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INTERACTIONS OF THE GIANT FIBRES AND MOTOR GIANT NEURONES OF THE HERMIT CRAB

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SUMMARY

A recent claim that the giant fibre of the hermit crab excites its contralateral motor giant neurone through a chemical rather than an electrical synapse (Stephens, 1986) was re-examined. We found that the reported increased latency (relative to the electrical ipsilateral synapse) was postsynaptic in origin, as was the increased spike 'jitter'. There was no difference in synaptic latency between the electrical synapse and the supposed chemical one. We did not find a consistent resistance to *N*-ethylmaleimide (an uncoupler of electrical synapses) by the supposed chemical synapse, but the synapse was resistant to 2 mmol l^{-1} cadmium, which blocks known chemical synapses in the system. Sub-threshold depolarizing current passed from the presynaptic giant fibre to the postsynaptic contralateral motor giant, and hyperpolarizing current passed antidromically. We conclude that the synapse is electrical and not chemical in nature.

INTRODUCTION

A striking feature of the neuronal circuitry underlying the escape tail-flip of crayfish is that the giant fibres (GFs) activate all of the excitatory abdominal flexor motor neurones by way of electrical synapses (for a review see Wine & Krasne, 1982). Hermit crabs too have an escape behaviour (rapid withdrawal into their mollusc shells) which is homologous in many respects to that of the crayfish (Chapple, 1966). There is a single pair of GFs which are homologous to the crayfish medial giant (Chapple & Hearney, 1974; Stephens, 1985). Each GF drives the ipsilateral member of a pair of large neurones, the segmental giants (SGs), through a rectifying electrical synapse (Heitler & Fraser, 1986). The SG in turn excites the fast flexor (FF) motor neurones, also probably through electrical synapses. There is one large flexor motor neurone which usually receives suprathreshold input directly from the ipsilateral GF *via* a rectifying electrical synapse. This is the homologue of the crayfish motor giant (MoG) neurone (Umbach & Lang, 1981). The hermit crab MoG, unlike that of the crayfish, also receives input from the SGs (like the unspecialized FF neurones), but this input is usually subthreshold (Heitler & Fraser, 1986).

Key words: hermit crab, electrical synapse, giant fibre, escape.

It has recently been reported that in the hermit crab the GF excites the MoG *contralateral* to itself by way of a *chemical* synapse (Stephens, 1986). In our own previous work we noted the existence of this contralateral excitatory path, but tacitly assumed that the excitation was mediated electrically. If the excitation is indeed chemical, it would be the only known case of functional excitatory chemical output from a crustacean GF, and the only known case where an FF motor neurone received its primary GF excitation *via* a chemical synapse.

The evidence adduced to support the claim of chemical transmission was four-fold.

(1) The contralateral GF–MoG synaptic delay was longer than the ipsilateral GF–MoG delay.

(2) There was greater latency jitter with contralateral excitation and, with repeated stimulation, the contralateral excitation failed much more quickly than the ipsilateral excitation.

(3) Application of *N*-ethylmaleimide (NEM; an uncoupler of electrical synapses; Spray *et al.* 1984) disrupted ipsilateral but not contralateral excitation.

(4) The dye Lucifer Yellow was transferred from the GF to the ipsilateral MoG but not the contralateral one.

This evidence is certainly consistent with chemical transmission, but is by no means conclusive proof. We have re-examined transmission between the GF and the contralateral MoG and, although some of our results are similar to those of Stephens, we suggest that they are as consistent with electrical as with chemical transmission. We support this by presenting additional data, including demonstration of the direct passage of current between the two neurones. We conclude that the MoG receives excitation from the contralateral GF through an *electrical* synapse which is similar to, but weaker than, the electrical synapse which connects it to the ipsilateral GF.

MATERIALS AND METHODS

Hermit crabs (*Eupagurus bernhardus*) were collected locally from St Andrews bay and kept in tanks of circulating sea water. Prior to dissection a crab was removed from its mollusc shell by carefully crushing the latter in a vice. The abdomen was cut from the anterior body, and the fourth ganglion was dissected free from the abdominal ventral nerve in association with its roots and interganglionic connectives. The nerve cord was pinned dorsal surface upwards on a Sylgard platform, and submerged in saline (Atwood & Dorai-Raj, 1966, but with Tris substituted for $NaHCO_3$). The saline was kept chilled to 8°C. Where required, 5 mmol l^{-1} *N*-ethylmaleimide (NEM) and 2 mmol l^{-1} cadmium chloride were added to the saline without adjusting the other ionic components.

Hook and pin electrodes were used to record extracellularly and to stimulate the connectives and the first and third roots of the fourth abdominal ganglion. Intracellular recordings were made with glass microelectrodes (initial resistance 20–40 MΩ) filled either with potassium acetate or with 5 % Lucifer Yellow dissolved in 1 mol l^{-1} lithium chloride. Penetrations were all made from the dorsal aspect of the

ganglion into axonal or neuropile processes. Prior to penetration the ganglionic sheath was first softened by applying a few specks of protease (Sigma Type XIV) for about 60 s, and then partially removed. In some experiments a computer-based signal averager was used to reduce noise (mainly spontaneous activity) in the response to stimulation. Neurones were injected with Lucifer Yellow using 0·5-s negative current pulses of 10–100 nA delivered at 1 Hz for a period of 30 min to 3 h. Tissue was fixed in 4 % formaldehyde in saline, dehydrated in alcohol and cleared in methyl salicylate. Preparations were examined in whole mount with epi-illumination (Zeiss filter set 06), and drawn using a *camera lucida* attachment.

Abbreviations

Abbreviations used in the text are as follows: GF, giant fibre; MoG, giant motor neurone; SG, segmental giant neurone; G4, fourth ganglion of the abdominal nerve cord; AC, interganglionic connectives anterior to G4; PC, interganglionic connectives posterior to G4; R1, first root innervating swimmerets and containing the SG axon; R3, third root innervating the flexor muscles and containing the MoG axon; i, ipsilateral; c, contralateral; l, left; r, right; x, extracellular stimulation (see below).

In the text, laterality of neurones will usually be referenced as ipsilateral or contralateral to the axon of the MoG in question (e.g. iGF for the GF ipsilateral to the MoG axon and contralateral to its cell body, cGF for the other GF). In the figure legends an exact designation (left or right, e.g. lAC, rAC) is given where possible. No consistent difference was observed between the neurones on the left side of the ganglion and those on the right.

Figure conventions

To avoid confusion, certain conventions have been adhered to throughout the figures and their legends. The artefacts caused by extracellular stimuli are all marked with filled curved arrows, while the duration of intracellular current pulses is marked with dots. Where a giant fibre is activated by extracellular stimulation of its axon in one of the connectives, this is termed a stimulus applied to the GFx.

Current injection

The neurones into which current was injected had a low input impedance, and the Lucifer Yellow-filled microelectrodes had a tendency to block during injection of depolarizing current, which meant that in order to pass sufficient current to achieve significant changes in membrane potential we often had to bypass the recording amplifier and apply voltages (10–100 V) directly into the microelectrode. Thus, in these cases we were not able to monitor the membrane potential of the injected neurone. The bath potential was stabilized using a virtual-ground current monitor with separate voltage-recording and current-passing indifferent electrodes (Purves, 1981). With this configuration there was little or no artefactual voltage change in the recording microelectrode when large currents were passed, other than transients at the onset and offset of the pulse. (See Fig. 7F for physiological evidence of the lack of coupling artefact.)

A current pulse is described as bracketing an event when the event fell within the duration of the current pulse.

RESULTS

Is there a connection between the GF and contralateral MoG?

In an earlier study (Stephens, 1985) no connection was observed between the cGF and the MoG, although a connection was described between the MoG and a contralateral non-GF through-conducting axon (Stephens, 1985). This conclusion was not in agreement with our own preliminary work (which was a factor in initiating the present study), but was subsequently modified when a chemical synapse from the cGF to the MoG was described (Stephens, 1986). The first task in the present report is thus to confirm the existence of the cGF–MoG connection.

Physiology

Extracellular recording shows that in most preparations stimulation of the cAC induces a MoG spike at exactly the cGF threshold (Heitler & Fraser, 1986). Intracellular electrodes were used to test the necessity and sufficiency of a cGF spike for MoG activation (Fig. 1). Depolarizing current injected into a GF would cause it to spike, and this usually resulted in a spike in both MoGs. Conversely, if the GF was induced to spike by extracellular stimulation of the AC, a pulse of negative current injected into the GF could block its spike. When the GF spike failed, the MoG spikes also failed. Thus a cGF spike is sufficient to activate the MoG, and there is no axon in the AC which activates the MoG and has a threshold less than or identical to that of the GF.

Anatomy

Lucifer Yellow stains of the MoG (Fig. 2) usually revealed two groups of short dendrites sprouting from either side of the neurite, which were suitably located to make contact with the GFs on their respective sides. The dendritic group on the side of the MoG axon was usually slightly posterior to the other group. Thick sections of preparations in which the MoG was stained with horseradish peroxidase (HRP) showed that the two groups of dendrites did indeed make close contact with their respective GF axons.

We did not observe dye-coupling to either MoG after injecting Lucifer Yellow into a GF axon, even after injection times of several hours. However, the staining was not as intense as that produced by a similar regime in a crayfish GF, in our experience.

Latency of GF–MoG interaction

When the two GFs were stimulated with extracellular electrodes which had been carefully positioned on the ACs to be equidistant from the ganglion, there was a greater latency of response recorded extracellularly in the R3 contralateral to the stimulated AC compared to that in the ipsilateral R3, as reported previously

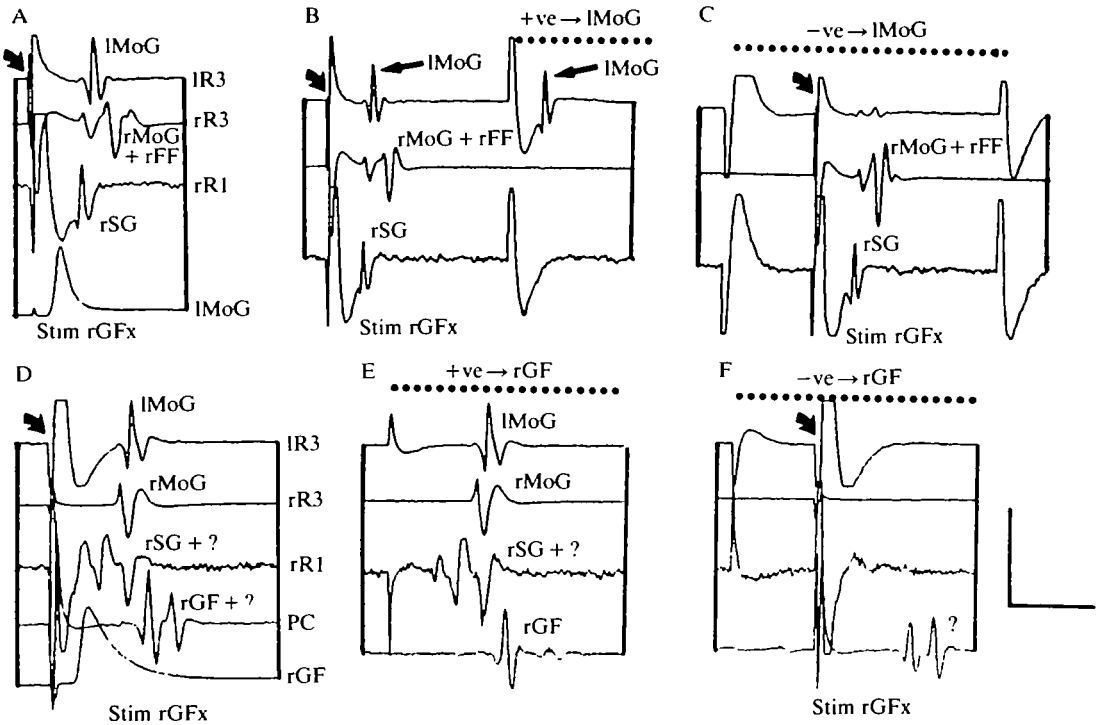

Fig. 1. (A–F) Identification of the MoG extracellular spike (A–C) and the cGF–MoG connection (D–F). Please refer to the Figure conventions and Abbreviations sections in Materials and Methods for this and subsequent figures. (A) The rGFx was stimulated while recording extracellularly from lR3 (top trace), rR3 (second trace), rR1 (third trace) and intracellularly from the lMoG (fourth trace). The extracellular records show the spikes of the lMog, rMog + unidentified rFF units and the rSG in traces 1–3, respectively. (B) As A, except that the rGFx stimulus is followed by a pulse of depolarizing current to lMoG, which spikes. This confirms the identity of the extracellular lMoG spike in the lR3 trace (arrows). (C) As A, except the rGFx stimulus is bracketed by a hyperpolarizing current pulse to the lMoG, whose spike fails in the lR3 trace. (D) The rGFx is stimulated. Traces as A, except that the PC is recorded extracellularly (fourth trace), and the rGF is recorded intracellularly (fifth trace). (E) A pulse of depolarizing current injected into the rGF elicits an rGF spike. Note that the extracellular responses in the roots are identical to those elicited by rGFx stimulus (although the PC response is different). (F) As A, except the rGFx stimulus is bracketed by a pulse of hyperpolarizing current to the rGF. The root responses are abolished. These traces show that a GF spike is necessary and sufficient to elicit the root responses, including that of the contralateral MoG. Scale: vertical A 90 mV, D 80 mV; horizontal A,D–F 5 ms, B,C 8 ms.

(Stephens, 1986). However, intracellular recording from the MoG showed that much of this latency difference was caused by a change in the conduction latency between the peak of the spike recorded intracellularly, and the MoG spike recorded extracellularly in R3 (Fig. 3A,B). This suggests that an iGF spike initiates a spike in the MoG at a more distal location than a cGF spike, thus shortening the conduction distance to the extracellular recording electrodes. Since in all experiments the

microelectrodes were located close to the synaptic sites this in turn suggests that the iGF excites the MoG more powerfully than the cGF. Further evidence for this was obtained by simultaneous intracellular recordings from the MoGs. When care was taken to penetrate these neurones at exactly the same anterior–posterior location there was no detectable difference in the latency from a stimulus exciting either AC to the start of the MoG response (Fig. 3C,D). There was a slight difference in the latency from the stimulus to the peak of the intracellular spike, with the MoG spike stimulated by the cAC delayed relative to that stimulated by the iAC. This is clearly due to the faster rise time of the latter, again suggesting that the MoG receives more powerful iGF than cGF excitation. This was confirmed by stimulating the AC at high frequency to induce MoG spike failure, and examining the amplitude of the residual EPSP. No matter which AC was stimulated, the MoG ipsilateral to the stimulus had a larger EPSP than that contralateral to the stimulus (Fig. 3E,F).

Thus we conclude that the latency difference between the extracellular response of the MoG to iGF and cGF stimulation is due to the excitation that the MoG receives

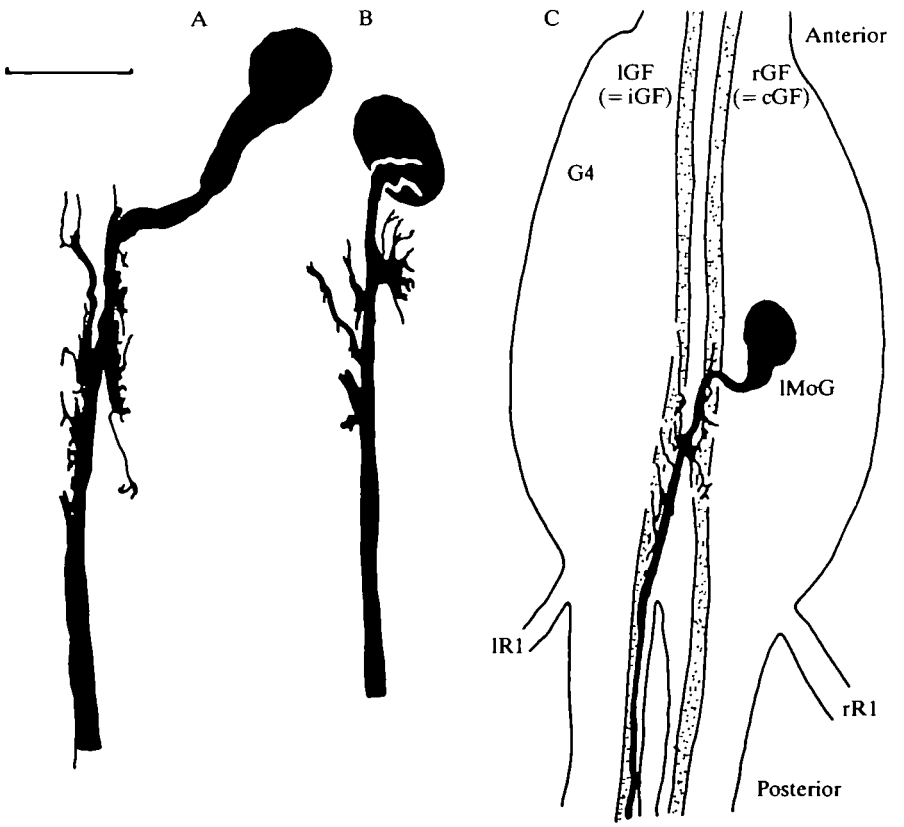

Fig. 2. (A–C) Anatomy of the MoG. *Camera lucida* drawings of the lMoG stained in three different preparations. In C the neurone is shown within the ganglion and in approximate relationship to the two GFs. The GFs were stained in another preparation because when stained with the MoG the dendritic morphology was obscured. Scale bar, A,B 155 μm, C 250 μm.

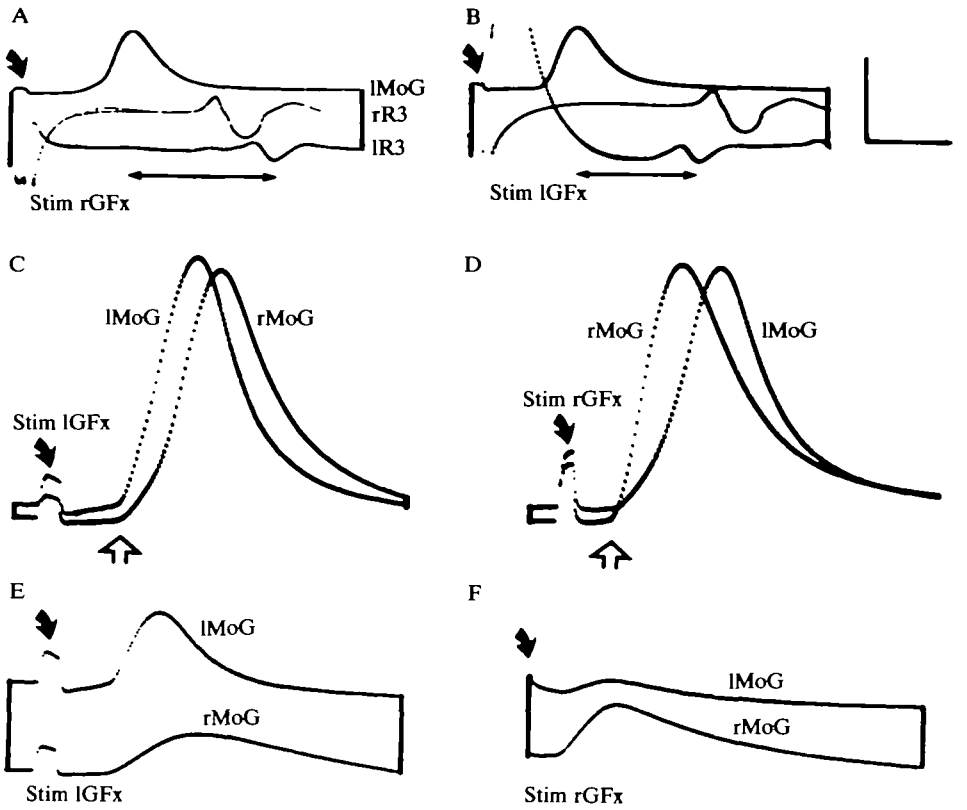

Fig. 3. (A–F) The latency of the MoG response to iGFx and cGFx stimulation. (A,B) Recordings were made intracellularly from the lMoG (top trace) and extracellularly from rR3 (second trace) and lR3 (third trace). (A) The rGFx is stimulated; (B) the lGFx is stimulated. The latency from the peak of the intracellular lMoG spike to the lMoG spike recorded extracellularly in lR3 is shorter with iGFx than cGFx stimulation (double-headed arrows). (C,D) Dual intracellular recordings from lMoG (top trace) and rMoG (second trace). (C) The lGFx is stimulated; (D) the rGFx is stimulated. The latency to the start of the response is identical in the two MoGs (open arrows), but the contralateral response has a slower rise time. (E,F) High-frequency GFx stimulation abolishes the MoG spike, revealing the underlying EPSP. A single sweep is shown after about 1 s of stimulation at 80 Hz. (E) The lGFx is stimulated; (F) the rGFx is stimulated. The EPSP resulting from ipsilateral stimulation is bigger than that from contralateral stimulation. Scale: vertical A,B 60 mV, C–F 20 mV; horizontal A,B 1·2 ms, C–F 1 ms.

from the iGF being more powerful and having a faster rise time than that it receives from the cGF, and is not due to a difference in the actual synaptic delay.

Synaptic jitter

When either GF was stimulated extracellularly at relatively high frequency (50–80 Hz) there was a gradual increase in the latency of MoG spikes recorded extracellularly in R3, followed by eventual spike failure, as described above. This

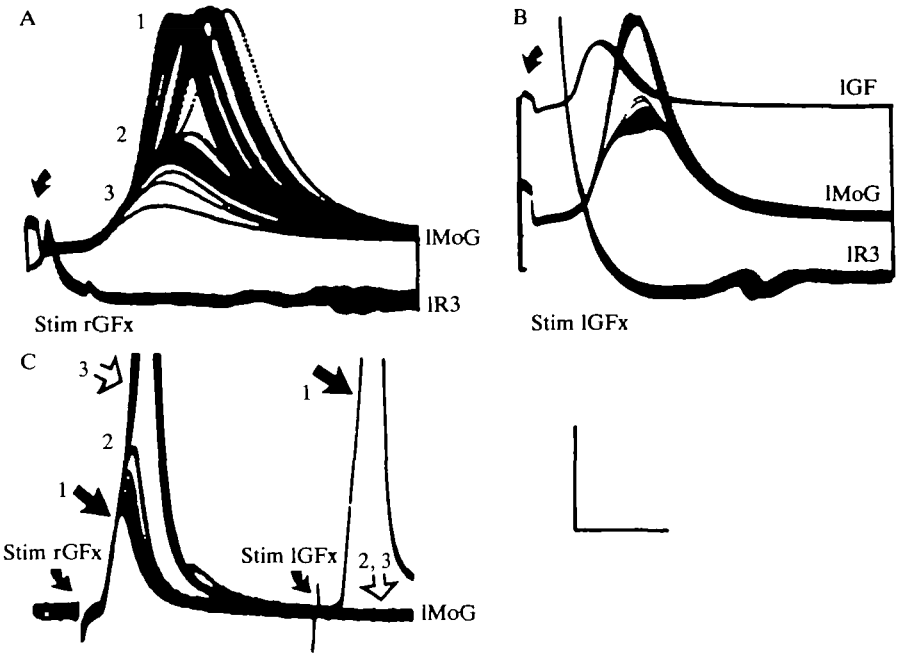

Fig. 4. (A–C) Synaptic jitter with high-frequency GFx stimulation is dependent on relative laterality and is postsynaptic in origin. (A) The rGFx was stimulated at about 80 Hz while recording intracellularly from the lMoG (top trace) and extracellularly from lR3 (second trace). Many sweeps are superimposed, showing the initial full-sized MoG spike (1), the progressive delay and eventual failure of the MoG spike (2), followed by the decline in amplitude of the residual EPSP (3). (B) Stimulation of the lGFx (intracellular recording, top trace) causes some delay and eventual failure of the lMoG spike recorded intracellularly (second trace) and extracellularly (third trace), but the failure is less complete and the jitter minimal. Note that the GF spike is little affected by the stimulus. (C) The lGFx and rGFx were stimulated in rapid alternation while recording intracellularly from the lMoG, but without displaying the response. After 1 or 2 s the MoG spike initiated by the cGF failed and the residual EPSP declined in amplitude as in A, although the iGF response was maintained. At this point the oscilloscope was triggered to display the residual response to the cGF stimulus followed by the full iGF response (filled arrows; 1). The iGF stimulus was then switched off, whereupon the 'residual' EPSP increased in amplitude (left-hand 2), and eventually initiated a full MoG spike again (left-hand 3), while the iGF response, of course, ceased (open arrows; right-hand 2, 3). The record thus shows *recovery* of response, and is the opposite of A. The cGF stimulus remained constant throughout. Scale: vertical A,B 30 mV, C 15 mV; horizontal A,B 1·25 ms, C 5 ms.

occurred much more rapidly, and at lower frequencies of stimulation, for the cGF than for the iGF (Fig. 4). The effect has been noticed previously (Stephens, 1986) and attributed to the MoG receiving chemical excitation from the cGF and electrical excitation from the iGF. Our intracellular recordings from the MoGs during high-frequency AC stimulation showed that the increasing latency was mainly due to a progressive delay in the time of occurrence of the spike relative to the start of the underlying EPSP (Fig. 4A,B). The difference in latency jitter with iGF and cGF stimulation can thus be explained by a gradually rising spike threshold in the MoG

combined with the difference in amplitude and rise time of the underlying EPSPs. In our experiments the effect was reversible, provided the stimulation was not too prolonged (*pace* Stephens, 1986).

A second effect was noticed with high-frequency cGF stimulation. If the stimulation was maintained after MoG spike failure, there was a progressive decrease in the amplitude of the residual EPSP to a baseline minimum. There was also some decrease in response to iGF stimulation, but this was much less marked (Fig. 4A,B). Prominent anti-facilitation of this sort is generally characteristic of chemical synapses, where it is a presynaptic phenomenon caused by a reduction in transmitter release. However, such effects could also be generated postsynaptically, for instance, by a gradual decline in membrane resistance. To gain further information on this point the two GFs were stimulated alternately with pulse trains of gradually increasing frequency. Eventually spike failure and a reduction in EPSP amplitude occurred in the MoG, similar to that occurring when the cGF alone was stimulated. Using these paired stimuli the cGF frequency at which failure occurred was about half that required when the cGF alone was stimulated. The stimulation to the iGF was then switched off, so that the total stimulation received by the MoG was halved, but the frequency of cGF–MoG synaptic activation did not change. This caused a rapid increase in the amplitude of the EPSP in the MoG, and a return of the MoG spike (Fig. 4C). The effect was the exact reverse of that seen with high-frequency cGF stimulation. This strongly suggests that the decline in EPSP amplitude with high-frequency stimulation is *not* due to anti-facilitation at a chemical synapse, since it can be induced by paired stimuli to different presynaptic neurones, and reversed without any change in presynaptic spike frequency of the supposed chemical synapse. Furthermore, addition of 2 mmol l^{-1} cadmium ions to the bathing medium significantly increased the duration and/or frequency of presynaptic stimulation required before MoG spike failure and EPSP anti-facilitation occurred. This was true for both cGF stimulation alone, and for the paired cGF–iGF stimuli, suggesting that a common mechanism underlies the anti-facilitation under the two stimulus conditions. This is certainly not what would be expected at a chemical synapse (see below for more details).

NEM application

N-ethylmaleimide (NEM) is reputed to be an uncoupler of electrical synapses (Spray *et al.* 1984). Like Stephens (1986), we found that NEM caused overall depolarization, and both the GF and MoG neurones eventually ceased to respond to direct extracellular stimulation. However, in some cases the EPSP caused by iGF stimulation was reduced in amplitude and eventually abolished while the GF spike was still maintained (Fig. 5). In these cases the EPSP caused by cGF stimulation decremented in a parallel fashion. We found no consistent difference in the effects of NEM on the response of the MoG to iGF and cGF stimulation, unlike Stephens (1986).

We do not wish to place too much emphasis on these results because of the variability introduced by the direct effects of NEM on membrane potential and spike

viability, but in our experiments the evidence, in so far as it goes, supports the proposal that both MoGs receive their input from the GF by electrical synapses.

Effects of cadmium ions

If the MoG receives its input from the cGF through a chemical synapse, the input ought to be blocked by substances, such as cadmium ions, which block calcium channels and hence presynaptic transmitter release. A convenient control exists in this system because there are axons in both the ACs which have thresholds close to the GF threshold and which elicit depolarizing IPSPs in the MoG. It seems reasonable to assume that these are homologous to the IPSPs induced in the crayfish MoG (Wine, 1977), which are chemical in origin and caused by an increase in chloride conductance (Ochi, 1969), although this has not been tested explicitly in the hermit crab.

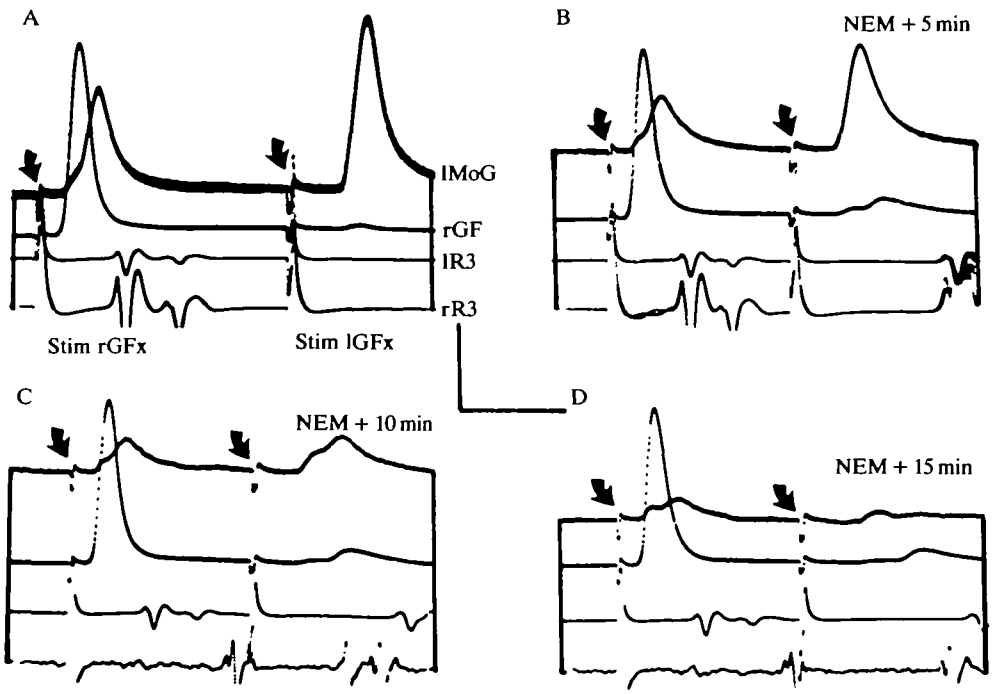

Fig. 5. (A–D) Effects of *N*-ethylmaleimide (NEM), a blocker of electrical synapses. Intracellular recordings were made from the lMoG (top trace) and rGF (second trace), while lR3 (third trace) and rR3 (fourth trace) were recorded extracellularly. The rGFx and lGFx were stimulated rapidly one after the other, with an overall frequency of about 1 Hz. In each record several sweeps are superimposed. (A) In normal saline the MoG spiked in response to both stimuli (in this preparation the spikes were of different amplitude). (B–D) Similar stimuli were delivered 5, 10 and 15 min after the application of 5 mmol l^{-1} NEM. The MoG spike initiated by both stimuli declined in amplitude, and this was accompanied by a decline in amplitude in both EPSPs. There was little difference in the response to the two GFx stimuli. Membrane potential was not monitored continuously in these records. Scale: vertical MoG 20 mV, GF 40 mV; horizontal 5 ms.

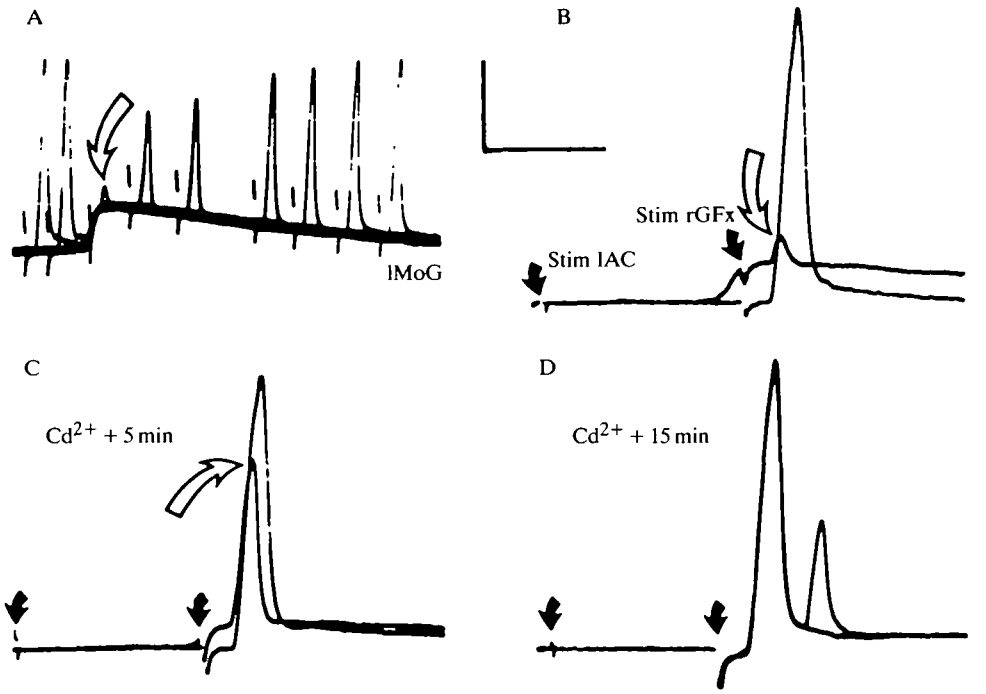

Fig. 6. (A–D) Cadmium ions block chemical inhibition of the lMoG caused by lAC stimulation, but not excitation caused by the contralateral rGF. (A) The lAC was stimulated below lGF threshold, and caused a depolarizing IPSP in the lMoG. The rGFx was stimulated at varying times relative to the lAC stimulus. When the rGFx stimulus coincided with the peak of the IPSP the lMoG response was inhibited (open arrow). Several sweeps are superimposed. The stimuli are not marked with filled curved arrows in this record. (B) Two sweeps at higher speed showing the lMoG response to rGFx stimulation (right-hand filled curved arrow) with and without preceding lAC stimulation (left-hand filled curved arrow). The IPSP virtually abolishes the lMoG response (open arrow). (C) As B, 5 min after applying 2 mmol l^{-1} cadmium chloride. The IPSP has decreased in amplitude, and the MoG response following lAC stimulation has increased (open arrow). (D) As B, 15 min after applying cadmium. The depolarization associated with the IPSP has been completely abolished, and the initial lMoG response is the same with and without preceding lAC stimulation. (The origin of the second peak in the MoG response is not known, but it only occurred with rGF stimulation alone.) Scale: vertical A 25 mV, B–D 15 mV; horizontal A 15 ms, B–D 6 ms.

To test the effects of cadmium ions on cGF–MoG transmission the iAC was stimulated at an intensity which induced a depolarizing IPSP in the MoG. In different preparations this intensity could be either above or below the GF threshold. In a preparation where the IPSP was elicited with an iAC stimulus just below the iGF threshold, the cAC was also stimulated, this time at exactly the cGF threshold. When this stimulus was timed to coincide with the peak of the IPSP, the cGF-induced MoG spike was abolished, leaving only a small residual EPSP (Fig. 6A,B). Saline containing 2 mmol l^{-1} cadmium was then applied to the system. Over a period of about 10 min the IPSP (induced by iAC stimulation) diminished in amplitude,

while the EPSP (induced by cGF stimulation) grew, and eventually gave rise to an MoG spike (Fig. 6C,D). Thus the chemically induced inhibition was abolished, while the cGF activation was not. This strongly suggests that the cGF activates the MoG through an electrical synapse rather than a chemical one.

Current coupling

Perhaps the most direct physiological test for electrical coupling is the ability to pass current from one neurone to the other. Depolarizing current injected into the cGF does indeed depolarize the MoG (Fig. 7B), while hyperpolarizing current has no effect. Depolarizing current injected into the MoG has no effect on the GF. The simplest explanation for these results is that the two neurones are connected by a rectifying electrical synapse. However, interpretation is complicated by two factors. First, both the cGF and the MoG tended to spike as a result of the current injection (Fig. 7C). Second, a similar effect could be obtained at a chemical synapse if the presynaptic neurone released transmitter in a graded manner in response to subthreshold depolarization. A more satisfactory test is to hyperpolarize the postsynaptic MoG, and see whether the hyperpolarization spreads to the presynaptic cGF. The experimental procedure was to stimulate the cGF extracellularly in the cAC, and bracket the stimulus with a pulse of hyperpolarizing current injected into the MoG. With gradually increasing levels of current the first effect observed in extracellular recordings was the failure of the MoG spike in iR3 (Fig. 7D). This was followed by the simultaneous failure of the cGF spike recorded extracellularly in the PC (not shown), and the cMoG and cSG spikes recorded extracellularly in cR3 and cR1, respectively (Fig. 7E). Simultaneous intracellular recording from the cGF showed that as the increasing hyperpolarizing current was injected into the MoG, the cGF hyperpolarized until its spike eventually failed (Fig. 7D–F). The absolute value of the peak voltage of the cGF spike remained approximately constant as the cGF membrane potential was increased, indicating that this hyperpolarization was not a coupling artefact.

The passage of hyperpolarizing current from a postsynaptic neurone to a presynaptic one strongly suggests that the two are connected by electrical synapses. However, it does not necessarily mean that the connection is monosynaptic. There is a disynaptic electrical pathway from the cGF to the MoG *via* the cSG, since the latter receives electrical input from its ipsilateral GF and makes electrical output to both MoGs (Heitler & Fraser, 1986, and see below). Thus hyperpolarizing current could pass from the MoG to the cSG and hence to the cGF. There are a number of reasons why this is unlikely to be a major pathway. First, the electrical EPSP produced in the MoG by cSG stimulation is nearly always subthreshold, and usually quite small (2–8 mV; Heitler & Fraser, 1986), suggesting that this pathway is not strong. Second, when hyperpolarizing current was injected into the MoG, the cSG spike did not fail at lower current levels than the cGF spike, as might be expected if the current was passing through the cSG on its way to the cGF (Fig. 7D,E). Third, when sufficient hyperpolarizing current was injected into the MoG to block the cGF and cSG spikes, a simultaneous recording from the cSG showed that it only

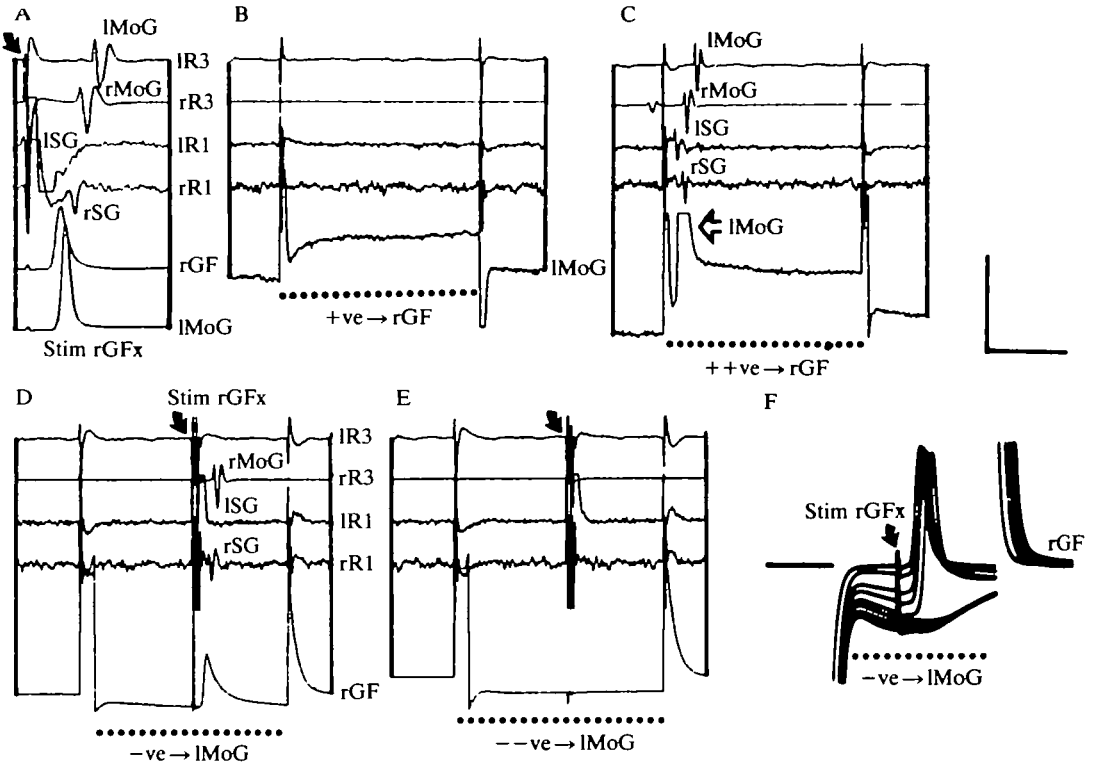

Fig. 7. (A–F) Current coupling between the cGF and the MoG. The rGF (fifth trace) and the lMoG (sixth trace) were recorded intracellularly, while extracellular recordings were made from the lR3 (top trace), rR3 (second trace), lR1 (third trace) and rR1 (fourth trace). (A) Stimulating the rGFx causes spikes in both MoGs and both SGs (note that activation of the contralateral SG is unusual, and that the cSG spike is only just visible in the stimulus artefact). (B) Subthreshold depolarizing current injected into the rGF causes a depolarization of the lMoG, but no spikes occur. (C) A slight increase in current causes the rGF to spike, and induces the same complement of extracellularly recorded spikes as in A. The intracellular lMoG spike (fifth trace) is clipped by the recording device (open arrow). (D) The rGFx is stimulated and bracketed by a pulse of hyperpolarizing current injected into the lMoG. This abolishes the lMoG spike in lR3, and hyperpolarizes the rGF (fifth trace), but does not abolish its spike. The other extracellular spikes are not affected. (E) A slight increase in hyperpolarizing current to the lMoG now abolishes the rGF spike, and all the extracellular spikes. (F) Experiment as in D, but from another preparation showing multiple sweeps with increasing levels of hyperpolarizing current injected into the lMoG. Only the intracellular rGF trace is shown. Note that the rGF spike has an approximately constant peak amplitude until it is abolished, showing that the voltage coupling is not an artefact. Scale: vertical A 100 mV, B,C 16 mV, D,E 140 mV, F 60 mV; horizontal A,F 5 ms, B–E 12 ms.

hyperpolarized by about 8 mV, compared to the 40 mV hyperpolarization recorded in the cGF in another experiment (Fig. 8A compared to Fig. 7F). This 8 mV was insufficient to block a cSG antidromic spike initiated by stimulating cR1 (Fig. 8B), which required at least 20 mV hyperpolarization of cSG. Finally, in one aberrant preparation, the iGF failed to connect to the MoG, leaving only the disynaptic

pathway iGF–iSG–MoG (Fig. 8C). The iSG–MoG connection was strong, producing an EPSP of about 13 mV, but even 1000 nA of hyperpolarizing current injected into the MoG failed to block the iGF spike, and only produced an iGF hyperpolariz-

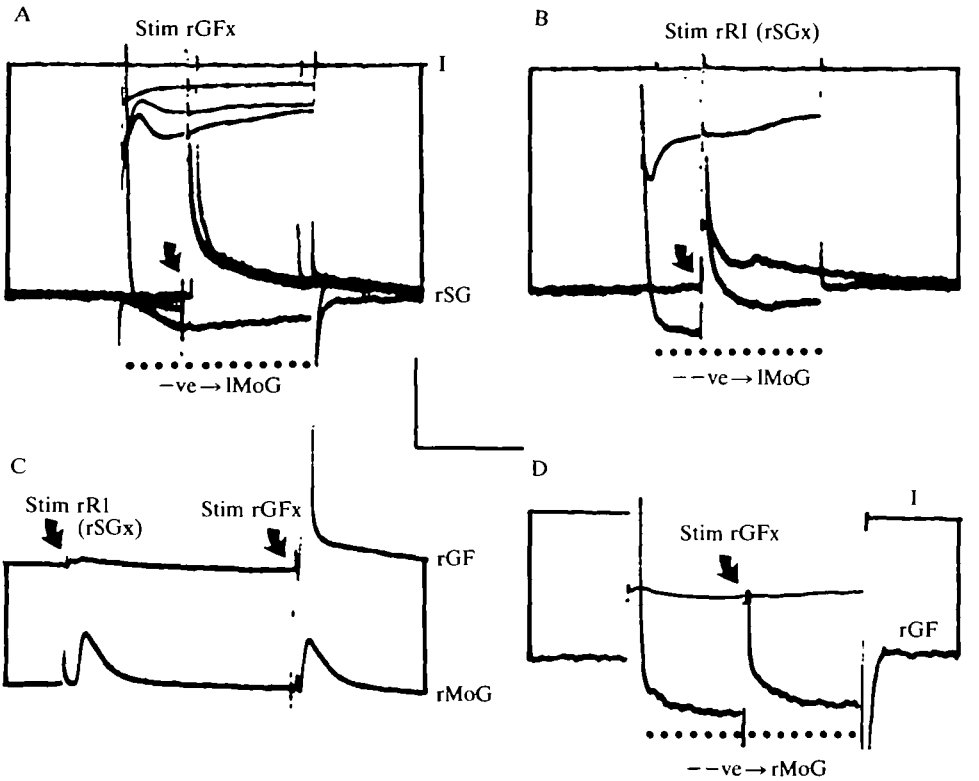

Fig. 8. (A–D) The cGF–cSG–MoG connection is not a significant pathway for the passage of current from the cGF to the MoG. A,B are from one preparation, C,D are from another. (A) The rGFx was stimulated to induce a spike in the rSG (second trace). Four superimposed sweeps are shown with increasing amounts of hyperpolarizing current (monitor in top trace, I) injected into the IMoG. Eventually the rSG spike fails, with no trace of a residual rGF-induced EPSP. This suggests that the SG failure is caused by failure of the rGF spike. (B) The rRI is stimulated to induce an antidromic spike in the rSG. Two sweeps are superimposed, in one of which hyperpolarizing current was injected into the IMoG. The rSG spike does not fail, even though the level of injected current and the consequent rSG hyperpolarization were greater than that which in the same preparation caused failure of the rGF-induced rSG spike (cf. A). C and D are from an aberrant preparation in which the rGF did not excite the rMoG directly, but only indirectly through the rSG. (C) Dual intracellular recordings from the rGF (top trace) and the rMoG (second trace) show the aberrant connectivity. First the rRI is stimulated, causing a relatively large unitary EPSP in the rMoG and a very small potential in the rGF. This is followed by stimulation of the rGFx. An EPSP identical to that caused by the rRI stimulus occurs in the rMoG, rather than the expected spike. The simplest explanation is that both EPSPs are caused by the rSG. (D) An rGFx stimulus is bracketed by a large amount of hyperpolarizing current (monitor in top trace) injected into the rMoG. This fails to block the rGF spike and only weakly hyperpolarizes it (cf. Fig. 7F). This indicates that the connection between the MoG and the iGF *via* the iSG is only weak. Scale: vertical A–C (rGF), D 12 mV, C (rMoG) 24 mV, A,B 100 nA, D 1000 nA; horizontal A,B,D 50 ms, C 25 ms.

ation of about 7 mV (Fig. 8D). Since the iSG–MoG synapse is usually stronger than the cSG–MoG synapse, these results suggest that the disynaptic cGF–cSG–MoG connections are not a significant pathway for the passage of hyperpolarizing current from the MoG to the cGF.

DISCUSSION

The synapse between the GF and MoG in crayfish is a classic example of an electrical synapse; indeed it was the first such synapse ever demonstrated (Furshpan & Potter, 1959). The suggestion that in hermit crabs the cGF makes a *chemical* synapse (Stephens, 1986) with the MoG is therefore of considerable interest. The aim of this report is twofold; first, to confirm that the hermit crab cGF does indeed activate the MoG, since there is some doubt in the literature about this (Stephens, 1985, 1986; Heitler & Fraser, 1986) and, second, to determine whether this activation is by a chemical synapse as claimed by Stephens (1986). The evidence for cGF activation of the MoG is clear-cut. Depolarizing current injected into a cGF to induce it to spike almost always induces a MoG spike. Conversely, if the cGF is induced to spike by extracellular stimulation and then injected with sufficient hyperpolarizing current, its spike is blocked and there is simultaneous spike failure of the MoG.

The question of whether the cGF–MoG synapse is chemical or electrical is more complex. Stephens worked on the first abdominal ganglion of *Pagurus pollicaris* (which is fused to the thoracic ganglionic mass), while we have been using the fourth (free) abdominal ganglion of *Eupagurus*. We do not think that this difference in preparations is significant, however, since our raw data are largely in agreement with those of Stephens. The difference lies in our interpretation of these data. We have extended the observations made by Stephens, and also performed new experiments, which lead us to conclude that the synapse is in fact electrical, not chemical.

Is the synapse electrical?

The most convincing physiological proof of the existence of an electrical synapse between two neurones is the demonstration of the direct passage of current from one neurone to the other. We show that depolarizing current injected into the cGF spreads to the MoG, while hyperpolarizing current injected into the MoG spreads to the cGF. If sufficient hyperpolarizing current is injected into the MoG it can actually cause cGF spike failure. The only known disynaptic path from the cGF to the MoG is *via* the SG, and simultaneous microelectrode penetrations have shown that this pathway is not strong enough to account for the observed effects.

Supplementary evidence for the nature of a synapse can be obtained from its response to synaptic blocking agents. The cGF–MoG synapse is resistant to the application of 2 mmol l^{-1} cadmium ions. In contrast, the depolarizing IPSP which is activated in the MoGs by extracellular stimulation of unidentified units in the

anterior connectives is abolished by this treatment, showing that cadmium does indeed block at least some chemical synapses in this system.

NEM, which is a blocker of electrical synapses, was applied to the preparation to try to distinguish between cGF and iGF activation of the MoG. In contrast to Stephens we found that NEM *could* block the cGF–MoG synapse, although the results were hard to interpret because of the direct effects of NEM on membrane potential and spike production. We found no consistent resistance of the cGF-initiated EPSP compared to that initiated by the iGF, and on at least some occasions the responses declined in parallel.

Thus, on the basis of direct current coupling, resistance to known blockers of chemical synapses and susceptibility to blockers of electrical synapses, we conclude that there is a strong electrical component to the cGF–MoG synapse.

Is the synapse also chemical?

The first line of evidence adduced to suggest that the cGF–MoG synapse is chemical was that the MoG spike recorded extracellularly has a greater latency to cGF stimulation than to iGF stimulation. We confirm that this latency differential exists, but show that it is due to a difference in delay between the initiation of the EPSP in the MoG and the passage of the spike past the extracellular recording electrodes on R3. This is partly due to the slower rise time of the cGF-initiated EPSP causing a delay in MoG spike initiation, and partly due to the cGF-initiated MoG spike having a greater conduction delay to the extracellular recording electrodes (presumably due to differences in the site of spike initiation). The overall latency difference is *not* due to a difference in synaptic latency.

We also confirm that high-frequency cGF stimulation causes greater MoG spike jitter and earlier spike failure than iGF stimulation. This is explained in part by the difference in amplitude and rise time of the EPSP underlying excitation from the two sources, and in part by the greater degree of anti-facilitation shown by the cGF-induced EPSP. This sort of anti-facilitation is a common characteristic of chemical synapses. However, similar anti-facilitation can be produced by paired stimuli to both the GFs. Under these conditions the cGF needs only to spike at about half the frequency that is required to produce the effect when the cGF alone is stimulated. This suggests that the important factor is the net stimulation received by the MoG, rather than the cGF spike frequency *per se*. An alternative possibility is that two separate phenomena are occurring – anti-facilitation when the cGF alone is stimulated, and some sort of presynaptic inhibition of the cGF by the iGF when paired stimuli are used. It seems unlikely that these two processes would exactly mimic each other, and it also seems unlikely that they would both be substantially reduced by the presence of cadmium ions. In particular, if the anti-facilitation is the result of a chemical synapse, the cadmium ions should tend to reduce the amplitude of the EPSP, rather than augment it as actually happens.

The cause of the decrement has not been investigated in detail, but one possibility is that it is caused by a reduction in postsynaptic membrane resistance due to corollary discharge neurones making chemical inputs to the MoG which are blocked

by cadmium. Another possibility is that there is normally a cumulative increase in postsynaptic calcium-activated potassium conductance which is blocked by cadmium.

Stephens (1986) found Lucifer Yellow dye-coupling between the iGF and the MoG but not between the cGF and the MoG, while in our experiments we were unable to obtain dye-coupling between either GF and the MoG. However, even if present, differential dye coupling could be explained by the difference in the strength of electrical coupling which we report, and not necessarily by a difference between electrical and chemical coupling. The absence of dye-coupling is a notoriously unreliable indicator for the absence of electrical coupling (Audesirk, Audesirk & Bowsher, 1982; Powell & Westerfield, 1984).

We conclude that there is no positive physiological evidence for a significant chemical component in the cGF–MoG synapse. It is, of course, extremely difficult, if not impossible, to prove that a minute chemical component does *not* exist, but, in the absence of any evidence that it *does* exist, we suggest that at present the data indicate that the cGF–MoG synapse is purely electrical in nature.

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