

VARIABILITY IN THE STRUCTURE OF AN IDENTIFIED INTERNEURONE IN ISOGENIC CLONES OF LOCUSTS

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(Received 6 September 1982 — Accepted 9 September 1982)

SUMMARY

Several studies have shown that there can be considerable variability in the morphology of identified neurones. In a recent investigation (Pearson & Goodman, 1979) a great degree of variability was observed in the axon branching patterns of the descending contralateral movement detector (DCMD) interneurons of locusts. Corresponding to the variation in the structure of DCMD was a large variation in the synaptic connections made by this interneurone; the absence of a monosynaptic connection always correlated with the lack of the appropriate axonal branch of DCMD.

Since this variability could be related to genotypic differences, we investigated the structure and synaptic connections of DCMD in individuals from several different isogenic clones of the locust *Schistocerca gregaria*. Within a single group of clones the variability in the axonal branching patterns and synaptic connections of DCMD was generally less than that between different clones or in sexually reproduced control animals. More significantly, a few of the clonal groups had consistently unique branching patterns and concomitant synaptic connections. Nevertheless, there was still some variability in the structure of DCMD within each clone. We conclude from these observations that differences in genotype can influence the morphology of individual neurones at the relatively refined level of axonal branching patterns and consequently the neurone's synaptic connections. However, due to the variability of DCMD structure within a single clone, epigenetic factors must also determine the pattern of axonal branching.

INTRODUCTION

Recent studies in invertebrates have shown that there can be duplications and deletions of identified neurones as well as considerable variability in the structure of individual neurones (Macagno, Lopresti & Levinthal, 1973; Kuffler & Muller, 1974; Goodman, 1974; Burrows, 1975; Triestman & Schwartz, 1976; Altman & Tyrer,

Key words: Isogenic locust clones, DCMD interneurone structure.

1977). One of the most extreme examples of variability occurs in the axonal morphology of the two descending contralateral movement detector (DCMD) interneurons in the locust. Each DCMD interneurone originates in the protocerebrum of the locust brain and its axon descends through the thoracic ganglia via the contralateral nerve cord. It responds to small moving objects within the visual field of the eye contralateral to the axon (Rowell, 1971; O'Shea, Rowell & Williams, 1974).

The variation in the axonal branches within the metathoracic ganglion is so great that it is not possible to specify a 'normal' structure (Pearson & Goodman, 1979). Since this variability was observed in colonies of sexually reproducing animals the question is raised as to how much variability is related to differences in genotype. In an attempt to answer this question we have investigated the variability in the structure of DCMD in isogenic clones of locusts. A further reason for this investigation was the possibility of isolating clones with distinctly different and relatively invariant structures. By correlating the behaviour with DCMD structure in these different clones, information regarding the function of specific axonal branches of DCMD may be gained.

METHODS

All experiments were performed on adult *Schistocerca gregaria*. Both sexually reproduced (control) insects and parthenogenically produced isogenic clones were examined. All locusts (clones and controls) were raised under identical environmental conditions.

Production of isogenic locust clones by parthenogenesis was similar to previously described methods (Goodman, 1977, 1978). Parthenogenesis in locusts is by automictic thelytoky (Hamilton, 1953). Several genetic and cytological studies of the genus *Schistocerca* suggest that the resumption of diploidy after meiosis is by fusion of the cleavage nuclei (endomitosis) (for review see Goodman, 1978). The fusion of cleavage nuclei would result in isogenicity within the first two generations of parthenogenesis.

The procedure for intracellular recording and staining of the DCMD interneurons has been previously described (Pearson & Goodman, 1979; Pearson, Heitler & Steeves, 1980). In short, intracellular injections of Lucifer yellow dye (Stewart, 1978) were made by impaling the main axon of DCMD, close to its point of entry into the metathoracic ganglion. Penetration was confirmed by the 1:1 correspondence of intracellular action potentials with the spikes recorded extracellularly from the DCMD axon descending through the promesothoracic connective of the ventral nerve cord.

Following injection (10–30 min) of Lucifer yellow with constant currents of approximately -5 nA, the metathoracic ganglion was removed, fixed in 4% paraformaldehyde (1 h), dehydrated, cleared in methyl salicylate and mounted, dorsal side up, on a depression slide. Drawings and/or photographs were taken using a Wild-Leitz Orthoplan microscope equipped with epi-fluorescence. Since we routinely stained axonal processes as fine as $1\ \mu\text{m}$, we have considerable confidence that the intracellular injections filled the entire metathoracic structure of DCMD.

In some insects we also recorded intracellularly from the fast extensor tibiae (FETi) motoneurone of the metathoracic ganglion. Penetration of the main process of FE

the lateral neuropile was confirmed by the 1:1 correspondence of each action potential with the rapid extension of the hindleg tibia. FETi is the only fast extensor motoneurone to innervate the extensor tibiae muscle and is known to receive monosynaptic excitatory postsynaptic potentials (EPSPs) from the ventral branches of DCMD (Burrows & Rowell, 1973; Pearson & Goodman, 1979). To ascertain whether FETi received monosynaptic EPSPs from DCMD it was usually not necessary to average the postsynaptic responses of FETi to DCMD activation. However due to the small size of the EPSP (0.5–2.0 mV) and the possibility that the monosynaptic connections could not be detected because of recording noise, a Nicolet (model 1072) signal averager was often used. An average of 128 synaptic events was more than sufficient to demonstrate a connection if it was present.

RESULTS

The structure of DCMD within the metathoracic ganglion was determined in six different isogenic clones, as well as in female locusts from our sexually reproducing colony which served as controls. The results described here are from third to seventh generation parthenogenic offspring. Since no changes in the extent of the variability in the structure or synaptic connections of DCMD were noted over successive generations, the data from all generations within a single clone were pooled. The structure in each insect can be broadly specified by noting the occurrence or absence of four identifiable branches (anterior, ipsilateral ventral, contralateral ventral and dorsal) arising from the main axon and projecting to localized regions within the metathoracic ganglion, and by noting whether the main axon projects through the ganglion to the next unfused ganglion (Fig. 1A).

Table 1 outlines the different patterns of variability in the axonal branching of DCMD within the metathoracic ganglion. Superficially, the variability in DCMD within an isogenic clone may not appear to differ greatly from the variability within the control group. On closer examination, however, each clone does have a predominant pattern of axonal branching for DCMD. Some of the clones also consistently exhibit peculiar morphological features rarely seen in other clones or controls.

Comparison of the six clones showed that the predominant structure of DCMD was similar in clones 3 and 12 (Fig. 1C), and that this structure differed from that found in the other clones. Likewise, clones 4 and 10 (Fig. 1D) were similar but distinct when compared to other clones. Finally, clones 7 (Fig. 1E) and 8 (Fig. 1B) had structures which differed both from each other and from all other groups of clones.

One distinguishing characteristic of the DCMD axon branching in clones 3 and 12 was the lack of a dorsal branch. In 75 % of clone 3 and in 87 % of the clone 12 insects, the dorsal branch was absent. A more uniform feature in clone 12 was the high percentage (93 %) of insects having the main axon projecting into the next unfused abdominal ganglion. This was not noted with such consistency in the other clones or controls. In 78 % of clone 4 and 65 % of clone 10 insects, the dorsal process arose from the ipsilateral ventral branch rather than projecting directly from the main axon. This feature was observed in only 15 % of control insects and very rarely in the other clones. Another prominent variation of the dorsal branch occurred in clone 7 where % had the dorsal branch arising from the contralateral ventral branch. This was

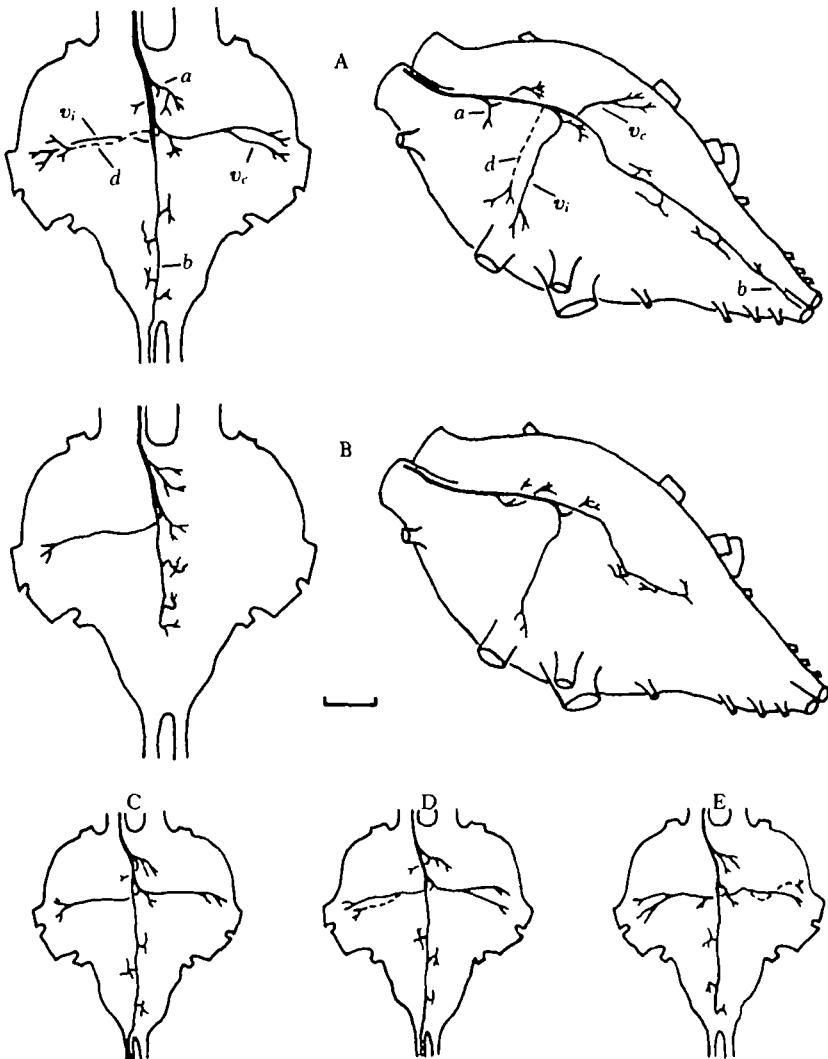


Fig. 1. Variability in the axonal branching patterns of DCMD in the metathoracic ganglion of *Schistocerca gregaria* clones. (A) Dorsal and oblique representations of the possible primary axon branches of DCMD in the metathoracic ganglion. The main axon enters the ganglion near the midline at the dorsal surface. The prominent axon branches projecting from the main axon are the anterior (*a*), dorsal (*d*, drawn with a dotted line), ipsilateral ventral (*vi*) and contralateral ventral (*vc*). The main axon may continue through the ganglion and exit via the ipsilateral abdominal connective (*b*). (B) The most common axonal branching pattern of DCMD in clone 8 animals; note the lack of dorsal, abdominal and contralateral ventral branches. (C) Dorsal view of the characteristic structure of clones 3 and 12 illustrating the absence of dorsal branches. (D) Typical DCMD in clones 4 and 10 showing the dorsal process (dotted line) arising from the ipsilateral ventral branch. (E) DCMD in clone 7 has the dorsal branch coming off the contralateral ventral process and lacks the projection of the main axon into the abdominal connective. For the sake of clarity, some of the small secondary and tertiary branches have been omitted. Calibration bar = 200 μm (A and B), 310 μm (C to E).

not a regular occurrence in the other clones or controls. A second interesting trait of DCMD in clone 7 was the abdominal axon terminating in 71% of the locusts before it reached the next unfused abdominal ganglion.

Table 1. Variability of DCMD axonal branching patterns in the metathoracic ganglion

Group	Number of insects	Anterior	Branch		Dorsal	Abdominal axon
			Ipsilateral ventral	Contralateral ventral		
Control	6	+	+	+	-	+
	4	+	+	+	-	-
	3	+	+	+	+i	+
	3	+	+	+	+c	+
	2	+	+	+	+	-
	2	+	+	+	+	+
Clone 3	10	+	+	+	-	+
	2	+	+	+	+c	+
	2	+	+	+	+i	-
	1	+	+	+	-	-
	1	+	+	-	-	-
Clone 4	8	+	+	+	+i	-
	5	+	+	+	+i	+
	2	+	+	+	-	+
	1	+	+	+	+c	+
	1	+	+	+	+i	+
	1	+	+	+	+	+
Clone 7	9	+	+	+	+c	-
	3	+	+	+	+c	+
	1	+	+	+	+i	+
	1	+	+	+	+i	-
Clone 8	7	+	+	-	-	-
	4	+	+	-	-	+
	2	+	+	+	-	-
	2	+	+	+	-	+
	1	+	-	+	-	-
	1	+	-	+	-	+
	1	+	+	-	+i	-
	1	+	+	-	+	-
Clone 10	9	+	+	+	+i	+
	4	+	+	+	+	+
	3	+	+	+	+i	-
	2	+	+	+	-	-
	1	+	+	+	-	+
	1	-	+	+	+i	+
Clone 12	12	+	+	+	-	+
	2	+	+	+	+i	+
	1	+	+	+	-	-

+ = present (for the dorsal branch, + = dorsal directly off the main axon)
 - = absent
 +i = dorsal arising from the ipsilateral ventral branch
 +c = dorsal arising from the contralateral ventral branch

The most interesting and distinctive clone was clone 8. As shown in Fig. 1B, the morphology of DCMD in clone 8 offspring departs dramatically from that seen in any other group. The absence of the contralateral ventral branch in the majority (68%)

of clone 8 insects is the most striking omission. The contralateral ventral branch was always found in the control group and was missing in only 2/102 (2%) of the individuals from all other clones. Two other characteristics of the DCMD structure in clone 8 are noteworthy: (1) the dorsal branch was absent in 90% of the insects; and (2) the main axon failed to project into the next unfused ganglion in 63% of the locusts.

Physiologically, the ventral branches of DCMD are known to evoke monosynaptic EPSPs in FETi motoneurons. As shown in a previous report (Pearson & Goodman, 1979) a failure to record monosynaptic EPSPs in FETi, in response to DCMD activation, was always associated with the absence of the appropriate ventral branch. In the present study we confirmed this finding in clone 8 insects by a failure to record EPSPs in the contralateral FETi in many insects (Fig. 2). Subsequent examination of the structure of DCMD revealed the contralateral ventral branch was absent in those locusts where a physiological connection was not detected.

DISCUSSION

The two main findings of this study are: (1) different structural features of DCMD were observed in different isogenic clones, including some unique branching patterns never or rarely observed in controls; and (2) there could be considerable variability in the DCMD axonal branching patterns within a single clone, although it was usually less than that for the control group. Essentially our results are similar to those reported

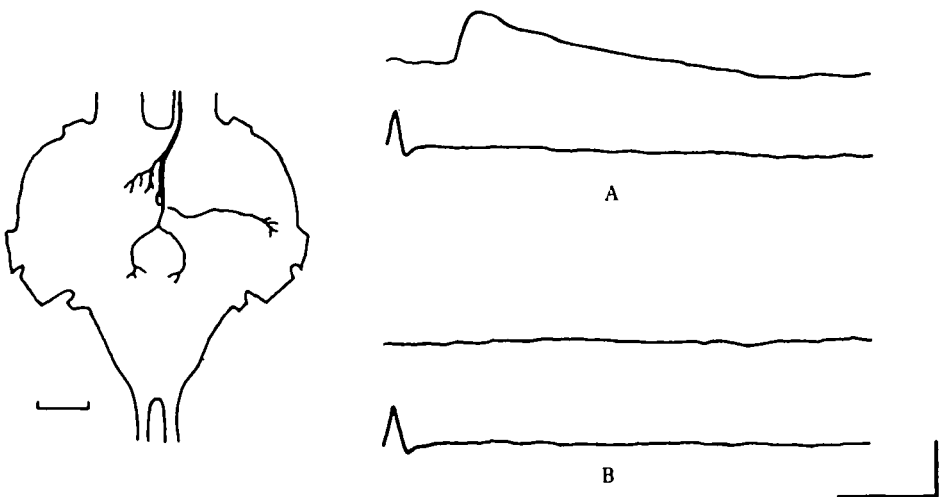


Fig. 2. Variability in the connection from DCMDs to (left side) FETi motoneurone in a clone 8 locust. (A) Monosynaptic EPSP (upper trace) in FETi from the left DCMD (lower trace, extracellularly recorded DCMD spike). (B) No EPSP from the right DCMD (illustrated at left). Each record is the average of 128 events. In both cases the FETi motoneurone was well penetrated, having an antidromically evoked spike of approximately 60 mV. The lack of an EPSP from the right DCMD was not due to a conduction block of DCMD activity, since 50 mV action potentials were recorded from the main axon in the metathoracic ganglion while the neurone was injected with Lucifer yellow dye. Subsequent examination of the right DCMD's structure (drawing on left) revealed that the contralateral ventral branch was missing. If present, the contralateral ventral branch would project to the left FETi. Calibrations: 0.5 mV, 3 ms (A and B), 200 μ m (drawing).

For the variability of ocellar interneurons (Goodman, 1978). The significant addition is that secondary axonal branches as well as the primary axonal path of DCMD can be correlated with genotypes.

Nevertheless, the fact that for both DCMD and ocellar interneurons there can be considerable variability in single clones means that the occurrence of identifiable differences in insect interneurons may depend to a large degree on epigenetic factors (Stent, 1981). A possible epigenetic determinant, which may or may not be directly influenced by the genome, is the segmental ganglion environment encountered by the neurites as they grow and develop. At the level of the finer patterns of synaptic interactions between neurons, stochastic processes may predominate although this remains to be elucidated.

It is of interest, however, to note that the DCMD axonal branches showing variability in occurrence also make very weak connections (usually <1 mV) to flight motoneurons (dorsal branch) and the FETi motoneurons (ventral branches) (Pearson & Goodman, 1979). During behaviour such as flight or jumping the depolarization in these motoneurons is usually greater than 15 mV (Pearson & Robertson, 1981; Robertson & Pearson, 1982). Thus it appears that the variable branches of the DCMDs (when present) make only minor contributions to behaviour. On the other hand the anterior branch shows very little variability, makes strong synaptic connections to interneurons, and conveys an important input for the initiation of the jump (Pearson *et al.* 1980; Steeves & Pearson, 1982). This correlation of variability with functional importance parallels findings in other animals where less important phenotypic structures often show more variability (cf. Hall, Greenspan & Harris, 1982).

An obvious question is why do the branches of DCMD showing variability occur at all? One possibility is that the branches are important in a juvenile behaviour not expressed in adults, and there is a regression of unnecessary connections with maturation. Another is that the branches are simply evolutionary relics having served important functions in progenitor species. Only by examining the structure and function of DCMD in juvenile instars and in other locust species can the merit of these, or other, possibilities be assessed.

One of the primary motivations for this study was the hope that isogenic locust clones might furnish the opportunity to correlate any notable alterations in behaviour with underlying variations in the anatomy or physiology of identified neurons. This does not appear to be likely, at least for DCMD, since no individual clone had a distinctly different DCMD structure which could be correlated with any alteration in behaviour. Instrumentally, it may be more favourable to try and induce mutations in isogenic organisms, since even recessive phenotypes could be expressed through parthenogenic breeding. Whether isogenic clones will be of value in the neuronal analysis of locust behaviour remains to be seen. What is certain is that any approach using isogenic locusts requires a large investment of time and facilities to establish and maintain the clones.

We thank Drs T. Grigliatti and R. M. Robertson for their constructive comments. We are indebted to Dr W. W. Stewart for his kind gift of Lucifer yellow dye. We also thank Elizabeth Bond and Hugo Bergen for their participation in some of the

experiments and Sam Gopaul for his skilled technical assistance. This work was supported by a grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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