

## NEURONAL INTERACTIONS MEDIATED BY NEURALLY EVOKED CHANGES IN THE EXTRACELLULAR POTASSIUM CONCENTRATION

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### SUMMARY

Neuronal interactions mediated by alteration of the extracellular  $K^+$  concentration  $[K^+]_o$  occur between populations as well as among single neurones in very restricted regions. The interactions mediated by  $K^+$  ions may range from low efficacy ones (in which the effects of increased  $[K^+]_o$  around the non-active cells can be recorded only after massive activity of a large population of neurones) to very effective interactions (in which a single action potential in a neurone is sufficient to produce a depolarization of several mV in a second one). Such efficient  $K^+$ -mediated interactions cannot be unequivocally distinguished by shape, amplitude or time course from postsynaptic responses induced by chemical or electrotonic synapses.

We review here experiments which demonstrate various levels of interactions mediated by changes in potassium ion concentration. The giant axons (Gax) and non-giant axons from the central nervous system of the cockroach *Periplaneta americana* were used. The types of interactions discussed are: (a) pathological interactions among populations of neurones induced by the convulsant drug picrotoxin; (b) restricted and limited interactions which are the consequence of the combination of the special geometry of Gaxs and increases in extracellular  $K^+$ ; and finally, (c) local and efficient interactions among Gaxs which are postulated to be mediated by  $K^+$  ions.

The experiments described in this review, as well as others, demonstrate that the extracellular spaces in the CNS serve as predetermined pathways for  $K^+$ -mediated neuronal communication. When the extracellular space between two adjacent neurones is very small, the  $K^+$ -mediated interaction may resemble the PSPs of chemical or electrotonic synapses. It is possible that because of this resemblance, other  $K^+$ -mediated interactions in the CNS have not been identified as such.

### INTRODUCTION

It is well established that the concentration of potassium ions can be transiently altered in the restricted extracellular spaces of the central nervous system (CNS), as a result of neuronal activity (Nicholson, 1980a,b; Somjen, 1979; Orkand, Nicholls & Kuffler, 1966; Orkand, 1980; Sykova, Hník & Vyklíček, 1981). The changes in the

concentration of  $K^+$  ions are a direct consequence of two of the main design features of the nervous system: (a) rapid transmission of signals (i.e. action potentials) along axons is associated with the influx of  $Na^+$  (and in some cases  $Ca^{2+}$ ) into a neurone, and the efflux of  $K^+$  from it; (b) the narrow and restricted extracellular spaces (which appear to be a consequence of the evolutionary trend to concentrate nerve cells into tightly packed groups) separating neurones, as well as non-excitabile cells, enable the rather minute  $K^+$  efflux during the falling phase of the action potential to alter effectively the extracellular potassium concentration ( $[K^+]_o$ ). Despite the various mechanisms which control  $[K^+]_o$  and prevent its increase, various degrees of  $K^+$ -mediated interactions between neurones are inevitable. Moreover, despite mechanisms that prevent free  $K^+$  diffusion, the spread of these interactions may be quite significant, involving elements that are not nearest neighbours.

The interactions between active neurones and their inactive neighbours produced by the efflux and redistribution of  $K^+$  in the extracellular spaces may exhibit a wide range of efficiency. Thus in low efficacy interactions, the effects of increased  $K^+$  around the inactive neurones can be detected only after massive activity of a large population of neurones, or following the activity of a few neurones firing at very high rates. On the other hand, high efficacy interactions in which a single spike or a short train of action potentials in a single neurone is sufficient to produce a substantial depolarization in an inactive neurone have been demonstrated (Spitzer, 1976; Alkon & Grossman, 1978; Yarom & Spira, 1982; Spira & Yarom, 1983*a,b*). The efflux of  $K^+$  from an active axon may also alter the properties of the active axon itself (Frankenhaeuser & Hodgkin, 1956) and thereby act as a self modulator. For example, it can block propagation along an axon with special geometry by reducing the safety factor (Parnas, Spira, Werman & Bergman, 1969; Spira, Yarom & Parnas, 1976), or it can block the propagation of impulses into one branch of a bifurcating axon and thereby channel impulses in one branch only (Grossman, Spira & Parnas, 1973; Grossman, Parnas & Spira, 1979*a,b*; Parnas, 1972, 1979).

The extent to which the activity of neurones affects inactive ones depends on a large number of factors which include: (a) the amount of  $K^+$  liberated from the active neurones; (b) the dimensions, shape and properties of the extracellular space; (c) the activity of neurones and glial cells in regulating the  $[K^+]_o$  and, (d) the membrane properties of the affected neurones, especially their sensitivity to  $K^+$  concentration gradients across their membrane (Somjen, 1979; Nicholson, 1980*a,b*).

The highly ordered organization of neurones in brains and ganglia into defined aggregates and axonal tracts is associated with the formation of a highly structured network of extracellular spaces. As mentioned previously the three dimensional structure of these extracellular pathways, and their properties, define the efficiency of potassium-mediated interactions. Moreover they define the spatial specificity of the interactions. Accordingly, the spatial specificity of neuronal interactions mediated by  $K^+$  ions may exhibit some variety. Thus we find local interactions, for example, the interaction between A photoreceptors and hair cells of *Hermisenda* (Alkon & Grossman, 1978) or the interactions between adjacent giant interneurones of the cockroach (Yarom & Spira, 1982). Less restricted but spatially defined interactions between a given group of neurones can also be observed, as in the case of the primary afferent terminals in the spinal cord of various vertebrates (Barron & Mathews, 1938; Eccles,

Magni & Willis, 1962; Kriz, Sykova, Ujec & Vyklicky, 1974; Krnjevic & Morris, 1972; Vyklicky, Sykova & Kriz, 1972; Vyklicky, Sykova, Kriz & Ujec, 1975; Rudomin, Stefani & Werman, 1979). The defined extracellular pathways that normally restrict communication between neurones may not function under various pathological conditions. For example, during epileptic episodes,  $K^+$  accumulation may affect a large population of neurones which are normally isolated from one another (Moody, Futamachi & Prince, 1974; Prince, 1974, 1978).

We will describe experiments which demonstrate various levels of interactions mediated by local changes in potassium ion concentration. The giant axons (Gax) and non-giant axons from the central nervous system of the cockroach *Periplaneta americana* were used. The types of interactions discussed are: (a) interactions among populations of neurones induced by superfusion of the CNS with the convulsant drug picrotoxin, (b) restricted and limited interactions which are the consequence of the combination of the special geometry of the Gaxs in the metathoracic ganglion ( $T_3$ ) and the accumulation of  $K^+$  in the periaxonal space; and finally, (c) local interactions among the Gaxs in  $T_3$  which are postulated to be mediated by  $K^+$  ions. The experiments on which most of the present paper is based have been published previously (Spira *et al.* 1976; Spira & Yarom, 1983*a,b*; Yarom & Spira, 1982, 1983; Yarom, Grossman, Gutnick & Spira, 1982).

#### ANATOMICAL CONSIDERATIONS

Each connective from the ventral nerve cord of the cockroach *Periplaneta americana* contains eight giant axons (Gax), each with a relatively large diameter (20–50  $\mu\text{m}$  in adults). Their cell bodies are in the last abdominal ganglion ( $A_6$ ), and it is here that their dendrites receive synaptic inputs from afferents originating in the cerci (Pumphrey & Rawdon-Smith, 1937; Roeder, 1948; Callec, Guillet, Pichon & Beistel, 1971). The afferents are connected to filiform hairs that are extremely sensitive to changes in direction and velocity of air movements (Westin, Langberg & Camhi, 1977). The axon of each giant is continuous through the ventral nerve cord (Fig. 1) (Farley & Milburn, 1969; Spira, Parnas & Bergman, 1969*a,b*), the suboesophageal ganglion, and terminates in the supraoesophageal ganglion (D. Zeldes & M. E. Spira, unpublished observations). In the abdominal connectives and ganglia the Gaxs are organized into a dorsal and a ventral group, each containing four fibres. The axons can be individually identified, histologically, since each assumes a characteristic position within the group (Fig. 1B) (Harris & Smyth, 1971). In each ganglion the fibres send off several neurites which extend and branch into the neuropile (Fig. 1C). A particular Gax has a similar number of neurites in each ganglion with similar patterns of branches from cockroach to cockroach (Yarom & Spira, 1983). The axon of a giant interneurone is wrapped by a glial envelope, often several layers thick (Fig. 2A,B) (Castel, Spira, Parnas & Yarom, 1976; Meiri, Dormann & Spira, 1983; Lane, 1974, 1981). The base of the neurites which extend from the axon are also covered by glial processes, but the distal branches of the neurites are virtually devoid of glial covering (Fig. 3A,B) (Castel *et al.* 1976). Other unidentified axonal profiles which either run parallel to the longitudinal axis of the Gax or cross the ganglia are in close proximity with the Gax neurites (Fig. 3A). The

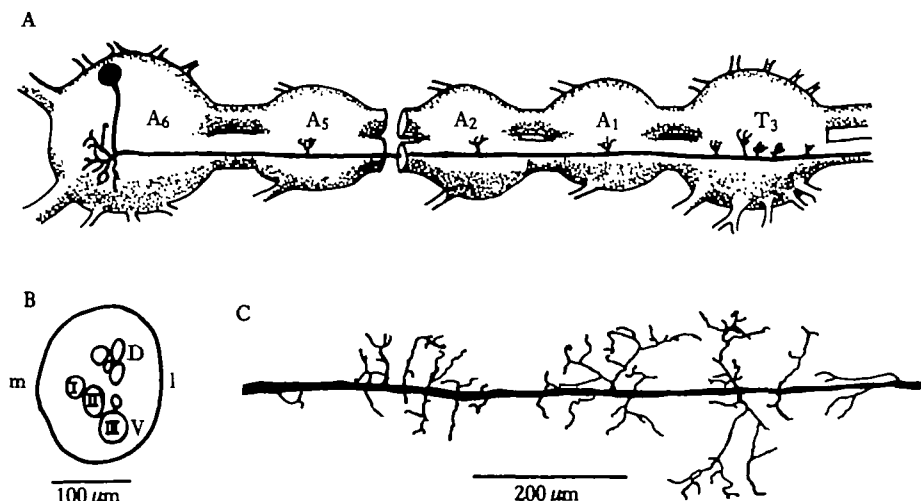


Fig. 1. (A) Schematic drawing of the ventral nerve cord and a giant interneurone. The cell body of the giant interneurone is in the last abdominal ganglion ( $A_6$ ). Its axon extends continuously through the cord, and in each ganglion emits neurites which branch into the neuropile. (B) Schematic drawing of a cross section to illustrate the typical organization of the giant axons in the connectives. D, dorsal group; V, ventral group; m, medial; l, lateral. Three of the ventral axons are labelled I, II, III. (C) *Camera lucida* tracing of a cobalt sulphide-filled ventral giant interneurone within the metathoracic ganglion ( $T_3$ ). Note the large number of neurites which extend into the neuropile.

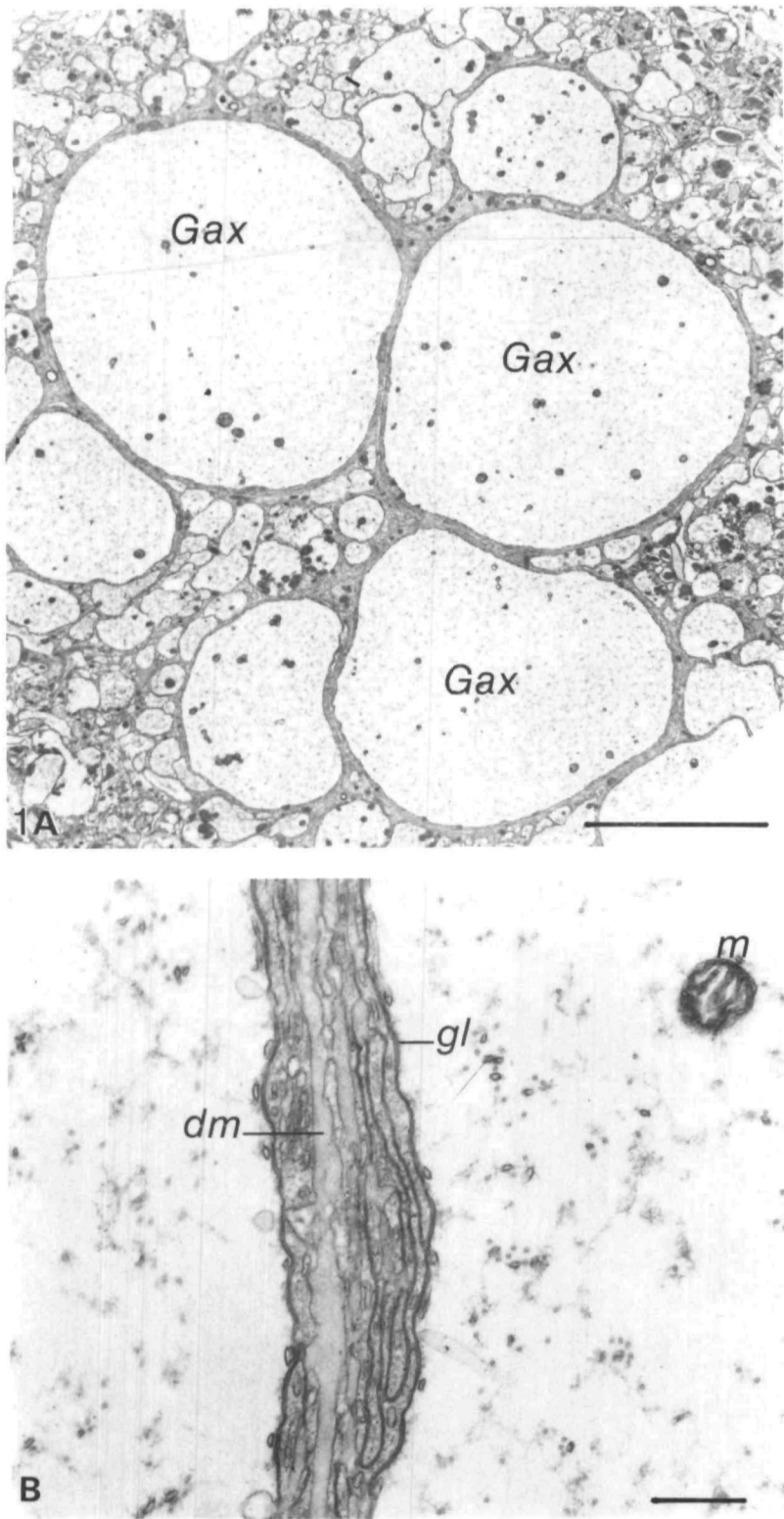
gap between the Gax neurites and other axonal profiles within the neuropile typically ranges between 15–20 nm. However, regions in which the extracellular space between the Gax's neurites and other axonal profiles is reduced to 8–10 nm are also observed (Fig. 3B) (Castel *et al.* 1976; Yarom & Spira, 1982).

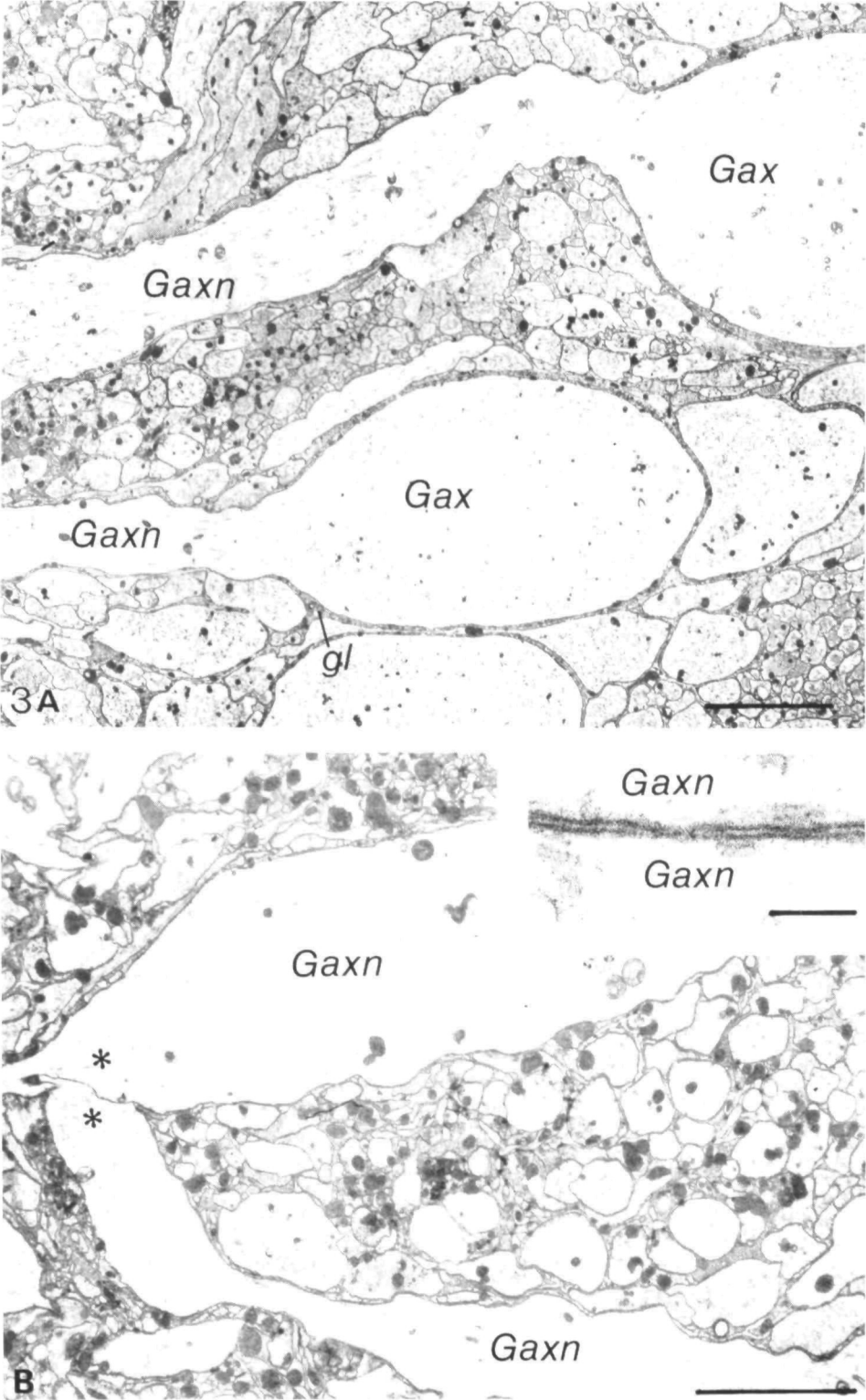
#### PHYSIOLOGY

The isolated ventral nerve cord of late nymphal, or adult male cockroaches *Periplaneta americana* was fixed to the bottom of a Perspex chamber, ventral side up. The metathoracic ganglion ( $T_3$ ) and the connectives at its base were mechanically desheathed. Intracellular recordings were made from one or two giant axons at the caudal or rostral edge of  $T_3$ . Extracellular stimulation and recording were made by either suction or hook electrodes.

Recording from a Gax while stimulating either the ipsi- or contralateral connectives at intensities below the threshold of the recorded Gax itself or at low frequencies (1–10 Hz), reveals that it is functionally well insulated from other axons. The membrane of the impaled Gax is not depolarized despite the activation of a large number of axons and measurable build up of  $[K^+]_o$  (Grossman & Gutnick, 1981). However, this insulation becomes insufficient under conditions which promote massive activation of the nerve cord, such as high frequency stimulation, or following superfusion with a convulsive agent.

Fig. 2. (A) Cross section through a group of dorsal giant axons (Gax) in the abdominal connective of an adult cockroach. Each axon is covered by a multilayer of glial processes. (B) The glial insulation between two adjacent giant axons in the connective. *gl*, glial processes; *dm*, dense extracellular material between processes of glial cells; *m*, mitochondrion. Calibration bar in A, 10  $\mu m$ , in B, 0.5  $\mu m$ .





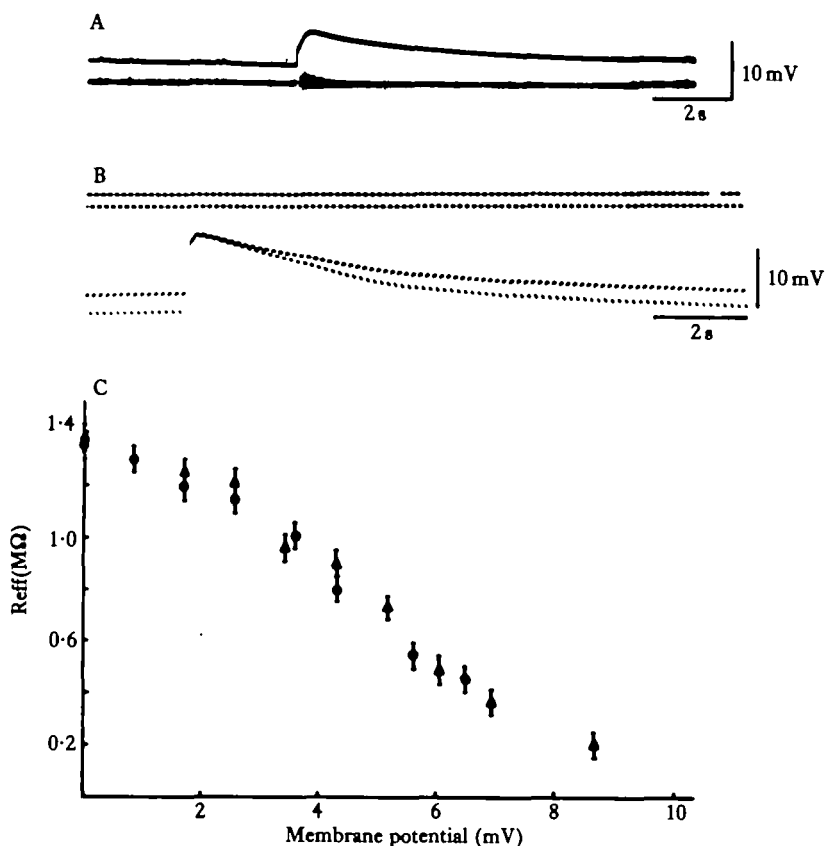


Fig. 4. Characterization of the prolonged depolarization shifts (PD) of a giant axon following superfusion of the nerve cord by picrotoxin. (A) Six minutes after superfusion of the isolated CNS, stimulation of the connective triggers a 1-s discharge of non-giant axons, recorded extracellularly between abdominal ganglia  $A_4$  and  $A_5$  (lower trace in A). This discharge is associated with a prolonged depolarization of a Gax recorded intracellularly at the base of  $T_3$  (upper trace). The PD is associated with a decreased conductance. Upper traces in B, are current pulses injected through a microelectrode placed in a Gax at the rostral edge of  $T_3$ . The recorded PD was evoked by stimulation of the contralateral connective and recorded by an intracellular electrode placed at the caudal base of  $T_3$ . The changes in effective input resistance ( $R_{eff}$ ) of the Gax in  $T_3$  as a function of membrane potential during a PD (triangles) and during applied DC depolarizing current (circles) are plotted in C. The change in  $R_{eff}$  induced by DC current injection and during a PD are almost identical (after Yarom, Grossman, Gutnick & Spira, 1982).

#### POTASSIUM ACCUMULATION PRODUCED BY SUPERFUSION WITH PICROTOXIN

After several minutes of superfusion of the isolated ventral nerve cord with the convulsant agent picrotoxin ( $10^{-5} \text{ mol l}^{-1}$ ), a single extracellular stimulus (which under normal conditions produces a short burst of impulses) produces a very long (1 s)

Fig. 3. (A) Section through giant axons (Gax) and the base of a neurite (Gaxn) in the metathoracic ganglion. Note that the glial (gl) insulation between the neurite of the giant axons and other axonal profiles is thinner than around the main axon. (B) Longitudinal section through two identified neurites extending from two Gaxs into the neuropile in  $T_3$ . The two branches come into close proximity ( $\bullet$ ). The space between the neurites in this region is only 7–10 nm (inset in B). Calibration bar  $10 \mu\text{m}$  in A,  $5 \mu\text{m}$  in B, and  $0.1 \mu\text{m}$  in the inset.

burst of activity in many fibres (Fig. 4A). This burst is associated with a prolonged depolarization (PD) of the giant axon. The amplitude of the PD can reach 10 mV and last for 10 s (Yarom *et al.* 1982) and is associated with a large increase in membrane conductance of the Gax (Fig. 4B,C).

The observed conductance increase can be explained by either rectification of the Gax's membrane (i.e. to a voltage-dependent decrease in membrane resistance; Spira *et al.* 1976), or to prolonged synaptic activity in  $T_3$ , (i.e. to a voltage-independent conductance). To distinguish between these possibilities, the effective membrane resistance of the Gax was measured at different steady state membrane potentials induced by current injection. The plot in Fig. 4C demonstrates that the conductance change during the PD can be entirely explained by membrane rectification.

Since the PD is associated with intense burst activity in the connectives and since the conductance change during the PD can be entirely accounted for by a voltage-

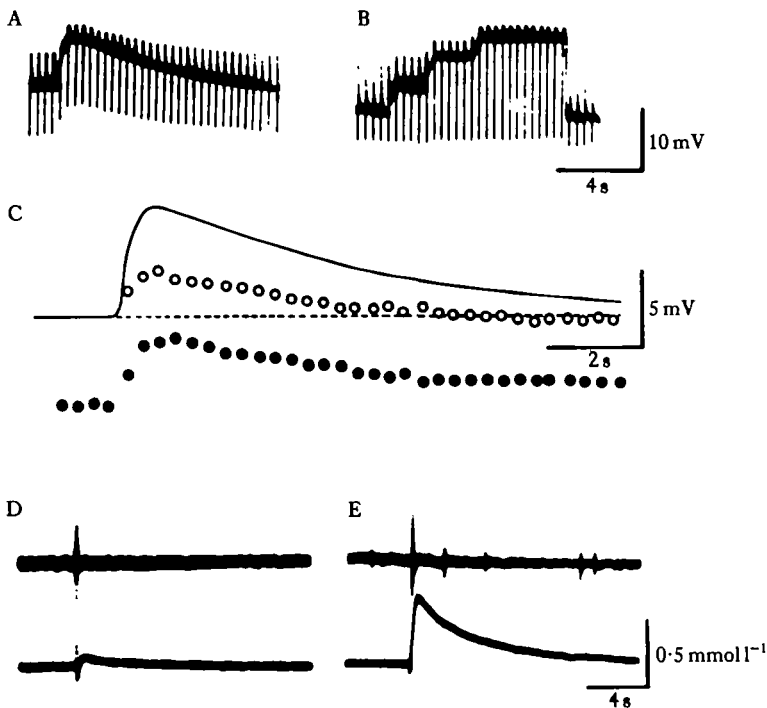


Fig. 5. Changes in extracellular potassium concentration associated with a PD. (A) PD activated by contralateral stimulation is recorded by a microelectrode inserted into a Gax at the base of  $T_3$ . Action potentials superimposed on the PD were triggered by just threshold ipsilateral stimulation. (Note that only the AHP and the depolarizing after-potential are recorded.) The peak of the AHP superimposed on the PD appears at a more depolarized level indicating that  $E_K$  is changed. (B) Superposition of action potentials triggered as in A on a DC depolarization induced by current injection through a second intracellular electrode. Note that under these conditions the peak value of the AHP is not changed. (C) The solid line is a tracing of the membrane potential during a PD. Filled circles are the values of the AHP peaks, the open circles were obtained after subtraction of the PD from the peaks of the AHP. (D) and (E) Upper trace, extracellular recording from the connectives between  $T_2$  and  $T_3$ ; lower trace, recording of potassium activity by an ion-sensitive microelectrode. (D) Control. (E) After 4 min of superfusion with  $10^{-4}$  mol l<sup>-1</sup> picrotoxin (after Yarom, Grossman, Gutnick & Spira, 1982).



dependent conductance increase, we speculated that the PD is a consequence of accumulation of  $K^+$  in the periaxonal space. This possibility was confirmed by direct measurements of  $[K^+]_o$  using ion-sensitive electrodes placed as close as possible to the Gax (Fig. 5D,E). An additional estimate of the changes in  $[K^+]_o$  in the immediate vicinity of a Gax membrane was made by examination of the amplitude and absolute peak value of the Gax spike after-hyperpolarization (AHP) before, during and after the PD (Fig. 5). The AHP peaks at a membrane potential ( $V_m$ ) close to the reversal potential of potassium ions ( $E_K$ ) (Frankenhaeuser & Hodgkin, 1956; Baylor & Nicholls, 1969; Brodwick & Junge, 1972). In the experiment illustrated by Fig. 5A, action potentials were triggered during the course of a PD. The peaks of the AHPs were more depolarized, indicating that  $E_K$  is changed during the PD. These changes, which closely followed the PD time course, are not due to changes in the membrane potential (Fig. 5B). Subtraction of the PD from the changes in  $E_K$  (as measured from the shift of the AHP) reveals that the PD cannot be fully accounted for by the change in  $E_K$  (Fig. 5C). The mechanism underlying this discrepancy is not known. However, several independent mechanisms can account for it: (a) contribution of chemical synaptic potentials to the PD amplitude; (b) a direct action of increased  $[K^+]_o$  on other ionic conductances (Adelman & Palti, 1969); (c) the depolarization of the membrane by raised  $[K^+]_o$  itself increases conductance to other ions, such as sodium or calcium and thereby increases the membrane potential change; (d) assuming that the resting potential of a Gax is to some extent (a few mV) influenced by an electrogenic pump, then the large conductance increase during the PD would shunt the hyperpolarizing current generated by the pump. It is unlikely that the discrepancy between the PD amplitude and the change in  $E_K$  is due to activation of chemical synapses, since a similar discrepancy is seen when potassium ions are iontophoresed in the vicinity of a Gax in the connective, where chemical synapses are absent and the nearest ones are more than 1 mm away in the ganglion. Thus the most likely possibilities to explain the added depolarization involve direct or indirect activation of cationic conductances in addition to the changes in  $E_K$  produced by  $K^+$  accumulation.

The effects of picrotoxin in producing the PD were far more prominent in nymphal than in adult stages. This difference may be mainly attributed to the fact that the insulation to  $K^+$  diffusion between Gaxs and other axons is better in the adult than in nymphal stages (Grossman & Gutnick, 1981).

These experiments illustrate that, as a consequence of treatment which alters the normal pattern of neuronal activity and produces massive synchronized activity of large populations of neurones, the glial envelope that normally provides insulation between neurones is no longer capable of doing so. Under such conditions, interactions among groups of neurones that are normally isolated take place, and phenomena such as epileptogenic foci may be seen (Moody *et al.* 1974; Prince, 1974, 1978).

#### ALTERATION OF SAFETY FACTOR FOR IMPULSE PROPAGATION AS A CONSEQUENCE OF $[K^+]_o$ ACCUMULATION

A more localized interaction due to change in  $[K^+]_o$  is observed around a Gax at  $T_3$  when the connectives are electrically stimulated at high frequencies.

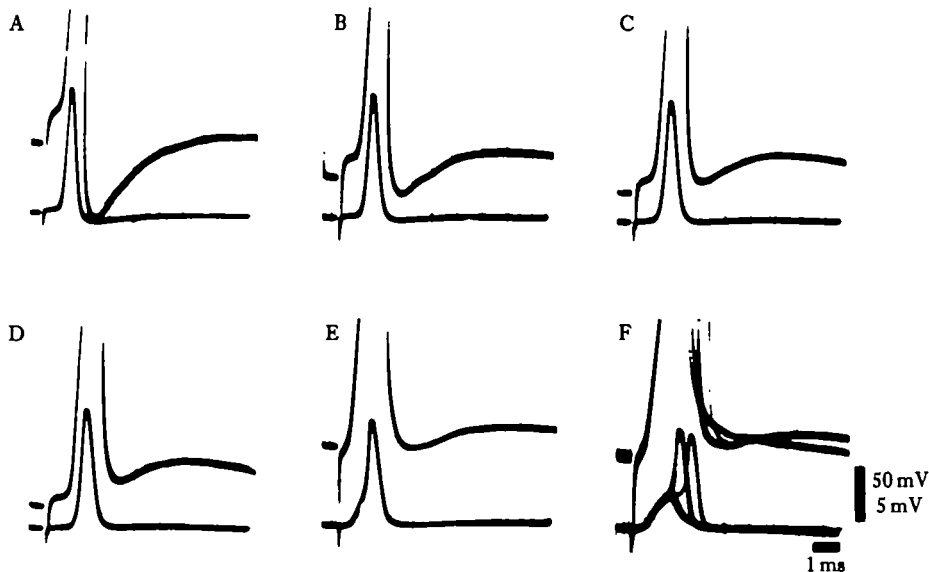


Fig. 6. Conduction block of descending impulses at  $T_3$ . Recordings were made at the caudal base of  $T_3$  while extracellular stimuli were delivered to  $T_2$ – $T_3$  connective (50 Hz). The upper and lower traces show the same events at two gains (5 mV, upper trace; 50 mV, lower trace). (A) During the first second of stimulation at  $33\text{ s}^{-1}$ , (B) 12 s, (C) 24 s, (D) 36 s and (E) 50 s after the onset of stimulation. Note the gradual reduction in the after-hyperpolarization (AHP) preceding the conduction block in F. The gradual depolarization associated with the reduction in AHP is not shown (after Spira, Yarom & Parnas, 1976).

In the isolated adult ventral nerve cord, the giant axons can transmit single action potentials in both ascending and descending directions. When the stimulus frequency is increased above 30–40 Hz, however, intermittent conduction failures are observed at the level of the thoracic ganglia but not at the abdominal ones. Complete blockage of impulse propagation at  $T_3$  can be produced after stimulation at frequencies around 100 Hz, or after long periods of stimulation at 30 Hz.

The events leading to, and associated with the conduction block are illustrated in Figs 6 and 7. In general, during stimulation at high frequency, the following changes are observed: (a) consecutive reduction in the amplitude of either ascending or descending action potentials approaching ganglion  $T_3$  (Figs 6, 7); (b) a gradual decrease in the amplitude of the after-hyperpolarization associated with membrane depolarization of 10–20 mV (Figs 6, 8); (c) a gradual appearance of a pre-potential and a delay in the initiation of the action potential across the ganglion (Fig. 6) (under these conditions the delayed action potential spreads back across the ganglion and is seen as a reflection on the falling phase of the pre-ganglionic action potential) (Fig. 7); (d) intermittent failure of the action potential to propagate across the ganglion (Figs 6, 7) (under these conditions only a decremental potential, resulting from electrotonic spread can be seen on the far side); (e) after cessation of stimulation and a period of rest, the conduction of action potentials across  $T_3$ , and the resting potentials recover as normal.

The development of conduction block at restricted and defined regions along a Gax at the thoracic ganglia is a consequence of the combination of two factors; the inhomogeneous geometry of the giant fibres in  $T_3$  and the increase in Gax membrane

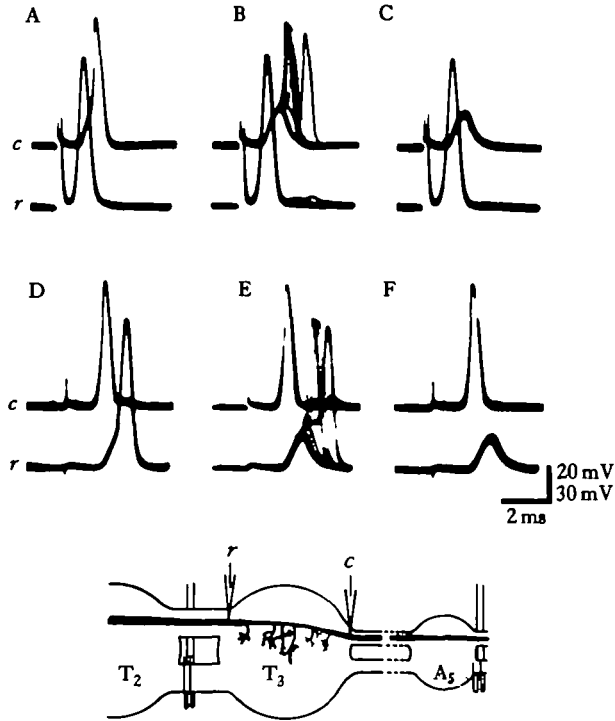


Fig. 7. Conduction block of impulses at  $T_3$ . Simultaneous recording of action potentials at the caudal (c) and rostral (r) edge of ganglion  $T_3$  (see drawing). Descending impulses were evoked by extracellular stimulation of a connective between  $T_2$  and  $T_3$ , and ascending ones by stimulation between ganglia  $A_5$  and  $A_6$ . A–C, descending impulses; D–F, ascending impulses; stimulation frequency, 50 Hz. Note the gradual appearance of a pre-potential and the delay of spike initiation in the caudal electrode for descending impulses, and (B) in the rostral electrode (r) for ascending impulses. As the spike initiation is delayed, a back reflection of the spike is recorded by the other electrode. Conduction across  $T_3$  was completely blocked after 15 s of descending stimulation, and after 40 s of ascending stimuli (after Spira, Yarom & Parnas, 1976).

conductance at these regions. While passing through the metathoracic ganglion the giant axon diameter is reduced to about half of its value in the connectives. This narrowing provides the morphological basis for the formation of a low safety region for impulse propagation (Parnas, Hochstein & Parnas, 1976; Yarom & Spira, 1983). During high frequency stimulation of the connectives, the Gax membrane is depolarized and the membrane conductance is increased. These changes further decrease the safety factor for impulse propagation leading eventually to conduction block (Spira *et al.* 1976). The increased conductance at  $T_3$  can be attributed to two independent factors: activation of chemical synapses which terminate on Gax's neurites (Spira *et al.* 1976; Yarom & Spira, 1983), and Gax membrane rectification following depolarization induced by accumulation of  $K^+$  ions.

If high frequency conduction block is mainly due to voltage-dependent membrane rectification and not to voltage-independent changes (i.e. chemical synaptic inputs), it would be expected that membrane hyperpolarization to resting level should restore the membrane resistance and restore conduction. The experiment illustrated in Fig. 9 confirms this assumption: the conduction blockade produced by descending

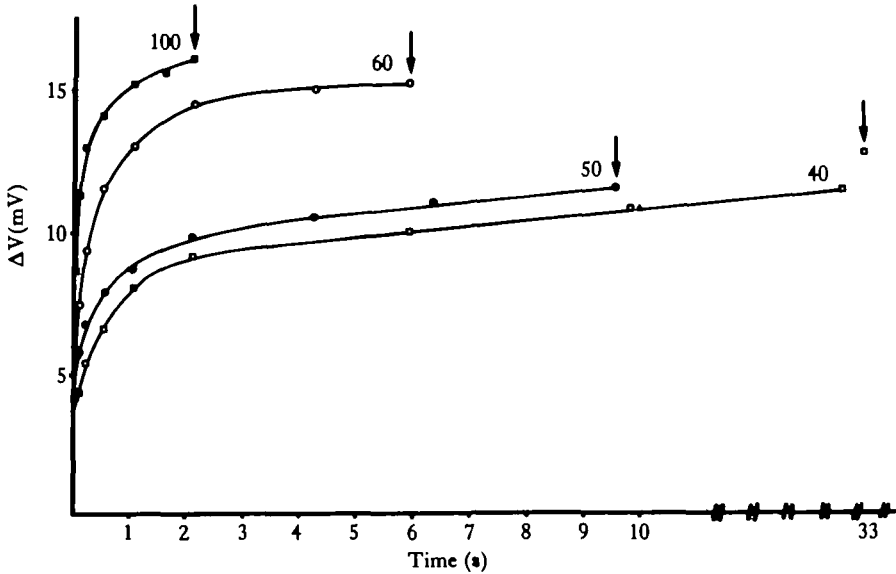


Fig. 8. Depolarization as a function of time after onset of stimulation at different stimulation frequencies. Arrows indicate the time at which conduction block appeared. Numbers represent stimulation frequencies (after Spira, Yarom & Parnas, 1976).

impulses at 50 Hz was released by membrane hyperpolarization. Further support for the contention that the block is not produced by voltage-independent processes is provided by the observation that conduction block can be produced under conditions in which chemical synapses are unlikely to be activated, namely at low  $\text{Ca}^{2+}$  ( $1 \text{ mmol l}^{-1}$ ), high  $\text{Mg}^{2+}$  ( $9 \text{ mmol l}^{-1}$ ) concentrations.

Finally, direct recording of potassium activity with ion-sensitive electrodes in the vicinity of a Gax in  $T_3$  revealed that the high frequency conduction block is associated with an increase in extracellular  $\text{K}^+$  concentration (Grossman & Gutnick, 1981).

The phenomenon of conduction block in the thoracic ganglia is attributed to a combination of local increase in Gax membrane conductance and the changes in the shape of the axons in  $T_3$  (Parnas *et al.* 1969, 1976; Spira *et al.* 1976). Evidence for

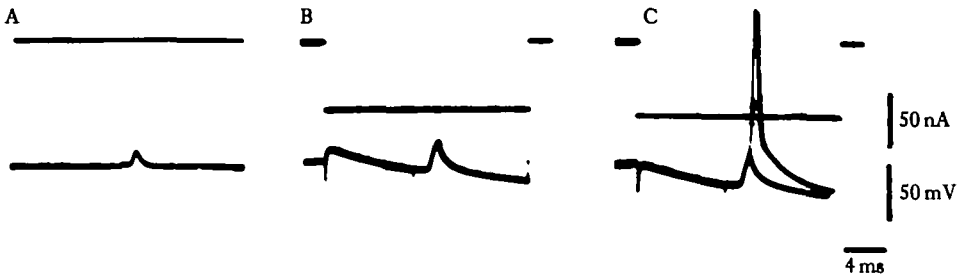


Fig. 9. Effects of hyperpolarization of a Gax in  $T_3$  on conduction block. The amplitude of a decremental potential after descending conduction block occurred (A) is increased by hyperpolarization (B). When the hyperpolarizing current is further increased the amplitude of the decremental potential is increased and a spike is initiated (C) (after Spira, Yarom & Parnas, 1976).

The importance of the special structure of the Gax in producing the low safety region for impulse propagation was recently provided by comparison of the properties of Gaxs from adult and nymphal cockroaches (Spira & Yarom, 1983a; Yarom & Spira, 1983). The Gaxs of nymphal cockroaches do not narrow extensively while traversing  $T_3$ . In spite of the fact that the rate of  $K^+$  accumulation around the Gax in nymphal stages is faster, and the extent of  $K^+$  accumulation is larger, conduction block cannot be produced in early nymphal stages. The safety factor for spike propagation is reduced gradually as the nymphs develop into the adult form (Spira & Yarom, 1983a). Thus the phenomenon of conduction block at  $T_3$  is a localized one in which the efflux of  $K^+$  ions from many fibres in the ganglia affects the capacity of a single Gax to conduct impulses at high frequencies. It is not known yet whether this interaction plays any physiological role. However, mean frequencies of 300 spikes  $s^{-1}$  and instantaneous frequencies of up to 900  $s^{-1}$  have been recorded from Gaxs in response to physiological stimuli (Westin *et al.* 1977). These frequencies are far beyond those required to produce conduction block in the isolated ventral nerve cord.

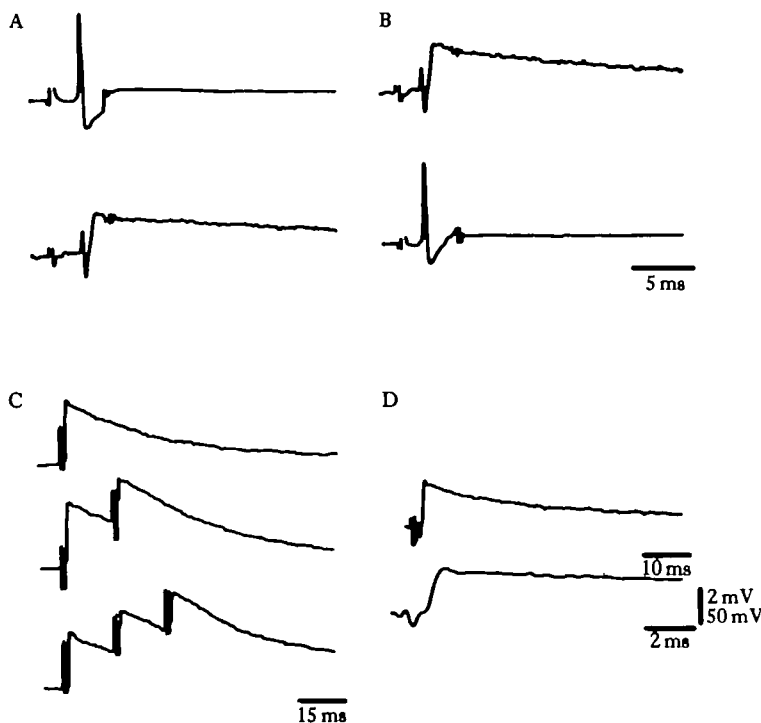


Fig. 10. Reciprocal synaptic interactions between two adjacent Gaxs. Two adjacent Gax were impaled at the caudal edge of ganglion  $T_3$ . Intracellular stimulation of one Gax (A) (upper trace) produced a synaptic potential (GGSP) in the other (lower trace). Stimulation of the second Gax (B) (lower trace) produced a synaptic response in the first (upper trace). (C) The GGSPs summate but no sign of facilitation is seen. (D) The field potential generated by a giant axon (intracellularly stimulated at  $A_5$ – $A_6$ ) is picked up by the intracellular electrode ('postsynaptic') in an adjacent Gax in  $T_3$ . The delay between the field potential generated by the 'presynaptic' Gax and the onset of the postsynaptic response is about 0.2 ms, 23 °C. (Upper and lower traces in D are the same GGSP at two sweep speeds.)

## LOCAL INTERACTIONS MEDIATED BY POTASSIUM IONS

Adjacent Gaxs interact with each other in  $T_3$  by a reciprocal synapse (Gin-to-Gin synaptic potential; GGSP). These interactions appear to be due to the efflux of potassium ions during the falling phase of an action potential of one Gax into a restricted and defined extracellular space between it and an adjacent Gax (Yarom & Spira, 1982). The reciprocal interaction is illustrated in Fig. 10. In this experiment two adjacent Gaxs were impaled by microelectrodes at the base of  $T_3$ ; each electrode could serve for either current injection or voltage recording. Generation of an action potential in one Gax (Fig. 10A) evokes a synaptic response in the other, and *vice versa* (Fig. 10B). The GGSPs differ in several features from chemical synapses recorded in the same region ( $T_3$ ) and from the chemical synapses between the cercal nerve and the Gax in the last abdominal ganglion ( $A_6$ ). The GGSP has a shorter latency than potentials produced by monosynaptic chemical junctions (0.1–0.2 ms, and 0.5–1 ms respectively at 23°C, Bennett 1972, 1974) (Fig. 10D). Furthermore the GGSP has a faster rise time (1–1.5 ms) than chemical PSPs (2.8–3 ms) recorded in the same region and its decay time is slower (60–100 ms) than the decay of potentials produced by chemical synapses (about 5–10 ms) (Fig. 10).

The GGSP differs from conventional chemical synapses in three additional major

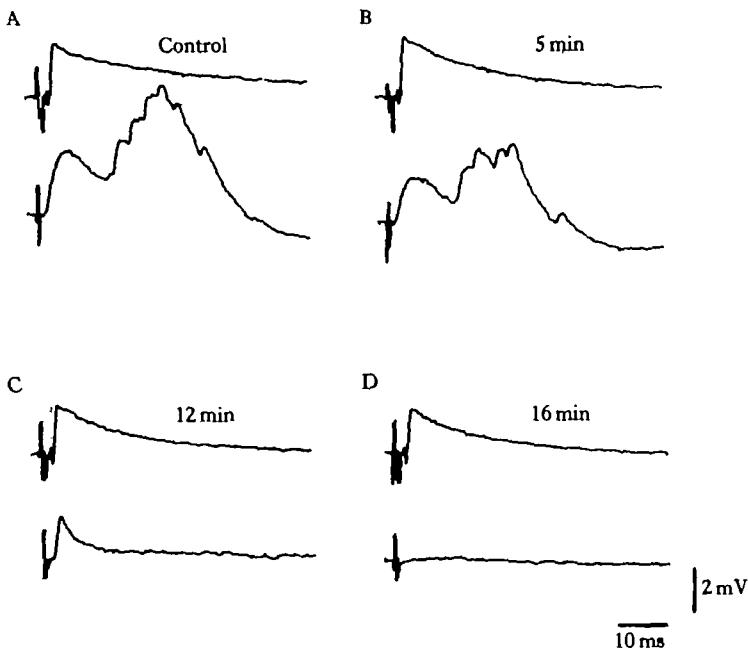


Fig. 11. Insensitivity of the GGSP to cobalt ions in the bathing solution. Upper traces are GGSPs evoked by intracellular stimulation of a second Gax impaled between ganglia  $A_3$  and  $A_6$ . Lower traces show a compound chemical synaptic potential initiated by stimulation of the ipsilateral cercal nerve and recorded at the rostral edge of the last abdominal ganglion. Whereas the chemically-mediated PSP is blocked after 16 min of superfusion with physiological solution containing  $5 \text{ mmol l}^{-1}$  cobalt, the GGSP is almost unaffected.

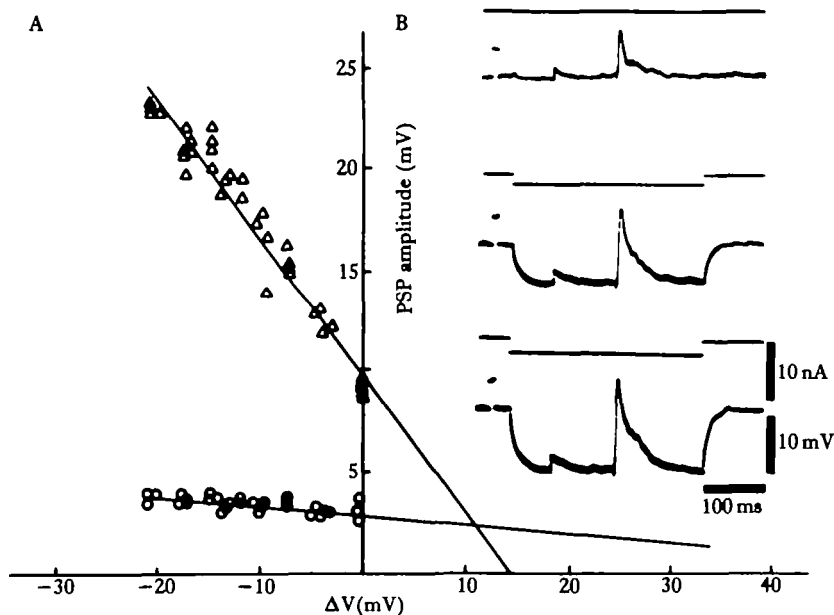


Fig. 12. (A) The extrapolated reversal potential of a chemical synapse and the GGSP (circles, GGSP; triangles, chemical PSPs). Whereas the amplitude of a chemically-mediated PSP (evoked by stimulation of the contralateral thoracic connective) increases when the Gax membrane potential is hyperpolarized, the GGSP amplitude is only slightly affected (A and B) (after Yarom & Spira, 1982; Spira & Yarom, 1983a,b).

aspects. First, it is insensitive to cobalt ions. A compound chemical PSP generated by cercal nerve stimulation is blocked after 10 to 15 min of superfusion with  $5 \text{ mmol l}^{-1}$  cobalt ions, the GGSP is almost unaffected (Fig. 11), though in some experiments the GGSP decay time becomes brief and its amplitude is slightly reduced. The mechanisms underlying these changes are still not clear. They can be attributed to an effect of cobalt ions on a delayed release of transmitter, or to delayed  $\text{Ca}^{2+}$ -dependent potassium currents in the neurites (Meech & Strumwasser, 1970; Meech, 1978; Yarom & Spira, 1982; Hounsgaard & Nicholson, 1983). Second, the amplitudes of depolarizing PSPs generated by stimulation of the contralateral thoracic connective are largely increased by hyperpolarization of the Gax membrane, the GGSP is almost unaffected (Fig. 12). The extrapolated reversal potential of the GGSP is between 0 and  $-10 \text{ mV}$ , and that of other synapses is between  $-57$  and  $-65 \text{ mV}$ . An apparent reversal potential of 0 to  $-10 \text{ mV}$  is expected if the GGSP results from an increase in the extracellular  $\text{K}^+$  concentration (Rudomin *et al.* 1979). Third, it is insensitive to change in temperature. By contrast the synaptic delay of a chemical synapse is extremely sensitive to temperature, with a  $Q_{10}$  as high as 3–4 (Katz & Miledi, 1965; Datyner & Gage, 1980). The experiment of Fig. 13 illustrates the effect of lowering the bath temperature from 21 to  $12^\circ\text{C}$  on a GGSP. Unlike the chemical responses (see Fig. 11), no clear delay between the peak inward current of the 'presynaptic' Gax spike and the GGSP can be recorded. Lowering of the temperature caused: (1) a decrease in the conduction velocity of the action potential along the Gax, (2) an increase in the peak amplitude of the action potential, (3) an increase in duration of the Gax action

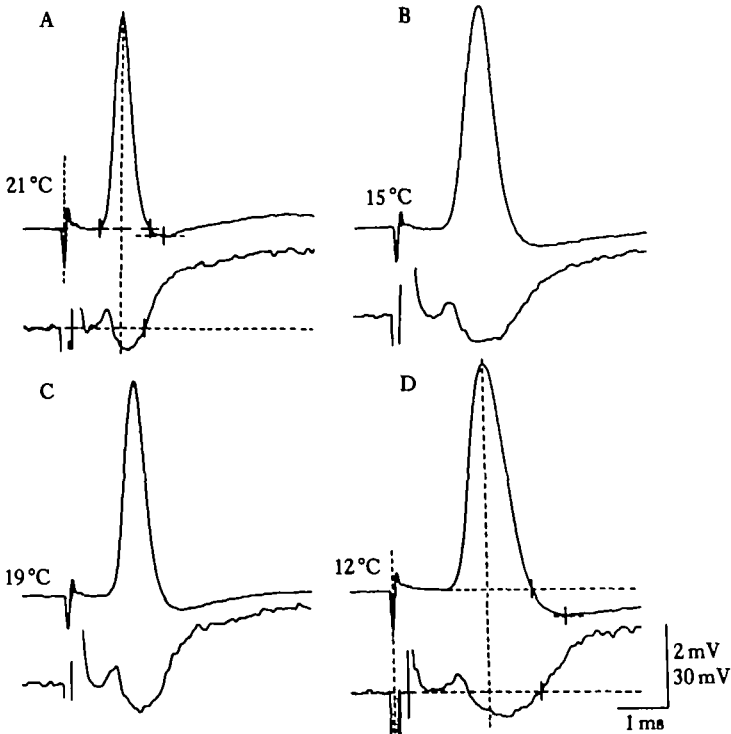


Fig. 13. The effects of bath temperature on the shape of a Gax's action potential, and the delay of GGSP initiation. An action potential in one Gax was generated by just threshold stimulation of the ipsilateral connective. The GGSP initiated by this Gax was intracellularly recorded at the caudal base of  $T_3$  from a second Gax. Lowering the temperature increased the action potential duration, amplitude and peak AHP. No discernible synaptic delay was observed, when the temperature was lowered, but the GGSP rate of rise was slowed.

potential and (4) an increase in the peak value of the AHP. These changes are also reflected in the extracellular recording of the 'presynaptic' action potential by the intracellular electrode in the second Gax. The alterations in the shape of the action potential can be attributed to a decrease in the rate of activation of the potassium conductance. Unlike conventional chemical synapses, lowering the temperature did not produce changes that reveal a clearly discernible synaptic delay. The increased duration between the peak of the inward current of the 'presynaptic axon' and the GGSP can be entirely attributed to the effect on the rate of  $K^+$  efflux during the repolarizing phase of the action potential. The decreased rate of  $K^+$  efflux from the 'presynaptic' Gax may also be the cause of the slowing of the GGSP rate of rise.

The reciprocity, relatively short latency, fast rise time, insensitivity to cobalt ions and insensitivity to temperature and transmembrane voltage are inconsistent with the known properties of conventional chemical synapses. These properties resemble to some extent the properties of electrotonic synapses (Bennett, 1977). Three lines of evidence, however, argue against this possibility. First, hyperpolarizing or depolarizing current pulses sufficient to produce  $-40$  to  $+20$  mV shifts in membrane potential of one Gax did not spread to a second Gax (Yarom & Spira, 1982; Spira & Yarom, 1983b). Second, intracellular injections of the fluorescent marker Lucifer Yellow, known to traverse gap junctions in invertebrates, do not reveal evidence of spread



From one Gax to another. Third, TEA, which can cross invertebrate low resistance junctions (Deschenes & Bennett, 1974), does not cross between Gaxs that produce GGSPs (Yarom & Spira, 1982).

Through elimination of the possibilities that the GGSP is mediated either by a conventional chemical or an electrotonic synapse, it has been suggested that the GGSP is the result of efflux of potassium ions during the falling phase of a spike in one Gax into a narrow gap between two Gaxs (Yarom & Spira, 1982). This possibility was supported by the observation that intracellular injection of TEA, which decreases the outflow of  $K^+$  during an action potential (Armstrong, 1969; Hille, 1967), decreases the rate of rise and amplitude of the GGSP (Yarom & Spira, 1982).

In previous sections we have emphasized that the glial envelopes around the Gaxs in the connectives and the ganglia prevent cross-talk. However, distal regions of the neurites which emerge from two Gaxs do come into close proximity. The space between the neurites in these regions is only 8–10 nm (Fig. 3B). Several such junctions have been observed among neurites of adjacent Gaxs. These contact regions may serve as the morphological substrate for the  $K^+$ -mediated reciprocal interactions among the Gaxs.

#### CONCLUSIONS AND SPECULATIONS

We have described three levels of neuronal interactions mediated by changes in extracellular potassium concentration. The first level is non-specific interactions which are produced as a consequence of abnormal and massive activation of neuronal networks in the CNS. Under these conditions the amount of potassium liberated from a large number of neurones overcomes the regulating capacity of the highly organized insulating structures of the glial envelopes (Lane, 1974, 1981) as well as the capacity of the extracellular matrix to regulate the concentration of ions in extracellular spaces (Treherne & Schofield, 1981).

The second level is interactions that take place in a more restricted fashion and were demonstrated by the blockade of impulse propagation following high frequency stimulation of the Gaxs. Whereas impulse propagation along the abdominal ganglia is insensitive to stimuli at high frequency, in  $T_3$  it is blocked as a consequence of two factors: (a) the non-homogeneous geometry of the Gaxs in  $T_3$  and (b) the local conductance increase of a Gax membrane due to potassium accumulation. Although a high frequency of impulses in the abdominal connectives was recorded in response to physiological stimuli, it is not clear that the conduction block appears, or plays any role in the intact animal. It is possible that the non-specific electrical stimulation of the connectives activates pathways which are normally not activated at such frequencies or in such patterns in the intact animal. In any event, conduction block is a reflection of the structural relations between the various neurones, glial envelopes and extracellular spaces in the thoracic ganglia only. It is of importance to note however, that conduction block and information channelling in bifurcating axons as a consequence of potassium ion accumulation in the periaxonal space of peripheral neurones occur in isolated preparations and in semi-intact and even whole animals (Grossman *et al.* 1973, 1979a,b; Parnas, 1972, 1979; Parnas & Segev, 1979).

The third level of interactions are specific and local ones, like those provided by

conventional chemical and electrotonic synapses. Local interactions of this type mediated by potassium ions have been demonstrated in several animals; between type A photoreceptors and hair cells in *Hermisenda* (Alkon & Grossman, 1978); between the post- and presynaptic elements of the squid giant synapse (Erulkar & Weight, 1977); between the giant axons of the cockroach (Yarom & Spira, 1982), and, under certain conditions, between the presynaptic nerve terminal and muscle fibres of the frog (Katz & Miledi, 1981, 1982).

Based on these observations and on the ultrastructural studies of various neuronal tissues (for example see Boyne & Tarrant, 1979, 1982; DiFiglia, Pasik & Pasik, 1979) it seems possible that  $K^+$ -mediated interactions are prevalent. As pointed out by Katz & Miledi (1982) 'One may wonder whether some of the brief synaptic potentials that have been attributed to electric coupling between neurons were not due to potassium release'. It is possible that  $K^+$ -mediated interactions were not identified as such because phenomenologically the  $K^+$  PSPs are similar to electrotonic and chemical PSPs (Spira & Yarom, 1983b).

Some of the basic characteristics of a hypothetical synapse in which potassium ions serve as the mediator may be very similar to those of chemical and electrotonic junctions. Therefore the conventional manipulations that are used to differentiate between chemical and electrotonic junctions may be insufficient to identify the existence of a potassium-mediated interaction. We will describe briefly some of the features that may be common to all these modes of transmission.

#### *Directionality of propagation*

Unidirectional conduction is a basic property of a chemical synapse and may be possible in electrotonic junctions (rectifying electrotonic synapses, for review see Bennett, 1972, 1974, 1977). Basically, a potassium-mediated synapse should be bidirectional (for example the GGSP). Unidirectional transmission, however, could be produced if the density of  $K^+$  channels is larger on one side of the junction than on the other, or if more  $K^+$  is liberated from one neurone than from the other. That the release of  $K^+$  during an action potential may be strongly dependent on local membrane properties was recently demonstrated by Hounsgaard & Nicholson (1983). Another condition which may produce unidirectional propagation in a  $K^+$ -mediated synapse is when the two interacting neurones are asymmetrical in terms of their input resistances (Bennett, 1972, 1974, 1977). Thus directionality of transmission is not a diagnostic feature of any type of synapse.

#### *Delay*

A very short synaptic delay usually indicates the presence of an electrotonic junction. However, it would be difficult to differentiate between an electrotonic synapse and a  $K^+$ -mediated one on this basis (Katz & Miledi, 1982 and see the latency of the GGSP).

#### *Calcium ions*

Low  $Ca^{2+}$ , high  $Mg^{2+}$  solutions block chemical synapses but have little effect on electrotonic ones (Bennett, 1977). If calcium ions are not involved in the generation of the action potential of a  $K^+$ -mediated interaction, then lowering the  $Ca^{2+}$  concentration

will not affect the transmission. On the other hand if  $Ca^{2+}$  ions are involved in the spike generation, reduction in  $[Ca^{2+}]_o$  may lower the efflux of  $K^+$  from the neurone and thereby reduce the amplitude of a  $K^+$ -mediated PSP (Meech & Strumwasser, 1970; Meech, 1978; also see Hounsgaard & Nicholson, 1983). Thus again this conventional tool for identification of the mode of synaptic transmission may not be very useful in recognizing a  $K^+$ -mediated synapse.

### *Reversal potential of the postsynaptic response*

The inversion of a chemical PSP by shifting the postsynaptic membrane potential is a most powerful diagnostic tool. This is because at most chemical synapses the generation of synaptic conductance is almost entirely independent of transmembrane voltage. However, in non-isopotential neurones where the synapses are located far from the point of current injection, it may be impossible to invert the PSP. In most electrotonic junctions the changes in PSP amplitude are restricted by the extent to which the presynaptic membrane potential is influenced by postsynaptic current injection (Bennett, 1972, 1974, 1977).  $K^+$ -mediated potentials are only slightly dependent on transmembrane voltage (Rudomin *et al.* 1979 and see for example the GGSP) and therefore behave in a similar manner to either an electrotonic synapse or a remote chemical synapse.

To summarize, this partial list demonstrates some of the expected experimental difficulties in identification of localized potassium interactions. We expect, however, that additional studies will provide sufficient information and experimental criteria that will enable this mode of transmission to be identified.

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