ELECTRICAL PROPERTIES AND ANION PERMEABILITY OF DOUBLY RECTIFYING JUNCTIONS IN THE LEECH CENTRAL NERVOUS SYSTEM

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Summary
A study has been made of the electrical connections between touch sensory (T) neurones in the leech central nervous system (CNS) which display remarkable double rectification: depolarization spreads in both directions although hyperpolarization spreads poorly. Tests were made to determine whether this double rectification was a property of the junctions themselves or whether it resulted from changes in the length constants of processes intervening between the cell body and the junctions. Following trains of action potentials, T cells and their fine processes within the neuropile became hyperpolarized through the activity of an electrogenic sodium pump. When any T cell was hyperpolarized by 25 mV by repetitive stimulation, hyperpolarization failed to spread to the T cells to which it was electrically coupled. Further evidence for double rectification of junctions linking T cells was provided by experiments in which Cl⁻ was injected electrophoretically. Cl⁻ injection into one T cell caused inhibitory potentials recorded in it to become reversed. After a delay, Cl⁻ spread to reverse IPSPs in the coupled T cell. Movement of Cl⁻, like current flow, was dependent on membrane potential. When the T cell into which Cl⁻ was injected was kept hyperpolarized, Cl⁻ failed to move into the adjacent T cell. Upon release of the hyperpolarization in the injected T cell, Cl⁻ moved and reversed IPSPs in the coupled T cell. Together these results indicate that the gating properties of channels linking T cells are voltage-dependent, such that depolarization of either cell allows channels to open whereas hyperpolarization causes them to close.

Introduction
Electrical junctions with distinctive rectifying properties occur between neurones in a variety of species. At junctions in the crayfish, leech, teleost, frog, rat or chicken, current passes equally well in both directions (Eckert, 1963; Nicholls & Purves, 1970, 1972; Stuart, 1970; Bennett, Aljure, Nakajima & Pappas, 1963; Grinell, 1970; Korn, Sotelo & Crepel, 1973; Martin & Pilar, 1963). At certain other junctions, in crayfish and leech, current passes better in one direction than in the other (Furshpan & Potter, 1959; Auerbach & Bennett, 1969; Nicholls &
In the leech, for example, at an electrical junction between a touch sensory neurone (T) and a motoneurone (L), depolarizing current flows better from the sensory neurone to the motoneurone, although hyperpolarization spreads better in the opposite direction. This corresponds to the normal direction for transfer of excitation in this reflex pathway. A third, unusual, type of electrical connection has been described for ipsilateral touch sensory (T) neurones in the leech CNS, between which double rectification occurs: depolarizing current can spread from T cell to T cell in both directions, but hyperpolarizing current does not spread well in either direction (Baylor & Nicholls, 1969b; see also Fig. 1C).

The properties of the junctions between the ipsilateral T cells could in theory be brought about by two mechanisms: (a) double rectification and voltage-sensitivity of the channels linking T cells electrically or (b) rectification by extrajunctional membranes between the neurones. As in other invertebrate nervous systems, the neurones are not coupled at their somata but through distant junctions in the neuropile. Hence if hyperpolarization caused a decrease in the length constant of T cell processes, hyperpolarizing currents applied to the cell body could be attenuated before reaching the junctions.

The first aim of this study was to test whether double rectification between touch sensory cells in the leech occurred at the junctions or whether it was the result of rectifying properties of cell membranes of neurites leading to the junctions within the neuropile. From morphological studies it is known that the connections of ipsilateral T cells are direct and do not include an interneurone (Muller & Scott, 1981; De Riemer & Macagno, 1981). Accordingly, experiments were made to observe hyperpolarizations that would be initiated throughout the fine process in the neuropile and reach the junctions. A second aim was to assess the permeability of the junction to ions and the voltage-dependence of the ion permeability. It is known that K⁺ moves between heart muscle fibres and TEA⁺ moves between neurones (Deschenes & Bennett, 1974; Weidmann, 1966), but limited information is available about other ions that can cross electrical junctions. A notable exception is dye studies using Lucifer Yellow and 5,6-carboxyfluorescein. These negatively charged dyes spread across certain electrical junctions but not others: for example, in leech ganglia Lucifer Yellow spreads from S cells to coupling interneurones but not from T cell to T cell (Macagno et al. 1981). In the present experiments hyperpolarizing inhibitory potentials arising from T cell processes within the neuropile provided a sensitive and reliable assay for changes in intracellular [Cl⁻]. The procedure was to inject Cl⁻ into the cell, recording its IPSPs while observing IPSPs in the coupled T cell. Once it had been shown in this way that Cl⁻ and Br⁻ moved from T cell to T cell, the voltage-dependence of these fluxes was measured.

**Materials and methods**

Experiments were made on touch sensory (T) neurones in ganglia of *Hirudo medicinalis* (Fig. 1A). Dissection of ganglia, identification of T cells and recording
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procedures have been described previously (see Nicholls & Baylor, 1968; Baylor & Nicholls, 1969b). The way in which two T cells are connected in the ganglion and their common inhibitory input are shown in Fig. 1B. To illustrate double rectification, Fig. 1C shows the electrical coupling between T cells in the ganglion: depolarizing currents spread from T₁ to T₂ and vice versa but hyperpolarizing currents spread only poorly between the two coupled cells. In all experiments

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**Fig. 1.** (A) Schematic illustration of the positions of the three touch sensory neurones (T₁, T₂ and T₃) on each side of a leech ganglion. (B) The electrical junctions are not made between cell bodies but lie in the neuropile. The cell body is connected to the junctions by processes whose membrane properties cannot be studied directly since all recordings are made with intracellular electrodes in the cell soma. An inhibitory interneurone gives rise to synchronous inhibitory synaptic potentials in all three T cells on one side of the ganglion (see Baylor & Nicholls, 1969b). (C) Double rectification between two electrically coupled touch sensory neurones in the leech ganglion. Depolarizing pulses spread in both directions between T₁ and T₂ whereas hyperpolarizing pulses spread in neither direction. Left panels show records from T₁ (above, low gain) and T₂ (below, high gain) when current was injected into T₁. Right panels (T₁ high gain, T₂ low gain) show effects of injecting current into T₂. The bathing solution contained 10 mmol l⁻¹ Mg²⁺ to block chemically mediated transmission. These results are similar to those described by Baylor & Nicholls (1969b).
the cell in which either currents or anions were injected was called the 'presynaptic' cell, and the cell in which responses were recorded was called the 'postsynaptic' cell.

**Electrical recording**

Recordings from ipsilateral T cell pairs were made by impaling each cell with a single, 20–30 MΩ resistance glass microelectrode (Haer Ultratip Omegadot capillary tubing). The electrodes were usually filled with 4 mol l⁻¹ potassium acetate. In experiments where Cl⁻ (or Br⁻) flux through the junction was studied, the presynaptic electrode was filled with 3 mol l⁻¹ KCl or KBr and the postsynaptic electrode was filled with 4 mol l⁻¹ potassium acetate. Cl⁻ or Br⁻ was injected by giving hyperpolarizing pulses (50 ms) to the presynaptic cell. To reduce capacitative coupling between the electrodes a grounded shield was interposed. In all experiments coupling artefacts were tested for by withdrawing one electrode.

**Recording solutions**

Leech Ringer's fluid contained (in mmol l⁻¹): NaCl, 115; KCl, 4; CaCl₂, 1.8; glucose, 12.3; Tris-maleate, 10; adjusted to pH 7.4 with 10 mmol l⁻¹ NaOH. To reduce chemical synaptic transmission in some experiments MgCl₂ (10 mmol l⁻¹) was added to the recording solution. When inhibitory potentials were recorded, the CaCl₂ concentration in the Ringer's bathing solution was increased to 10 mmol l⁻¹. MgCl₂ or CaCl₂ was added to the recording solution by isotonic substitution for NaCl. When necessary 90% (103.5 mmol l⁻¹) of the external sodium was replaced by sucrose. The isotonic sucrose solution contained (in mmol l⁻¹): sucrose, 230; KCl, 4; CaCl₂, 1.8; glucose, 11; Tris-maleate, 10; adjusted to pH 7.4 with 10 mmol l⁻¹ NaOH.

**Results**

**Spread of hyperpolarization following a train of impulses**

In leech sensory neurones, repetitive stimulation activates an electrogenic pump and causes a prolonged hyperpolarization (Baylor & Nicholls, 1969a; Van Essen, 1972). This hyperpolarization arises in the fine processes of the cell as well as in the soma, is initiated throughout the neuropile (Van Essen, 1972) and reverses inhibitory potentials that arise in the neuropile. Accordingly, trains of impulses were used to test whether a hyperpolarization of this sort that had reached the electrotonic junctions would spread from T cell to T cell. In the experiment shown in Fig. 2, a train of impulses (20 s⁻¹) in the presynaptic T cell led to a hyperpolarization of 15 mV. At the same time recordings from the postsynaptic T cell showed no sign of hyperpolarization, although clear depolarizing potentials occurred with each impulse. Similar results were obtained in nine other experiments in which the presynaptic T cell became hyperpolarized by 20–30 mV. Such hyperpolarizations reversed IPSPs in the presynaptic T cell (see Fig. 2) but never
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Fig. 2. Absence of response in T₂ following after-hyperpolarization of T₁. A series of depolarizing stimuli (20 s⁻¹) evoked action potentials in T₁ (bottom trace) and clear depolarizing responses in T₂ (top trace). The after-hyperpolarization of about 15 mV which arose in T₁ after the train of stimuli through the action of a Na⁺ pump did not spread to T₂. (The black area in the record from T₁ consists of action potentials not photographed separately at this sweep speed.)

spread across from one touch sensory neurone to the next or influenced IPSPs in the coupled cell.

These results suggested that rectification was due to the voltage-sensitivity of junctional channels rather than to anomalous rectification of extrajunctional processes running from the cell bodies through the neuropile. In five other experiments the membrane resistance was increased by a factor of 2.2 ± 0.4 (S.D.) by replacing 90% of the sodium in the recording solution with sucrose. Under these circumstances (which would presumably allow a greater fraction of the hyperpolarizing current injected into the cell body to spread to the junction) doubly rectifying properties between touch sensory neurones were not affected. Had the rectifying properties of coupling disappeared or diminished under these conditions, the possibility of anomalously rectifying extrajunctional membranes playing a part might have seemed more likely.

Junctional permeability for chloride

All three T cells on one side of the ganglion are supplied by a common inhibitory interneurone that gives rise to synchronous inhibitory postsynaptic potentials (IPSPs) (Baylor & Nicholls, 1969b). These inhibitory potentials are caused by an increase in Cl⁻ conductance and are reversed by changes in intracellular or extracellular [Cl⁻]. Hence they provide a sensitive assay for small changes in intracellular [Cl⁻].
In the experiments described below, tests were made to determine whether \( \text{Cl}^- \) crossed the junction between T cells. KCl electrodes were used to inject chloride into the presynaptic cell and to record spontaneous IPSPs. At the same time, IPSPs arising in the postsynaptic cell were recorded with potassium acetate electrodes. The membrane potentials of both cells were held at their normal resting value of \(-45\) mV. Chloride was injected electrophoretically by giving small hyperpolarizing pulses through the KCl electrode. Thirty seconds after starting the chloride injection, the IPSPs arising in the presynaptic cell were reversed, although the simultaneous IPSPs arising in the postsynaptic cell remained normal. After about 7 min of chloride injection, the IPSPs in the postsynaptic cell became reversed; first some, then others, and eventually all, reversed (Fig. 3). In eight experiments the mean time for all IPSPs to reverse in the coupled T cell was \(7.4 \pm 0.9\) min (s.d.).

Using the reversal potential as an index it was possible to estimate the increase in intracellular [Cl\(^-\)] that had occurred as a result of Cl\(^-\) flux through the junctions. After Cl\(^-\) injection into the presynaptic cell, the reversal potential for IPSPs in the postsynaptic cell was shifted in a positive direction (Fig. 4). In five experiments the mean change in Cl\(^-\) concentration in the postsynaptic cell was estimated as \(16.7 \pm 6.1\) mmol\(\cdot\)l\(^{-1}\) (s.d.). It should be noted that the true value of \(E_{\text{Cl}}\) cannot be measured and the contribution of ions other than chloride (albeit small) has not been measured.

\(\text{Br}^-\) like Cl\(^-\) reverses IPSPs when electrophoretically injected into certain neurones (Coombs, Eccles & Fatt, 1955). In seven experiments, after KBr had been injected into the presynaptic T cell, inhibitory potentials became reversed in 30 s. Within 7 min IPSPs became reversed in the postsynaptic cell. These results show that junctions between T cells are permeable to Br\(^-\) as well as to Cl\(^-\).

![Diagram](image)

Fig. 3. Chloride movement through junctions between T cells. Chloride was injected into T\(_1\) via a KCl electrode. At the same time, synchronous IPSPs were recorded from T\(_1\) and from T\(_2\). At the beginning of the experiment (0 min), the IPSPs in both T cells were hyperpolarizing (A). Thirty seconds after starting the Cl\(^-\) injection, the IPSPs arising in T\(_1\) became reversed, although the IPSPs in T\(_2\) were not. Four minutes later, the IPSPs in T\(_2\) started to reverse (B) and 7 min after starting Cl\(^-\) injection IPSPs in T\(_2\) were all reversed (C).
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Fig. 4. Determination of the reversal potential for chloride in T2 before (arrow) and after (arrow with asterisk) injection of Cl\(^-\) in T1. The diagram shows amplitudes of IPSPs arising in T2 measured at different membrane potentials before (▲) and after (△) Cl\(^-\) injection. After Cl\(^-\) injection in T1, the reversal potential in T2 shifted to a more positive value, indicating that the Cl\(^-\) concentration in T2 had increased and that Cl\(^-\) had moved through the junction. Approximate estimates of changes in intracellular [Cl\(^-\)] in T2 could be made from these results.

Voltage-dependence of chloride flux

Do Cl\(^-\) fluxes between T cells have the same voltage-dependence as current flow? To investigate this, Cl\(^-\) was injected into the presynaptic cells using KCl electrodes. Inhibitory potentials were recorded simultaneously from the pre- and postsynaptic cells. The membrane potential of the postsynaptic cell (recorded with a potassium acetate electrode) was held constant at the normal resting value of -45 mV. In the experiment shown in Fig. 6, inhibitory potentials in the presynaptic cell reversed within 1 min of Cl\(^-\) injection. Four minutes later

Fig. 5. Bromide movement through junctions between T cells. Br\(^-\) was injected into T1 via a KBr electrode. At the same time, synchronous IPSPs were recorded from T1 and T2. The inhibitory potentials in T1 reversed almost immediately after the start of the Br\(^-\) injection, whereas the IPSPs in T2 were not reversed. Only after 7 min of Br\(^-\) injection into T1 were the IPSPs in T2 also reversed.
Fig. 6. Voltage-dependence of Cl⁻ flux through the doubly rectifying junctions between T cells. Cl⁻ was injected into T₁. During the experiment the membrane potential of T₁ was held at −45 mV or at −95 mV (upper panel). Simultaneously the IPSPs in T₂ were monitored (lower panel) and used as an indicator for Cl⁻ flux through the junctions. At the beginning of the experiment, T₁ was held at the resting potential (−45 mV), after 4 min the potential was changed to −95 mV and held there for 23 min. As long as T₁ was hyperpolarized, the IPSPs in T₂ remained unchanged (no chloride flux from T₁ to T₂). Shortly after the membrane potential in T₁ had been brought back to −45 mV the IPSPs in T₂ started to reverse, as Cl⁻ moved through the junction.

Inhibitory potentials in the postsynaptic cell had not reversed. The presynaptic cell was then hyperpolarized from its normal resting potential of −45 mV to −95 mV and held at this value for 23 min. During this time, the inhibitory potentials in the postsynaptic cell did not reverse, indicating that Cl⁻ had not moved from cell to cell. Twenty-seven minutes after the beginning of the experiment the membrane potential of the presynaptic cell was repolarized to the original resting value of −45 mV. After a short delay of 2 min the inhibitory potentials in the postsynaptic cell reversed. Thus, enough Cl⁻ had moved to reverse the inhibitory potentials arising in the postsynaptic cell. In four experiments the movement of Cl⁻ to the postsynaptic cell (following a period of prolonged hyperpolarization of the presynaptic junction) occurred with a delay of 2.3 ± 0.4 min (s.d.). This is considerably shorter than the delay in experiments such as those shown in Fig. 3. Cl⁻ had presumably accumulated in the presynaptic T cell close to the junctions during the period of injection and hyperpolarization. Similar results were obtained when Cl⁻ was injected into the presynaptic cell while the postsynaptic T cell was hyperpolarized. Only after the hyperpolarization had been released did Cl⁻ move.

As expected from electrical recordings, hyperpolarization of either side blocked Cl⁻ fluxes from T cell to T cell. Together these results show that chloride not only passes between touch sensory neurones, but that chloride fluxes display the same voltage-dependence as current flow through the junctions.
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Discussion

The site of double rectification between T cells

In the original studies on electrical coupling of T cells (Baylor & Nicholls, 1969b) it was not possible to establish whether double rectification depended on special features of the junctions or on influences of extrajunctional membranes on current spread. A third possibility at that time was that rectifying interneurones might connect ipsilateral T cells, but this was ruled out later by selective destruction of the coupling interneurone between contralateral T cells, which did not uncouple ipsilateral T cells, and by light-microscopic analysis of labelled pairs of T cell processes in the neuropile (Muller & Scott, 1981; De Riemer & Macagno, 1981). Several lines of evidence now show that, as one might expect, any contribution of extrajunctional membranes to double rectification is only minor and that the junctions themselves are the sites of double rectification between T cells. Thus, hyperpolarization that is initiated throughout the neuropile and even in distant peripheral T cell axons (Van Essen, 1972) fails to spread to the next T cell. Moreover, T neurones in leech ganglia are connected with the longitudinal (L) motoneurones by singly rectifying electrical junctions (Nicholls & Purves, 1970) that enable hyperpolarization in the L cell to spread through the junction and extrajunctional membranes to the T cell soma. It seems unlikely that T cells could have neurites with such different properties in different regions electrically isolated from each other. The present finding that Cl⁻ movement is completely blocked by hyperpolarizing the T cell body further indicates that such hyperpolarization actually reaches junctions and closes channels.

Voltage-dependence and ionic permeability of the junction

A peculiar feature of double rectification is that current flow is not simply related to potential differences across the junction. Depolarization of one cell does not affect coupling resistance in the same way as hyperpolarization of the other cell. The coupling therefore depends on the absolute membrane potentials (i.e. the voltage differences between inside the pre- or postsynaptic cell and the external bathing solution). As predicted, the voltage-dependence for Cl⁻ moving through the doubly rectifying junction is the same as for current. At hyperpolarized potentials, neither current nor Cl⁻ moves through the junctions. The experiments showing that T cell electrical junctions are permeable to the negatively charged ions Cl⁻ and Br⁻ represent a novel finding. K⁺, TEA⁺ and anion dyes such as Lucifer Yellow and 5,6-carboxyfluorescein are known to pass through certain electrical junctions but until now no description of Cl⁻ movement between cells has been reported. The concentration changes that were produced were of the order of several millimolar in the postsynaptic cell. The far more rapid transfer, 2 min compared with 7 min, when a hyperpolarization was released suggested that Cl⁻ which was being continually injected into the presynaptic T cell had diffused to reach junctions, where it accumulated.
What structural features might a doubly rectifying junction have? What types of channel-like structures bridge the gap between the synaptic membranes, allowing passage of ions from one cell to the other, as proposed in other electrical junctions (Bennett et al. 1963; Rose, 1971)? It will be of interest to explore the structure and the ion specificities of these channels and correlate structure with function.

Possible functions of double rectification between touch sensory neurones

What effects could rectification have for communication among T cells, particularly in relation to higher integration of information? Spread of depolarization in both directions between T cells plays a part in the shortening reflex of a leech. Touch sensory neurones make singly rectifying electrical synapses with L motoneurones (responsible for the shortening of the animal). Action potentials in one T cell can therefore spread to the other T cells and through their junctions to the L cells. Similarly they would spread to the S cell, which also plays a part in shortening (Muller & Scott, 1981). The general effect might then be a more effective shortening reaction through a single T cell using many available junctions. Why would hyperpolarization not spread at all between T cells? One possible function might be to prevent spread of after-hyperpolarization from one T cell to another. For leech Retzius cells in culture the amount of transmitter released by an action potential is reduced when the cell is hyperpolarized (Fuchs, Henderson & Nicholls, 1982; Dietzel, Drapeau & Nicholls, 1986). One could imagine that, in this way, doubly rectifying synaptic potentials between T cells might prevent T cells that have been active from inhibiting other T cells with neighbouring receptive fields (Nicholls & Baylor, 1969b). It will be of interest to explore further the functional role of such connections and to determine whether they exist between neurones in animals other than the leech.

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References


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