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PROPERTIES OF DESCENDING DORSAL UNPAIRED MEDIAN (DUM) NEURONS OF THE LOCUST SUBOESOPHAGEAL GANGLION*

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A group of six dorsal unpaired median (DUM) neurons of the suboesophageal ganglion (SOG) of locusts was studied with neuroanatomical and electrophysiological techniques. The neurons are located posteriorly in the SOG and have axons that descend into the ganglia of the ventral nerve cord, some as far as the terminal abdominal ganglion. Within thoracic ganglia the neurons have profuse dendritic ramifications in many neuropiles, including ventral sensory neuropiles. Based on their projection patterns three different morphological types of neurons can be distinguished. These neurons receive excitatory inputs through sensory pathways that ascend from the thoracic ganglia and are activated by limb movements. They may be involved in the modulation of synaptic transmission in thoracic ganglia.

Keywords: Neuromodulation – insect – DUM neuron – octopamine – biogenic amine

INTRODUCTION

The large octopaminergic, efferent, dorsal unpaired median (DUM) neurons of locusts are the most intensely studied neuromodulatory cells of insects [reviewed in 5]. These neurons are located in each ganglion of the ventral nerve cord. In thoracic and abdominal segments they supply the segmental musculature, particular proprioceptors, and some also form neurohaemal ramifications on the surface of peripheral nerves [3, 4, 6].

In the suboesophageal ganglion (SOG), however, the 6–7 efferent DUM cells do not supply peripheral targets in the mouthparts as would be expected from their metameric origin. Instead, one group of these neurons send ascending axons into the cervical connectives to supply targets innervated by peripheral nerves of the brain. A second group, also has ascending axons that supply the principal neuropiles of the brain such as mushroom bodies, central complex and antennal lobes. While the mor-

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phology of both groups has been described in detail [1, 2, 3] a third group of six DUM neurons in the SOG has, however, not been investigated further since their first description [2, 9]. The somata of these neurons are located posteriorly in the ganglion and their axons descend into the ganglia of the ventral nerve cord.

Our study was motivated by previous findings that octopamine can modulate central circuits in locusts. Such effects range from the modulation of identified monosynaptic connections [10] to the recruitment of specific motor patterns [11, 12]. The identity of the octopaminergic neurons responsible for these effects *in vivo* is still an open question. We realized that the DUM neurons with axons descending from the SOG might be one possible group. Our initial stainings showed profuse dendritic ramifications in many neuropile regions of the prothoracic ganglion and this lead us to pose the following questions: How far posteriorly in the nervous system do these neurons project? Are there distinct morphological types within this group as observed with other DUM cells? Do they participate in the modulation of central synaptic connections in thoracic ganglia?

MATERIALS AND METHODS

Adult *Locusta migratoria* and *Schistocerca gregaria* were obtained from crowded laboratory cultures. Preliminary experiments showed no obvious interspecies differences in the organisation of the neurons studied here. To trace the axons pathways of these DUM neurons, the chain of ganglia from the suboesophageal ganglion to the 4th abdominal ganglion was dissected, pinned dorsal side up into a Sylgard-lined dish, and covered with locust saline. The main tracheal trunks supplying the CNS were left attached to the ganglia and opened to the air. In some experiments parts of the hind legs were also left attached. Motor neurons in the metathoracic ganglion were identified by antidromic stimulation of appropriate muscles or nerves in the hind leg. DUM cells of the SOG were identified by their characteristic soma position and their large soma spikes. Methods for recording and staining locust neurons have been described in detail in previous publications [3, 7].

RESULTS

Morphology

Some 30 neurons were stained successfully so that their dendritic ramifications were visible in the SOG, the prothoracic ganglion and, in a few exceptional cases, in the mesothoracic ganglion. By morphological criteria three types of neurons can be distinguished (two examples are shown in Fig. 1A). In exceptionally well-stained preparations the axons could be followed as far as the 2nd abdominal neuromere of the metathoracic ganglion (abdominal ganglia 1–3 are fused with the third thoracic gan-

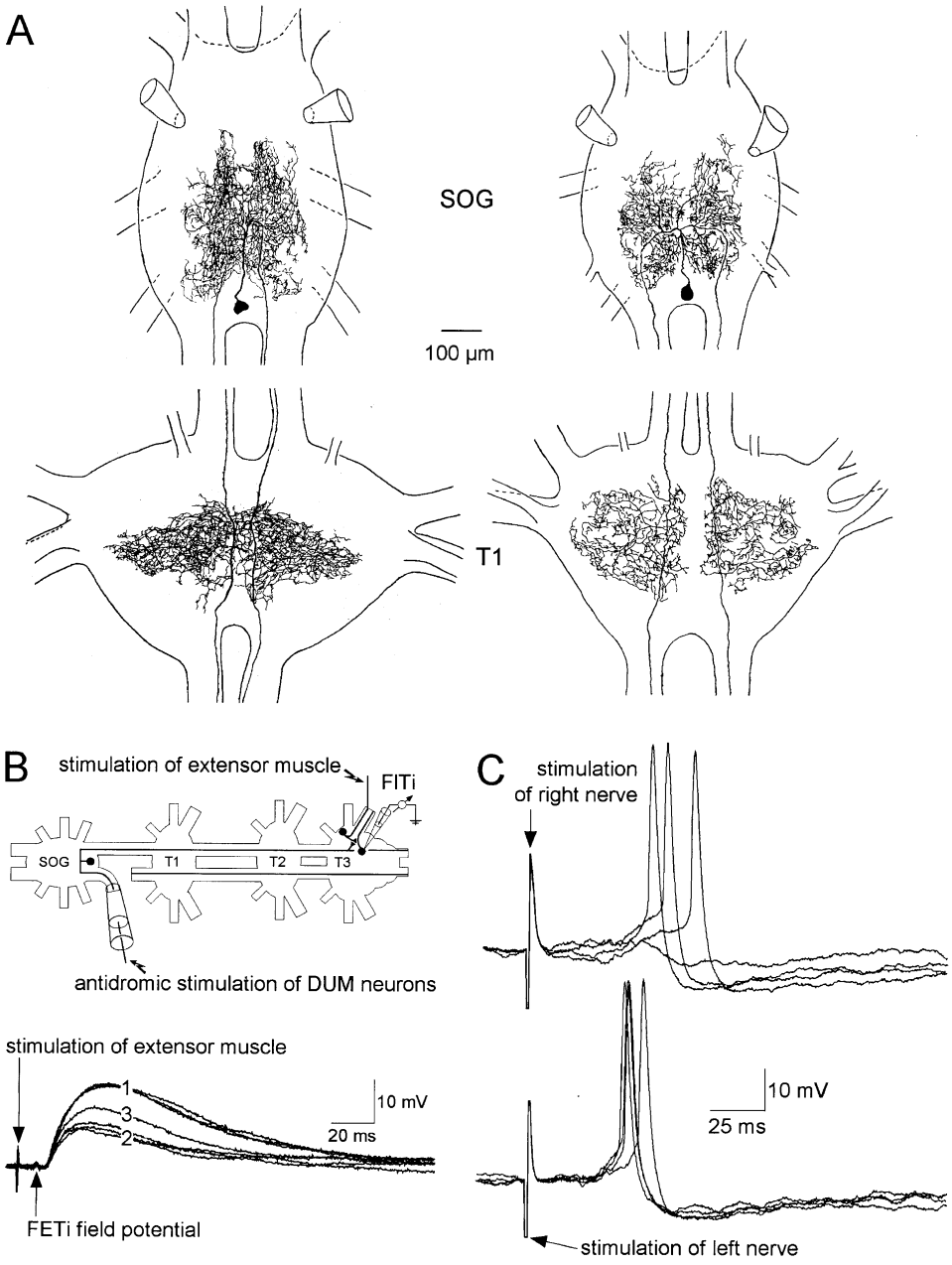
glion in locusts) where staining faded. In somata of the DUM cells in the SOG, however, it was possible to record antidromic spikes elicited by electrical stimulation of the connectives just in front of the terminal abdominal ganglion. These antidromic spikes followed the stimulus in a one-to-one fashion. Such experiments (data not shown) indicated that at least four and perhaps all of the DUM cells send axons through the entire ventral nerve cord. Their morphology suggests that they may have dendritic ramifications in the third thoracic and all abdominal ganglia.

Do the descending DUM neurons modulate central circuits?

To test the hypothesis that the SOG DUM neurons descending into the neuropiles of thoracic ganglia might represent a possible natural source for octopaminergic modulation of central synaptic connections, we modified the isolated nervous system preparation so that both hind legs were still attached. Stimulation of the extensor tibiae muscle allowed the identification of the fast extensor tibiae motor neuron (FETi) and evoked a well-characterised monosynaptic connection between FETi and flexor motor neurons [7]. Cutting a neck connective contralateral to the somata of the metathoracic motor neurons and stimulating the stump attached to the SOG activated the descending DUM cells antidromically (Fig. 1B). Such stimulation caused a reversible depression of the synaptic connection between the FETi motor neuron and flexor motor neurons (Fig. 1B).

SOG DUM cells receive excitatory inputs from the legs

Stimulation of a hind leg extensor muscle causes movements of the tibia and each stimulus caused a barrage of excitatory postsynaptic potentials (EPSPs) in DUM cells of the SOG. Similar effects can also be evoked by stimulating particular leg nerves on both sides of the body (Fig. 1C). Nerves innervating prominent mechanoreceptive sense organs such as the femoral chordotonal organ or the subgenual organ were ineffective. We have not yet been able to identify the exact source of this excitation. In principle there are two possible pathways. First, sensory neurons of the leg are connected to interneurons within the third thoracic ganglion that ascend towards the SOG. In the metathoracic ganglion we have found interneurons with ascending axons that respond to the same stimuli, but none that make direct connection to the SOG DUM cells. Second, neurobiotin backfills from those leg nerves which cause excitation in the DUM cells when stimulated show a few intersegmental afferent projections which ascend at least as far as the SOG. Such afferents might also cause excitation of the DUM cells, most likely mediated by local interneurons within the SOG.


A
SOG
100 µm
T1
B
stimulation of extensor muscle
FITi
SOG
T1
T2
T3
antidromic stimulation of DUM neurons
stimulation of extensor muscle
1
3
2
10 mV
20 ms
FETi field potential
C
stimulation
of right nerve
10 mV
25 ms
stimulation of left nerve

DISCUSSION

Our morphological characterization of the six descending DUM cells of the SOG shows three distinguishable types. Since we stained neurons at random and encountered each type with approximately the same frequency (each type was stained in 10 out of 30 preparations) most likely each type is represented by two morphologically similar neurons. This is not an unusual finding, since, within the SOG, for instance, there are two efferent DUM cells that send axons into the antennal nerves, the frontal connectives, the NCC III and the tritocerebral ventral nerve [1, 3]. Morphologically these two neurons are indistinguishable from each other. Likewise many efferent DUM neurons of the metathoracic ganglion cannot be distinguished from each other by morphological criteria [3, 8].

Evidence presented previously indicates that all six descending DUM cells are octopaminergic [2]. Another study [14], using antisera directed against octopamine, showed that ventral neuropiles of thoracic ganglia contain numerous immunoreactive profiles. The segmental efferent DUM neurons of thoracic ganglia do not project to these ventral sensory neuropiles. The DUM cells descending from SOG could contribute to such profiles. Some of them supply ventral, sensory neuropiles in the first and second thoracic ganglia and it is likely that they do so in other ganglia of the ventral nerve cord as well because some project to the terminal abdominal ganglion. The only other known potential source for octopamine-immunoreactive profiles in ventral neuropiles are the paired ventral cells observed in the thoracic ganglia [13].

Possible modulation of central synaptic pathways by descending DUM cells

Bath application of octopamine reduces the amplitude of EPSPs in flexor motor neurons caused by the monosynaptic connection from the FETi motor neuron in the metathoracic ganglion [7, 10]. Stimulating descending DUM cells of the SOG by antidromic activation from the neck connective caused a similar depression of this

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Fig. 1. Properties of descending SOG DUM cells. **A:** Two different morphological types of DUM cells of the locust suboesophageal ganglion (SOG) with descending axons. Their morphology in the suboesophageal and prothoracic ganglia is shown. **B:** Excitatory postsynaptic potentials (EPSPs) recorded from a hind leg flexor tibiae motor neuron (FlTi). These EPSPs are caused by spikes in the fast extensor tibiae motor neuron (FETi) that is antidromically activated by stimulation of the extensor muscle at 0.5 Hz. Activation of the DUM cells in the SOG by stimulation (100 pulses at 50 Hz) of the cervical connective (see inset) reduces the amplitude of these EPSPs in a reversible fashion: 1, three sweeps before stimulation of connective began. 2, three sweeps immediately after stimulation ended. 3, partial recovery of the EPSP 30 seconds later. **C:** Stimulation of nerve 5B2 in the left and right hind legs causes excitation in the DUM cells of the SOG. T1, T2, T3 = pro-, meso- and metathoracic ganglion

synaptic connection. These experiments, however, can only be taken as a first hint that these DUM neurons might be responsible for this modulation because stimulation of an entire connective, albeit the one contralateral to the neurons investigated, might cause the activation of other descending pathways.

Excitation of DUM neurons by leg afferents

DUM neurons located within the SOG that descend into thoracic ganglia and possibly are involved in the modulation of synaptic connections between neurons involved in leg movements themselves receive excitatory inputs caused by such movements. Although these pathways are most likely polysynaptic and we are just beginning to characterize their cellular components, these results indicate that the DUM cells of the suboesophageal ganglion represent integral but extended loops of locomotory circuits. Their influence on locomotory, and possibly other neuronal networks is most likely a very general one. This is illustrated by the fact that these DUM cells supply neuropiles in all ganglia of the ventral nerve cord, not only the thoracic ganglia that coordinate flight and walking. General, however, does not mean unspecific. The three types of neurons clearly supply specific neuropile regions within thoracic ganglia (and most likely abdominal ganglia as well). One of the future tasks will be to find out about the functional importance of this specificity. The other task, of course, is to further study the cellular sources responsible for the activation of these modulatory neurons. This latter task does not only apply to the cells studied here, but to all other DUM cells as well.

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