Development of Chemosensitivity of an Identified Insect Interneurone¹

J. M. BLAGBURN,* D. J. BEADLE,* AND D. B. SATTELLE†²

* A. F. R. C. Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, and † Department of Biological Sciences, Thames Polytechnic, Wellington St., Woolwich, London SE18, England

Abstract

Characteristic features of cockroach embryos (*Periplaneta americana*) are determined for different stages in development. Morphological changes in giant interneurone 2 (GI 2) in the terminal abdominal ganglion during embryogenesis are described based on cobalt injections. A sequential proliferation of branches preceded by filopodial extension is observed between 45 and 80% embryogenesis, by which time the characteristic arborization of the first instar cell is established. The cholinergic agonist carbamylcholine was ionophoretically applied to the cell body and dendritic region of GI 2 at various stages in development, and the responses were recorded intracellularly. Chemosensitivity of GI 2 appears at 40 to 45% embryogenesis, to a similar degree in both cell body and dendrites. The sensitivity of these areas subsequently diverges, and after the 60 to 65% stage, the dendrites are approximately 1000 times more sensitive than the cell body. At 80 to 85% embryogenesis, there is a temporary peak in dendritic sensitivity, and cell body sensitivity increases during postembryonic development. The changes in sensitivity take place before synaptogenesis, and the decline in dendritic sensitivity after the 80% stage may be related to synaptic maturation.

Identified neuronal pathways in the nervous system of insects are well suited for studies of synaptogenesis and neuronal interactions during development. The cercal afferent, giant interneurone pathways in Orthoptera and Dictyoptera, which play a part in mediating the escape response to mechanical stimulation of sensory receptors on the cerci, have been investigated by several laboratories (Roeder, 1948; Edwards and Palka, 1974; Mendenhall and Murphey, 1974; Matsumoto and Murphey, 1977; Ritzmann and Camhi, 1978; Camhi et al., 1978; Murphey et al., 1983). Although the development of the sensory afferent and interneurone branching patterns in *Schistocerca americana* have been described in most detail (Shankland 1981a, b; Shankland and Goodman, 1982a), the equivalent neurones in the cockroach *Periplaneta americana* are excellent candidates for developmental studies because, in the adult, aspects of their anatomy, physiology, and pharmacology and their role in behavior have been described (Callec 1974; Callec et al., 1980; Harrow et al. 1980; Daley et al., 1981; Sattelle et al., 1980, 1983). Moreover, it has been shown that the first instar nymphs of *P. americana* possess only

two functional filiform hair sensilla on each cercus (Bugnion, 1921; Dagan and Volman, 1982) giving rise to large identifiable axons (Blagburn and Beadle, 1982) which form synaptic connections with the giant interneurons (Blagburn et al., 1984).

At cercal afferent, giant interneurone synapses in the adult cockroach ACh is the likely transmitter (Shankland et al., 1971; Callec, 1974; Sattelle et al., 1976; Sattelle 1978; Sattelle et al., 1983). Since it has been widely postulated that receptors for transmitter substances are involved in the formation and maintenance of synapses (Changeux and Danchin, 1977; Freeman, 1977), it is important to compare the chemosensitivity of the postsynaptic neurones throughout their development with the temporal pattern of synapse formation. Here we investigate the suitability of the developing cercal afferent, giant interneurone system of *P. americana* for this type of study.

Binding studies employing α -bungarotoxin on the developing antennal lobes of *Manduca sexta* revealed a gradual rise in receptor density during development that was unaffected by deafferentation (Sanes et al., 1977; Hildebrand et al., 1979). Electrophysiological studies of chemosensitivity in dorsal unpaired median (DUM) neurones of *Schistocerca nitens* showed that ACh and GABA sensitivity first appears at 40% embryogenesis, along with axonal growth (Goodman and Spitzer, 1979; 1980; Goodman et al., 1979). At this time, the whole cell becomes sensitive, but chemosensitivity apparently decreases slightly as synaptic inputs appear at 75% embryogenesis. Recently, ionophoresis of ACh and related compounds has been used to quantify the sensitivity to these ligands of identified interneurons and a motoneurone in the cockroach (Sattelle et al., 1980; Harrow et al., 1982; Callec et al., 1982; David and Pitman, 1982; Harrow and Sattelle, 1983; David and Sattelle, 1984). In the present study on giant interneurone 2 (GI 2), the developing morphology of the cell during embryogenesis is investigated by intracellular cobalt injection, and the development of chemosensitivity is followed by the ionophoretic application of the cholinergic agonist carbamylcholine onto the cell body (soma) and the dendrites.

Materials and Methods

Oothecae projecting from female cockroaches were harvested daily and could be dated to within 24 hr. Each daily collection was stored in separate Petri dishes containing damp cotton wool, at a temperature of 30°C. Their age was expressed as a percentage of the total time to hatching (normally 31 days). The designations A1 to A6 are used for abdominal ganglia in the adult cockroach; A₁ to A₁₁ are used to refer to ganglion rudiments present in the embryonic abdominal CNS. The terminal ganglion refers to the postfusion ganglion (A6) formed from rudiments A₇ to A₁₁.

Newly hatched first instar nymphs or embryos of the desired age were placed in saline of the following composition: 150 mM NaCl, 3.1 mM KCl, 5.4 mM CaCl₂, 5 mM HEPES buffer, pH 7.4 (based on Callec and Sattelle, 1973). Following removal of the legs and gut, the nerve cord (still attached to the head, caudal segments, and cerci) was transferred to saline inside a rubber-walled chamber constructed on a glass microscope slide. The CNS was anchored by attaching the head together with the thoracic and caudal

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² To whom correspondence should be addressed.

segments to petroleum jelly. Tracheal trunks and the stumps of nerves 7 and 8 (nomenclature of Roeder et al., 1960) were removed from the terminal abdominal (A6) ganglion, which was held in position by maintaining the interganglionic connectives and nerves 9, 10, and 11 under slight tension. It was often necessary to crush or remove the remaining musculature of the caudal segments to prevent tissue movements. The ganglion was desheathed using fine forceps and finally secured in the experimental chamber, dorsal side uppermost. For studies on adult insects, a preparation including abdominal ganglia A5 to A6 was dissected out and anchored by the interganglionic connectives and cercal nerves, using petroleum jelly. A6 was then desheathed.

Isolated preparations were viewed with a Zeiss $\times 40$ water immersion lens using differential interference contrast (Nomarski) optics. Electrical isolation of the objective from the body of the microscope was achieved with a Perspex insert. A gravity perfusion system maintained a constant flow of saline (approximately $1 \text{ ml} \cdot \text{min}^{-1}$). The soma of GI 2 was identified by its characteristic size, location, and appearance. Although, in the adult, the thickness of the tissue provided optical conditions that were far from ideal, it was nevertheless possible to recognize GI 2. The cell body was impaled with 80- to 150-megohm glass microelectrodes containing $0.6 \text{ M K}_2\text{SO}_4 + 5 \text{ mM KCl}$ (Thomas, 1978) or $1.0 \text{ M KCl} + 5 \text{ mM Tris buffer (pH 7.3)}$.

The cholinergic agonist carbamylcholine was applied ionophoretically to the cell body and to the dendrites of GI 2. In the case of cell body applications, low-resistance glass micropipettes containing $1.0 \text{ M carbamylcholine chloride}$ (Sigma Chemical Co.) were moved to within $5 \mu\text{m}$ of the cell membrane after the recording microelectrode had been inserted. During investigation of dendritic chemosensitivity, the ionophoretic micropipette was first inserted into the center of the contralateral neuropile, opposite the cell body, which was subsequently impaled with the recording microelectrode. A small retaining current (45 nA) prevented leakage of the agonist. Carbamylcholine was expelled by application of 0.2- to 2.0-sec, square, positive current pulses, monitored by a virtual-earth circuit, and subsequent responses were recorded on a pen recorder and oscilloscope. Dose response curves for carbamylcholine-induced depolarizations were constructed for both cell body and dendritic membranes of GI 2 at various stages after 40% embryogenesis. The amount of carbamylcholine released from the ionophoretic micropipette was measured using a blunt microelectrode the tip of which was filled with K^+ -sensitive liquid ion exchange resin (Corning LIE 477319) which is also sensitive to acetylcholine and carbamylcholine. The drug release was found to be linear using current pulses up to 20,000 nA.

The membrane input resistance (R_{in}) was estimated by application of current pulses of 100 msec or more duration via a bridge-circuit, while monitoring the transmembrane voltage.

For intracellular cobalt injection, the cell body of GI 2 was impaled with 50- to 100-megohm microelectrodes containing 6% hexaminecobalt chloride (Sigma Chemical Co.). Square, positive current pulses 0.5 sec in duration (at a frequency of 0.5 Hz and of 5 nA amplitude) were passed through the electrode for 3 to 8 min. The preparation was left for 10 to 15 min in saline to allow for distribution of cobalt, followed by precipitation with $(\text{NH}_4)_2\text{S}$ and tissue fixation with alcoholic Bouin's fixative for 30 min. Cobalt-stained cells were silver-intensified using the whole-mount Timm's procedure of Bacon and Altman (1977), dehydrated in an alcohol series, cleared, and mounted in neutral Canada Balsam. The specimens were drawn with the aid of a Zeiss drawing tube.

Results

Embryonic staging in *Periplaneta americana*. The age of embryos is expressed as a percentage of the total time elapsed before hatching (cf. Bentley et al., 1979) and several stages in embryogenesis are depicted in Figure 1. At 35%, the embryo occupies the posterior half of the egg, having undergone katabolism at 30 to 35%. It is fully segmented, and the antennal and limb buds are undergoing elongation. Pleuropodia remain on the first abdominal segment. The proctodeum has invaginated as far as the sixth abdominal segment, and this region is folded under the embryo. The CNS is not yet separate from the ventral surface and is composed of segmentally arranged ganglion rudiments. Five of these, in segments 7 to 11, will form the terminal abdominal ganglion, and fusion between ganglion rudiments A_9 , A_{10} , and A_{11} has begun. Some intersegmental fibers are present.

By 45%, the embryo occupies approximately two-thirds of the embryo sac. The limbs are folded, and claws are developing. Segmentation of the antennae and cerci has begun. Peristaltic

contraction of the dorsal heart sinus and body wall are visible. At 50%, traces of pigment are present in the eyes. Occasional flexures of the caudal segments are observed. The ganglion rudiment in the first abdominal segment (A_1) fuses with that in the third thoracic segment, forming the metathoracic ganglion, while ganglion rudiment A_7 fuses with the terminal ganglion. Longitudinal and transverse fiber bundles are visible in the CNS, and peripheral nerves are present.

By 60%, the dorsal half of the eye is pigmented. The cuticle is ridged in appearance, the heart beats normally, and the legs twitch spasmodically. The yolk has been completely enclosed by the midgut by 55% embryogenesis, and Malpighian tubules are formed. In the CNS, interganglionic connectives are formed. At 70% embryogenesis, the secondary cuticle is apolysed from the underlying epidermis. The Malpighian tubules are mobile and contain a yellow fluid.

By 80%, the eyes are heavily pigmented, although the two most ventral rows of facets are only outlined by pigment. Faint pigmentation is observed at the base of the antennae. Bristles form at this stage. The gut becomes mobile and filled with yellow fluid; hepatic caecae develop. The CNS ganglia are similar in appearance to those in the first instar. By 90% embryogenesis, the eyes, mandible teeth, and bristles are fully pigmented. Brief muscle twitches and slow peristaltic contractions are noted occasionally. At 95%, opening the ootheca may be followed by a hatching reaction, but only after a long interval. Finally, at 100% embryogenesis, most individuals are competent to hatch; peristaltic body contractions and air-swallowing result in emergence from the ootheca and simultaneous first ecdysis.

Development of giant interneurone 2. Using Nomarski optics, the cell body of GI 2 can be identified by its characteristic size, position, and appearance. The soma in the first instar is $26 \pm 1 \text{ (SE)} \mu\text{m}$ in diameter, situated just posterior and dorsal to nerve 8 and its associated tracheal trunk. The cytoplasmic volume is greater than in surrounding neurones and has a distinctive granular appearance. The nucleolus is also conspicuous. The branching pattern of GI 2 at hatching (Fig. 2) is very similar to that of the adult cell (Fig. 2) described by Harrow et al. (1980) and Daley et al. (1981). The primary neurite projects from the cell body to the center of the contralateral neuropile, where it divides and gives rise to an anteriorly directed axon and a posteriorly directed dendrite. The axon extends as far as the brain and forms small medial branches in each abdominal ganglion. The main dendrite arborizes in a region of neuropile enclosed by branches of first instar cercal sensory axons (Blagburn and Beadle, 1982) which resembles the cercal glomerulus described in the grasshopper (Shankland, 1981b; Shankland and Goodman, 1982). The axon, neurite, and primary dendrite give rise to secondary dendrites in this region, and these branch profusely, forming an ovoid region of dendritic processes. Two other areas of dendrite arborization can be seen; the first arises from the neurite in the neuropile ipsilateral to the cell body and the second extends from the axon in the medial region of the anterior neuropile.

In early embryonic stages ($<45\%$), the cell body of GI 2 is more difficult to identify, since it is embedded in a cluster of similar cells. Its position is the most reliable criterion for identification, with the soma located between nerves 8 and 9 near the anterior of a cluster of similar cells, directly adjacent to the anterior commissure of ganglion rudiment 9. GI 2 is two cell layers below the dorsal surface and from the lateral edge of the ganglion, with an anterior neighboring cell body of similar diameter. At 45% embryogenesis (Fig. 2), the ganglion rudiment A_7 is not yet completely fused to the terminal ganglion, the axon of GI 2 extends into the anterior ganglion rudiments, and the primary dendrite is present. The neurite, axon, and dendrite bear fine processes which are almost certainly filopodia. These are particularly long and abundant in areas where branching will eventually be observed, such as the neurite in the medial ipsilateral neuropile and the axon in the neuropile of ganglion rudiment A_7 . By 55% (Fig. 2), at least six secondary dendrite branches are formed. These have swollen ends bearing filopodia and resem-

Figure 1. Embryogenesis of *Periplaneta americana*. Lateral view of embryos at various percentage stages throughout development. The scale bar represents 1 mm.

bling growth cones. The anterior collateral axon branch is also initiated at this time. A sequential proliferation of branches is observed from this stage until 80% (Fig. 2), by which time the characteristic branching pattern of the mature cell is established.

Postembryonic changes in Gl 2 appear to be confined to an increase in the size of the cell and an increase in the number of dendritic branches. Different regions of the neurone have different growth rates, with the cell body being proportionally smaller and the axon proportionally larger in the adult (Fig. 2). Similarly, the portion of the terminal ganglion occupied by the arborization of the adult cell is smaller, probably due to the massive postembryonic ingrowth of cercal afferent axons.

Electrical properties of giant interneurone 2 during development. The recorded resting potential of Gl 2 increases significantly from -58 ± 2 mV in the 35 to 42% period of embryogenesis to -71 ± 1 mV during the 80 to 85% stages. There is a subsequent significant increase to -77 ± 1 mV in the first instar, and we have detected no further postembryonic change (Table I). The R_{in} increases from 23 ± 5 megohms at 35 to 42% embryogenesis to 43 ± 6 megohms at 51 to 53%. There is a subsequent decrease to 20 ± 2 megohms in the first instar. For the adult cell, values for R_{in} of 3 to 4 megohms are obtained. Some degree of membrane rectification was observed after the 60 to 65% stage (Fig. 3).

Action potentials were not observed in Gl 2 before 55% embryogenesis. After this stage, nonovershooting action potentials (amplitude, 10 to 20 mV) were recorded from the cell body. Short bursts of spikes were elicited by suprathreshold (approximately 20 mV) depolarizations which follow the application of carbamylcholine to the dendrites. After 75% embryogenesis, many cells exhibited continuous spike activity for long periods, while others produced spontaneous depolarizing potentials of varying amplitude (1 to 10 mV; Fig. 3).

Chemosensitivity of giant interneurone 2 during development. Ionophoretic application of carbamylcholine to the soma and to the dendrites of Gl 2 resulted in transient depolarization of the cell membrane, the amplitude of which was dependent upon the dose (Fig. 4). Application of the agonist to either region of Gl 2 at the 35 to 42% stage of embryogenesis elicited no measurable response. A dose of 1 nC to the dendrites of Gl 2 in the first instar elicited a response of 1 mV with a very short latency and a duration of approximately 200 msec. The time taken to reach the peak of the response was 50 msec. Rise times were consistent with the agonist acting close to the site of application. The maximum depolarization amplitude (ΔV_{max}) ranged from +20 mV at the 50% stage to +44 mV at 85%.

The minimum dose (Q) of agonist required to produce a response of 1 mV amplitude can be estimated by the equation $Q = (1/F)CH$, where Q = amount of substance released (moles); F = the Faraday (96,400 coulombs/equivalent); C = coulombs passed; and H = the transport number (0.1 for acetylcholine, after Dionne, 1976). The value of Q for the dendrites drops from 2×10^{-14} mol at 50%

embryogenesis to 1×10^{-16} after 65%. The value of Q for the cell body varies from 2×10^{-14} mol at 80 to 85% to 2×10^{-13} mol at 60 to 65%; the value for the first instar cell body is 1×10^{-14} mol, and that for the adult is 5×10^{-14} mol. This compares with the value of 2.5×10^{-15} mol obtained for the application of ACh to the cell body in the presence of 1.0×10^{-6} M neostigmine (Harrow and Sattelle, 1983), suggesting that carbamylcholine is 20 times less effective an agonist than ACh. Similarly, the value for Q obtained for the dendrites of Gl 2 in the first instar (1×10^{-15} mol) is greater than that for the dendrites of the adult Gl 1 (3×10^{-17} mol of ACh; Callec et al., 1982), although this may also be due to differences in sensitivity between Gl 1 and Gl 2 and/or postembryonic changes.

The log-dose response curves (Fig. 5) for dendritic application of carbamylcholine to the later embryonic and first instar neurone show that maximal responses (ΔV_{max} 30 to 45 mV) are elicited by doses of 10 to 50 nC. The maximum sensitivity of Gl 2 dendrites at the 50% stage is 0.14 ± 0.04 mV/nC. This increases to 4.0 mV/nC at 55% and subsequently to 9.1 ± 2.4 mV/nC at 60 to 65%, from which value it does not deviate significantly until hatching (Table II).

However, analysis of data for the dendrites of Gl 2 is complicated by the cable properties of the neurite. Its small radius and many fine branches are likely to reduce the length constant, resulting in attenuation of the responses to a significant degree. It can be estimated that attenuation would be insignificant if the specific membrane resistance (R_m) of the neurite were ten times the value in the cell body. Thus, although it is not possible to give accurate values of dendrite chemosensitivity, the pattern of relative developmental changes can be discerned.

The maximum sensitivity of the soma decreased from 0.1 ± 0.05 mV/nC at 52% to 0.01 ± 0.002 mV/nC at 60 to 65% embryogenesis. The first instar neurone has the same sensitivity, 0.014 ± 0.005 mV/nC, but at 80/85%, the value is 0.07 ± 0.02 mV/nC. In the adult, the maximum sensitivity is 0.05 ± 0.01 mV/nC. The ΔV_{max} for the cell body exhibits no significant changes, remaining within the range of 25 to 42 mV throughout development. The mean value is 31 ± 2 mV. These maximal responses are elicited by doses of 2,000 to 20,000 nC.

The injection into the soma of 0.3 nA depolarizing current pulses of 250 msec duration at a frequency of 2 Hz before and during drug application allows a direct measurement of R_{in} and conductance change (Δg) to be made (Fig. 4). For small (≤ 10 mV) responses the reversal potential (V_r) can be estimated as -40 mV from

$$\Delta g = \frac{\Delta V}{R(E - V_r)}$$

where ΔV = voltage change, R = input resistance, and $E = V_r$ - resting potential. Since current pulses into the cell body cannot be used to measure Δg in the dendrites, it is not known if V_r is the same for the dendrites as for the cell body. For this reason, Δg_{max} cannot be calculated for the dendrite responses, and only the pattern

Figure 2. Development of GI 2. *Camera lucida* tracings were made of whole mounts of cobalt-injected, silver-intensified neurones at various stages of embryonic and postembryonic development. a, 45% of embryogenesis; b, 55%; c, 65%; d, 75%; e, 80%; f, first instar; h, adult; g is a photomicrograph of the 75% neurone represented in d. The scale bar represents 50 μ m for a to g and 150 μ m for h. Filopodia are present on dendritic branches up to 75%. At 80%, the branching pattern is complete.

TABLE I
Properties of giant interneurone 2 throughout development

	Developmental Stage							
	35–42%	48–50%	51–53%	55%	60–65%	80–85%	First Instar	Adult
Resting potential (mV)	-58 ± 2^a (6) ^b	-55 ± 3 (6)	-63 ± 2 (12)	-65 ± 3 (3)	-67 ± 1 (14)	-71 ± 1 (19)	-77 ± 1 (68)	-77 ± 1 (17)
Input resistance (megohms)	23 ± 5 (6)	30 ± 7 (5)	43 ± 6 (10)	40 ± 2 (3)	37 ± 3 (8)	23 ± 3 (11)	20 ± 2 (13)	3.5 ± 0.5 (3)
Soma diameter (μm)	12 ± 0.6 (4)	13 ± 0.4 (3)	14 ± 1 (3)	15 ± 0.6 (5)	19 ± 0.3 (5)	23 ± 0.5 (5)	26 ± 1 (5)	69 ± 2.5 (4)
Soma surface area (μm^2)	454 ± 50 (4)	530 ± 30 (3)	580 ± 70 (3)	710 ± 60 (3)	1080 ± 30 (5)	1660 ± 70 (5)	2130 ± 120 (5)	14980 ± 108 (4)
Contralateral dendrite surface area (μm^2)		1040 ± 200 (2)		1530 ± 500 (2)	2100 ± 300 (2)	2660 ± 330 (2)	3040 ± 500 (3)	
Estimated specific membrane resistance (ohm cm^2)	107 ± 34	161 ± 46	254 ± 65	285 ± 38	401 ± 44	384 ± 66	429 ± 67	530 ± 113

^a Mean $\pm$ SE.

^b Numbers in parentheses, number of experiments.

Figure 3. Electrical properties of GI 2. *a*, I/V curves for GI 2 at 51% embryogenesis (open circles) and in the first instar (solid circles). Each curve represents the mean of 4 neurons at each age. *b*, Spontaneous action potentials and possible synaptic activity, recorded from the soma of the first instar GI 2.

of relative changes in chemosensitivity can be discerned. Similarly, it is not possible to determine if the dendrites undergo greater or lesser changes in conductance than the cell body. Hill plots were

Figure 4. Intracellularly recorded responses of GI 2 to carbamylcholine. *a*, Applications (0.2 sec) of drug (4 and 12 nC, respectively) to the center of the contralateral dendritic field of first instar GI 2. Resting potential, -78 mV. *b*, Application (2 sec) of carbamylcholine (9,000 nC) to the soma of GI 2 at the 61% stage of embryogenesis. Depolarizing 0.3 nA pulses were applied throughout the response. Resting potential, -67 mV. *c*, 1s (2,000 nC) dose applied to the soma of first instar GI 2. Resting potential, -81 mV; calculated V_{Cl} , -44 mV. After 2 min, a 2-sec (12,000 nC) dose was applied. Depolarizing 0.3-nA pulses were applied throughout the responses. A 35-sec period has been removed from the latter part of the record. Vertical scale, 10 mV; horizontal scale, 10 sec.

constructed using the values of Δg obtained for the cell body at the stages shown in Table II. Values obtained for the Hill coefficient (n_H) approximated to 1.5, and no significant change was seen at any stage.

The surface area of the soma and of the contralateral dendrites can be estimated from the dimensions of the cells filled with cobalt. It is assumed that cobalt penetrates all the finest branches of the neurone and that all branches are circular in cross-section. Preliminary low-power electron-microscopical observations (J. M. Blagburn, D. J. Beadle, and D. B. Sattelle, unpublished observations) yield similar values for the dimensions of branches in horseradish peroxidase fills. The calculated area of the soma in later stages is likely to be a slight underestimate because of the ingrowth of glial trophosphingia. In the case of the dendrites, the number of processes of a certain diameter can be ascertained and, hence, value for the dendritic surface area can be estimated (Table I).

Discussion

The branching pattern of giant interneurone 2 in the sixth abdominal ganglion of *P. americana* is described, based on intracellular

Figure 5. Dose-response curves for ionophoretic application of carbamylcholine to Gl 2. The resultant depolarization (mV) is plotted against the ionophoretic charge applied (nC), for various developmental stages. A, Application of carbamylcholine to the centre of the contralateral dendritic field of Gl 2. B, Application of the agonist to the cell body membrane. Vertical bars represent SE.

cobalt fills performed at a series of embryonic stages. The neurone undergoes a sequential proliferation of dendrite branches between the 45% and the 80% stages of embryogenesis, resulting in a miniature version of the morphology of the adult cell. Growth of branches is preceded by the appearance of transient filopodia, and apparently no inappropriate branches are formed during this period. The morphology and the developmental timetable of this neurone closely resemble those of the medial giant interneurone (MGI) in *S. americana* (Shankland and Goodman, 1982). In both neurones, there is a well-defined region of dendrites contralateral to the cell body and an ipsilateral region of small branches on the neurite. However, in MGI, the contralateral dendrites give rise to branches which cross the midline of the ganglion, but in Gl 2, such branches

are not detected. Gl 2 forms a small axonal branch in the region of neuropile formed from ganglion rudiment A₇; in *Schistocerca*, A₇ does not fuse with the terminal ganglion, and this branch is absent. The common origin of the cells in ganglion rudiment A₉ suggests that there may be some degree of interspecific homology between the cockroach Gl 2 and the grasshopper MGI.

The recorded resting potential of Gl 2 increases throughout the last half of embryogenesis, from -58 to -77 mV. No subsequent postembryonic change in resting potential was noted. The input resistance of the cell increases from 35 to 40% embryogenesis to 60% then decreases until hatching. A further postembryonic decrease in R_{in} takes place. Estimates of the specific membrane resistance (R_m) suggest that this property increases from the 35 to

TABLE II
Chemosensitivity of giant interneuron 2 throughout development

	Developmental Stage							
	35–42%	48–50%	51–53%	55%	60–65%	80–85%	First Instar	Adult
Maximum sensitivity (mV nC ⁻¹)								
Soma	0 (3) ^a	— ^b	0.098 ± 0.051 ^c (5)	—	0.010 ± 0.002 (9)	0.067 ± 0.002 (6)	0.014 ± 0.005 (4)	0.047 ± 0.0014 (8)
Dendrites	0 (1)	0.14 ± 0.04 (3)	—	4.0 (1)	9.1 ± 2.4 (4)	10.9 ± 4.9 (3)	5.6 ± 0.8 (3)	—
Maximum conductance change (nmho)								
Soma	—	—	144 ± 52 (10)	—	98 ± 21 (9)	127 ± 20 (9)	249 ± 42 (11)	920 ± 260 (8)
Apparent K ₅₀ (nC)								
Soma	—	—	1440 ± 830 (5)	—	6090 ± 1350 (9)	1670 ± 460 (6)	6400 ± 900 (4)	3760 ± 990 (8)
Dendrites	—	108 ± 26 (3)	—	9 (1)	12.1 ± 2.5 (2)	8.5 ± 1.5 (3)	11.1 ± 3.6 (3)	—

^a Numbers in parentheses, number of experiments.

^b Bar, not tested.

^c Mean ± SE.

42% stage up to approximately 60% embryogenesis and then remains constant. The apparent initial increase in R_m is probably due to progressive electrical uncoupling of GI 2 from its neighboring cells. The true value of R_m at this time is probably ~400 ohm cm². A similar embryonic increase in R_m appears to take place in grasshopper DUM neurones where the oldest progeny of the DUM neuroblast become uncoupled at 55% embryogenesis (Goodman and Spitzer, 1981). Electrical excitability appears by 55%, and spontaneous depolarizing potentials of varying amplitude probably representing synaptic input are present by 75%. This sequence of events is approximately equivalent to that in MGI and DUMet (Goodman and Spitzer, 1981; Shankland and Goodman, 1982).

Carbamylcholine has proved to be a potent ligand at the insect ACh receptor characterized by [¹²⁵I]α-bungarotoxin binding (Schmidt-Nielsen et al., 1977; Sattelle, 1980). It has also been shown to be a potent agonist at ACh receptors that participate in transmission at the cercal afferent, GI synapses (Sattelle et al., 1976; Sattelle, 1978) which are blocked by nanomolar concentrations of α-bungarotoxin (Harrow et al., 1979; Sattelle et al., 1980; 1983; Harrow et al., 1982). In addition, carbamylcholine is not hydrolyzed by cholinesterase (Koelle, 1975), which is present in the neuropile of the terminal ganglion from 55% (Blagburn, unpublished observation).

Throughout the development of GI 2, a general pattern of changes in chemosensitivity can be discerned. Sensitivity to carbamylcholine first appears at approximately 40 to 45% embryogenesis to a similar degree over the whole cell. In GI 2, the carbamylcholine sensitivities of the dendrites and soma rapidly diverge in value, with that of the dendrites increasing at least 100-fold and that of the soma decreasing 10-fold by 60 to 65% embryogenesis.

During late embryogenesis (near 80%), there is apparently a peak in cell body and dendritic chemosensitivity. In both regions of the cell, there is a subsequent decline in sensitivity prior to hatching. In the first instar, the dendrites are 500 to 1000 times more sensitive to carbamylcholine than the cell body. During postembryonic development, the soma sensitivity triples. It is not known whether this increase also takes place in the dendrites.

Chemosensitivity in DUMet appears at a similar stage in development in all regions of the cell and decreases slightly after synaptic input appears at 75% embryogenesis, although this may be due to a decrease in input resistance (Goodman and Spitzer, 1980). A similar rise in receptor numbers before and during synaptogenesis has been detected in the antennal lobes of *M. sexta* (Sanes et al.,

1977) and in the chick retina (Vogel and Nirenberg, 1976). A subsequent decline in α-bungarotoxin binding activity has been detected in the chick ciliary ganglion which has been related to synaptic maturation (Wang and Schmidt, 1976).

It is interesting to note that the difference in chemosensitivity between the dendrites and the soma is 500- to 1000-fold. A large proportion of the dendritic membrane is probably postsynaptic, whereas no synapses have been reported to occur on adult insect neuronal cell bodies (Smith and Treherne, 1965). In comparison, the ACh sensitivity of synaptic regions of frog parasympathetic ganglion cell bodies is 276 mV/nC, whereas that of extrasynaptic regions is no more than 164 mV/nC (Harris et al., 1971). At the vertebrate neuromuscular junction, the sensitivity of the postsynaptic surface ranges from 1500 to 8000 mV/nC (Fambrough, 1979); in the rat diaphragm, extrasynaptic regions are 1000 times less sensitive than the end-plate (Miledi, 1960), whereas in snake muscle there is a difference of only 50-fold (Kuffler and Yoshikami, 1975).

The maximum slope of the dose-response curve has been used as a measure of chemosensitivity (Harris et al., 1971; Kuffler and Yoshikami, 1975), and this is thought to be proportional to the square of the receptor density (Hartzell and Fambrough, 1972). Changes in the shape of the curve have thus been attributed to changes in receptor density. The present results can be interpreted in this way, in which case the changes in the maximum slope of the dose-response curves suggest that large changes in both soma and dendrite receptor density take place as the neurone develops.

However, Dreyer et al. (1978) have shown that a dose-response curve can be described by at least three parameters: n_H (the Hill coefficient); Δg_{max} (the maximum conductance change, proportional to the receptor density); and K_{50} (apparent affinity constant, equivalent to the dose at which a response of $\Delta g_{max}/2$ is elicited). In the present study, n_H for the cell body responses is approximately 1.5 at all the stages investigated; thus the maximum slope of the curves is determined by Δg_{max} and K_{50} . If Δg_{max} is a measure of the receptor density, there is little significant variation in Δg_{max} and receptor density throughout embryogenesis until the period between 85% and the first instar in which Δg_{max} approximately doubles. This change may take place in the last part of embryonic development or in the posthatching period, when the giant interneurons become behaviorally functional. A further 3- or 4-fold increase takes place during postembryonic development.

Comparison of the K_{50} values (Table II) apparently shows that the

values at the soma differ at 51 to 53% and 80 to 85% from those at 60 to 65% and the first instar. These relatively small variations could be caused by differing degrees of glial investment remaining around the cell bodies. On the other hand, glial layers develop progressively, around the neuronal somata during embryogenesis and postembryonic development (J. M. Blagburn, unpublished observation), whereas there is no such age-dependent shift in cell body K_{50} values.

In addition, there are apparently 200- to 500-fold differences between the soma K_{50} and those estimated for the dendrites. Large shifts in the apparent value of K_{50} can be produced by the removal of a "receptor reserve" using an irreversible antagonist (Triggle and Triggle, 1976). Carbamylcholine is a strong agonist, and the total number of receptors in the dendrites may be in excess of that required to produce a maximal response. At the soma, this excess may be absent, resulting in the same maximal response but giving a higher value for the K_{50} . The possibility exists that access of carbamylcholine to the cell body membrane may be restricted by glial layers remaining around the soma after desheathing, whereas access to dendritic membranes is relatively unrestricted. The difference in time course of the responses recorded from the two regions of the neurone may support this idea. Alternatively, the values of Δg_{max} for the dendrites may be orders of magnitude greater than those measured during the cell body responses; in this case, the values of K_{50} may not in fact be significantly different.

In Gl 2, there is a rapid divergence in the chemosensitivities of synaptic areas (represented by the dendrites) and extrasynaptic areas (represented by the cell body). This divergence takes place by 55% embryogenesis, at which stage only the primary dendrite branches and numerous filopodia are present and morphologically mature synaptic contacts have not formed (Blagburn et al., 1985). It seems that any mechanism by which receptors are preferentially localized in dendritic membranes must be initiated before the onset of synapse formation, perhaps by transient filopodial contact. In *Manduca*, clustering of receptors in synaptic areas of the antennal lobe takes place even in the absence of afferent antennal innervation (Hildebrand et al., 1979), although the possible influence of nonantennal neurones cannot be ruled out. In contrast, during the *in vivo* development of neuromuscular junctions, ACh receptor clusters only form on myotubes after innervation and do not occur in extrajunctional areas (Bevan and Steinbach, 1977). *In vitro* ACh receptors cluster before innervation, but there is no tendency for nerves to grow to preexisting clusters, and receptors subsequently accumulate at the newly formed synapses (Frank and Fischbach, 1977). The available evidence suggests that changes in receptor density in insect neurones take place before synapse formation, and a possible guiding role for receptor clusters cannot, as yet, be ruled out. Giant interneurone 2 in the terminal ganglion of *P. americana* emerges as a suitable candidate for development studies, since both synaptic and extrasynaptic regions of the cell are accessible to microiontophoresis of receptor agonists.

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