The Central Projections of the Stretch Receptor Neurons of Crayfish: Segmental Gradients of Synaptic Probability and Strength

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The 20 stretch receptor neurons (SRs) of the crayfish abdomen send axons into the CNS that then project both to the brain and to the last abdominal ganglion, G6 (Bastiani and Mulloney, 1988). In G6, we recorded intracellularly from different kinds of neurons postsynaptic to SR axons. In a sample of 100 postsynaptic neurons, 59 synapsed with both SR1 and SR2 axons, 19 synapsed only with SR1 axons, and 22 synapsed only with SR2 axons. Most monosynaptic connections in G6 were excitatory and behaved like typical chemical synapses. The EPSPs showed moderate facilitation but could be depressed about 50% by protracted stimulation at 20 Hz or more.

In individual postsynaptic neurons, comparisons of synapses made by SRs that originated from different abdominal segments and from each side of the abdomen revealed gradients of probability of synaptic connection and of relative sizes of EPSPs; SRs originating in anterior segments were less likely to synapse with most postsynaptic neurons than were SRs originating in posterior segments, and the EPSPs caused by these anterior SRs tended to be smaller. Similarly, SRs contralateral to the postsynaptic neuron were less likely to make a connection, and the EPSPs they caused tended to be smaller than those caused by ipsilateral SRs. Some local interneurons in G6 had reversed anterior–posterior gradients in EPSP amplitude. Calculations of shape indices for PSPs from SRs originating in different segments and measurements of the maximum shunting by preceding PSPs from other SR axons indicated that neither electrotonic decrement in the postsynaptic neurons nor shunting could account fully for the observed gradients in PSP strength.

These results are discussed in terms of the known gradients in structural variability, soma size, and axon diameter. A developmental hypothesis is proposed to explain both the normal and reversed gradients of PSP strength.

The stretch receptor neurons (SRs) convey to a crayfish's CNS information about the relative position and rate of movement of each of its abdominal segments (Alexandrowicz, 1951, 1967;

Fields and Kennedy, 1965; Wine, 1977). In each of the first 5 abdominal segments, there is one bilateral pair of SR1 neurons and one bilateral pair of SR2 neurons. Each neuron gives rise to an axon that enters the CNS by the second nerve, N2, of the abdominal ganglion in the adjacent anterior segment. Each SR axon projects from the ganglion where it first enters the CNS both anteriorly to the brain and posteriorly to the terminal abdominal ganglion, G6 (Alexandrowicz, 1951; Bastiani and Mulloney, 1988). In each of the ganglia they first enter, the SRs make a characteristic set of synapses with excitatory and inhibitory motor neurons that contribute to orderly extension and flexion movements (Wine, 1977; Edwards and Mulloney, 1987). Wine (1977) demonstrated that the pattern of synapses made in the ganglion of entry is repeated by each SR in adjacent ganglia but that these synapses in neighboring ganglia were weaker than those in the ganglion of entry. Thus, each SR axon makes a series of synaptic connections as it traverses the segmental ganglia, and the strength of these synapses declines as the axon gets farther from its ganglion of entry both in the anterior and the posterior directions.

We had observed gradients of structural variant frequency in the projections to the terminal abdominal ganglion, G6, made by both kinds of SR neuron (Bastiani and Mulloney, 1988). SR axons originating in more anterior ganglia, those farther from G6, were more apt to have unusual structural features in G6 than were SR axons from more posterior ganglia. These variant structures included unusual shapes of primary axons, long primary axons, and large terminal varicosities. We therefore investigated the synaptic targets of the SR axons in G6 and the properties of these synapses to see if any physiological correlates of these structural variations could be discovered.

In G6, SR neurons synapse directly with motor neurons, intersegmental and local interneurons, and other types of sensory neurons (Bastiani, 1981). All the monosynaptic connections we recorded were excitatory, and most behaved like chemical synapses. Synapses made by SR1 axons and SR2 axons had similar properties. They facilitated at moderate stimulus frequencies but depressed if stimulated for relatively long periods.

Three sorts of gradient became apparent when we compared PSPs caused by different SRs in the same postsynaptic neuron. In most of the postsynaptic neurons from which we recorded, anterior SRs were less likely to make a synaptic connection than were posterior SRs, and when a synapse with an anterior SR did occur, the PSP was smaller than that caused by more posterior SRs. Comparing SRs originating on the ipsi- and contralateral sides of the same segment showed that the PSPs caused by contralateral SRs were smaller than those caused by ipsilateral SRs. In some postsynaptic neurons, however, these ante-

rior–posterior gradients in probability of connection and synaptic strength were clearly reversed; the more anterior SRs caused larger PSPs. These postsynaptic neurons with reversed gradients were all local interneurons. These results are discussed in the context of the normal development of arthropod CNS. A preliminary account of these results has already appeared (Bastiani and Mulloney, 1981).

Materials and Methods

Male and female crayfish, *Procambarus clarkii*, were obtained from Monterey Bay Hydroculture Farms (Santa Cruz, CA). Animals were cooled on ice, and a window was cut in the dorsal carapace that overlies the 2 pairs of muscle-receptor organs (MROs) in each segment. The SR neurons originate in the MROs. The MROs were dissected free of the extensor muscles, and the remaining dorsal carapace, extensor muscles and deep flexor muscles were removed. The second nerve (N2) on each side of each abdominal ganglion was freed up to the ganglion, and the ventral nerve cord with the MROs attached by the N2s was removed from the abdomen and transfered to a Sylgard-lined petri dish. The nerve cord was pinned out in the dish under saline. Two pairs of pin electrodes (Mulloney and Selverston, 1974a) were placed on each N2. One pair, used for stimulation, was placed as far distally as possible. The other, used for recording, was placed near the ganglion. This arrangement allowed selective stimulation of the SR axons, normally the largest axons in N2.

We used 2 different preparations to study the patterns and properties of synapses made by SR axons with diverse targets in G6. In most experiments, we prepared the ventral cord with stimulating and recording electrodes on from 3 to 5 N2s on one side and 1 or 2 N2s on the other. From 6 to 10 SR ipsilateral SR neurons, chosen for a large anterior to posterior spread, and at least 2 contralateral SRs were stimulated to determine the pattern of synaptic connections made by SR neurons with other postsynaptic neurons in G6.

In 11 experiments, we prepared the ventral cord with each N2 of G1–G4 intact and equipped each N2 with both recording and stimulating electrodes. This arrangement permitted us to test each postsynaptic neuron's response to each of 16 SR neurons—4 SR1s and 4 SR2s on each side of the cord. Because of technical difficulties in dissecting G5's MROs, this segment was passed over in these 11 more difficult experiments.

Intracellular recordings. Physiological recordings were made from the neuropil processes of 87 SR neurons and over 300 neurons postsynaptic to the SR neurons in 136 crayfish. Intracellular recordings were made from the axons of SR neurons in G6, using microelectrodes and methods described in Bastiani and Mulloney (1988).

Physiological identification of the SR neurons. The axons of SR neurons in G6 were identified by correlating the extracellular spike recorded from an N2 of a selected abdominal ganglion with an intracellular spike that rose from a flat baseline and that followed each extracellular spike at a constant latency at frequencies as high as 200 Hz. SR2 axons could be distinguished from SR1 axons in the same N2 by SR2's lower threshold to extracellular stimulation, its faster conduction velocity, and the larger amplitude of its extracellularly recorded action potential (see review, Alexandrowicz, 1967).

Classification of cells postsynaptic to the SR neurons. Neurons were considered to receive monosynaptic input from the SR neurons if the postsynaptic potentials (PSPs) followed each stimulation of the SR neurons at up to 200 Hz without failure, at a constant latency, independent of the frequency of stimulation or the number of SR neurons stimulated. Postsynaptic cells were classified as motor neurons if an antidromic impulse could be elicited in them by stimulating one of the motor nerves of G6 or if the cell had an axon projecting out of one of G6's peripheral nerves. Interneurons could only be defined anatomically; cells filled with Lucifer yellow (LY) that had an axon projecting anteriorly in the connective were defined as intersegmental interneurons; cells with structures confined to G6 were defined as local interneurons.

Quantitative description and normalization of PSPs. The peak amplitudes and 3 shape indices—time-to-half-peak amplitude, time-to-peak amplitude, and halfwidth (Rall, 1967)—of selected PSPs were measured with a HIPAD Digitizer (Houston Instruments) and a laboratory computer. Each point was measured twice, and the mean value was used in further calculations. Each of the shape indices was paired with
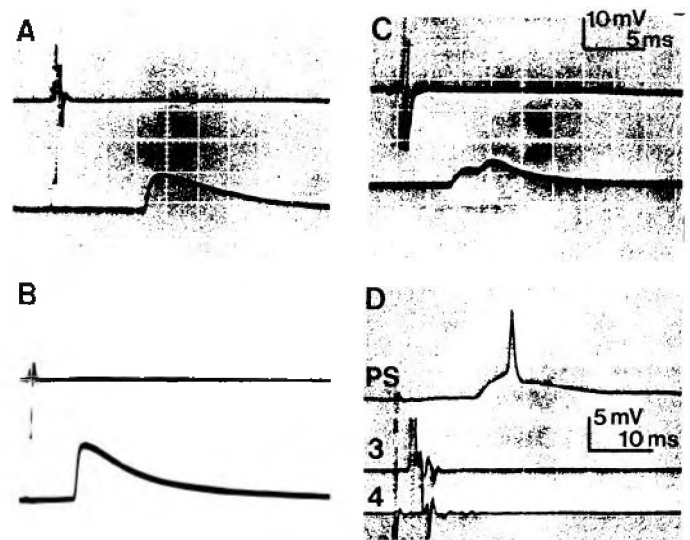

Figure 1. PSPs in neurons of the terminal abdominal ganglion that received synaptic input from SR neurons. *A,* Neuron receiving input only from SR1s. *B,* Cell receiving input only from SR2s. *C,* Cell receiving input from both SR1s and SR2s. In *A–C,* the *upper traces* are extracellular records of SR impulses in the nerves through which they first entered the CNS; the *lower traces* are intracellular records of PSPs in neurons in G6. *D,* Postsynaptic neuron (*PS*) fired an action potential in response to 4 EPSPs from SR1 and SR2 of G3 and G4, summed with 3 nA injected current. *PS,* intracellular record from a neuron in G6; *3, 4,* Extracellular records of SR1 and SR2 impulses in N2 of G3 and G4.

the peak amplitude of the PSP. The Wilcoxon test for paired samples was used to determine the significance of the results (Zar, 1974).

The measured amplitudes of the largest EPSPs from SR1 and SR2 in each postsynaptic cell were used to normalize the other EPSPs from the respective SR neurons. The amplitude of each of these EPSPs was divided by the amplitude of the largest EPSP to generate a normalized synaptic strength. This normalization corrected for differences in the input resistances of different postsynaptic neurons and for the effects of different recording sites when the same neuron was encountered in different animals. The sum of all the normalized synaptic strengths for each type of SR neuron in each hemisegment was divided by the total number of postsynaptic neurons sampled to get the mean normalized synaptic strength for each SR neuron of each hemisegment.

Cytology and morphometry were done with the methods described in Bastiani and Mulloney (1988).

Results

This section first describes the properties of individual SR synapses with other neurons in the terminal abdominal ganglion, G6, then compares synapses made by SR neurons that originated in different segments.

Synaptic connections of the SR neurons in G6

Different postsynaptic cells could receive input from either both SR1s and SR2s, SR1s alone, or SR2s alone (Fig. 1). In all cases, where a cell received input from both SR2 and SR1, the first PSP recorded in the postsynaptic cell was from SR2 and the second from SR1, which had a slower conduction velocity than SR2. Most connections behaved like typical excitatory chemical synapses. EPSPs could sum with each other (Fig. 1C) and with depolarizing currents to initiate an action potential in the postsynaptic cell (Fig. 1D). The size of each PSP was influenced by membrane potential; depolarizing current injected into the postsynaptic cell caused the EPSP to get smaller, while hyperpolarizing current caused it to get bigger (data in Bastiani, 1981).
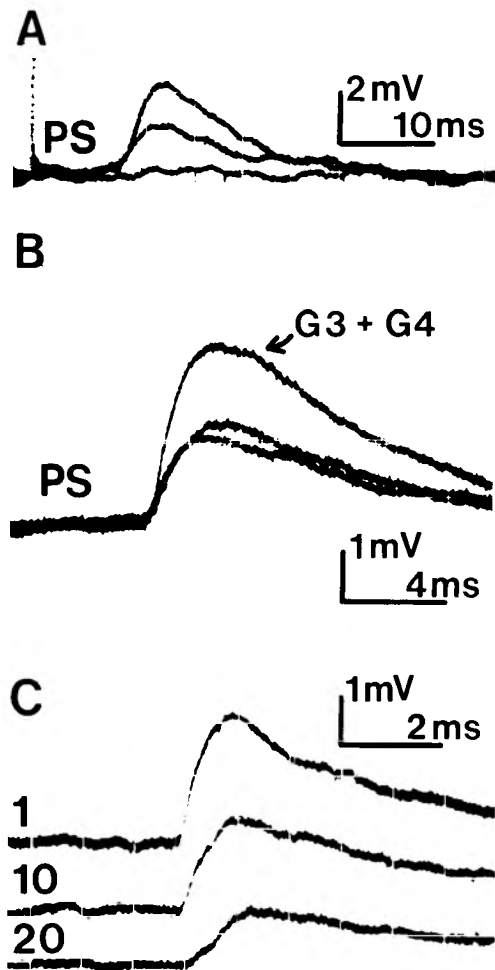
Figure 2. PSPs from SR axons onto postsynaptic (*PS*) cells in G6 exhibited spatial and temporal summation. *A,* Three responses in the PS cell showing spatial summation. *Smallest PSP,* Stimulation of SR2 of G4; *middle PSP,* stimulation of SR2 and SR1 of G4; *largest PSP,* stimulation of SR1 and SR2 in G3 and G4. *B,* Another postsynaptic neuron. *Small PSPs,* Separate stimulation of SR2 axons from G3 and G4; *G3 + G4,* simultaneous stimulation of the same SR2 axons. *C,* Depression of the EPSP from SR2 after 5 sec of stimulation at 1, 10, and 20 Hz. Depression is approximately 50% at 20Hz.

EPSPs from SR neurons exhibited both spatial and temporal summation (Fig. 2, *A, B*). They also showed moderate synaptic depression when stimulated above 20 Hz (Fig. 2*C*).

Some SR axons appeared to be weakly coupled by gap junctions to other SR axons; small PSPs that were insensitive to postsynaptic membrane potential and showed no synaptic depression occurred in these axons when other SR axons fired impulses. Some were also dye-coupled to other SR axons (see Giaume and Korn, 1984), but this was uncommon, and when it occurred only a small subset of the SR axons were coupled (data in Bastiani, 1981).

Gradients in probability of synaptic connections

Neurons in G6 could be postsynaptic to SR neurons from both ipsi- and contralateral sides of the crayfish. In different motor neurons and in different animals, the exact number of presynaptic SR neurons and the absolute magnitude of the PSPs varied considerably, but the probability that more posterior SR neurons innervated a postsynaptic cell was greater than that of more
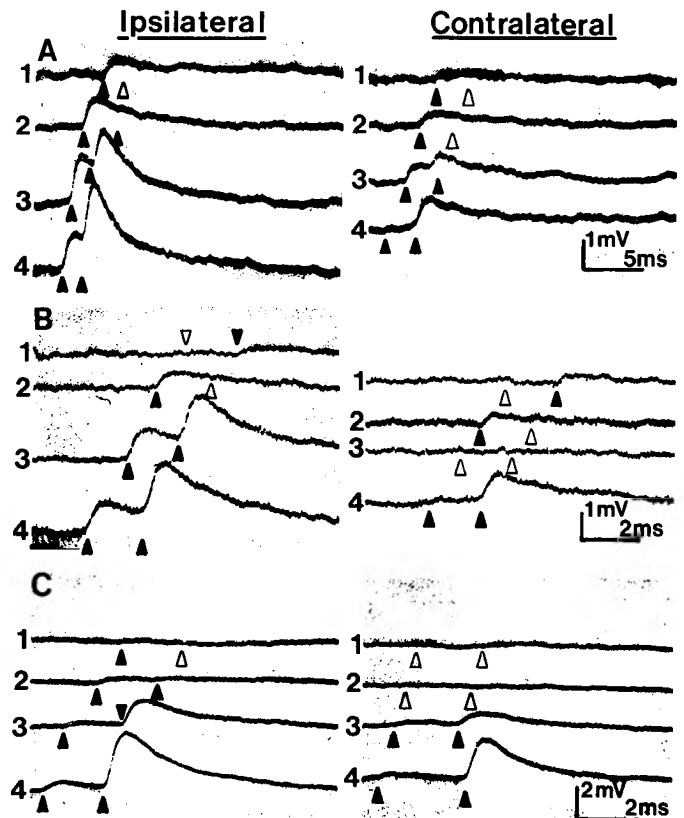

Figure 3. PSPs recorded from 3 neurons in G6 (*A–C*) that were postsynaptic to SR neurons. Each panel shows the results of stimulating separately each N2 of G1 through G4. To separate the traces triggered by the different stimuli, the position of the trace on the oscilloscope screen was changed between each sweep; the membrane potentials of the postsynaptic neurons were not altered. ▲, PSPs caused by an SR neuron; △, time when a missing PSP was expected.

anterior SR neurons. The probability of synapses onto all neurons postsynaptic to the SR neurons was greater for the ipsi- than for contralateral SR neurons originating in the same segment. This gradient of probability occurred in all motor neurons and most interneurons postsynaptic to the SR neurons.

The patterns of synapses between the ipsi- and contralateral SR neurons from G1 through G4 and 3 postsynaptic cells—A, B, and C—are shown in Figure 3. SR1 and SR2 neurons from abdominal ganglia G1–G4 were each stimulated on the side ipsi- and contralateral to the postsynaptic cell in G6. The PSPs from SRs in more anterior segments arrived later than those from more posterior SRs because of the time needed for their impulses to reach G6, but the delays of SR PSPs from the posterior segments are good predictors of the times when PSPs from the more anterior SRs should have arrived. In cell A, both SR1 PSPs from G1 and one contralateral SR1 PSP from G2 were missing. In cell B, both SR2 PSPs from G1 and both SR1 PSPs from G2 were missing; the contralateral SR1 and SR2 PSPs from G3 were also missing. In cell C, the contralateral PSPs from G1 and G2 were all missing, and the ipsilateral SR1 PSP from G1 was missing.

To measure the probability of synaptic connections with different SRs, we measured the responses of each postsynaptic neuron encountered in 11 preparations to stimulation of 16 different SR neurons from G1 through G4. SR1 and SR2 neurons from both sides of G1–G4 were stimulated separately to
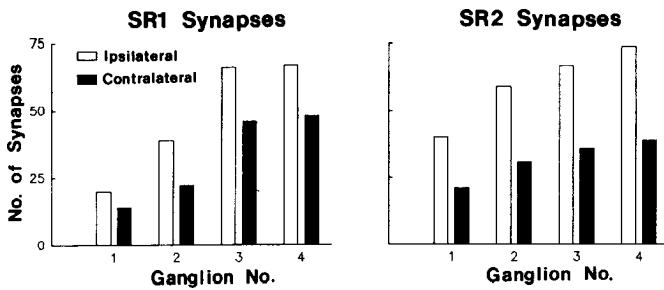
Figure 4. Distribution of numbers of synapses made in G6 by SR axons originating in different segments. A sample of 100 neurons in G6, from 11 crayfish, was tested for synaptic connections with each of the SR1 and SR2 axons entering each side of G1 to G4. *Open bars,* ipsilateral SRs; *filled* bars, contralateral SRs.

Figure 5. Relative strengths of synapses (mean ± SE) made by SR neurons originating on each side of the first four abdominal segments. The number of PSPs in each category is given in Figure 4. *Open symbols,* ipsilateral SRs; *filled symbols,* contralateral SRs.

determine if the neuron in G6 synapsed with that SR neuron. Data like those shown in Figure 3 were collected for each of 100 postsynaptic neurons (mean rest potential = -84 mV ± 9 SD). Fifty-nine neurons synapsed with both SR1 and SR2 axons, 19 synapsed only with SR1s, and 22 synapsed only with SR2s.

Both anterior-to-posterior and contralateral-to-ipsilateral gradients of increasing numbers of synapses occurred for SR1 and SR2 axons in this sample (Fig. 4). A cell in G6 had a greater probability of receiving an EPSP from the more posterior SR neurons and from the ipsilateral SRs within any segment. In this sample, 15 postsynaptic neurons synapsed with every one of the 16 SR neurons. Seven postsynaptic neurons synapsed with every ipsilateral SR tested but with none of the contralateral SR neurons. No postsynaptic neurons synapsed exclusively with contralateral SR neurons.

Gradients in the strength of synapses

When SR1 and SR2 axons originating in different ganglia were stimulated in turn, the EPSPs caused by SRs originating in the more posterior segments were commonly bigger (Fig. 3). The amplitude of the PSP caused by stimulating individual SR neurons from different segments was measured and taken as an index of synaptic strength. The largest EPSP from SR1 and SR2 recorded from each cell was used to normalize these amplitudes so that differences between animals and between input resistances of the various postsynaptic cells would not affect the analysis. These normalized amplitudes were used to calculate the mean relative size of PSPs caused by each SR from each side of each segment in the sample of 100 neurons. The mean relative sizes of these EPSPs (Fig. 5) showed a trend similar to the gradients of probability of synapses. The strength of synapses made by more posterior SR neurons was greater, except for SR2 axons from G3 and G4. Ipsilateral SR neurons from any given segment made stronger synapses than their contralateral homologs.

Reversed gradients of synaptic strength

Some of the neurons postsynaptic to SR axons in G6 showed reversed patterns of PSP sizes (Fig. 6). In this example, from a local interneuron recorded in a preparation that lacked stimulating electrodes on both N2s of G1 and the contralateral N2 of G3, the neuron received synaptic input both from SR1s and from SR2s. The usual gradient of synaptic strength can be seen for the EPSPs from SR2 neurons, but the gradient of SR1 PSPs was reversed. EPSPs from the more anterior SR1 neurons were larger than EPSPs from more posterior SR1s. In this example,
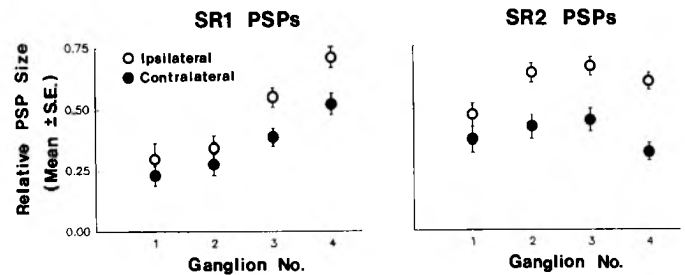
the contralateral-to-ipsilateral gradient was the same for both SR2 and SR1, despite the reversed anterior-to-posterior pattern. The interneuron itself is illustrated in Figure 8*F*. In our sample of 100 neurons whose PSPs from each of 16 SR neurons were measured, 9 had reversed SR1 gradients and 15 had reversed SR2 gradients.

Differences in relative synaptic strength are not caused by missing synapses

Our assessment of the relative strengths of PSPs from different SRs was complicated by the occasional absent connection (e.g., Fig. 3). To eliminate this potential source of error, we isolated the data from those postsynaptic neurons that had measurable PSPs from each ipsilateral SR1 or SR2 from G1–G4. To define and identify cells with reversed gradients, we set a criterion for PSPs from different segments. A cell had a normal gradient unless the amplitudes of its ipsilateral PSPs had these relations:

$$A_1 \geq A_3$$

and

$$A_1 > A_4$$

and

$$A_2 > A_4$$

where A_n means the amplitude of the PSP recorded in the cell when the SR axon from abdominal ganglion n was stimulated. Most showed normal gradients (cf. Figs. 7 with 5). A minority of these postsynaptic neurons showed reversed gradients of PSP size; the mean ipsilateral PSPs from G1 in these neurons were larger than those from G4. Three neurons of this kind were postsynaptic to SR1 neurons; 4 were postsynaptic to SR2 neurons. The gradients of PSP sizes in these neurons contrast with those in the other neurons that received 4 ipsilateral SR PSPs (Fig. 7). This difference was apparent when we considered either relative PSP sizes (Fig. 7) or absolute PSP sizes (data not shown).

Differences in relative synaptic strength are not caused by shunting

The interpretation of the pattern of EPSP size for SR1 might be confused in some instances, especially those of reverse patterns, if the EPSP from SR2, which arrived first, affected the amplitude of the EPSP from SR1. To test this possibility, different SR neurons from the same or adjacent segments were stimulated at selected intervals of time (Fig. 6, control). In this example, the size of the G4 PSP decreased when it arrived shortly after the peak of the G3 PSP; compare the sizes of G4
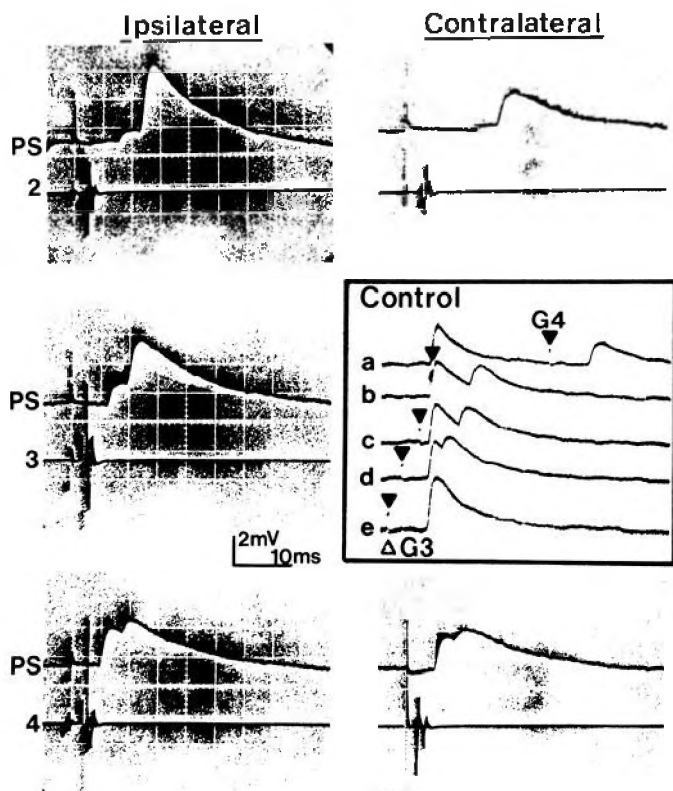
Figure 6. PSPs, recorded in a local interneuron in G6 (Fig. 8*F*), from SR neurons originating in segments 2–4. Each panel shows the result of stimulating an N2 entering an anterior ganglion. The first EPSP in each record was from SR2, the second from SR1. *Control,* Summation of EPSPs in a different postsynaptic cell from 2 SR2 neurons from G3 and G4. In these 5 sequential recordings (*a–e*), one SR2 of G3 was stimulated at the start of the sweep (△) and one SR2 of G4 was stimulated at different latencies (▼). *Trace a* shows the EPSPs from SRs in G3 and G4 stimulated at times sufficiently different that no summation occurred; *traces b–e* show the summation of these PSPs when they were stimulated at shorter intervals.

PSPs in traces a and d. The maximum shunting occurred when the second PSP arrived 2.7 msec after the first: the average maximum reduction of the second PSP was 26% ± 14 SD (*n* = 16). This shunting decreased sharply as the temporal separation of the PSPs increased. The separation of PSPs from the more anterior SRs was greater because of the different conduction velocities of SR1 and SR2 axons, so shunting is unlikely to be the cause of the observed gradients in synaptic strength.

Differences in strength do not reflect distances from recording site

Cells postsynaptic to the SR neurons normally received EPSPs of different sizes from SR neurons originating in different ganglia (Figs. 5, 7). This situation might arise in at least 2 ways. The number of synaptic contacts made with a postsynaptic cell might be the same for all SR neurons, and the larger EPSPs would result from SR neurons that made their synapses closer to the recording site. Electrotonic decrement would then create the size differences. Alternatively, the sites of synaptic contact might be electrically equivalent for all SR neurons, and the larger EPSPs might result from a larger number of synaptic contacts made by some SR neurons. These 2 alternatives could be distinguished by comparing the shapes of PSPs from different SRs.
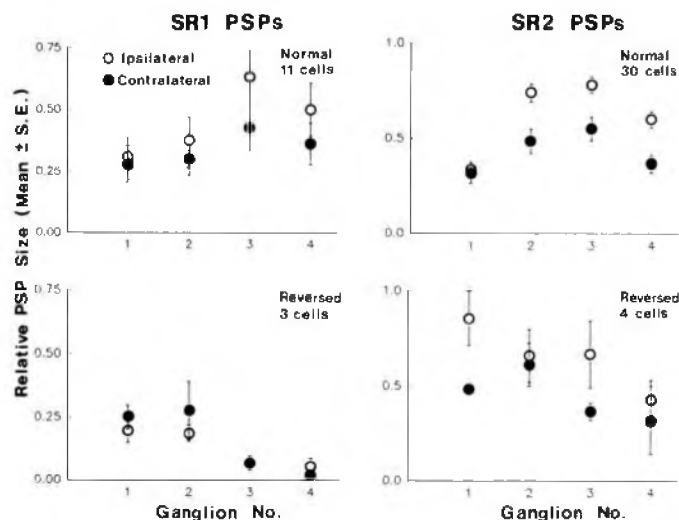

Figure 7. Relative strengths of synapses (mean ± SE) made by SR neurons originating on each side of the first 4 abdominal segments. These results include only data from neurons that synapsed with every one of the 4 ipsilateral SR neurons. Postsynaptic neurons that had reversed gradients of synaptic strength were analyzed separately (*lower plots*). *Open symbols,* ipsilateral SRs; *filled symbols,* contralateral SRs.

If a larger EPSP resulted from the same synaptic current arising with the same time course as a smaller EPSP, but simply at a site closer to the recording electrode, then the shape indices (Rall, 1967) for the larger EPSP should differ from those for a smaller EPSP in the same cell. To make these comparisons, a large and a small EPSP were recorded in each of 16 cells postsynaptic to the SR neurons. The larger EPSP (mean = 2.28 mV ± 1.24 SD) was from a more posterior or from an ipsilateral SR neuron and the smaller EPSP (mean = 0.77 mV ± 0.36 SD) was from a more anterior or from a contralateral SR neuron. The peak amplitudes and 3 shape indices—time-to-half-peak amplitude, time-to-peak amplitude, and halfwidth—were measured. Three hypotheses that would be true if the different PSPs arose at electrotonically separated sites were tested: that the time-to-half-peak amplitude of the larger EPSP was less than that of the smaller EPSP ($p = 0.10$), that the halfwidth of the larger EPSP was less than that of the smaller EPSP ($p = 0.25$), and that the time-to-peak of the larger EPSP was shorter than that of the smaller EPSP. This last hypothesis was false ($p = 0.0005$). These results imply that larger EPSPs are not larger because they arise at synapses closer to the recording site than do smaller PSPs.

Classification of postsynaptic targets of SR neurons in G6

To determine if different types of postsynaptic neurons had characteristically different patterns of connections and synaptic strength, cells that had been shown physiologically to be postsynaptic to SR neurons were then filled with LY to discover their structures. For this analysis, neurons were classified as motor neurons, intersegmental interneurons, and local interneurons. Other sensory neurons were excluded from the sample. Six neurons were characterized and filled at least twice (Fig. 8). Several other neurons of each type were identified and filled only once. Different motor neurons postsynaptic to the SR neurons projected axons out nerves 1, 2 (not shown), 3, and 6. All of the motor neurons and most interneurons received PSPs from both SR1 and SR2. The pattern of PSPs in all the motor neurons
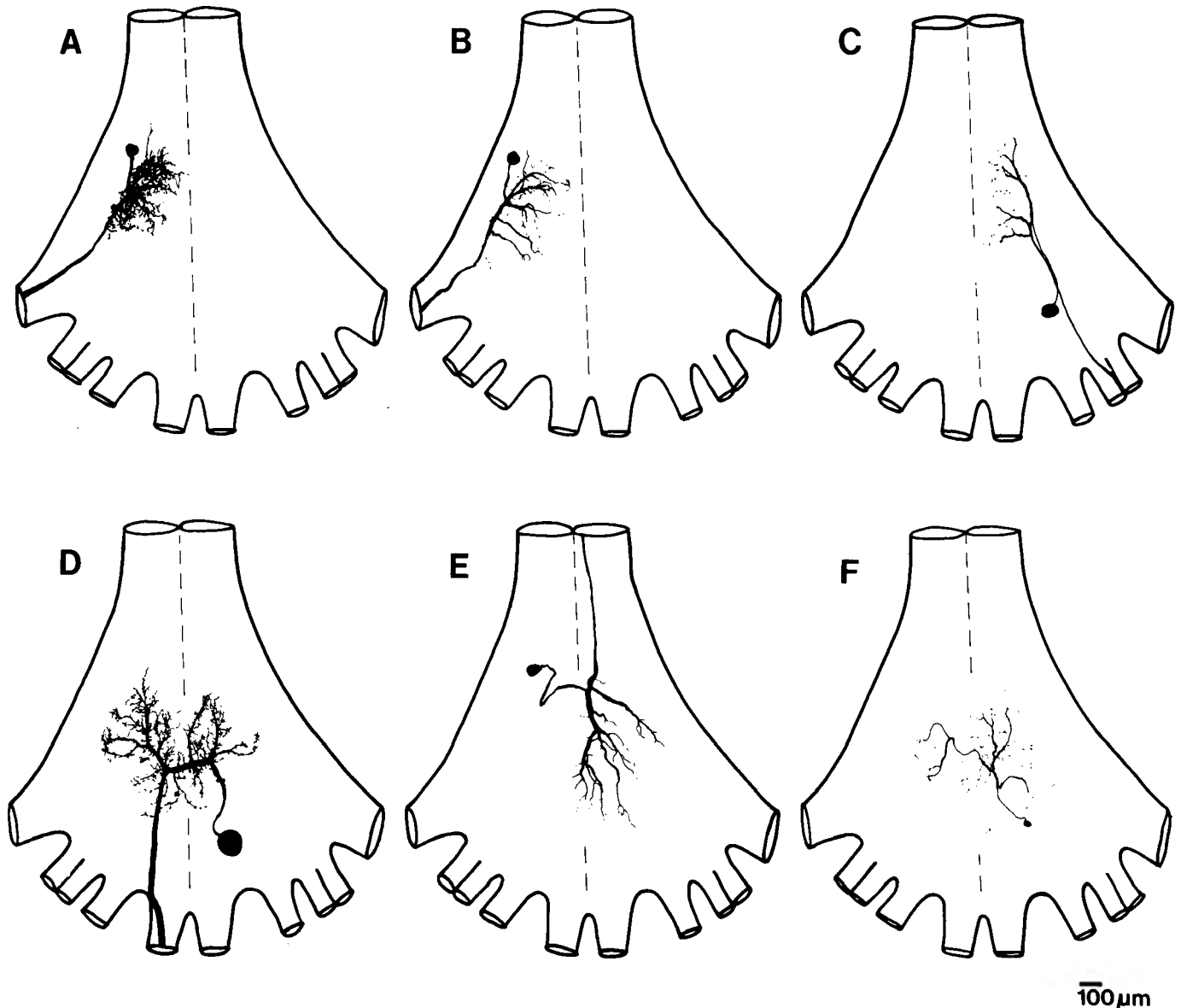
Figure 8. Neurons postsynaptic to the SR neurons in G6. Each cell was filled with dye at least twice. *A–D,* Motor neurons. *E,* An intersegmental interneuron. *F,* Local interneuron.

was so similar that it could not be used to distinguish among them. Two cells that received input from only one kind of SR neuron, one from SR1s and one from SR2s, were interneurons. All the motor neurons and intersegmental interneurons had the usual anterior-to-posterior gradients of connectivity and synaptic strength. One neuron with a reverse pattern of connectivity and synaptic strength was filled more than once, a local interneuron (Fig. 8*F*).

The neurons shown in Figure 8 are probably not a complete catalog of the cells postsynaptic to the SR neurons in G6, or even of the neurons from which we recorded more than once. Only if the cell was filled with LY could it be classified, and not all neurons were held long enough to fill them successfully. Wiersma (1958) described 2 interneurons, one that summed the activity of all the SR1 neurons and another that summed the activity of the SR2 neurons. He found these units at several levels in the CNS and suggested that they integrated SR activity in G6. We recorded from several intersegmental interneurons

that summed the activity of the SR neurons but none with properties like those Wiersma described. Had these interneurons been brought near threshold by other synaptic input, it is possible that their spike frequency would have reflected the summed activity of the SR neurons. It might be that tonic input to these cells normally present in intact crayfish was removed by severing their connection with the cephalothorax and the periphery, so that in our preparations these interneurons could not be identified as the neurons that Wiersma studied.

Discussion

The SR neurons from different abdominal segments converge onto a varied group of postsynaptic neurons in G6. Each SR, however, has only a certain probability of synapsing with any given target neuron. The exact number of SR neurons that synapsed with a given target neuron, and the magnitudes of its EPSPs, varied from animal to animal. Although the SR neurons on each side of each segment can be identified and although we
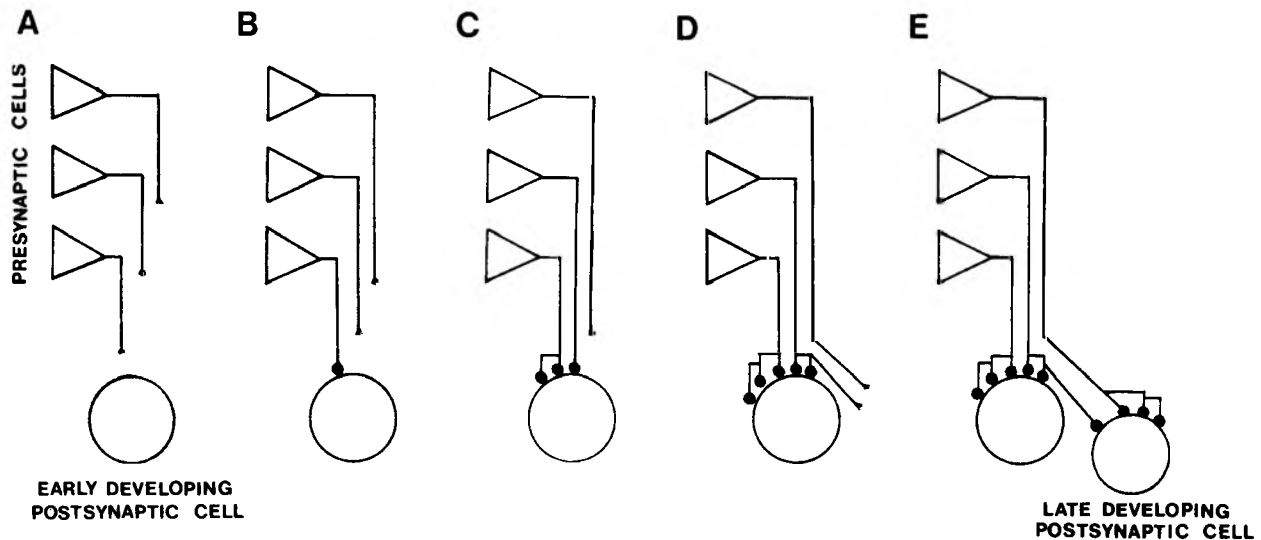
Figure 9. Diagram of a hypothesis to account for the patterns of synaptic connectivity and relative strength observed between the SR neurons and their postsynaptic targets in G6. Here, 3 SR neurons from different starting points project axons toward G6. Each SR can form 3 synaptic contacts (●). The SR axons encounter potential postsynaptic targets when they reach G6, but these target neurons differentiate at different times and can support at most 5 synaptic contacts. These limitations on the abilities of pre- and postsynaptic neurons to form synaptic contacts interact with the different times of arrival of SR axons and differentiation of targets to generate the different patterns of synaptic connections made by SR axons from different segments of the animal (see text).

have never seen duplications or omissions of SR neurons, this set of converging synapses is not as tightly determined as are those in other well-characterized circuits in arthropods, such as the stomatogastric ganglion (Mulloney and Selverston, 1974a, b; Selverston and Mulloney, 1974; Mulloney, 1977, 1987) or the projections of the compound eyes (Meinertzhagen and Frohlich, 1983).

We recorded from over 300 cells postsynaptic to the SR neurons in G6, a ganglion that contains about 650 neurons (Reichert et al., 1982). Their synapses with these targets were all qualitatively similar, but 40% of the postsynaptic neurons synapsed selectively with either SR1 or SR2 neurons. SR1s and SR2s conduct different information about abdominal movements, despite the similarity of their structures in G6, so it is not surprising that the 2 kinds of SR neurons were distinguished by some of their postsynaptic targets. The observation of a reversed gradient for SR1 in the presence of a normal gradient for SR2 in the same postsynaptic neuron (Fig. 6) points to the possibility of even finer specification of connections with some neurons.

Our analysis of the data recorded from the 100 postsynaptic neurons encountered in the 11 experiments that had every SR axon from G1 to G4 prepared for testing treated these neurons as independent samples drawn from a population. This treatment might be misleading if the same neuron was sampled repeatedly in one experiment; the pattern of PSPs seen in this neuron would unduly influence the resulting statistics. To estimate the number of repeated penetrations within each experiment, we counted the occurrences of identical patterns of connectivity. Identical patterns occurred in 5 of the 11 experiments; 4 experiments had 2 penetrations each with the same pattern, the fifth had 2 penetrations with one pattern and 3 others with another pattern. Taking the view that every occurrence of identical patterns was actually a repeated penetration of the same postsynaptic neuron, our sample was contaminated by at most 6 neurons recorded repeatedly in the same experiment, and the actual sample size was 93, not 100. Since there is no reason to

expect that every postsynaptic neuron will have a unique pattern of connectivity, the actual number of repeats is probably less than 6.

Structural correlates of the gradients of probability

We have shown that the mean number of ipsilateral branches made by SR neurons from each abdominal segment in G6 was significantly larger than the mean number of contralateral branches (Bastiani and Mulloney, 1988). The numbers of ipsilateral synapses made by these neurons are also greater than the numbers of contralateral synapses (Fig. 4), differences that occurred in SR axons from every abdominal segment. This correlation suggests that the numbers of branches made by each SR axon are indicators of the numbers of synapses they make. If this suggestion were correct, then our previous observation that SR neurons from G1 and G5 make similar numbers of branches in G6 (Bastiani and Mulloney, 1988) would predict that SR neurons from G1 and G5 make similar numbers of synapses in G6. Our sample of these synapses, for both SR1 and SR2, indicated that this was not so; SR neurons from posterior segments made more synapses than those from anterior segments (Fig. 4).

Two possible explanations of this contradiction would be either that the correlations of numbers of branches and synapses on each side of G6 are not causally related or that our sample of postsynaptic neurons was not an unbiased sample of SR target neurons in G6. For example, in our sample, 24 of 100 postsynaptic neurons had at least one reversed gradient. However, this may not mean that 24% of postsynaptic neurons have reversed gradients; our estimate might have been lowered if our sampling methods were biased. All these neurons with reversed gradients were probably local interneurons. Local interneurons are abundant in G6 (Takahata et al., 1981; Reichert et al., 1982; Nagayama et al., 1984), and some are known to integrate sensory information there, but these small neurons are the most difficult to record from with microelectrodes. If our methods made suc-

cessful recordings from larger neurons more probable than from smaller neurons, we may have undersampled the gradients in local interneurons. If small local interneurons normally have reversed gradients of synaptic strength, then we may have underestimated the frequency with which reversed gradients occur in G6, and so underestimated the numbers of synapses made by neurons from anterior segments.

The gradient of probability of synapses might have another structural basis. SR neurons from anterior ganglia are more likely to have axonal projections of unusual shape in G6 than are SRs from posterior ganglia, and these unusual shapes apparently develop during embryogenesis (Bastiani and Mulloney, 1988). Therefore, the gradients of synaptic probability might arise in the face of a relatively constant number of SR branches if some of these branches develop in inappropriate parts of G6 and fail to find their normal targets. Certainly, individual variants in the shapes of SR axons in G6 might be the cause of missing PSPs in a given target neuron (e.g., Fig. 3); in locusts, missing branches of descending interneurons have been correlated with missing PSPs in target neurons (Pearson and Goodman, 1979; Steeves and Pearson, 1983).

Structural correlates of the gradients of synaptic strength

From our comparisons of the shape indices of PSPs from different segments, we conclude that the ipsilateral–contralateral and anterior–posterior gradients of synaptic strength cannot be explained by electrotonic decrement of PSPs that arise in different sites. Shunting of a PSP by other PSPs that arrived shortly before is also inadequate to account fully for the observed gradients in strength. Furthermore, the gradient in probability of unusual shapes of SR axons in G6 (Bastiani and Mulloney, 1988) does not suggest an explanation for these gradients, particularly not for the reversed gradients that were measured. The most common gradient in synaptic strength runs counter to 2 other structural gradients: SR neurons from more anterior segments have larger cell bodies (Wiersma and Pilgrim, 1961) and axons of larger diameter in the CNS (Bastiani and Mulloney, 1988), but make weaker synapses in G6.

Another segmental gradient of synaptic strength has been reported in the crayfish abdominal cord. In each abdominal ganglion, a pair of segmental interneurons (SG) act as relays between the lateral giant interneurons (LG) and the non-giant fast flexor motor neurons (FF) of that segment (Kramer et al., 1981). The strength of this relay decreases from anterior to posterior segments (Miller et al., 1985). This gradient differs in interesting ways from the one we describe here. The synapses between SG and FF are electrical, and the FFs do not receive input from SGs other than the pair in its own segment. Miller et al. (1985) also described a gradient of increasing amplitude of PSPs in FFs in posterior abdominal ganglia caused by a pair of intersegmental descending interneurons. Like the SG-FF gradient, individual postsynaptic neurons that receive these synapses do not see a gradient of PSP amplitudes. The structural correlates of these gradients have not been described.

A hypothesis to account for development of gradients of synaptic strength

The patterns of normal neuronal differentiation and pathfinding that occur in arthropods provide some insight into underlying mechanisms that could generate gradients like those we have observed. Peripheral sensory neurons differentiate from the ectoderm and epidermis of each segment, and the SR neurons do

so early during embryonic development; all the SRs are present and their projections to G6 are in place at hatching (Allen, 1894; Bastiani and Mulloney, 1988). Neurons in the CNS of crayfish and insects differentiate from a segmentally repeating pattern of neuroblasts (Bate, 1976; Thomas et al., 1984) that spin off progeny in a fixed order: first, long projection neurons and, later, local interneurons (Goodman et al., 1979). Thus, the different classes of targets of SR axons in G6 arise in a temporal sequence. We propose that the sequential arrival in G6 of SR axons from different segments and cellular limitations on their abilities to form synaptic contacts lead to the development of the observed gradients of synaptic strength.

Lateral differences. The observation that neurons ipsilateral to the SR neurons's cell body made stronger synapses with the SR than did contralateral neurons (Fig. 5) suggests that the time at which different SRs first reach a given target neuron may influence the strengths of the synapses they can form. The hook-shaped course through G6 followed by most SR axons (Bastiani and Mulloney, 1988) implies that the first targets encountered by each SR axon as it enters G6 during embryogenesis are ipsilateral. Only after its axon crosses the midline and turns again anteriorly will it encounter targets on the contralateral side. If developing segmental pairs of SR neurons extend their axons on the same schedule, then each should reach its ipsilateral targets before its contralateral homolog does. The normal development of many nervous systems involves processes that can be interpreted as competition for synaptic sites (e.g., Hume and Purves, 1983; Forehand and Purves, 1984; Shepherd and Murphey, 1986). In these competitions, late arrivals might be at a disadvantage, and so make weaker synapses.

Segmental gradients. The temporal sequence hypothesis can be extended to account for the segmental gradients of synaptic strength in G6. From our data on the number of branches made by each SR in G6 (Bastiani and Mulloney, 1988) and the number of points of contact between presynaptic SRs and postsynaptic neurons (Bastiani, 1981), we can predict that each SR can make 65 branches and that each branch will make between 2 and 8 points of contact with a given postsynaptic neuron. We assume that each SR can make a limited number of synaptic contacts and will try to make as many as it can up to that limit. We also assume that the ability of any postsynaptic neuron to accept a synapse from the SR neurons is limited, and this postsynaptic restriction sets an upper limit on the total number of SR synaptic contacts with that neuron. These cellular limitations, which are common to all the SRs and are independent of any temporal gradients, then interact with the postulated differences in the times of arrival of SRs from different segments and from different sides of the same segment to generate the differences in synaptic strength. The interactions of these assumptions also predict the existence of reversed gradients in those postsynaptic neurons that differentiate late, after the first SRs to reach G6 have already made most of their synaptic contacts.

A concrete example may clarify both the assumptions and the predictions of the model (Fig. 9). For the sake of clarity, the example assumes that each presynaptic branch has an intrinsic ability to make 3 synaptic contacts and each postsynaptic cell has 5 receptive sites for synaptic contacts. In Figure 9A, 3 equivalent presynaptic branches from different abdominal segments begin to grow towards an early-developing postsynaptic cell. In Figure 9B, the first branch arrives at the postsynaptic cell and makes a synaptic contact. By the time the second branch arrives and makes its first synaptic contact, the first branch has made

2 synaptic contacts (Fig. 9C). In Figure 9D, when the third branch finally reaches the postsynaptic cell, the first branch has made its limit of 3 synaptic contacts and the second branch has made 2 synaptic contacts, so all the sites for SR synapses on the postsynaptic cell are taken. Therefore, the last branch to arrive is unable to synapse with the early-developing postsynaptic cell. An anterior-to-posterior gradient in connectivity and synaptic strength between the early-developing postsynaptic cell and the SRs results. In Figure 9E, both the second and the last branch to arrive go in search of other neurons with which to synapse and discover a late-developing postsynaptic cell. Both cells arrive at the same time and begin making synaptic contacts. The most recently arrived SR has not previously made any synaptic contacts and so makes all 3 on the late-developing target cell. The other cell has already made 2 synaptic contacts on the early-developing postsynaptic cell and so makes its last synaptic contact on the late-developing cell. The gradients of probability and synaptic strength are reversed in this late-developing neuron. By such interactions, the rather complex patterns of connectivity and synaptic strength in a heterogeneous population of postsynaptic cells might be the consequence of the spatiotemporal patterns of maturation in the pre- and postsynaptic populations and the limited abilities of each neuron to form synapses.

References

Alexandrowicz, J. S. (1951) Muscle receptor organs in the abdomen of *Homarus vulgaris* and *Palinurus vulgaris*. Q. J. Microsc. Sci. *92:* 163–199.

Alexandrowicz, J. S. (1967) Receptor organs in the thoracic and abdominal muscles of crustacea. Biol. Rev. *42:* 288–326.

Allen, E. J. (1894) Studies on the nervous system of crustacea: I. Some nerve-elements of the embryonic lobster. Q. J. Microsc. Sci. *36:* 461–482.

Bastiani, M. (1981) The central anatomy and physiology of the stretch receptor neurons of the crayfish. Ph.D. Thesis, University of California, Davis.

Bastiani, M. J., and B. Mulloney (1981) Patterns of connectivity and synaptic strength in cells of the sixth abdominal ganglion post-synaptic to the abdominal stretch receptor neurons of the crayfish. Soc. Neurosci. Abstr. *7:* 403.

Bastiani, M. J., and B. Mulloney (1988) The central projections of the stretch receptor neurons of crayfish: Structure, variation, and postembryonic growth. J. Neurosci. *8:* 1254–1263.

Bate, C. M. (1976) Pioneer neurons in an insect embryo. Nature *260:* 54–56.

Edwards, D. H., and B. Mulloney (1987) Synaptic integration in excitatory and inhibitory crayfish motoneurons. J. Neurophysiol. *57:* 1425–1445.

Fields, H. L., and D. Kennedy (1965) Functional role of muscle receptor organs in crayfish. Nature *206:* 1232–1237.

Forehand, C. J., and D. Purves (1984) Regional innervation of rabbit ciliary ganglion cells by the terminals of preganglionic axons. J. Neurosci. *4:* 1–12.

Goodman, C. S., M. O'Shea, R. McCaman, and N. C. Spitzer (1979) Embryonic development of identified neurons: Temporal pattern of morphological and biochemical differentiation. Science *204:* 1219–1222.

Giaume, C., and H. Korn (1984) Voltage-dependent dye coupling at a rectifying electrotonic synapse of the crayfish. J. Physiol. (Lond.) *356:* 151–167.

Hume, R. I., and D. Purves (1983) Apportionment of the terminals from single preganglionic axons to target neurons in the rabbit ciliary ganglion. J. Physiol. (Lond.) *338:* 259–275.

Kramer, A. P., F. B. Krasne, and J. J. Wine (1981) Interneurons between giant axons and motoneurons in crayfish escape circuitry. J. Neurophysiol. *45:* 550–573.

Meinertzhagen, I. A., and A. Frohlich (1983) The regulation of synapse formation in the fly's visual system. Trends Neurosci. *6:* 223–228.

Miller, L. A., G. Hagiwara, and J. J. Wine (1985) Segmental differences in pathways between crayfish giant axons and fast flexor motoneurons. J. Neurophysiol. *53:* 252–265.

Mulloney, B. (1977) Organisation of the stomatogastric ganglion of the spiny lobster. V. Coordination of the gastric and pyloric systems. J. Comp. Physiol. *122:* 227–240.

Mulloney, B. (1987) Neural circuits. In *The Crustacean Stomatogastric Nervous System: A Model for the Study of Central Nervous Systems*, A. I. Selverston and M. Moulins, eds., pp. 59–75, Springer, Heidelberg.

Mulloney, B., and A. I. Selverston (1974a) Organization of the stomatogastric ganglion of the spiny lobster. I. Neurons driving the lateral teeth. J. Comp. Physiol. *91:* 1–32.

Mulloney, B., and A. I. Selverston (1974b) Organization of the stomatogastric ganglion of the spiny lobster. III. Coordination of the two subsets of the gastric system. J. Comp. Physiol. *91:* 53–78.

Nagayama, T., M. Takahata, and M. Hisada (1984) Functional characteristics of local nonspiking interneurons as the premotor elements in crayfish. J. Comp. Physiol. *154:* 449–510.

Pearson, K. G., and C. S. Goodman (1979) Correlation of variability in structure with variability in synaptic connections of an identified interneuron in locusts. J. Comp. Neurol. *184:* 141–166.

Rall, W. (1967) Distinguishing theoretical synaptic potentials computed for different soma-dendrite distributions of synaptic input. J. Neurophysiol. *30:* 1138–1168.

Reichert, H., M. R. Plummer, G. Hagiwara, R. L. Roth, and J. J. Wine (1982) Local interneurons in the terminal abdominal ganglion of the crayfish. J. Comp. Physiol. *149:* 145–162.

Selverston, A. I., and B. Mulloney (1974) Organization of the stomatogastric ganglion of the spiny lobster. II. Neurons driving the medial tooth. J. Comp. Physiol. *91:* 33–51.

Shepherd, D., and R. K. Murphey (1986) Competition regulates the efficacy of an identified synapse in crickets. J. Neurosci. *6:* 3152–3160.

Steeves, J. D., and K. G. Pearson (1983) Variability in the structure of an identified interneurone in isogenic clones of locusts. J. Exp. Biol. *103:* 47–54.

Takahata, M., T. Nagayama, and M. Hisada (1981) Physiological and morphological characterization of anaxonic non-spiking local interneurons in the crayfish motor control system. Brain Res. *226:* 309–314.

Thomas, J. B., M. J. Bastiani, M. Bate, and C. S. Goodman (1984) From grasshopper to *Drosophila*: A common plan for neuronal development. Nature *310:* 203–207.

Wiersma, C. A. G. (1958) On the functional connections of single units in the central nervous system of the crayfish, *Procambarus clarkii* (Girard). J. Comp. Neurol. *110:* 421–471.

Wiersma, C. A. G., and R. L. Pilgrim (1961) Thoracic stretch receptors in crayfish and rock lobster. Comp. Biochem. Physiol. *2:* 51–64.

Wine, J. J. (1977) Crayfish escape behavior. III. Monosynaptic and polysynaptic pathways involved in phasic extension. J. Comp. Physiol. *121:* 187–203.

Zar, J. H. (1974) *Biostatistical Analysis*, Prentice-Hall, Englewood Cliffs, NJ.