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LOW-CALCIUM FIELD BURST DISCHARGES OF CA1 PYRAMIDAL NEURONES IN RAT HIPPOCAMPAL SLICES

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SUMMARY

1. Incubation of rat hippocampal slices in solutions containing low Ca^{2+} and increased Mg^{2+} rapidly blocked synaptic responses and increased spontaneous firing of all the principal neurones. More remarkably, a rhythmic and synchronous bursting discharge developed, which was restricted to the CA1 population of pyramidal neurones. These 'field bursts' or 'spreading excitation' were rapidly abolished by restoring the Ca^{2+} to 2 mM, by increasing the Mg^{2+} to 6 mM or by decreasing K^+ from 6 to 3 mM.

2. The CA1 pyramidal cells depolarized after the change to the low-Ca^{2+} solution by about 10–20 mV. Individual field bursts were associated with a further depolarization of 10–12 mV surmounted by a burst of action potentials at about 20/s. This transient depolarization shift, recorded extracellularly as a negative field, could be attributed to the increase of $[K^+]_o$ during the bursts, reaching 9–10 mM as measured by ion-sensitive electrodes. The bursts were followed by a hyperpolarization, seen extracellularly as a small soma-positive field, which was attributed to an electrogenic pump and/or a Ca^{2+}-activated K^+ conductance.

3. Stimulation of the tightly packed pyramidal cell axons in the alveus elicited a train of population spikes, instead of the single spike normally seen, and could trigger a full field burst. Recordings of the alvear tract volley suggested that the repeated spikes arose within the pyramidal cells.

4. Multiple recordings from CA1 revealed that field bursts usually, but by no means always, started near the caudal (subicular) end of the area. They spread through the cell layer at 0·04–0·12 m/s. The most rapid propagation was seen when the bursts had an abrupt onset; slower propagation (1–10 mm/s) occurred when the bursts started gradually, which generally was the case near the sites of burst initiation and termination. Usually the action potentials within each burst were synchronized into population spikes which spread across CA1 at 0·04–0·15 m/s. The site of initiation and the extent of the spread of these population spikes varied during each burst, as did their amplitude. The degree of spike synchronization was enhanced by various treatments expected to increase neuronal excitability. Measurements of transmembrane potential during the burst confirmed the role in the generation of population spikes of ephaptic or field interactions between the pyramidal cells.

5. It is proposed that the increased firing of all neurones is due to the block of tonic inhibition, depression of after-hyperpolarization and to increased membrane excitability. A number of mechanisms are considered which might account for the organization into synchronous bursts: field 'ephaptic' interactions, fluctuations in $[K^+]_o$, the activity of ion pumps and Ca^{2+}-activated K^+ conductance. The synchronization of the discharge within bursts into population spikes, and the most rapid burst propagation are attributed to electrical (field) interactions between pyramidal cells.

INTRODUCTION

The discharge of the pyramidal cells of the CA1 region can become abnormally synchronous after chemical synaptic transmission has been blocked by incubating hippocampal slices in low-Ca^{2+} solutions for about 30 min. This can cause spontaneous bursts of population spikes to occur within a fraction of a second throughout CA1 (Jefferys & Haas, 1982); the remarkable regularity of these 'field bursts' provides a simple means of investigating the pharmacology of pyramidal neurones (Haas, Jefferys, Slater & Carpenter, 1984). A single antidromic stimulus normally causes a single population spike, due to the synchronous discharge of many neurones in this laminar structure (Andersen, Bliss & Skrede, 1971), but under low Ca^{2+} a train of population spikes results (Andersen, Gjerstad & Langmoen, 1978), which can become markedly prolonged (Taylor & Dudek, 1982).

Where it has been investigated in detail, neuronal synchronization has generally depended on synaptic mechanisms, for instance the synchronization of neocortical e.e.g. is attributed to the organization of inhibition in thalamic nuclei (Andersen & Sears, 1964; Avoli & Gloor, 1981). In the hippocampus, the epileptiform bursts induced by penicillin have been attributed to recurrent excitatory connexions between CA3 pyramidal cells (Traub & Wong, 1982). In contrast, different mechanisms must be at work in the present phenomenon which is induced in hippocampal CA1 pyramidal cells by low Ca^{2+}; in particular this work should focus interest in non-synaptic mechanisms such as field interactions in laminar structures (Gardner-Medwin, 1976; Jefferys, 1981; Schwartzkroin, 1983), and fluctuations in extracellular ions (Heinemann & Lux, 1977).

METHODS

The 400 μm hippocampal slices were cut using a McIlwain tissue chopper or a Vibroslice (Jefferys, 1982) from brains obtained from adult male Sprague–Dawley or Wistar rats (150–370 g), under halothane anaesthesia or shortly after cervical dislocation. Sprague–Dawley rats from one supplier proved resistant to low-Ca^{2+} field bursts; this may be related to the relatively high levels of noradrenaline and adrenoceptors found in these particular rats (C. Stanford & J. G. R. Jefferys, unpublished). Slices were maintained in chambers at a controlled temperature under a moist, warmed mixture of 95 % O_2/5 % CO_2, either in a thin film of artificial cerebrospinal fluid (c.s.f.) (Haas, Schaerer & Vosmansky, 1979) or on a nylon mesh at the surface of a larger volume of the liquid. The artificial c.s.f. was equilibrated with the same gas mixture, and had the following composition (mM): Na^+, 150; K^+, 6·26; Ca^{2+}, 2·0 or 0·2; Mg^{2+}, 2·0 or 4·0; Cl^-, 137; HCO_3^-, 26; HPO_4^-, 1·25; glucose, 10. Recording pipettes were filled with 1 M-NaCl (1–20 MΩ) for extracellular recording, and with 3 M-K acetate or KCl (50–100 MΩ) for intracellular recording.

K^+ activity was measured with double-barrelled electrodes, one barrel containing a 200 μm column of liquid K^+ ion exchanger (a gift from W. Simon, Zürich) and the reference barrel 0·2 M-NaCl.

Arrays of up to eight extracellular electrodes were used to follow the spread of excitation through the slice. They were positioned independently with conventional micromanipulators (Prior Ltd.) under visual guidance.

RESULTS

Post-synaptic responses were blocked by the 0·2 mM-Ca^{2+}, 4·0 mM-Mg^{2+} solution within 5–10 min. The field bursts took longer to develop, needing 1–2 h to reach the stage illustrated in Fig. 1, though smaller irregular bursts were seen earlier; the process was accelerated in the presence of EGTA. The spontaneous activity of neurones throughout the slice increased markedly (typically 10-fold), but under the

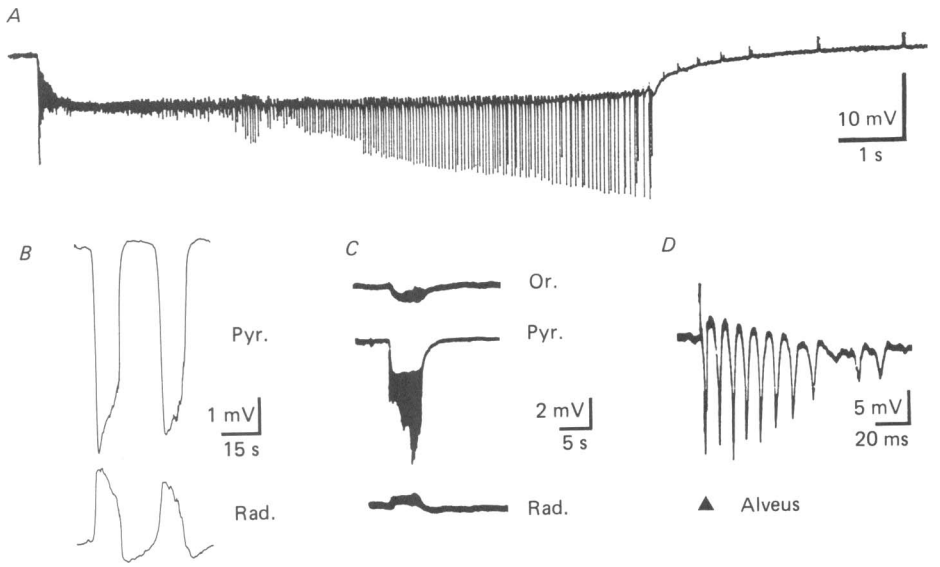

Fig. 1. Extracellularly recorded field bursts in the CA1 area. *A*: in stratum pyramidale, *B*: in strata pyramidale (Pyr.) and radiatum (Rad.), *C*: in strata oriens (Or.), pyramidale and radiatum, *D*: repetitive population discharge in stratum pyramidale in response to alveus (antidromic) stimulation. *A*, *C* and *D* are oscilloscope pictures, *B* is from a chart recorder, too slow to show the population spikes. Negative is downward in this and all other Figures.

present conditions spontaneous rhythmic activity was restricted to CA1 and was not recorded in CA3 or the dentate area. Field bursts recurred regularly in each slice for periods exceeding 12 h; the interburst interval varying from 15 s to several minutes in different slices. The bursts generally lasted 0·5–25 s, and had a stable morphology at any site, but this varied greatly between different slices and between recording sites on each slice (Figs. 9 and 10). Each field burst consisted of a potential shift, negative in the cell body layer, on which was usually superimposed a larger number of population spikes, each of which was due to the discharge of many pyramidal cells (Fig. 1 *A*). Potential shifts reversed in stratum radiatum (Fig. 1 *B* and *C*).

Extracellular cations. The bursts did not develop in 3 mM-K^+, and were rapidly and reversibly accelerated when the [K^+] was increased from 6·25 to 9·25 mM (Fig. 2);

adding Li^+ (2–5 mM) had a similar effect. Increasing the concentration of Mg^{2+} from 4 mM slowed the frequency of the bursts, and abolished them at 6 mM (Fig. 2); restoring Ca^{2+} to 2 mM also abolished the bursting. Lowering the pH (using acetic acid) by up to 0·5 units slowed the bursts, and raising pH (by adding NaOH or stopping the flow of CO_2) accelerated them.

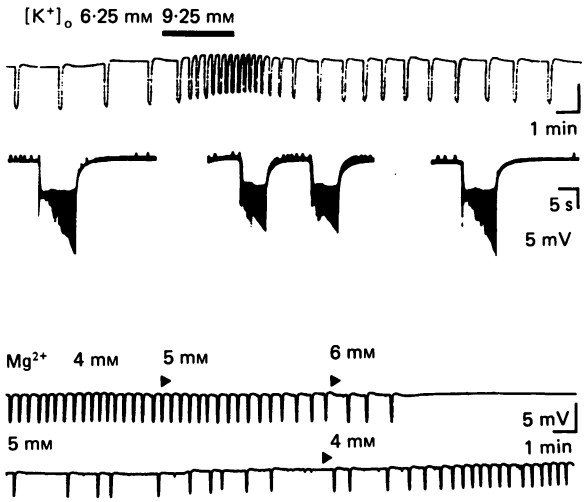

Fig. 2. The influence of changes in K^+ and Mg^{2+} ions in the bath on field burst frequency. Upper trace shows the acceleration by a 3 mM increase of $[K^+]_o$ during the bar above the continuous chart record. The oscilloscope photographs below are before (left), during (middle) and after (right) the $[K^+]$ increase. Lower traces are a chart record illustrating the effects of an increase and subsequent decrease of $[Mg^{2+}]$ in the bath.

The pattern of the population spikes during the field bursts was also affected by extracellular cations. The pattern illustrated in Fig. 2 under 6·25 mM-K^+ was often recorded during this study; there was an initial group of spikes, followed by a period of smaller population spikes which then grew progressively during the remainder of the burst. Following the change to 9·25 mM-K^+, the transient attenuation of the population spikes no longer occurred (the burst had 'filled out'). We have found that a similar enhancement of the population spike component of field bursts was produced by drugs such as DL-homocysteic acid and histamine (Fig. 3; Haas *et al.* 1984). Thus it appears that treatments that would be expected to increase neuronal excitability increase the frequency of bursting and also enhance the synchronization of the population spikes within bursts. A 10 % dilution of the perfusate also enhanced the synchronization of the population spikes. Conversely, γ-aminobutyric acid (GABA), taurine and adenosine, drugs which are inhibitory through increasing Cl^- and K^+ conductances, reduced the burst frequency (Fig. 3; Hood, Siegfried & Haas, 1983).

Role of the alveus in synchronization. The axons of the CA1 pyramidal cells gather in a dense sheet, the alveus, on the dorsal surface of the hippocampus, and it is possible that interactions between the tightly packed axons could contribute both to

the synchronization of the field bursts and of the population spikes within them. Stimulation of the alveus under the present conditions elicited a burst of population spikes instead of the single antidromic population spike normally seen (Fig. 1). The tract volley caused by the stimulus was recorded from an alvear electrode as a negative potential, preceding the first population spike at the pyramidal cell layer.

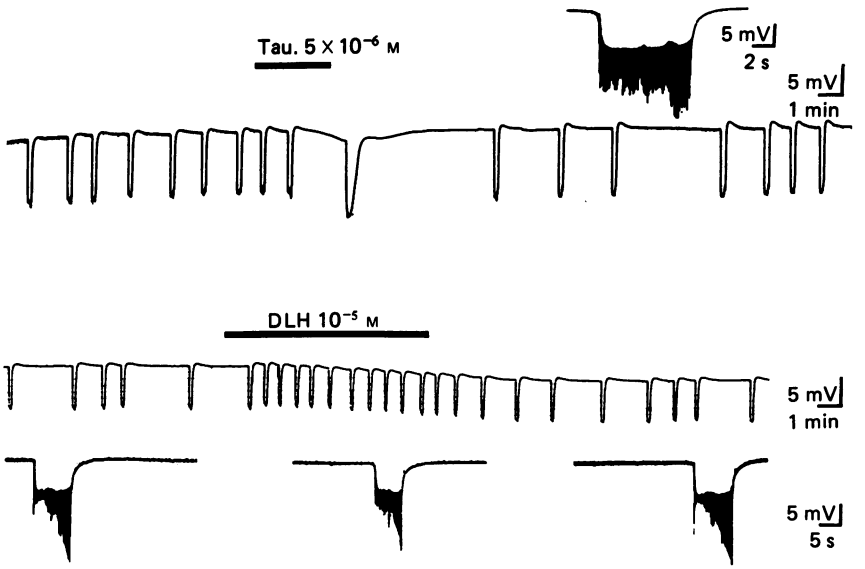

Fig. 3. Effects of inhibitory and excitatory amino acids on field bursts. Taurine (Tau., 5 μM) and DL-homocysteic acid (DLH, 10 μM) were added to the perfusion fluid during the time indicated by bars. Chart records and oscilloscope pictures are shown.

Subsequent spikes in the burst were followed, but not preceded, by alvear fibre potentials, and thus did not arise in the alveus. Similarly, population spikes recorded during spontaneous bursts were not preceded by alvear fibre volleys. The alveus also is not essential for the spread of field bursts through the CA1 region. Lesions made with scissors or a razor in CA1, but sparing the alveus, generally resulted in two independent foci of burst generation. When lesions were restricted to the alveus, the burst frequency remained the same either side of the lesion, but the propagation delay was much greater than normal, typically several seconds instead of tens of milliseconds.

Intracellular recordings. A total of five neurones in the CA1 pyramidal layer were impaled while slices were bathed in the solution containing 2 mM-Ca^{2+} and recordings were maintained during the change to low Ca^{2+} and the subsequent development of the field bursts. Compatible data were obtained from a further twenty-four cells penetrated during low-Ca^{2+} incubation. Following the change of solution, the cells depolarized, by about 20 mV by the time the burst discharges started after some tens of minutes. In their depolarized state, input resistance was little changed (compare ○ and ●, Fig. 4*D*) or was slightly reduced; however, when the membrane potential was restored to its initial value the input resistance was found to have increased by 25% (Fig. 4*D*, ▼). The slow after-hyperpolarization, attributed largely to a Ca^{2+}-

activated K^+ conductance, which normally limits the rate of firing, was almost abolished by incubation in the low-Ca^{2+} solution. This was seen most clearly following a burst of action potentials produced by injection of current through the recording pipette (Fig. 4*C*).

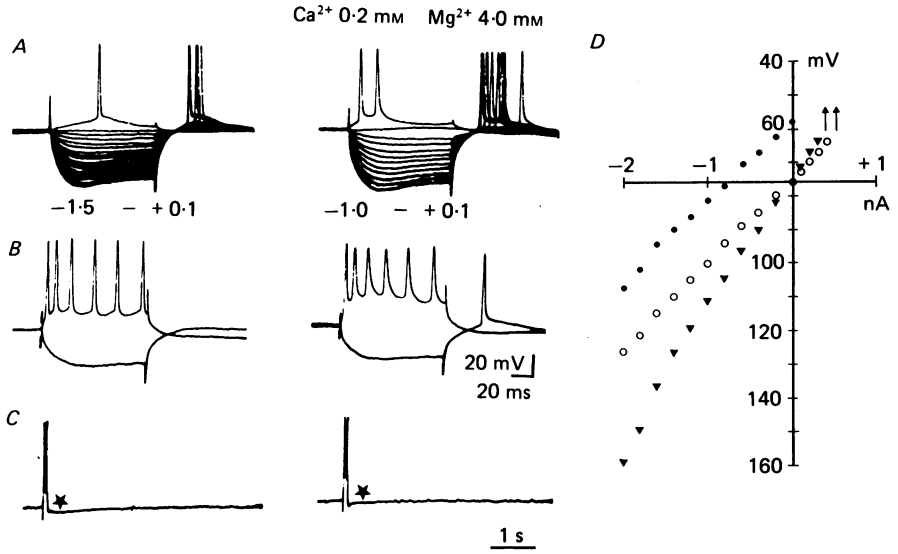

Fig. 4. Series of current injection pulses in a CA1 pyramidal cell before and 1 h after changing to low-Ca^{2+}, high-Mg^{2+} solution. The cell was forced to its initial resting potential by current injection during the right-hand records in *A*, *B* and *C*. *A*: −1·5 to +0·1 nA and −1·0 to +0·1 nA in steps of 0·1 nA; *B*: + −0·5 nA; *C*: +0·5 nA, stars indicate the long lasting after-hyperpolarization (Ca^{2+}-activated K^+ conductance) which is markedly reduced in low-Ca^{2+}. *D* illustrates the current–voltage relationship plotted from a similar experiment in another cell. ○, before; ●, ▼, during low Ca^{2+} high Mg^{2+}.

Intracellular recordings of individual bursts revealed a depolarization of about 10 mV, surmounted by a burst of action potentials at a rate of about 20/s, which started at or before the onset of the depolarizing shift (Fig. 5*A*). The size and frequency of the action potentials during the bursts did not reflect the variation in population spike size found in the extracellular recordings of the field bursts, which thus can be attributed to varying synchrony of discharge. Intracellular recordings during the bursts often revealed that action potentials arose from brief negative waves (Fig. 5*B* and *D*). Subtracting the extracellular from the intracellular record showed that these brief negative waves corresponded to depolarizations of the membrane which were sufficient to trigger action potentials (Fig. 5, *i*−*f*). The net depolarization during population spikes is seen most clearly where the intracellularly recorded action potential has failed (Fig. 5*C*).

Fluctuations in extracellular ion activities. Fluctuations in extracellular ion activities could modulate the excitability of large groups of neurones and thus participate in synchronizing or triggering the field burst discharges. Ca^{2+} does not appear to be involved, however, as field bursts persisted in, and were accelerated by, 1 mM-EGTA

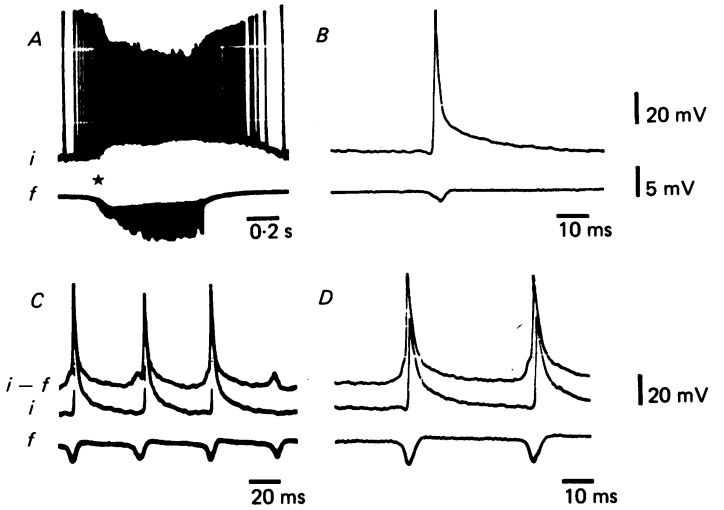

Fig. 5. Synchronous intra- and extracellular recording of field burst with two electrodes in close apposition (less than 50 μm). *B* is an expanded trace from the point marked with a star in *A*. *C* and *D* show intracellular (*i*) and field (*f*) recording and the difference (*i* − *f*).

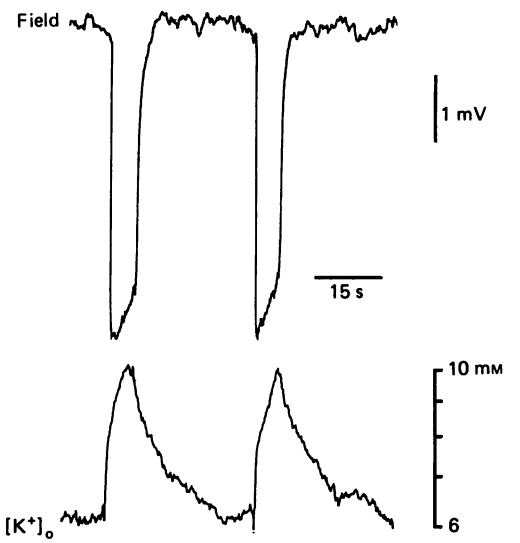

Fig. 6. Field bursts and $[K^+]_o$ recorded with a K^+-sensitive electrode. A slight increase of $[K^+]_o$ can be seen before the bursts begin. Note the difference in the time courses of the field (recorded with the reference barrel) and the K^+ signal.

(seven slices), which buffers Ca^{2+} activity to very low values (see also Yaari, Konnerth & Heinemann, 1983; Konnerth, Heinemann & Yaari, 1984). The putative Ca^{2+}-channel blockers, verapamil and D-600, reduced burst size and frequency at 10–100 μM; but either drug at a concentration of 300 μM always abolished the bursts irreversibly, probably because of their general (non-selective) channel-blocking properties (Bregestovski, Miledi & Parker, 1980; Slater, Haas & Carpenter, 1984).

Extracellular K^+ is known to depolarize neurones, and is also released by their activity, which suggests that fluctuations in $[K^+]_o$ could be involved in the generation of epileptic activity. However, this has only been shown clearly in the case of spreading depression (Leao, 1972; Van Harreveld & Stamm, 1953). Measurements with ion-selective electrodes showed that K^+ activity increased during the field bursts,

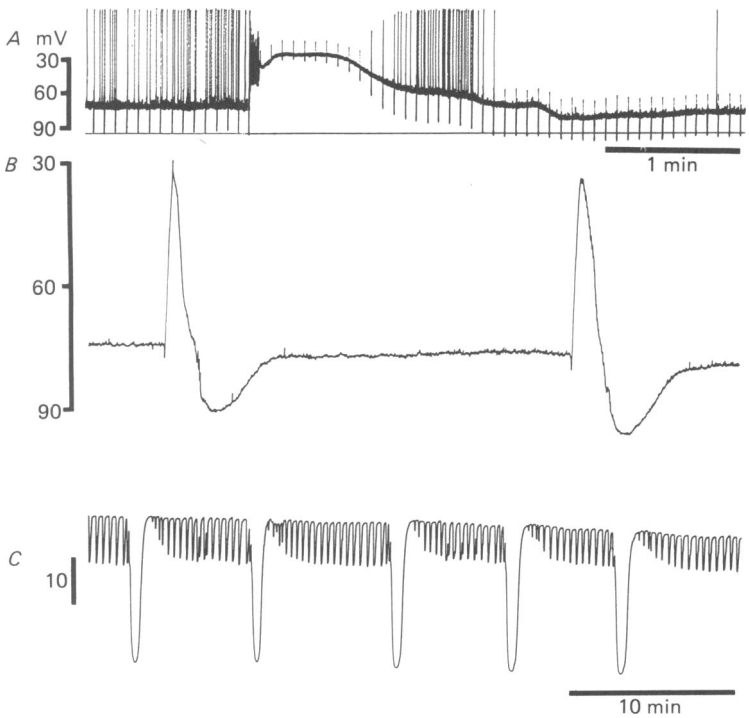

Fig. 7. Spreading depression and excitation. *A* and *B* are intracellular records. *A* is from an oscilloscope camera, upstrokes are action potentials, downstrokes are voltage deflexions from 0·5 nA current injection. *B*: two phases of spreading depression in a different cell registered on a pen-writer. Note the initial hyperpolarization and the long lasting after-hyperpolarization (which was blocked by ouabain later in this cell). *C* is an extracellular record in low Ca^{2+} high Mg^{2+} showing field bursts and five phases of spreading depression. Time calibration 10 min for *B* and *C*.

reaching 10 mM, but, at least for bursts with a fast onset, this generally did not start until the electrical burst had begun. Thus in these cases increased K^+ activity did not initiate the bursts. A slow and small increase in $[K^+]_o$ was however often observed just before the explosive burst (Fig. 6); this could move a cell population to the burst threshold and is probably responsible for the slowly spreading bursts with gradual onset (Konnerth *et al.* 1984).

Occasionally a burst triggered an even larger extracellular negative shift (30–40 mV), lasting for about 30 s, which we interpret as spreading depression and which differed clearly from the field burst described above. It generally was followed by a prolonged period (up to 20 min) of reduced excitability (Fig. 7; see also Haas & Ryall, 1980), and usually once started it recurred at intervals of 10–30 min for many

hours. As would be expected for spreading depression, these large shifts propagated slowly across the slice (a few millimetres per second) and were associated with large increases in $[K^+]_o$ (much greater than 10 mM). Intracellular recording revealed an initial burst of action potentials, followed by a depolarization to −30 mV (close to 0 mV if the extracellular field was subtracted), spike inactivation and a massive drop

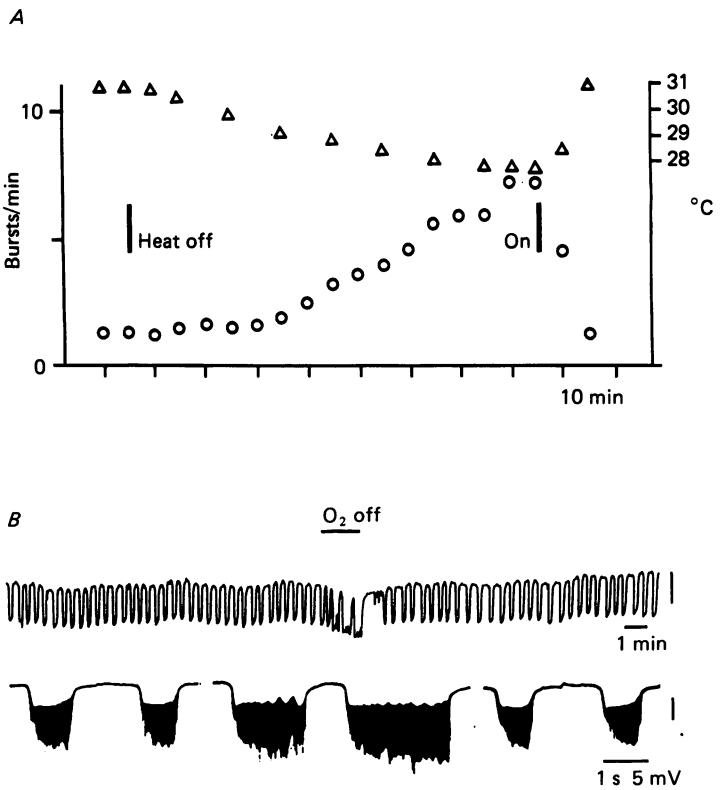

Fig. 8. The influence of temperature and anoxia on field bursts. *A*: average field burst frequency was determined every 5 min (○) and the bath temperature registered (△) before, during and after a period of 80 min when the heating was switched off. *B*: continuous chart record (upper trace) and oscilloscope traces. The oxygen superfusion was discontinued during the time indicated by a bar.

in input resistance. This spreading depression could also begin with a brief negative intracellular potential (Fig. 7*B*) resembling those corresponding to transmembrane depolarizations in Fig. 5*B* and *D*, which suggests that field potentials may also have a role in the propagation of this disturbance.

Field bursts recorded from the pyramidal layer were followed by a small positive potential, which corresponded to the membrane hyperpolarization that followed the bursts (Figs. 2 and 3). The only treatment that both accelerated and/or greatly prolonged the bursts was hypoxia, produced by interrupting the gas flow or by replacing some of the O_2 in the mixture with N_2. The initial effect of O_2 deprivation was an acceleration of the bursts followed by a marked prolongation and increase in amplitude (Fig. 8), often leading to spreading depression. Other treatments which

might have effects similar to hypoxia were tested: dinitrophenol (DNP, 100 μM) slowed the frequency of bursts, while ouabain (1 μM) and Li^+ (2 mM) and lowering the temperature by 3 °C accelerated them considerably. Warming the slices slowed the burst frequency, but above about 37–38 °C they speeded up again (Fig. 8).

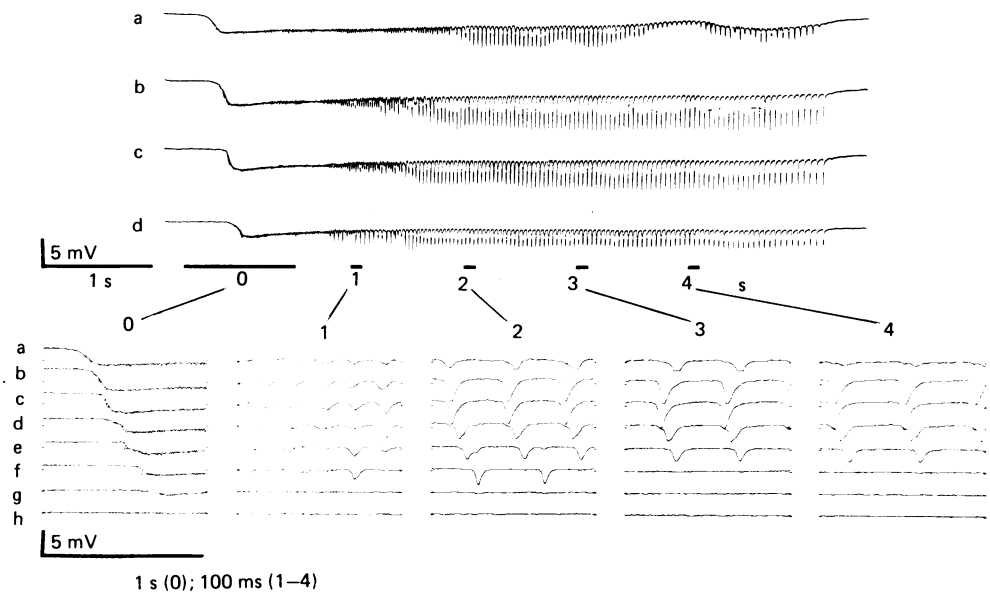

Fig. 9. Detailed structure of a burst. Simultaneous extracellular recordings were made from eight electrodes, 0·3 mm apart in the pyramidal layer (a–h, as shown in Fig. 10*A*). The burst is shown in full (upper panel) for channels a–d to illustrate the development of population spikes. Segments to the eight channel recordings are shown below, the first slow segment centred on the start of the burst at e, and the remaining faster segments 1, 2, 3 and 4 s later.

Propagation of field bursts. The rate at which the field bursts spread through CA1 must depend to some extent on the mechanism of synchronization. In order to measure this systematically groups of up to eight extracellular electrodes were positioned on the cell body layer at intervals of approximately 300 μm. Field bursts most commonly started at the caudal or subicular end of CA1, as occurred in the burst illustrated in detail (Fig. 9). However, this was by no means invariable, as may be seen in the two successive bursts illustrated in Fig. 10*A*. Once initiated, field bursts spread through most, if not all, of CA1. The propagation velocity varied; at its slowest it could be 1 mm/s, though 4–10 mm/s was more typical for bursts with a gradual onset (Fig. 10*A*). Faster propagation, up to 0·1 m/s, was recorded when the bursts had a more abrupt onset (Fig. 10*B*).

Shortly after their onsets, bursts often went through a period of desynchronized action potentials (Figs. 1–3). Subsequently the action potentials synchronized into population spikes, which initially covered a restricted range of recording sites (Fig. 9, 1 s), before all the sites invaded became entrained (2 s). The population spikes spread across CA1 at about 0·1 m/s, which interestingly is similar to the fastest burst propagation velocity.

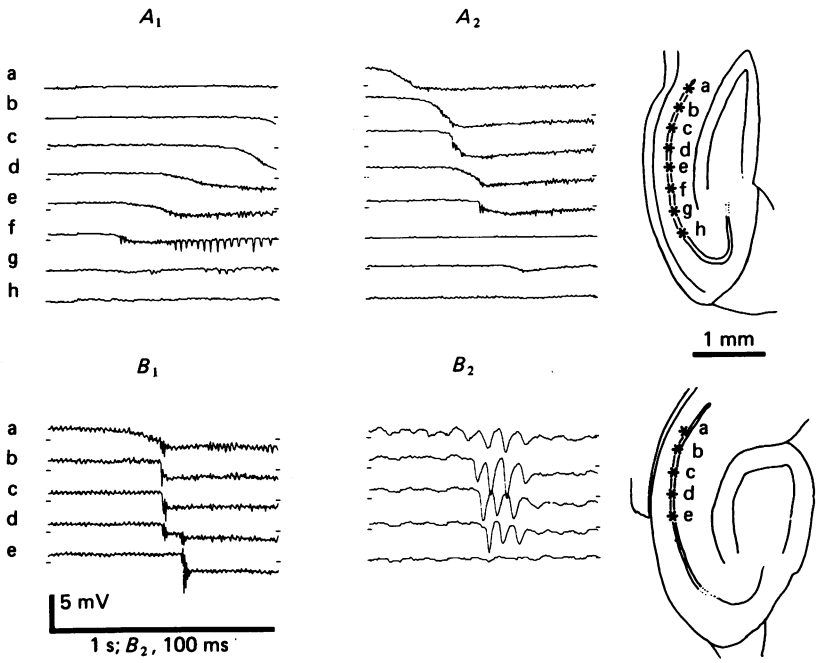

Fig. 10. Burst propagation. Simultaneous extracellular recordings made at the start of bursts in two slices (*A*, top row; *B*, bottom row). The eight electrodes in *A* (a–h) and the five electrodes in *B* (a–e) were spaced at 0·3 mm along the CA1 pyramidal layer as shown in the drawings of the two slices. The two consecutive bursts A_1 and A_2 illustrate slow propagation, while B_1 illustrates rapid propagation (shown at a faster sweep in B_2).

DISCUSSION

The abnormally synchronous 'field burst' or 'spreading excitation' discharges described in the present work developed in the CA1 pyramidal cells some tens of minutes after synaptic responses had been blocked by incubating the hippocampal slices in low-Ca^{2+} solutions. They developed more quickly in very low concentrations of external Ca^{2+}. Thus it seems likely that the field bursts required not only the increase in Mg^{2+} that blocked synaptic transmission, but also the equilibration to a low level of Ca^{2+}, which is known to be relatively slow (Hubbard, Jones & Landau, 1968; Yaari *et al.* 1983). Although CA1 is the region most sensitive to this activity, it has proved possible to provoke rhythmic activity in CA3 and the dentate area by lowering the Ca^{2+} still further (Snow & Dudek, 1983).

Effects of low $[Ca^{2+}]_o$ *on neuronal function.* Our intracellular records revealed that, prior to the first field bursts, the change to low-Ca^{2+} solution caused a progressive depolarization, which corresponded to the increased firing rate of extracellularly recorded units. It has long been known that neuronal membranes become more excitable when bathed in low concentrations of divalent cations (Frankenhaeuser & Hodgkin, 1957; Brismar & Frankenhaeuser, 1975); certainly the field bursts were abolished by increasing Mg^{2+} to 6 mM, which would more nearly balance the excitatory effect of low $[Ca^{2+}]_o$. However, this action of divalent cations would not

affect the measured membrane potential and thus should lower the action potential threshold, while we detected a substantial depolarization with little change in threshold. The steady depolarization was associated with an increased input resistance measured at the original resting potential, which indicates the closure of one or more ion channels; this may be due to a reduction of a Ca^{2+}-activated K^+ conductance, or to a loss of tonic inhibition.

Prolonged exposure to very low $[Ca^{2+}]_o$ can damage cells irreversibly (Frankenhaeuser & Hodgkin, 1957; Alvarez-Leefmans, deSantis & Miledi, 1979); however, this was unlikely to have occurred here as only moderate reductions in $[Ca^{2+}]_o$ were necessary for the bursts (to 0·2 mM in this report and to 0·5 mM in that of Taylor & Dudek (1982)), and restoring the Ca^{2+} quickly abolished them.

Synchronization. Whatever its basis, the slow depolarization would have the effect of making the neuronal population more sensitive to excitatory influences that might otherwise be subliminal. Three categories of synchronizing mechanism that might operate in the absence of chemical synaptic transmission are: fluctuations in extracellular ion concentrations, electrical (electrotonic) synapses, and field (ephaptic) interactions. More than one may be involved in the various aspects of the bursts.

Fluctuations in extracellular ions. While Ca^{2+} may have a central role in some epilepsies (Heinemann, Lux & Gutnick, 1977) it is unlikely that fluctuations in Ca^{2+} triggered the field bursts here as they still occurred in the presence of EGTA which would buffer $[Ca^{2+}]_o$ at very low levels (below micromolar). We did record increases in $[K^+]_o$ from 6 to 10 mM during field bursts; from the Nernst equation we obtain an upper limit to the depolarization shift that would be caused by this change in $[K^+]_o$ of 13 mV, which is consistent with the 10–12 mV we actually recorded. Thus fluctuations in $[K^+]_o$ could explain the depolarization shift, the rapid discharge recorded intracellularly and the negative field potential shift. However, they were not necessary to trigger the field bursts as the activity often increased during, but not before, the bursts. This situation is reminiscent of pentylenetetrazol- and penicillin-induced epileptiform activity (Heinemann *et al.* 1977). The phenomenon most clearly dependent on $[K^+]_o$ for its propagation is spreading depression (Leao, 1972), and the present field bursts differ from it in several respects: see Fig. 7 and Results. While it is unlikely that extracellular K^+ alone can be responsible for the fastest propagation of the field bursts, it probably does have a role which becomes most significant when the bursts propagate slowly, for instance, when bursts had a gradual onset, between sites separated by lesions or at the extremes of their travel where speeds of the order of 1 mm/s were recorded.

Two mechanisms dependent on ion movements may have helped to terminate the bursts in the absence of recurrent inhibition: Ca^{2+}-activated K^+ conductance and electrogenic ion pumps. Neither is entirely satisfactory. The bursts terminated with a hyperpolarization lasting several seconds, which was associated with a small conductance increase. The only factors that clearly affected the timing of termination were hypoxia, which greatly prolonged the field bursts, and treatments that increased the frequency of bursting beyond about 1/min, which tended to shorten the bursts. Drugs that have been reported to modify Ca^{2+}-activated K^+ conductance (Benardo & Prince, 1982; Madison & Nicoll, 1982; Haas & Konnerth, 1983; Haas, 1984) affected the burst frequency; those that depressed this conductance reduced the burst interval

but did not prolong the bursts (Haas *et al.* 1984). The Ca^{2+}-activated K^+ conductance clearly was depressed by incubation in low Ca^{2+}. However, the bursts were not prolonged by the addition of EGTA, which would reduce $[Ca^{2+}]_o$ and hence Ca^{2+} influx by a factor of 100 or more, though we cannot exclude the involvement of Ca^{2+} that might be released from intracellular stores by some consequence of the bursts. In the alternative hypothesis the electrogenic ion pump is stimulated by the increase of $[K^+]_o$ and the decrease of $[Na^+]_o$ during the bursts (Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969). Together with the spatial buffering of $[K^+]_o$ (Gardner-Medwin, 1983*a*, *b*), this hypothesis would help to explain why field bursts are often more closely synchronized in their termination than in their onset (Fig. 9). The duration of the post-burst hyperpolarization and the prolongation of the bursts by hypoxia were both consistent with the pump hypothesis, but it remains unclear why ouabain did not prolong the bursts.

Electrical synapses. The evidence for low resistance junctions between CA1 neurones derives from intracellular dye injection and from paired intracellular recordings (MacVicar, Ropert & Krnjevic, 1982; Andrew, Taylor, Snow & Dudek, 1982). Electrical synapses might be expected to provide a short-range synchronizing influence on small clusters of cells (usually two to three were stained by dye injection). Thus it is difficult to conceive of how such interactions could synchronize the whole population; computer modelling suggests they are not sufficient for synchronization either in the present case nor in penicillin bursts, but that they can modulate the strength of synchronization (R. D. Traub, F. E. Dudek, C. P. Taylor & W. D. Knowles, unpublished observation; Traub & Wong, 1982). There is no reason to suppose that electrical junctions would be enhanced under the present condition, as incubation in low concentrations of divalent cations tends to disrupt them (Alvarez-Leefmans *et al.* 1979).

Field interactions occur when extracellular current generated by activity in one set of excitable cells modifies the behaviour of another set, without the intervention of physical junctions. Some fraction of any current flowing through the extracellular space will pass through neurones, and in crossing membranes will modify their potentials. The balance between the parallel intra- and extracellular paths depends on their relative impedances: effectively on the conductivity of the extracellular fluid, the shape of the space it occupies, and the space constant of the neuronal processes in the direction of the current. For field interactions to be significant depends on the active neurones generating enough current, with the correct orientation to the 'receiving' neurones, and on the receiving neurones admitting sufficient of this current to affect their membrane potentials.

'Ephaptic interaction' and 'ephapse' are terms first used by Arvanitaki (1942) to describe transmission between two touching axons of *Sepia* (similar phenomena were described by Jasper & Monnier (1938) and Katz & Schmitt (1940) in the crab, and by tenBruggencate & Schulte (1963) in nerve fibres of hypocalcaemic cats): the derivation was from the Greek for touching, and was intended to contrast with synapses, also from the Greek, which implies a strong contact, linkage or union. Ephaptic transmission in its original sense has been described more recently between single nerve fibres in spinal nerve roots of dystrophic mice (Rasminski, 1980). There has been a tendency to include any electrical interaction between neurones under the term 'ephaptic'; we agree with Korn & Faber (1980) that interactions mediated by gap junctions should be excluded (equal to electrical synapses or electrotonic junctions), but we also believe that the original use of ephapse was not restricted to 'accidental or experimentally induced situations of interactions' and that the term

should include electrical interactions due to the close proximity or touching of excitable cells in the absence of anatomically differentiated and specialized junctions. Therefore a case can be made for describing the following two examples of electrical inhibition as ephaptic: close proximity of the basket cell axon plexus to the initial segment of the rat Purkinje cell causes the latter to be inhibited by the currents generated when an action potential invades the plexus (Korn & Axelrad, 1980; Korn & Faber, 1980); high extracellular resistance at the axon cap of the teleost Mauthner cell makes the neurones in that region a relatively low resistance path for the Mauthner cell's action currents, and again results in inhibition (Furukawa & Furshpan, 1963; Korn & Faber, 1975, 1980). However, the kind of interaction that concerns us here depends solely on the common orientation and lamination of the population of hippocampal pyramidal cells, and thus does not fall within the original scope of the term 'ephaptic'; for clarity we prefer a different term, such as field interaction.

Structures with a laminar organization, such as the hippocampus, generate large, laminar field potentials during synchronous activity. It has long been speculated that these large field potentials, or more strictly, the currents that underly them, could modulate neuronal excitability (Grundfest, 1959; Green, 1964; Gardner-Medwin, 1976). Experimental evidence in the mammalian brain has been limited in the past, apparently because of problems in controlling the direction of the current flow in intact preparations (Purpura & Malliani, 1966; Purpura, 1974), but using the hippocampal slice preparation it has proved possible to demonstrate clearly that neuronal excitability can be altered by imposed measured electric fields of a few millivolts per millimetre (Jefferys, 1981), which is lower than many endogenous field potentials, including those of population spike discharges recorded during field bursts (Jefferys & Haas, 1982; this paper). Data presented here on spontaneous bursts, and by Taylor & Dudek (1982) on stimulated low-Ca^{2+} burst discharges, show directly that population spikes were associated with depolarization of the transmembrane potential (i.e. intracellular with respect to nearby extracellular recordings) and could trigger action potentials.

The timing of the synchronization of population spikes across CA1 during field bursts is significant here. The net depolarizations during these population spikes reach their peaks within 0·5–1·0 ms, which is the time at which driven neurones would be most likely to fire. CA1 pyramidal cell bodies are approximately 20 μm apart, so if the group of neurones generating a population spike only recruited the immediately adjacent neurones, the population spike would propagate at about 20 mm/s. The velocity recorded experimentally was of the order of 100 mm/s which would be consistent with field effects acting over a slightly longer distance and recruiting more than the immediately adjacent shell of neurones (Fig. 9). The most rapid field bursts, which start with substantial population spikes, also spread at this velocity and probably share the same mechanism in contrast with the slower propagation attributed to $[K^+]_o$ (see above). Various treatments which tend to increase neuronal excitability enhanced both the more rapid form of the field burst and the synchronization of action potentials into population spikes.

Initiation of field bursts. Bursts often started near the subicular or caudal end of CA1, but this was by no means invariable, and it was not unusual to record changes in the lead site between consecutive bursts (Fig. 10). The initial burst generally had a gradual onset, though because of the close linkage between fluctuations in $[K^+]_o$ and neuronal firing rate it is difficult to determine which increases first. Bursts often occurred with remarkable regularity for long periods; hippocampal neurones do

possess the components of the pace-maker cycle described by Gorman, Hermann & Thomas (1982): a slow inward Ca^{2+} current and Ca^{2+}-activated K^+ conductance. However, our intracellular recordings revealed no pace-maker potentials, and the field bursts persisted, and indeed accelerated, in the presence of EGTA. The frequency of bursts was very sensitive to a wide range of treatments which alter neuronal firing rates (Jefferys & Haas, 1982; Haas *et al.* 1984; this paper), and it could be that field bursts start when a sufficient number of neighbouring neurones fire within a short time and are then able to recruit adjacent neurones, by field effects and by release of K^+, progressively building up into the field burst.

The low-Ca^{2+} field bursts confirm that a variety of mechanisms can cause hypersynchronous neuronal discharges. In particular, they have illustrated that non-synaptic processes, such as field interactions, and fluctuations in $[K^+]_o$, can exert a significant influence over neuronal activity. Low-Ca^{2+} conditions can arise *in vivo*, for instance early during epileptic discharges (Heinemann *et al.* 1977), so that the mechanisms underlying the field burst may be significant in some pathological situations. This work also emphasizes that such non-synaptic mechanisms should be more widely considered under more normal conditions where they could have a role to play, if in less dramatic form than illustrated above.

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