J. exp. Biol. (1981), 90, 283–305
With 9 figures
Printed in Great Britain

FURTHER OBSERVATIONS ON THE SEROTONERGIC CEREBRAL NEURONES OF *HELISOMA* (MOLLUSCA, GASTROPODA): THE CASE FOR HOMOLOGY WITH THE METACEREBRAL GIANT CELLS

BY BONNIE GRANZOW

Department of Zoology, University of Iowa, Iowa City, IA 52242*

AND C. H. FRASER ROWELL

Department of Zoology, University of California, Berkeley, CA 94720

(*Received* 29 *April* 1980)

SUMMARY

A bilaterally symmetrical pair of serotonergic neurones in the cerebral ganglia of the snail *Helisoma trivolvis* has major features in common with the serotonergic cerebral cells of other gastropods, including those of *Helix* and *Aplysia*. The cells were found to receive a high level of inhibitory synaptic activity which is important in determining their firing level. In the cells of isolated cerebral ganglia, tonic inhibitory synaptic potentials, uniform in frequency and amplitude, occur continually at a rate of about 2/s. In the cells of cerebral ganglia left intact with the periphery and buccal ganglia, phasic inhibitory activity occurs in addition to the tonic activity. Phasic inhibitory input could be observed at regular intervals, correlated with feeding motor activity in the buccal ganglia. Other properties of these cells include: a linear relationship between membrane potential and current injected into the cell; communication between bilateral cells in about 50% of the preparations studied; and sodium-based somatic action potentials. Some of these properties of *Helisoma* SCCs are different from those of *Helix* and *Aplysia*. We propose that the *Helisoma* SCCs are homologous to those in other gastropods and that the differences observed are the result of evolutionary adaptation of the cells to different functional roles.

INTRODUCTION

By relating properties of homologous neurones to behavioural characteristics, we may be able to determine the cellular bases of behavioural adaptation. The similarities between homologous structures are traceable to a common ancestry, whereas the differences are due to subsequent adaptation to different functions. Comparisons of apparently homologous neurones have been undertaken in a number of invertebrates including annelids (Keyser & Lent, 1977), arthropods (Paul, 1979) and gastropod molluscs (Blankenship & Coggeshall, 1976; Dickinson, 1979; Dorsett, 1974; Rózsa

* Present address: Department of Biology, B-022, University of California, San Diego, La Jolla, 92093.

et al. 1980; Willows & Dorsett, 1975). Identifiable neurones of invertebrates could well afford new opportunities for studying homologies of the nervous system at the cellular level.

In the gastropods, a bilaterally symmetrical pair of large serotonin-containing neurones has been identified in the cerebral ganglia of *Aplysia*, *Ariolimax*, *Helix*, *Lymnaea*, *Limax*, *Planorbis*, *Pleurobranchaea* and *Tritonia* (Berry & Pentreath 1976; Pentreath Osborne & Cottrell, 1973; Osborne & Cottrell, 1971; Sakharov & Zs-Nagy, 1968; Sedden, Walker & Kerkut, 1968; Weinreich *et al.* 1973). These neurones have four features in common which suggest that they are homologous: (*a*) large size, (*b*) serotonin content, (*c*) a complex projection with axons extending down the cerebrobuccal connectives and into buccal nerve trunks (Berry & Pentreath, 1976; Dorsett, 1967; Gillette & Davis, 1977; Kandel & Tauc, 1966*a*; McCrohan & Benjamin, 1980*a*; Pentreath & Cottrell, 1974; Senseman & Gelperin, 1974; Weiss & Kupfermann, 1976), and (*d*) the ability to influence neurones in the buccal ganglia (Berry & Pentreath, 1976; Bulloch & Dorsett, 1979; Gillette & Davis, 1977; Senseman & Gelperin, 1974). These cells were originally described in *Helix* and because of their large size and position in the metacerebral lobe of each ganglion they were designated the Metacerebral Giant Cells, or MCGs (Kandel & Tauc, 1966*a*,*b*). We have chosen to use a more general teminology, serotonergic cerebral cell (SCC), when referring to cells other than those of *Helix*. Whereas serotonin content is a feature of these cells across all gastropods so far examined, 'giant' cell size is not; furthermore, in most genera, pro-, meso-, and metacerebral lobes cannot be distinguished so that the term 'metacerebral' is also not appropriately descriptive. Weiss & Kupfermann (1976) have already put forward a strong case for homology between the SCCs of *Aplysia* and the MCGs of *Helix*.

A pair of cerebral cells in the aquatic snail *Helisoma trivolvis* (Granzow & Kater, 1977) also possess the four major features of the SCCs of other gastropods. In this paper we reveal some major physiological differences between these neurones and the SCCs of the most thoroughly characterized genera, *Aplysia* and *Helix*. We conclude that the anatomical and chemical similarities support homology, and that the numerous physiological differences might be related to behavioural and ecological diversification among genera.

MATERIALS AND METHODS

The experimental animals were laboratory-raised *Helisoma trivolvis* of the *Oregon Red* strain (albinos). Electrophysiological recordings were made from either the completely isolated cerebral ganglia or the semi-intact brain preparation described by Granzow & Kater (1977). Conventional recording and display methods were employed. Intracellular recordings were made from buccal ganglia neurones with 4 M potassium-acetate electrodes (DC resistance = 30–60 MΩ). 4 M potassium-chloride electrodes (DC resistance = 10–20 MΩ) were used for recording from SCCs since these electrodes consistently maintained lower resistances following repeated passage of stimulating currents. Penetration of SCCs was usually facilitated by softening the cerebral ganglion sheath with a 3–4 min treatment in 0·5 % protease (type IV, Sigma Chemical Co.), a procedure which did not noticeably alter neural activity when compared to non-proteased preparations. The physiological saline used consisted of 51·3 mM-NaC

7 mM-KCl, 4·1 mM-$CaCl_2$, 1·5 mM-$MgCl_2$, and 2·4 mM-$NaHCO_3$. In some experiments the normal saline was replaced by salines of different ionic concentrations: o Ca^{2+}—high Mg^{2+} saline ($CaCl_2$ omitted and Mg^{2+} concentration raised to 10 × normal by adding $MgSO_4$), o Ca^{2+} saline ($CaCl_2$ replaced with NaCl), and o Na^+ saline (NaCl replaced with sucrose).

RESULTS

Cell size and appearance

The SCCs of *Helisoma* are the largest cells in the cerebral ganglia as in other gastropods. Their size does not, however, much exceed that of the next largest cells and in this they differ from the SCCs of, for example, *Aplysia*, *Tritonia*, *Helix* and *Limax* (Kandel & Tauc, 1966*a*,*b*; Dorsett, 1967; Pentreath *et al.* 1973; Weinreich, *et al.* 1973; Bulloch, 1977) which have been described as giant cells. In *Helisoma* the diameter of SCC somata range from 80–90 μm. The cells are located at the anterior edge of the dorsal surface of each cerebral ganglion, and can often be recognized by a yellowish colour which contrasts with the much paler colour of surrounding cells. Thus, while the SCCs are not as easily distinguished on cellular size alone, as are the SCCs of some gastropods, they are still readily recognizable; in approximately 95% of all preparations in which an SCC was sought it was the first cell impaled with an intracellular electrode, as verified by its characteristic activity.

Firing patterns

SCC activity was recorded mostly in semi-intact preparations. Immediately following penetration, a SCC in such preparations usually displayed spontaneous action potentials with frequencies up to 1·5 impulses/s. The pattern of firing was usually modified by apparently inhibitory synaptic input. This frequently occurred in bursts of one to several seconds in duration, producing a low-frequency periodicity in the firing of SCCs (Fig. 1 A). Generally, the firing frequency declined over the first 20–30 minutes after penetration with the recording electrode, and then the cells often became silent. This was apparently due to an increase in firing threshold of the cell since the membrane potential remained stable and the cell could be fired by the intracellular injection of depolarizing current.

In contrast, when SCC activity was recorded from completely isolated cerebral ganglia (i.e. all peripheral nerves and all connectives joining the cerebral ganglia to the rest of the central nervous system cut), the activity was very regular and at a higher frequency, reaching 2·5 impulses/s (Fig. 1 B and C). Furthermore, this firing frequency was often maintained over relatively long periods of time (i.e. greater than 40 min).

Synaptic activity in isolated ganglia

The low-frequency i.p.s.p. bursts of semi-intact preparations occurred only rarely in isolated ganglia preparations and were completely absent from most. The SCCs of isolated ganglia did, however, display tonically occurring unitary potential fluctuations. These were generally very regular in both amplitude and frequency, as revealed by slightly hyperpolarizing the SCC to reduce the number of action potentials generated (Fig. 1 D). These unitary potential fluctuations occurred synchronously in both cells

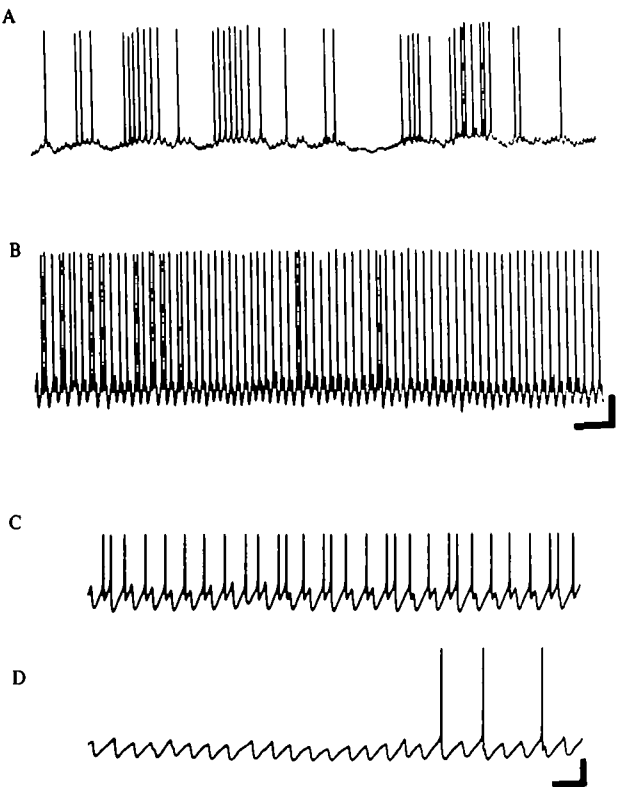

Fig. 1. Spontaneous activity of SCC in a semi-intact brain preparation (A) and in an isolated cerebral ganglia preparation (B). The SCCs of isolated cerebral ganglia display regular membrane potential fluctuations. These are shown for another cell which is at resting potential in (C) and slightly hyperpolarized in (D) to reduce firing of action potentials. Tops of action potentials are clipped in (C). Calibrations: (A) 25 mV, 2 s; (B) 20 mV, 2 s; (C) 20 mV, 1 s; (D) 20 mV, 1 s.

(Fig. 2A) indicating that they are inhibitory post-synaptic potentials (i.p.s.p.s) derived from a tonically-firing intracerebral source supplying both SCCs. The evidence that these potential fluctuations are chemical i.p.s.p.s is as follows: (1) the fast downward phase and slower rising phase of individual fluctuations were typical of the form of the classical chemical i.p.s.p. (2) The potentials were completely abolished in 0 Ca^{2+} high Mg^{2+} saline, suggesting a chemical synaptic origin. (3) Depolarization from resting potential augmented their amplitude and hyperpolarization diminished it, without affecting their frequency. (4) The potentials could be reversed by hyperpolarization (Fig. 3). In this experiment, the left cell was hyperpolarized with increasing steps of current while the right cell was either maintained at resting potential, or hyperpolarized just enough to reduce firing and thus more clearly reveal the i.p.s.p.s which were synchronous in the two cells. This procedure allowed the positive-going potentials in the hyperpolarized cell to be identified as reversed i.p.s.p.s by matching them with the negative-going potentials in the control cell. Gradually increasing the amount of hyperpolarization applied to the left cell first diminished th

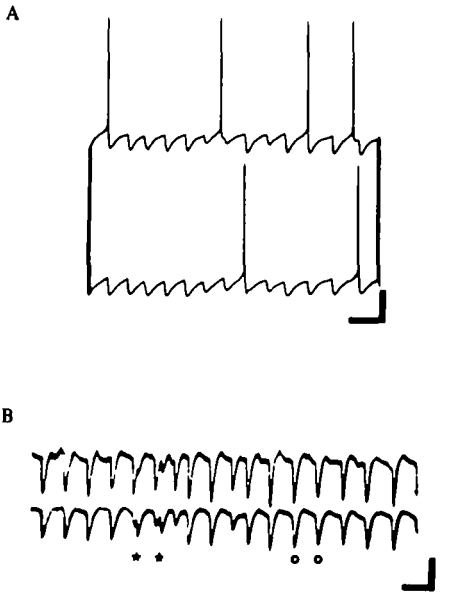

Fig. 2. Simultaneous intracellular recordings from right and left SCCs in isolated cerebral ganglia showing that regular membrane potential fluctuations occur synchronously in the two cells. (A) Activity shortly after penetration with an intracellular electrode. Both cells are being held slightly hyperpolarized to reduce firing of action potentials. (B) Activity approximately 60 min after electrode penetration. The cells are no longer generating action potentials and it appears that each of the larger i.p.s.p.s (o) actually consists of the summed results of two smaller i.p.s.p.s. (*). Calibrations: (A) 20 mV and 1 s; (B) 10 mV and 2 s.

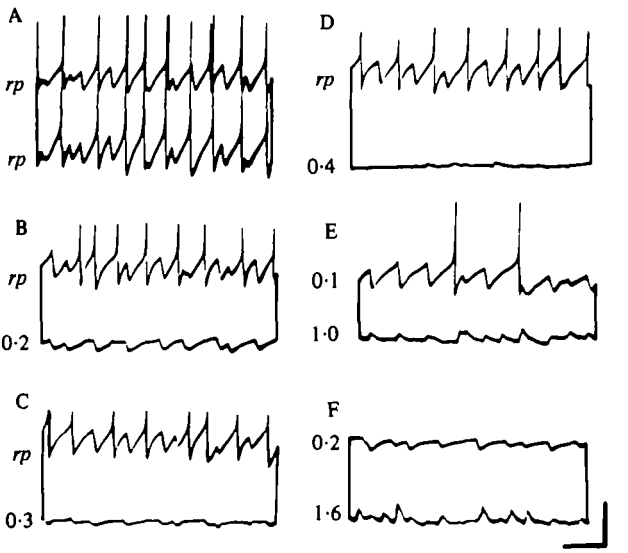

Fig. 3. Reversal of presumed i.p.s.p.s. in SCCs by the injection of hyperpolarizing current. Activity was recorded intracellularly from both R-SCC (top trace of each record) and L-SCC (bottom trace of each record) in isolated cerebral ganglia. (A) Both cells are at resting potential (*rp*). (B–F) Increasingly greater amounts of hyperpolarizing current are injected into L-SCC as indicated to the left of each trace in nAmps. In (E) and (F) R-SCC is also slightly hyperpolarized as indicated in order to reduce firing and better reveal the baseline fluctuations. The bridge was out of balance during current injection so the records do not indicate the true membrane potential at hyperpolarized levels. Tops of action potentials have been clipped. Calibrations: 10 mV and 0·5 s.

amplitude of the p.s.p.s (Fig. 3B–C), then abolished them (Fig. 3D) and subsequently reversed them (Fig. 3E–F). Although in these records extra positive-going deflexions are apparent in the hyperpolarized left cell, each downward deflexion in the right cell is matched by an upward deflexion in the left cell.

Action potentials in the SCC of the isolated ganglia appeared to be generated off the rising phase of the recurrent i.p.s.p.s (Fig. 1C and D). In each case the slope of the rising phase was the same, but the cell did not always reach threshold before the next i.p.s.p. The firing frequency of the SCC is therefore apparently determined by the frequency at which the tonic i.p.s.p.s occur. In some cases two or more action potentials may follow an i.p.s.p. if the next i.p.s.p. is sufficiently delayed. Thus, it appears that the normal 'resting' potential of a SCC is actually above firing threshold, but that the cell is under tonic inhibition, with the frequency of the i.p.s.p.s determining the actual firing level. Alternatively, it might be that each individual i.p.s.p. momentarily lowers the threshold of the cell and that action potentials are generated as a result of post-inhibitory rebound. We have observed post-inhibitory rebound following hyperpolarization of the cell with the intracellular injection of DC current.

After relatively long time periods of recording from the SCCs of isolated ganglia (usually >40 min) the cells became less excitable and the i.p.s.p.s no longer gave rise to action potentials. Sometimes when this occurred, two different sizes of i.p.s.p.s could be observed (Fig. 2B). The larger i.p.s.p.s seem to be the summed result of two of the smaller i.p.s.p.s which become apparent when they occur slightly asynchronously. This suggests that each of the regularly occurring i.p.s.p.s is composed of input from two, probably bilaterally symmetrical, intracerebral sources which are usually strongly coupled.

Although the phasic bursts of i.p.s.p.s seen in the semi-intact preparations (e.g. Fig. 1A) were absent from most SCCs of isolated cerebral ganglia, they did occur rarely in a few preparations (Fig. 4). This suggests that the presynaptic source(s) for at least some of the phasic i.p.s.p.s is located within the cerebral ganglia, but that activation of this source is usually dependent on inputs from either the periphery or other ganglia. The phasic i.p.s.p.s observed in isolated ganglia appeared as if they might be generated by the same source as the tonic i.p.s.p.s; the phasic nature resulting from a short duration increase in the firing frequency of that source. The small amplitudes of the unitary potentials in such barrages would be accounted for by their increased frequency which results in non-linear summation as the cell hyperpolarizes toward the reversal potential of the i.p.s.p.s.

Synaptic activity in semi-intact preparations

Synaptic activity in semi-intact preparations consisted of both the tonic i.p.s.p.s seen in isolated ganglia, and additional phasic bursts of i.p.s.p.s. No spontaneous excitatory synaptic activity was apparent. The phasic inhibitory input in these preparations generally resulted in an irregular bursting of the cell (Fig. 1A). In about 15% of all semi-intact brain preparations the SCCs received prominent cyclical bursts of i.p.s.p.s (Fig. 5). Simultaneous recordings made from feeding motoneurones of the buccal ganglia revealed that these cyclical hyperpolarizations in SCCs were correlated with the rhythmic activity of these motoneurones which comprises the feeding motor program. In particular, occurrence of the cyclical hyperpolarizations in SCCs was

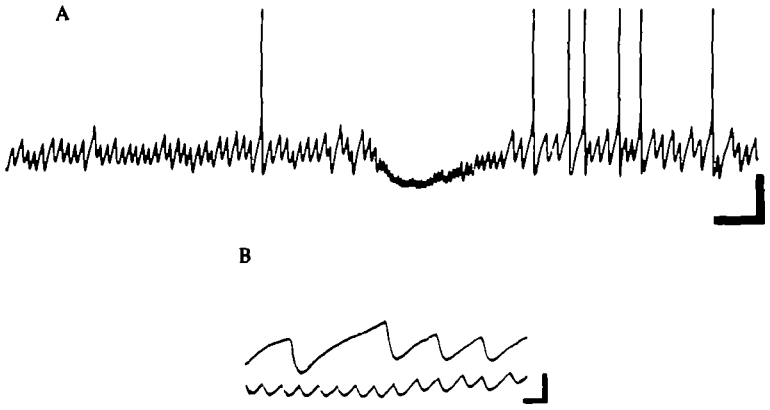

Fig. 4. (A) Spontaneous synaptic potentials in an SCC of an isolated cerebral ganglia preparation. Tonic i.p.s.p.s which are observed in all preparations of this type occur throughout the record and a phasic i.p.s.p. barrage which is observed only rarely in some preparations of this type occurs at the middle of the record. (B) Tonic i.p.s.p.s (top trace) and phasic i.p.s.p.s (bottom trace) seen at higher gain and faster sweep speed. (B) is from the same preparation as (A). Calibrations: (A) 20 mV and 2 s; (B) 200 ms and 4·5 mV.

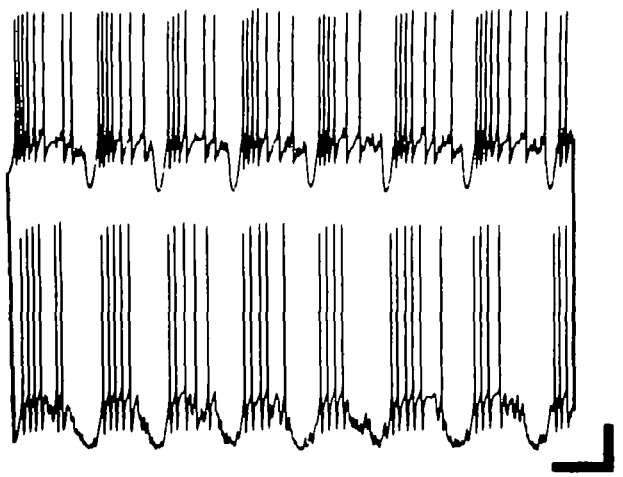

Fig. 5. Correlation of recurring phasic i.p.s.p.s in SCC with recurring hyperpolarizations in a protractor motoneurone of the buccal ganglia. Top trace, intracellular recording from identified protractor motoneurone. Bottom trace, intracellular recording from SCC. SCC is being held slightly depolarized by constant injection of DC current. Calibrations: 10 mV, top trace; 15 mV bottom trace; and 2 s.

correlated with the occurrence of the cyclical hyperpolarizations recorded from identified protractor motoneurones (Fig. 5). The onset of each SCC hyperpolarization generally preceded the onset of the shorter duration hyperpolarization in the protractor motoneurone by approximately 0·5 s, but the two terminated almost simultaneously and bursting in the two cells also occurred in the same phase of the cycle. Thus, one type of inhibitory input to the SCCs functions in correlating activity of SCCs with that of buccal ganglia feeding motoneurones. This may be due to direct or indirect feedback from the premotor pattern-generating neurones of the buccal ganglia feeding motor program.

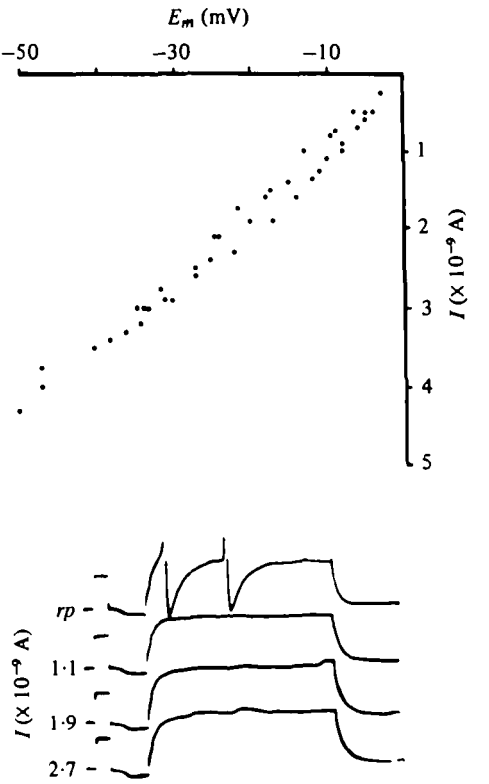

Fig. 6. Membrane potential is a linear function of the amount of current injected into SCC. An SCC has been penetrated with two intracellular electrodes. (A) Relationship between amount of current (I) injected into SCC through one electrode and membrane potential (E_m) recorded with a second electrode. (B) SCC is hyperpolarized to various levels through current injected through one electrode, the amount of current indicated at the left. Test pulses (0·7 nA) were then delivered through the second electrode. The voltage response to the test pulse was the same at all levels of membrane potential. Calibrations: (A) 10 mV and 10 ms.

Compound synaptic potentials could be evoked in SCCs by electrically stimulating the cerebral nerves in semi-intact preparations. These potentials were predominately inhibitory with the exception of those derived from the large frontal lip nerve. Stimulation of this nerve produced an antidromic action potential followed by a compound p.s.p. which had an early excitatory component and a later inhibitory component.

The absence of anomalous rectification in SCCs

Anomalous rectification has been reported as a property of the MCGs of other gastropods in which the current-voltage (I–V) relationship of the cell's membrane has been studied, including *Helix* (Kandel & Tauc, 1966*b*), *Aplysia* (Weiss & Kupfermann, 1976) and *Pleurobranchaea* (Gillette & Davis, 1977). We analysed the I–V relationship of the SCCs of *Helisoma* in several different preparations, by penetrating cells with two intracellular electrodes, one for passing current and one for measuring voltage. As shown in Fig. 6A, membrane potential (E_m) was a linear function of the amount of current injected, i.e. the membrane resistance remained constant at a

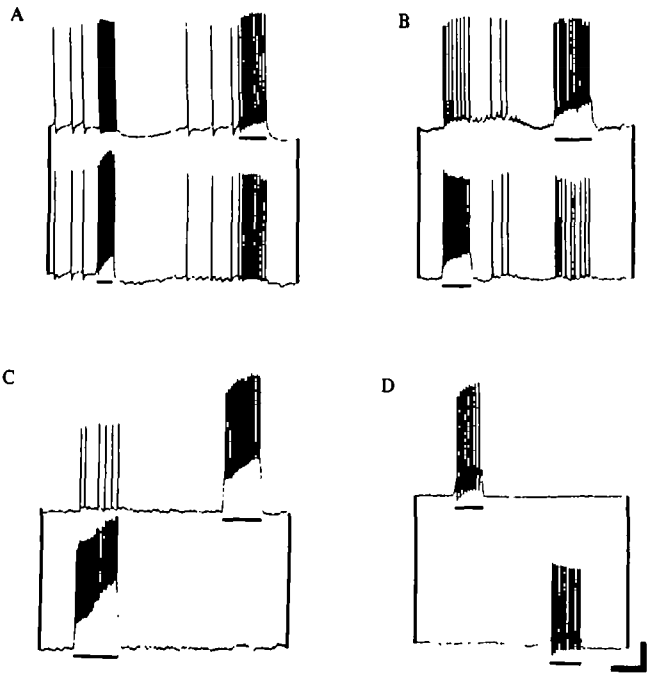

Fig. 7. Variability in communication between bilateral SCCs. Each record is taken from a different semi-intact brain preparation. Top trace in each record, R-SCC. Bottom trace in each record, L-SCC. Injection of DC depolarizing current is indicated by the bars below each trace. (A) Strong communication between SCCs. (B) Weak bidirectional communication. (C) Weak unidirectional communication. (D) No communication. Calibrations: (A) and (D) 30 mV and 2 s; (B) and (C) 25 mV and 2 s.

levels of membrane potential (Fig. 6B). Thus, anomalous rectification is *not* a property of the SCCs. We considered the possibility that the large amount of synaptic activity which occurs in these cells may have masked their anomalously rectifying properties. However, the same result – a linear I–V relationship – was also obtained in preparations bathed in 0 Ca^{2+} high Mg^{2+} saline which blocked all signs of synaptic input to the cells.

Communication between SCCs

Interactions occur between the two SCCs in *Helisoma* (Granzow & Kater, 1977). In 30% of the preparations, each action potential evoked in one cell could drive an action potential in the other cell at up to 10 impulse/s (Fig. 7A). In 20% of the preparations this communication was weaker, with post-synaptic spikes failing to follow when the pre-synaptic cell was driven at frequencies greater than 2 or 3 impulse/s. Usually this weak communication was bidirectional (Fig. 7B), but two cases of unidirectional weak communication were found (Fig. 7C). In 50% of the preparations, the SCCs did not communicate at all (Fig. 7D). We have also noted changes in the efficacy of communication within individual preparations, both from strong to weak and from weak to strong communication. Sometimes this occurred 'spontaneously' and at other times the change occurred during various experimental manipulations, such as stimulating cerebral nerves. However, attempts to alter pre-

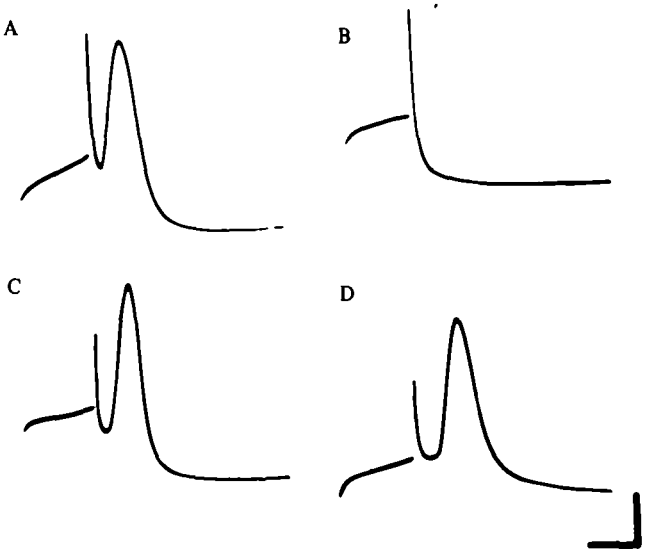

Fig. 8. Ionic basis of SCC action potential. (A) Action potential evoked with depolarizing pulse in normal saline. (B) In o Na^+ saline, action potential can no longer be evoked. (C) Return to normal saline following o Na^+ saline. (D) The action potential remains in o Ca^{2+} saline. Calibrations: 20 mV and 10 ms.

dictably the communication with such manipulations were unsuccessful. The absence of communication in preparations did not appear to be attributable to damage to the cells since activity was normal in all other respects.

The nature of the communication between SCCs was difficult to ascertain. Injection of D.C. current of either polarity into one SCC never resulted in a D.C. membrane potential shift in the other SCC, even when the two cells were strongly communicating with one another. However, communication between the two SCCs survived long periods of exposure to o Ca^{2+} high Mg^{2+} saline which abolished all signs of synaptic input to these cells. This suggests that the bilateral SCCs which do communicate are electrically coupled (but at a site far removed from the soma) as has been reported in some other genera including *Planorbis* (Berry & Pentreath, 1976), *Ariolimax* (Senseman & Gelperin, 1974) and *Lymnaea* (McCrohan & Benjamin, 1980*a*).

Each SCC apparently has more than one spike initiation zone. This conclusion follows from the observation that spontaneously occurring action potentials, recorded from the soma, i.e. those associated with the i.p.s.p.s described above, could be abolished with very small amounts of hyperpolarizing current (less than 0·1 nA). However, action potentials in one SCC which were driven by action potentials in the contralateral SCC were suppressed only with much larger amounts of hyperpolarizing current (greater than 2 nA). This indicates that spontaneous action potentials are initiated at a site which is located near the soma, but that action potentials driven by the contra-lateral SCC are initiated at a site which is relatively distant from the soma.

Ionic basis of SCC action potential

Bathing the cerebral ganglia in o Na^+ saline reversibly abolished all ongoing SCC action potentials, and action potentials could not be evoked by the injection of depolarizing current (Fig. 8A). However, SCC action potentials survived repeate

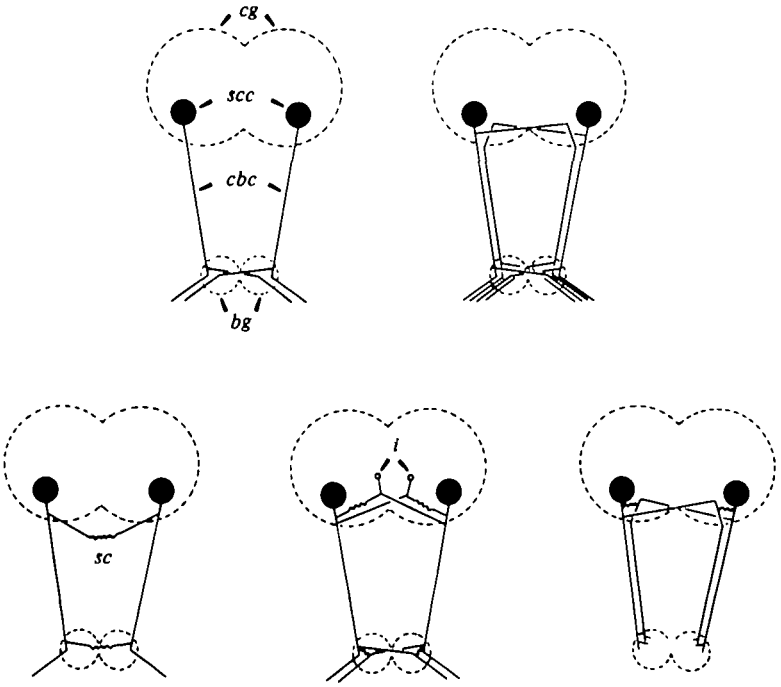

Fig. 9. A schematic, generalized representation of SCCs' (MCGs') innervation of the buccal ganglia in different gastropod genera. (A) *Aplysia*, *Limax*, *Pleurobranchaea* and *Tritonia hombergi*. (B) *Helix* and *Tritonia diomedea*. (C) *Lymnaea*. (D) *Helisoma* and *Planorbis*. (E) *Ariolimax*. (For references, see text.) No attempt has been made to indicate the laterality of the extension of the process, i.e. ipsilateral versus contralateral. In *Lymnaea* (C) there is evidence for the electrical junction (-WW-) indicated in the buccal ganglia (McCrohan & Benjamin, 1980*a*). The other electrical junctions indicated: in the subcerebral commissure (*sc*) of *Lymnaea* (C), in the buccal ganglia of *Helisoma* and *Planorbis* (D) and in the cerebral ganglia of *Ariolimax* (E) as well as the electrically coupled interneurones (*i*) in *Helisoma* and *Planorbis* (D) are hypothetical, based on the morphology of the cells and taking into consideration putative sites of contact between bilateral cells. In (E) the pathways in the buccal ganglia are omitted since no data in *Ariolimax* is available at this level. (*bg*, buccal ganglia; *cbc*, processes in cerebrobuccal connectives; *cg*, cerebral ganglia; *i*, interneurones; *sc*, subcerebral commissure; *scc*, serotonergic cerebral cells.)

washings in o Ca^{2+} saline with little or no reduction in amplitude (Fig. 8D). Thus, the currents underlying somatic action potentials of SCC are apparently due to the influx of sodium ions with little or no contribution by calcium ions.

DISCUSSION

I. *Comparison of SCCs of Helisoma and other gastropods*

In Table 1, features of *Helisoma* SCCs are compared with those of SCCs (MCGs) of several other gastropods. This table extends the comparisons made between the MCGs of *Helix* and the SCCs of *Aplysia* by Weiss & Kupfermann (1976). We have not included data on certain features such as responses to pharmacological agents and heterosynaptic facilitation as did Weiss & Kupfermann (1976) since these areas have so far not been investigated in *Helisoma* and no new data is available in other gastropods.

Table 1. *Comparison of* SCC *properties in nine genera of gastropods*

	Contains serotonin	Large soma size	Monopolar cell	Descending CBC axons: Ipsilateral	Descending CBC axons: Contralateral	Axons in other cerebral ganglia nerve trunks: Ipsilateral	Axons in other cerebral ganglia nerve trunks: Contralateral	Axons in buccal ganglia nerve trunks: Ipsilateral	Axons in buccal ganglia nerve trunks: Contralateral
Gastropoda									
Pulmonata									
Basommatophora									
Helisoma	+ (12, 13)	+ (12, 13)	+ (12, 13)	+ (12, 13)	— (12, 13)	Frontal Lip N. (12, 13)	— (12, 13)	+ (12)	+ (12)
Lymnaea	+ (24)	+ (17)	+ (17)	+ (17)	— (17)	Labial artery N. (17)	— (17)	+ (17)	— (17)
Planorbis	+ (1, 16)	+ (1)	+ (1)	+ (1)	— (1)			+ (1)	+ (1)
Opisthobranchia									
Stylommatophora									
Helix	+ (7, 22, 25)	+ (14, 22)	+ (22)	+ (21)	+ (21)	External & internal lip N. (21)	External & internal lip N. (21)	+ (21)	+ (21)
Limax	+ (7, 22, 25)	+ (22)	+ (22)	+ (26)	— (26)	External lip N. (26)			
Ariolimax		+ (26)		+ (26)	+ (26)	External lip N. (26)			
Anaspidea									
Aplysia	+ (21, 28)	+ (28)	+ (30)	+ (30)	— (30)	Posterior lip N. (30)	— (30)	+ (30)	+ (30)
Nudibranchia									
Tritonia	+ (28)	+ (8)	+ (2)	+ (2, 8)	•• (2, 8)	— (8)	— (8)	+ (8)	+ (8)
Notospidea									
Pleurobranchaea	+ (11)	+ (11)	+ (11)	+ (11)	— (11)	Mouth N. (11)	— (11)	+ (11)	+ (11)

	Communication (electrical coupling) between R & L cells	Anomalous rectification	Ionic basis of action potential	Spontaneous firing levels in dissected preparation	Spontaneous synaptic activity	Recurring synaptic input correlated with cyclical motor activity in BG
Helisoma	+ (*)	— (*)	Na^+ (*)	1·5–2·5 i/s (*)	Tonic i.p.s.p.s, unitary p.s.p.s common to both cells Phasic i.p.s.p.s, unitary p.s.p.s common to both cells (*)	Inhibitory during retraction (*)
Lymnaea	+ (16)			0·5–2·0 i/s (17)	Two types phasic compound i.-e.p.s.p.s common to both cells Tonic e.p.s.p.s, common to both cells Long duration compound e.p.s.p.s., common to both cells (17)	Excitatory during protraction, inhibitory during retraction (17)
Planorbis	+ (1)	+ (1)		~1–2 i/s (1)	Phasic e.p.s.p.s and i.p.s.p.s, some common to both cells Some independent in two cells (1)	
Helix	— (14)	+ (15)		Quiescent (14)	Tonic e.p.s.p.s, unitary potentials common to both cells Phasic e.p.s.p.s, unitary potentials common to both cells (14)	
Limax	— (26)					Excitatory during retraction and inhibitory during protraction (9)
Ariolimax	— (26)					
Aplysia	— (30)	+ (30)		Quiescent (30)	Tonic e.-i.p.s.p.s, unitary potentials *not* common to both cells Phasic e.p.s.p.s, unitary potentials common to both cells (30)	
Tritonia	— (2, 8)			1·1–1·7 i/s (8)		
Pleurobranchaea	— (11)	+ (11)		~0·5 i/s (11)		Excitatory during retraction and inhibitory during protraction (11)

	Synaptic responses to cerebral nerve stimulation	Connexions with BG Neurones	
		Monosynaptic	Polysynaptic via BG interneurones
Helisoma	I; E–I; predominately I (*)	Excitatory with protector motoneurone (13)	With protractor and retractor motoneurones (12, 13) with cell 4 which innervates salivary gland (12) and cell 5, the largest BG cell (unpublished data)
Lymnaea		Excitatory with some protractor and retractor motoneurones and with cell 1 which innervates salivary gland (17)	With protractor and retractor motoneurones (17)
Planorbis	E (1)	Excitatory with a few small BG cells (1)	With feeding motoneurones (1)
Helix	E (28); E–I, predominately E (6, 27)	Excitatory with 2 of 3 largest BG cells (4, 5)	With large medial BG cell (4, 5, 26)
Limax			With large medial BG cell With salivary burster (23, 26)
Ariolimax		Excitatory with 2 of 3 largest BG cells (26)	With large medial BG cell (26)
Aplysia	E–I, predominately E (30)	Some excitatory and some inhibitory with many BG cells including motoneurones (10, 29)	
Tritonia	I at resting potential (8)	Some excitatory and some inhibitory with a few BG cells including motoneurones (2, 3)	With feeding motoneurones (2, 3)
Pleurobranchaea		Excitatory with a few BG cells including motoneurones (11)	With feeding motoneurones (11)

	Effects on feeding motor output of BG	Modulates at level of muscle
Helisoma	Can initiate and maintain rhythmical BG motor output, can speed up ongoing rhythm, can increase intensity of protractor motoneurone bursts (12, 13)	
Lymnaea	Can increase intensity of retractor motoneurone bursts; does not initiate rhythmical BG motor output nor influence rate of ongoing rhythm (17)	
Planorbis	Can initiate and maintain rhythmical buccal mass movements, although not complete feeding movements (1)	
Helix		+ Lip muscle (20)
Limax	Can sometimes activate a few cycles of organized motor output from BG, but does not maintain rhythmical output (19)	
Ariolimax		
Aplysia	Can increase intensity of motoneurone bursts; in excitable preparations can speed up ongoing rhythmical BG motor output, can activate a single 'feeding cycle' but cannot maintain rhythmical BG motor output in otherwise quiescent preparations (29)	+ Buccal mass muscle (29)
Tritonia	Can activate multicomponent post-synaptic responses in identified BG motoneurones typical of feeding motor output (2, 3)	
Pleurobranchaea	Can speed up ongoing rhythmical BG motor output; can activate a single 'feeding cycle' but cannot maintain rhythmical BG motor output in otherwise quiescent preparations (11)	

+ indicates that a particular cell is characterized by the property listed at the head of the column; − indicates that a particular cell is known *not* to exhibit such a property; numbers in parentheses indicate the source of information as listed below. * refers to data presented in the present paper. **Bulloch (1977) has reported contralateral CBC axons in some specimens of *Tritonia hombergi*, but they are absent in most; Dorsett (1967) has reported evidence for contralateral CBC axons in *Tritonia diomedea*. (1) Berry, M. S. & Pentreath, V. W., 1976; (2) Bulloch, A. G. M., 1977; (3) Bulloch, A. G. M. & Dorsett, D. A., 1979; (4) Cottrell, G. A., 1977; (5) Cottrell, G. A. & Macon, J. B., 1974; (6) Cottrell, G. A., Macon, J. & Szczepaniak, A. C., 1972; (7) Cottrell, G. A. & Osborne, N. N., 1970; (8) Dorsett, D. A., 1967; (9) Gelperin, A. & Forsythe, D., 1976; (10) Gerschenfeld, H. M. & Paupardin-Tritsch, D., 1974; (11) Gillette, R. & Davis, W. J., 1977; (12) Granzow, B., 1979; (13) Granzow, B. & Kater, S. B., 1977; (14) Kandel, E. R. & Tauc, L., 1966*a*; (15) Kandel, E. R. & Tauc, L., 1966*b*; (16) Marsden, C. & Kerkut, G. A., 1970; (17) McCrohan, C. R. & Benjamin, P. R., 1980*a*; (18) McCrohan, C. R. & Benjamin, P. R., 1980*b*; (19) Osborne, N. N. & Cottrell, G. A., 1971; (20) Pentreath, V. W., 1973; (21) Pentreath, V. W. & Cottrell, G. A., 1974; (22) Pentreath, V. W., Osborne, N. N. & Cottrell, G. A., 1973; (23) Prior, D. J. & Gelperin, A., 1977; (24) Sakharov, D. A. & Zs- Nagy, I., 1968; (25) Sedden, C. B., Walker, R. J. & Kerkut, G. A., 1968; (26) Senseman, D. & Gelperin, A., 1974; (27) Szczepaniak, A. C. & Cottrell, G. A., 1973; (28) Weinreich, D., *et al.* 1973; (29) Weiss, K. R., Cohen, J. L. & Kupfermann, I., 1978; (30) Weiss, K. R. & Kupfermann, I., 1976.)

(A) *Synaptic activity*

Spontaneous levels of firing which occur in *Helisoma* SCCs in semi-intact brain preparations are similar to those recorded from the SCCs of semi-intact brain preparations of *Planorbis* (Berry & Pentreath, 1976) and *Lymnaea* (McCrohan & Benjamin, 1980*a*). On the other hand, the high level of firing in *Helisoma* SCCs of isolated cerebral ganglia is in contrast to the low level of activity recorded from the MCGs of isolated ganglia in *Helix* (Kandel & Tauc, 1966*a*,*b*) and the SCCs of isolated ganglia in *Aplysia* (Weiss & Kupfermann, 1976). Both the *Helix* and *Aplysia* cells are characteristically silent unless excitatory synaptic input is evoked by stimulation of cerebral nerve trunks.

In *Helisoma* SCCs, spontaneous synaptic activity is predominately, if not exclusively, inhibitory, whereas in *Helix* and *Aplysia* spontaneous activity consists largely of e.p.s.p.s. In all three genera the spontaneous p.s.p.s occur in completely isolated cerebral ganglia at relatively high frequencies. Two intracerebral sources of p.s.p.s have been postulated for both *Helix* (Kandel & Tauc, 1966*a*) and *Aplysia* (Weiss & Kupfermann, 1976), one source producing tonic p.s.p.s and the other phasic p.s.p.s. In *Helix*, individual e.p.s.p.s of both the phasic and tonic type are common to both right and left MCGs (Kandel & Tauc, 1966*a*). In *Aplysia*, individual e.p.s.p.s of the phasic type are also common in both right and left SCCs. However, the tonic type of p.s.p. (actually an e.-i.p.s.p.) occurs continuously in bursts which are synchronized in the two cells but individual p.s.p.s within these bursts are not matched one for one in the two cells (Weiss & Kupfermann, 1976). In *Helisoma* our data suggests a dominant intracerebral source for the spontaneous synaptic input to the SCCs. In the isolated cerebral ganglia this source generates tonic i.p.s.p.s which are common to both right and left cells. This source sometimes appears to consist of two components (presumably bilaterally symmetrical cells) which are usually tightly coupled to one another. The phasically occurring barrages of i.p.s.p.s seen in the semi-intact brain preparation could either derive from sources outside the cerebral ganglia such as primary sensory neurones or result from inputs which modify the activity of the intracerebral source

which otherwise gives rise to the tonic i.p.s.p.s in the isolated cerebral ganglia. In the MCGs of *Helix*, both the tonic and phasic e.p.s.p.s, which have their pre-synaptic sources in the cerebral ganglia, can be activated by electrical stimulation of cerebral nerve trunks (Kandel & Tauc, 1966*a*).

In some semi-intact brain preparations of *Helisoma* cyclically recurring barrages of i.p.s.p.s are found to correlate with cyclical hyperpolarizations occurring in identified protractor motoneurones of the buccal ganglia during generation of the feeding motor output. These cyclical hyperpolarizations could be the result of feedback from components of the buccal ganglia feeding motor program. Primary candidates for the source of this feedback would be the premotor pattern-generating neurones, the cyberchrons, since bursts in these neurones generate the cyclical hyperpolarizations in protractor motoneurones (Kater, 1974; Kaneko, Merickel & Kater, 1978). Because the onset of the hyperpolarizations in SCC actually precede the onset of the hyperpolarizations in the protractor motoneurones, another likely explanation might be that a separate source for the SCC hyperpolarizations is located in the cerebral ganglia and becomes phase-locked with cyberchron bursting during feeding. The cyclical hyperpolarizations in *Helisoma* SCCs serve to phase-lock the SCC bursts to those occurring in protractor motoneurones. Such phasic activity in the SCC is not required to generate rhythmical buccal ganglia motor output (Granzow & Kater, 1977). However, evidence has been obtained that the SCCs also excite protractor motoneurones directly (B. Granzow, in preparation) and in this way intensify the protractor motoneurone bursts. Phasic synaptic input to the SCCs also has been found to be correlated with rhythmical motor activity in the buccal ganglia of *Limax* (Gelperin & Forsythe, 1976; Prior & Gelperin, 1976), *Lymnaea* (McCrohan & Benjamin, 1980*b*) and *Pleurobranchaea* (Gillette & Davis, 1977). In both *Limax* (Gelperin & Forsythe, 1976) and *Pleurobranchaea* (Gillette & Davis, 1977) the cyclical synaptic input to SCCs consists of two phases—an excitatory component during the retraction phase and an inhibitory component during the protraction phase of the feeding motor program. Thus, firing of the SCCs tends to occur during the retraction phase of the cycle. In *Lymnaea* the SCCs also receive a two phase cyclical input–excitation followed by inhibition–but in this case the SCCs tend to burst during the protraction phase of the feeding motor program (McCrohan & Benjamin, 1980*b*). It therefore appears that phasic firing in SCCs may act to intensify one phase of the feeding cycle relative to another but that the particular phase affected is different in different genera. In *Helisoma* it has now been found that activity in protractor motoneurones can excite the premotor pattern generating neurones of the buccal ganglia (B. Granzow, in preparation). Thus, intensification of the protractor phase of the cycle could thereby excite the rhythm generating neurones and in this way account for the SCCs' ability to initiate or accelerate the whole motor program.

Compound inhibitory synaptic potentials are evoked in SCCs by electrically stimulating most of the peripheral cerebral nerves in *Helisoma*. In contrast, synaptic potentials evoked by cerebral nerve stimulation are predominantly excitatory in *Helix* and *Aplysia* (Kandel & Tauc, 1966*a*; Weiss & Kupfermann, 1976), although a later inhibitory component can be detected following stimulation of the tentacular nerve in *Helix* (Szczepaniak & Cottrell, 1973) and following stimulation of the lip nerves in *Aplysia* (Weiss & Kupfermann, 1976). This inhibitory component has a

reversal potential near resting potential and can therefore be detected if the MCG is held slightly depolarized during nerve stimulation (Weiss & Kupfermann, 1976).

The difference in signs of both the spontaneous and evoked synaptic potentials between SCCs of *Helisoma* and those of *Helix* and *Aplysia* constitutes the most puzzling finding derived from our comparisons. It is possible that the balance of inhibitory and excitatory inputs which can be evoked by cerebral nerve stimulation are the same in all three genera but that the inhibitory inputs have a lower threshold to electrical stimulation in *Helisoma* and the excitatory inputs have the lower threshold in *Helix* and *Aplysia*. In *Limax*, inhibitory potentials are the predominant responses of SCC to cerebral nerve stimulation; however, chemosensory stimulation of the lip epithelium has an excitatory effect on the cell (A. Gelperin, personal communication). It is also possible that recordings made from the somata of the SCCs reflect different synaptic inputs although all are present in the different genera. For example, the location of synapses onto the cells may vary.

It is most interesting, however, to consider that the SCCs of *Helisoma*, *Helix* and *Aplysia* might integrate inputs from different sources or that the same inputs have different post-synaptic effects on the cells. Perhaps in some gastropods such as *Aplysia* and *Helix*, SCC activity requires excitation by stimuli relevant to feeding whereas in *Helisoma* the SCCs normally tend to fire and thus excite the buccal ganglia feeding motor program unless they are actively inhibited, for instance when aversive stimuli are encountered. It was found that light prods to the head epithelium of *Helisoma* resulted in strong inhibitory synaptic input to the SCCs (B. Granzow and C. H. F. Rowell, unpublished observations). Thus, we may speculate that a basic difference between the SCCs of *Aplysia* and *Helix* and those of *Helisoma* is the active initiation of SCC firing at appropriate times in the former two animals and the active termination at appropriate times in *Helisoma*. Such a difference could reflect an ecological necessity for gastropods such as *Aplysia* to be alerted to the presence of a discontinuous food source, whereas for *Helisoma*, a microherbivore, the food source is more widely distributed and thus feeding behaviour initiated at almost any time could possibly result in food ingestion. However, on the basis of results on intact snails, one would predict that food stimuli might initiate activity in the SCC, since quiescent snails do commence feeding behaviour and already feeding snails increase the rate and vigour of feeding movements in response to novel food stimuli, such as 'Trout Chow', applied nearby in the aquarium (B. Granzow and C. H. F. Rowell, unpublished observations). The excitatory component which can be evoked in the SCC of *Helisoma* by stimulating the frontal lip nerve, therefore, may be the result of activity in chemosensory receptors which impinge on the SCC.

Unfortunately, little data is available on the specific inputs to the serotonergic cerebral cells in any of these genera although excitatory effects of chemosensory stimuli to the lip epithelium have been reported in both *Limax* (Gelperin & Forsythe, 1976) and *Pleurobranchaea* (Gillette & Davis, 1977). Recent work in *Aplysia* has demonstrated the existence of a chemical synaptic connection from another identifiable cerebral ganglion neurone, C-2 (Weiss *et al.* 1978); however, the function of C-2 as yet remains to be determined. The cellular analysis of sensory systems in gastropods has in the past been hindered by problems associated with locating and recording from the cell bodies of sensory neurones, many of which are located in the peripheral

body tissues (e.g. Kater & Rowell, 1973; Janse, 1974; Emery & Audesirk, 1977). However, an increasingly large number of primary sensory neurones are now being identified in gastropod central ganglia (e.g. Byrne, Castellucci & Kandel, 1974; Getting, 1974; Alkon, Akaide & Harrigan, 1978; Audesirk, 1979; Audesirk & Audesirk, 1979; Rosen, Weiss & Kupfermann, 1979). Thus, a thorough examination of the cerebral ganglia in *Helisoma* as well as in other gastropods may well reveal primary sensory neurones which could be tested for their effects on the serotonergic cerebral cells. Such an approach seems essential for furthering our understanding of the factors which determine the level of activity in these cells.

(B) *Anomalous rectification*

Anomalous rectification presumably involves a voltage-dependent change in membrane conductance to potassium and is related to the external potassium concentration [K_o] (Marmor, 1971; Apland & Livengood, 1979). Marmor (1971) has demonstrated that the G cell of the marine gastropod *Anisorodis* manifests anomalous rectification when bathed in artificial sea water ([K_o] = 10 mM) but that anomalous rectification was absent when [K_o] was reduced to zero. Thus, the presence of anomalous rectification in the MCGs of the terrestrial snail *Helix* (Kandel & Tauc, 1966*b*), and the marine gastropods *Aplysia* (Weiss & Kupfermann, 1976) and *Pleurobranchaea* (Gillette & Davis, 1977) and the absence of anomalous rectification in the SCCs of the freshwater snail *Helisoma* may be related to the differences in the potassium concentration of the bathing salines which in turn reflect the potassium concentrations of the haemolymph of these animals. The [K_o] in the snail physiological saline used to bathe *Helisoma* cerebral ganglia is low (1·7 mM) compared to that in the artificial sea water used to bathe *Aplysia* and *Pleurobranchaea* cerebral ganglia (10 mM) (Weiss & Kupfermann, 1976; Gillette & Davis, 1977) or the physiological saline used for *Helix* cerebral ganglia (5·8 mM) (Cottrell & Macon, 1974). Thus, whether or not an SCC in a particular genus manifests anomalous rectification may be a consequence of that genus' external ionic environment. Whether there are also differences in the ionic channel constituents of the membranes of these cells remains to be determined. In either case, the presence of anomalous rectification in the SCCs of some genera and its absence in others will cause the cells to respond differently to the same types of synaptic inputs, all other factors being equal. Such a difference may be especially important in influencing the interactions between synaptic potentials when they occur in close temporal proximity. For example, heterosynaptic facilitation is thought to be a consequence of the anomalously rectifying properties of the SCC in *Aplysia* (Weiss & Kupfermann, 1976).

(C) *Ionic basis of the action potential*

We have found that the generation of somatic action potentials in the SCC of *Helisoma* involves a large contribution by sodium ions and little or no contribution by calcium ions. Whereas little comparable data is available on the SCCs in other gastropods, Cottrell (1977) reported that somatic action potentials in the MCG of *Helix* remained in reduced external sodium and were probably mediated by calcium. In *Planorbis*, action potentials are propagated in zero calcium high magnesium saline just as in *Helisoma* (Berry & Pentreath, 1976). Further comparisons of this type could indicate similarities and differences among SCCs at the membrane level.

(D) *Communication between SCCs in Helisoma*

Another notable difference between the SCCs of *Helisoma* and those of *Helix* and *Aplysia* is the existence of communication between these cells in *Helisoma*. There is also electrical coupling between the bilateral serotonergic cerebral cells of *Ariolimax* (Senseman & Gelperin, 1974), *Planorbis* (Berry & Pentreath, 1976) and *Lymnaea* (McCrohan & Benjamin, 1980*a*). The variability in communication seen among preparations in *Helisoma* has also been reported for the serotonergic cerebral cells of *Planorbis* (Berry & Pentreath, 1976). In *Ariolimax*, the serotonergic cerebral cells could be directly coupled to one another in the cerebral ganglia since each cell sends a process into the contralateral ganglion. However, in *Helisoma* repeated intracellular injections of Lucifer Yellow CH into the SCCs have so far revealed no processes of the SCC extending towards the contralateral cerebral ganglion. Similar results have been found in *Lymnaea* where the site of electrical coupling has been localized to the buccal ganglia. In this case cutting the buccal commissure usually abolished the coupling, although some weak coupling sometimes remained (McCrohan & Benjamin, 1980*a*). The coupling contacts between the two cells are apparently made within the buccal commissure itself since axon processes do not extend across the commissure into the contralateral buccal ganglion. The weak coupling which survives cutting of the buccal ganglia commissure may be mediated via contacts made between the two cells in the subcerebral commissure since axon processes extend along this pathway. In *Helisoma*, putative sites of contact between SCCs also exist within the buccal ganglia since each cell does send processes into the contralateral buccal ganglion and out contralateral as well as ipsilateral buccal nerve trunks (Granzow, 1979). We have also found that in some preparations cutting a cerebrobuccal connective will abolish communication, but that in other preparations the communication remains relatively strong. Thus, while it is highly likely that the neurones are coupled within the buccal ganglia it is also necessary to postulate another pathway, e.g. an interposed interneurone which mediates coupling within the cerebral ganglia, since no direct sites of interaction occur outside the buccal ganglia.

The existence of communication between SCCs in *Helisoma* appears redundant considering that the synaptic inputs to the two cells are usually so similar. In this regard it is also relevant to consider the range of apparent redundancy across genera in the serotonergic cerebral cells' innervation of the buccal ganglia (Fig. 9). In all genera, the serotonergic cerebral cells send an axon branch down the ipsilateral cerebrobuccal connective (CBC). In *Ariolimax* (Senseman & Gelperin, 1974), *Helix* (Kandel & Tauc, 1966*a*; Pentreath & Cottrell, 1974) and *Tritonia diomedea* (Dorsett, 1967) the cells also send processes down the contralateral CBC, whereas in *Aplysia* (Weiss & Kupfermann, 1976), *Helisoma* (Granzow, 1979), *Limax* (Senseman & Gelperin, 1974), *Lymnaea* (McCrohan & Benjamin, 1980*a*), *Planorbis* (Berry & Pentreath, 1976) and *Pleurobranchaea* (Gillette & Davis, 1977) processes neither extend to the contralateral cerebral ganglion nor down the contralateral CBC. In *Tritonia hombergi*, based on intracellular injections of cobalt chloride, Bulloch (1977) found that eight out of ten SCCs filled lacked contralateral CBC axons whereas the other two had contralateral CBC axons in addition to the ipsilateral CBC axons. In all genera but *Ariolimax* (for which no data is available) and *Lymnaea*, axons continue into both

ipsilateral and contralateral buccal ganglia and out ipsilateral and contralateral buccal nerve trunks (although not every nerve trunk). In *Lymnaea*, axon processes extend out ipsilateral buccal nerve trunks but apparently do not cross the contralateral ganglion (McCrohan & Benjamin, 1980*a*). Considering the presence of communication between bilateral SCCs in *Ariolimax*, *Helisoma*, *Lymnaea* and *Planorbis* and the different number of parallel descending CBC axons of the SCCs of various genera as well as their extensions into both ipsilateral and contralateral buccal ganglia, there are varying degrees of apparent redundancy in the SCCs' innervation of the buccal ganglia among the gastropod genera (Fig. 9). Such redundancy seems to be a common feature of the bilaterally symmetrical neural systems in gastropods (Bahls, Kater & Joyner, 1980) apparently assuring that bilaterally symmetrical elements function in effect as a single unit (McCrohan & Benjamin, 1980*a*).

(E) *Functional role in buccal ganglion activity*

The SCCs of *Helisoma* can excite the buccal ganglion to produce the motor program for food ingestion (Granzow & Kater, 1977). The role of the SCCs in modulating components of the feeding motor output of the buccal ganglia has been demonstrated to various extents in a number of different gastropod genera including *Planorbis* (Berry & Pentreath, 1976), *Pleurobranchaea* (Gillette & Davis, 1977), *Aplysia* (Weiss, Cohen & Kupfermann, 1978) and *Tritonia* (Bulloch & Dorsett, 1979).

II. *The case for homology*

Since selection pressures act on behaviour, evolutionary relationships among animal groups should be reflected in homologies in the nervous system. The strength of the case for homology between identifiable neurones in different animal groups depends not only on the number of shared properties, but also on the extent to which those cells are characterized by properties which make them unique from other neurones (Weiss & Kupfermann, 1976). On the other hand, the functional role of homologous neurones can differ among animal groups, and this implies structural or chemical differences between the cells or thier associated neurones.

We conclude that the gastropod SCCs are homologous because of their unique combination of structural and chemical features. However, there are an impressive number of differences, especially apparent between *Helisoma* and the best-studied genera, *Helix* and *Aplysia*. These differences all appear to be relevant to the integrative role of the cells and we believe they will ultimately be explicable in terms of adaptive function. There are already indications of such a link between life style and physiological specialization in the above comparison of *Aplysia* and *Helisoma*.

The gastropods differ widely in the habitats which they occupy and include marine, freshwater and terrestrial groups. In addition they exploit widely disparate food sources which require the use of different feeding modes. In genera already studied neurophysiologically these range from grazing on macroalgae (*Aplysia*) or microalgae (*Helisoma*) to rapid ingestion of relatively large pieces of animal matter (*Pleurobranchaea* and *Tritonia*) (Kandel, 1979). This class of molluscs therefore offers a unique opportunity to compare across many diversified genera an identifiable neurone whose basic behavioural relevance is already known. It will be especially interesting to determine how cellular properties might be related to physiological

or behavioural properties across genera. Such a comparison might reveal the means by which natural selection acting at the behavioural level influences the evolution of the nervous system at the level of individual neurones.

This study was supported by NIMH predoctoral traineeship MH16109 to B. G. The authors are grateful especially to Dr S. B. Kater, in whose laboratory this work was carried out, for assistance in some phases of the study, as well as constructive criticisms throughout all phases, and also to Dr A. G. M. Bulloch for a critical reading of the manuscript.

REFERENCES

ALKON, D. L., AKAIKE, T. & HARRIGAN, J. (1978). Interaction of chemosensory, visual and statocyst pathways in *Hermissenda crassicornis*, *J. gen. Physiol.* **71**, 177–194.

AUDESIRK, T. C. (1979). Oral mechanoreceptors in *Tritonia diomedea*. I. Electrophysiological properties and location of receptive fields. *J. comp. Physiol.* **130**, 71–78.

AUDESIRK, T. E. & AUDESIRK, G. J. (1979). Oral mechanoreceptors in *Tritonia diomedea*. II. Role in feeding. *J. comp. Physiol.* **130**, 79–86.

BAHLS, F., KATER, S. B. & JOYNER, R. W. (1980). Neuronal mechanisms for bilateral coordination of salivary gland activity in *Helisoma*. *J. Neurobiol.* (In the Press.)

BERRY, M. S. & PENTREATH, V. W. (1976). Properties of a symmetric pair of serotonin-containing neurons in the cerebral ganglia of *Planorbis*. *J. exp. Biol.* **65**, 361–380.

BULLOCH, A. G. M. (1977). A neurobiological study of feeding behaviour in the nudibranch mollusc *Tritonia hombergi*. Ph.D. thesis, University of Wales.

BULLOCH, A. G. M. & DORSETT, D. A. (1979). The integration of the patterned output of buccal motoneurons during feeding in *Tritonia hombergi*. *J. exp. Biol.* **79**, 23–40.

BYRNE, J., CASTELLUCCI, V. & KANDEL, E. R. (1974). Receptive fields and response properties of mechanoreceptor neurons innervating siphon skin and mantle shelf in *Aplysia*. *J. Neurophysiol.* **37**, 1041–1064.

COTTRELL, G. A. (1977). Identified amine-containing neurons and their synaptic connexions. *Neuroscience* **2**, 1–18.

COTTRELL, G. A. & MACON, J. B. (1974). Synaptic connexions of two symmetrically placed giant serotonin containing neurones. *J. Physiol., Lond.* **236**, 435–464.

COTTRELL, G. A., MACON, J. & SZCZEPANIAK, A. C. (1972). Glutamic acid mimicking of synaptic inhibition on the giant serotonin neuron of the snail. *Brit. J. Pharmacol.* **45**, 684–688.

COTTRELL, G. A. & OSBORNE, N. N. (1970). Subcellular localization of serotonin in an identified serotonin-containing neurone. *Nature, Lond.* **225**, 470–472.

COTTRELL, G. A. & POWELL, B. (1971). Formation of serotonin by isolated serotonin-containing neurones and by isolated non-amine-containing neurones. *J. Neurochem.* **18**, 1695–1697.

DICKINSON, P. W. (1979). Homologous neurons control movements of diverse gill types in nudibranch molluscs. *J. comp. Physiol.* **131**, 227–283.

DORSETT, D. A. (1967). Giant neurones and axon pathways in the brain of *Tritonia*. *J. exp. Biol.* **46**, 137–151.

DORSETT, D. A. (1974). Neuronal homologies and the control of branchial tuft movements in two species of *Tritonia*. *J. exp. Biol.* **61**, 639–654.

EMERY, D. G. & AUDESIRK, T. E. (1977). Chemosensory neurons in *Aplysia*. *Soc. Neurosci. Abstr.* **3**, 175.

GELPERIN, A. & FORSYTHE, D. (1976). Neuroethological studies of learning in molluscs. In *Simpler Networks and Behaviour* (ed. by J. C. Fentress), pp. 239–246. Sunderland, Mass.: Sinauer Associates, Inc.

GERSCHENFELD, H. M. & PAUPARDIN-TRITSCH, D. (1974). On the transmitter function of 5-hydroxytryptamine at excitatory and inhibitory monosynaptic junctions. *J. Physiol., Lond.* **243**, 457–481.

GETTING, P. A. (1976). Afferent neurons mediating escape swimming of the marine mollusc, *Tritonia*. *J. comp. Physiol.* **110**, 271–286.

GILLETTE, R. & DAVIS, W. J. (1977). The role of metacerebral giant neuron in the feeding behaviour of *Pleurobranchaea*. *J. comp. Physiol.* **116**, 129–159.

GRANZOW, B. (1979). Identified higher-order neurons which regulate the feeding motor program of *Helisoma trivolvis*. Ph.D. thesis, University of Iowa.

GRANZOW, B. & KATER, S. B. (1977). Identified higher-order neurons controlling the feeding motor program of *Helisoma*. *Neuroscience* **2**, 1049–1063.

JANSE, C. (1974). A neurophysiological study of the peripheral tactile system of the pond snail *Lymnaea stagnalis* (*L.*) *Neth. J. Zool.* **24**, 93–161.

KANDEL, E. R. (1979). Cellular and behavioural homologies, divergence and speciation. In *Behavior Biology of Aplysia*, pp. 387–405. San Francisco: W. H. Freeman and Company.

KANDEL, E. R. & TAUC, L. (1966*a*). Input organization of two symmetrical giant cells in the snail brain. *J. Physiol., Lond.* **183**, 269–286.

KANDEL, E. R. & TAUC, L. (1966*b*). Anomalous rectification in the metacerebral giant cells and its consequences for synaptic transmission. *J. Physiol., Lond.* **183**, 287–304.

KATER, S. B. & ROWELL, C. H. F. (1973). Integration of sensory and centrally programmed components in generation of cyclical feeding activity of *Helisoma trivolvis*. *J. Neurophysiol.* **36**, 142–155.

KEYSER, K. T. & LENT, C. M. (1977). On neuronal homologies within the central nervous system of leeches. *Comp. Biochem. Physiol.* **58**, 285–297.

KOESTER, J., MAYERI, E., LIEBESWAR, G. & KANDEL, E. R. (1974). Neural control of circulation in *Aplysia*. II. Interneurons. *J. Neurophysiol.* **37**, 476–496.

KUPFERMANN, I., COHEN, J. L., MANDELBAUM, D. E., SCHONBERG, M., SUSSWEIN, A. J. & WEISS, K. R. (1979). Functional role of serotonergic neuromodulation in *Aplysia*. *Fed. Proc.* **38**, 2095–2101.

MARMOR, M. F. (1971). The effects of temperature and ions on the current-voltage relation and electrical characteristics of a molluscan neurone. *J. Physiol., Lond.* **218**, 573–598.

MARSDEN, C. & KERKUT, G. A. (1970). The occurrence of monoamines in *Planorbis corneus*: a fluorescence microscopic and microspectrometric study. *Comp. Gen. Pharmac.* **1**, 101–116.

MCCROHAN, C. R. & BENJAMIN, P. R. (1980*a*). Patterns of activity and axonal projections of the cerebral giant cells of the snail, *Lymnaea stagnalis*. *J. exp. Biol.* **85**, 149–168.

MCCROHAN, C. R. & BENJAMIN, P. R. (1980*b*). Synaptic relationships of the cerebral giant cells with motoneurones in the feeding system of *Lymnaea stagnalis*. *J. exp. Biol.* **85**, 169–186.

OSBORNE, N. N. & COTTRELL, G. A. (1971). Distribution of biogenic amines in the slug *Limax maximus*. *Z. Zellforsch.* **112**, 15–30.

PAUL, D. H. (1979). Identification of homologous muscles and motoneurons in two species of sandcrab belonging to different taxonomic families suggests ancestry of swimming by uropod beating. *Soc. Neurosci. Abstr.* **5**, 145.

PAUPARDIN-TRITSCH, D. & GERSCHENFELD, H. M. (1973). Transmitter role of serotonin in identified synapses in *Aplysia* nervous system. *Brain Res.* **58**, 529–534.

PENTREATH, V. W. (1973). Effect of stimulating a central giant serotonin-containing neuron on peripheral muscles in the snail *Helix pomatia*. *Experientia* **29**, 540–542.

PENTREATH, V. W. & COTTRELL, G. A. (1974). Anatomy of an identified serotonin neurone studied by means of injection of tritiated transmitter. *Nature, Lond.* **250**, 655–658.

PENTREATH, V. W., OSBORNE, N. N. & COTTRELL, G. A. (1973). Anatomy of giant serotonin-containing neurones in the cerebral ganglia of *Helix pomatia* and *Limax maximus*. *Z. Zellforsch.* **143**, 1–20.

PRIOR, D. J. & GELPERIN, A. (1977). Autoactive molluscan neuron: reflex function and synaptic modulation during feeding in the terrestrial slug *Limax maximus*. *J. comp. Physiol.* **114**, 217–232.

ROSEN, S. C. WEISS, K. R. & KUPFERMANN, I. (1979). Response properties and synaptic connections of mechanoafferent neurons in cerebral ganglion of *Aplysia*. *J. Neurophysiol.* **42**, 954–974.

RÓZSA, S. K., SALÁNKI, J., VÉRÓ, M., KOVAČEVIČ, N. & KONJEVIČ, D. (1980). Neural network regulating heart activity in *Aplysia depilans* and its comparison with other gastropod species. *Comp. Biochem. Physiol.* **65**, 61–68.

SAKHAROV, D. A. & ZS-NAGY, I. (1968). Localization of biogenic monoamines in cerebral ganglia of *Lymnaea stagnalis*. *L. Acta. biol. hung.* **19**, 145–157.

SEDDEN, C. B., WALKER, R. J. & KERKUT, G. A. (1968). The localization of dopamine and 5-hydroxytryptamine in neurons of *Helix aspersa*. *Symp. Zool. Soc. Lond.* **22**, 19–32.

SENSEMAN, D. & GELPERIN, A. (1974). Comparative aspects of the morphology and physiology of a single identifiable neuron in *Helix aspersa*, *Limax maximus* and *Ariolimax californica*. *Malacolog. Rev.* **7**, 51–52.

SZCZEPANIAK, A. C. & COTTRELL, G. A. (1973). Biphasic action of glutamic acid and synaptic inhibition in an identified serotonin-containing neurone. *Nature New Biol.* **241**, 62–64.

WEINREICH, D., MCCAMAN, M. W., MCCAMAN, R. E. & VAUGHN, J. E. (1973). Chemical, enzymatic and ultrastructural characterization of 5-hydroxy-tryptamine-containing neurones from the ganglia of *Aplysia californica* and *Tritonia diomedea*. *J. Neurochem.* **20**, 969–976.

WEISS, K. R., COHEN, J. L. & KUPFERMANN, I. (1978). Modulatory control of buccal musculature by serotonergic neuron (metacerebral cell) in *Aplysia*. *J. Neurophysiol.* **41**, 181–203.

WEISS, K. R. & KUPFERMANN, I. (1976). Homology of the giant serotonergic neurons (metacerebral cells) in *Aplysia* and pulmonate molluscs. *Brain Res.* **117**, 33–49.

WEISS, K. R., MANDELBAUM, D. E., SCHONBERG, M. & KUPFERMANN, I. (1979). Modulation of buccal muscle contractility by serotonergic metacerebral cells in *Aplysia*: Evidence for a role of cyclic adenosine monophosphate. *J. Neurophysiol.* **42**, 791–803.

WEISS, K. R., SHAPIRO, E., KOESTER, J. & KUPFERMANN, I. (1978). A histaminergic synaptic potential produced by a voltage-dependent apparent decrease of conductance in the metacerebral cell of *Aplysia*. *Soc. Neurosci. Abstr.* **4**, 210.

WILLOWS, A. O. D. & DORSETT, D. A. (1974). Evolution of swimming behaviour in *Tritonia* and its neurophysiological correlates. *J. comp. Physiol.* **100**, 117–133.