in controlling feeding, walking, jumping and flying of insect.

The large crural nerve trunk (N5) is regarded as the motor nerve supplying the extensor muscle. Reinvestigation of its branching pattern in *Poekilocerus pictus* using nickel chloride staining revealed no variation in its branching pattern and innervation of extensor tibiae musculature (Fig. 3). Each N5 originates latero-posteriorly from the metathoracic ganglion and bifurcates in coxa. After passing into the femur, each the motor nerve gives off branches to the proximal, dorsal portion of the extensor muscle and then passes through its ventral surface close to the mid line (Fig. 3). The single motor nerve trunk gives off irregular branches on both sides and supply to the lower and upper rows of muscle units.

Innervation of extensor tibiae muscle in the hind leg of *Poekilocerus pictus* was the ultimate aim of this work. Femur of hind leg consists of two muscles: extensor tibiae muscle and flexor tibiae muscle. The mass of the extensor muscle is much more than the flexor muscle. Holye (1955, 1978) described the anatomy and neuromuscular physiology of extensor tibiae muscle in *Locusta migratoria.* We had reinvestigated the innervations of extensor tibiae muscle in *P. pictus* and described their origin, size, and shape of the muscle. The muscle fibers were arranged pinnately and their length is variable depending on the shape of the femur as was in the case of *Locusta migratoria* studied by Holye (1955, 1978). Snodgrass (1928) first described the extensor tibiae muscle of hind leg in Carolina locust and numbered it M135, and its origin was anterior and dorsal femur and the insertion was tibia. A similar pattern of origin and insertion was observed in *Poekilocerus pictus* in the current study.

Years later, the basic innervation and neuromuscular physiology of extensor tibiae muscle in migratory locusts were described by Hoyle (1955a, 1955b), and our observation in *Poekilocerus pictus* are in accordance with that study. From the coxa, a single large nerve trunk enters the femur, (which is formed by the fusion of nerve 3b and major branch 5b of nerve 5). These both nerves are from the metathoracic ganglion. Within the femur, the nerve divides into several branches to innervate various structures.

This repeated branching pattern of a common nerve supply marks the compartmentalization; the phenomenon observed in *Locusta migratoria* and *Schistocera gregaria* where extensor tibiae muscle was found to be innervated by slow and fast motor neurons. Ventral nerve cord consists of a chain of bilaterally symmetrical segmental ganglia involved in controlling feeding, walking, jumping and flying of insect. Stomatogastric nervous system consists of unpaired frontal and hypocerebral ganglia and paired ingluvial ganglia (Hoyle, 1955a, 1955 b).

The large crural nerve trunk (N5) is regarded as the motor nerve supplying the extensor muscle (Cook, 1951; Albrecht, 1953). Reinvestigation of its branching pattern in *Poekilocerus pictus* using nickel chloride staining revealed no variation in its branching pattern and innervation of extensor tibiae musculature. Each N5 originates latero-posteriorly from the metathoracic ganglion and bifurcates in coxa.

In invertebrates ultra-structure, physiological and biochemical properties of muscle fibers have been greatly affected by innervation (Close, 1972). Several combinations of innervation differences with four distinct axons innervating the extensor tibiae are possible. However, an arthropod muscle that is strongly differentiated into slow and fast muscle fibers, receives a single axon (Dorai-Ray, 1964), so muscle fiber differentiation can occur independently of innervation differences. However, the further electrophysiological investigation might reveal such phenomenon in our observed animal.

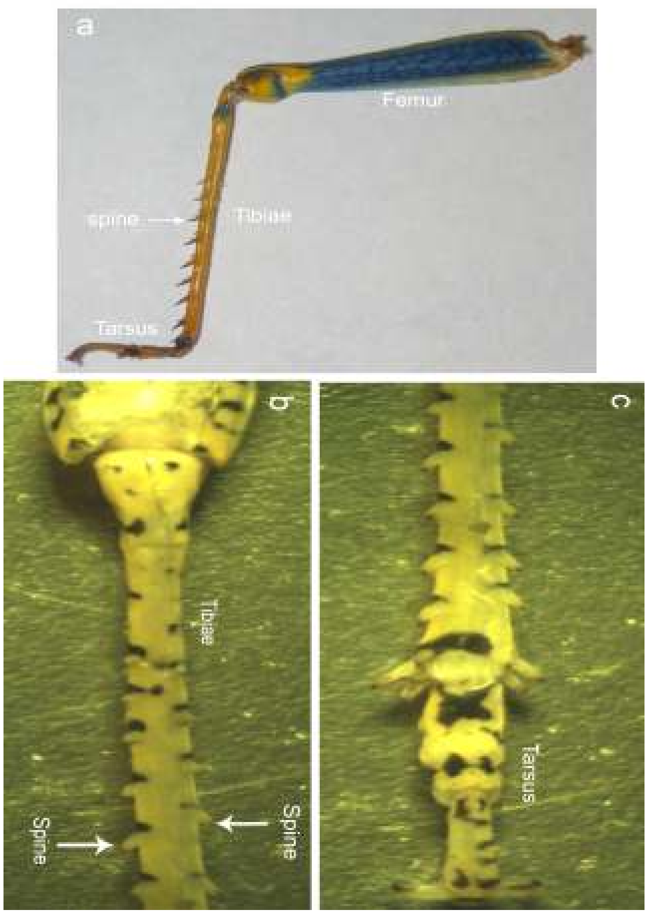

Fig 1.

Photographs of the hind leg of Ak Grasshopper showing external morphology. (a) Outer lateral view (b) ventral view of tibiae showing a double row of spines (c) ventral view of three tarsal segments with a claw at their end.

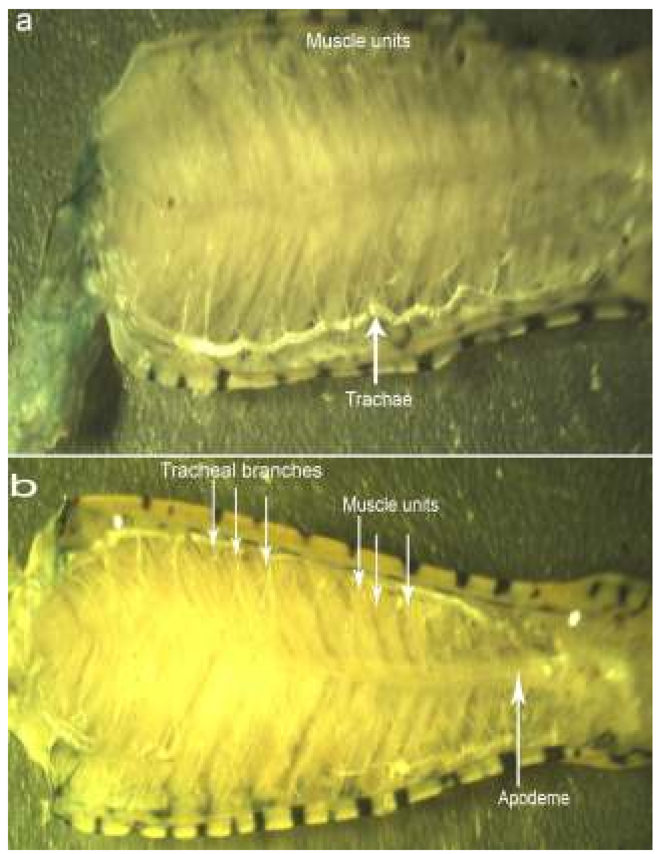

Fig 2.

Photographs of Musculature of Femur. (a, b) Photographs of extensor tibiae muscle taken by opening the femur from its ventral mid line show different muscle units arranged at common tendon. Each muscle unit is supplied by branches of trachea and nerve.

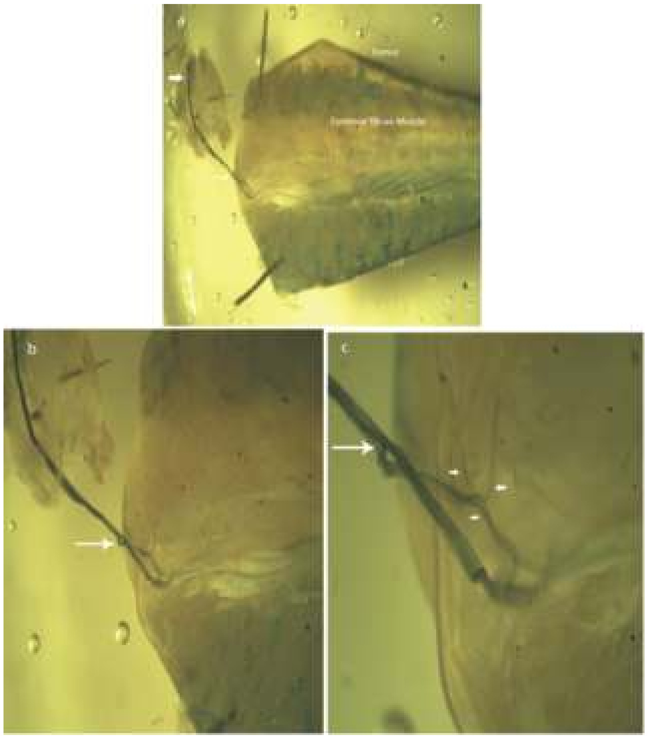

Fig 3.

(a-c) Photographs showing the innervation of extensor tibiae muscle, made after anterograde filling of N5 with 2% $NiCl_2$. Thick arrows mark anterograde filling of N5. Thin arrows mark the bifurcation of N5 in coxa that enters into the femur. (b, c) are photographs of (a) at higher magnification which show branching of one motor nerve (small arrows) to the proximal, dorsal portion of the extensor muscle while other passes with its ventral surface close to the mid line.

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