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# LOCAL CIRCUIT INTERACTIONS IN SYNCHRONIZATION OF CORTICAL NEURONES

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## SUMMARY

Under certain circumstances large numbers of neurones in the mammalian central nervous system (CNS) can discharge simultaneously. An example of such activity is recorded from a hippocampal slice in the presence of agents which block synaptic inhibition. This synchronized discharge occurs spontaneously in a rhythmic fashion or may be triggered by stimulation of any afferent pathway. Its generation appears to involve local circuit interactions. The favourable conditions offered by an *in vitro* preparation have allowed the cellular events during this activity to be examined in some detail. Three factors appear to be critically involved in the synchronization process. Firstly, the intrinsic ability of neurones to generate bursts, secondly, the existence of powerful recurrent excitatory connections, and thirdly the absence of inhibition which normally prevents the spread of bursting activity through the recurrent connections. Computer simulations show that in a sparsely connected network of bursting neurones activity initiated in a few cells may spread through recurrent connections until eventually the whole population discharges simultaneously. Rhythmic discharges similar to those described here also underly various CNS functions including centrally-originating motor patterns. It remains to be determined whether neuronal properties and connectivity found to be important in this hippocampal rhythm may also play a role in the generation of other rhythmic activities in the mammalian CNS.

## INTRODUCTION

The synchronized firing of neuronal populations is frequently observed in the mammalian central nervous system. Motor output for locomotion (Grillner, 1975) and respiration (Wyman, 1977) involves the simultaneous activation of many neurones, and synchronized firing also underlies prominent electroencephalographic waves such as the alpha (Andersen & Andersson, 1968) and theta rhythms (Fujita & Sato, 1964). At present, our knowledge of the synaptic and cellular processes leading to neuronal synchrony for these normal activities is extremely limited.

The brain slice preparation has been very useful for studying the properties of individual cortical neurones and the synaptic processes underlying their collective

Key words: Hippocampus, synchronization, neuronal oscillation.

behaviour. One of the most interesting observations from hippocampal slices is that groups of pyramidal cells when disinhibited can undergo spontaneous synchronized oscillations (Schwartzkroin & Prince, 1978; Wong & Traub, 1983). These events have stereotyped patterns that vary little from preparation to preparation and they can even be recorded from segments of a slice containing as few as 1000 pyramidal cells (Miles, Wong & Traub, 1984). The small size of the neuronal population involved has permitted detailed investigation of the cellular mechanisms underlying the generation of this activity. The data show that the initiation of synchronized discharge critically depends on the three following properties of the hippocampal network. (1) Pyramidal cells can generate intrinsic bursts. (2) These cells are interconnected by a sparsely distributed yet powerful network of excitatory synapses. (3) Synaptic inhibition plays an important regulatory function. The incorporation of these experimentally observed properties of hippocampal neurones into a computer model has allowed the formulation of a plausible mechanism for synchronization. In this review we will illustrate the operation of each of these processes and their involvement in the initiation of population discharge.

## CHARACTERISTICS OF SYNCHRONIZED EVENTS

Spontaneous and evoked synchronized activity of hippocampal neurones is consistently observed in the presence of agents which block gamma-aminobutyric acid (GABA) mediated inhibition (Dichter & Spencer, 1969; Dingledine & Gjerstad, 1980; Schwartzkroin & Prince, 1978; Wong & Prince, 1979; Alger & Nicoll, 1980). The occurrence of these events is indicated by a comb-shaped field potential in extracellular recordings. Characteristically spontaneous events occur rhythmically at intervals of 5–10 s (Fig. 1). Intracellular recordings suggest that all neurones

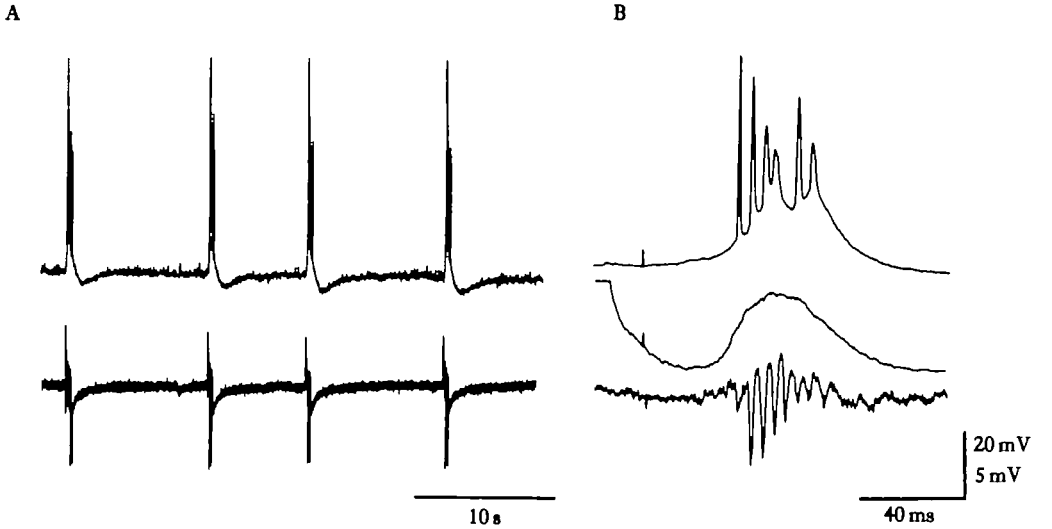


Fig. 1. Characteristics of synchronized activities. (A) Spontaneous synchronized discharge occurs rhythmically in the presence of GABA blockers. Top trace, intracellular recording; lower trace, extracellular recording. Deflections observed in the extracellular trace depend on a large population of neurones discharging simultaneously. (B) Synchronous discharge elicited by afferent stimulation. Top traces, intracellular recording; lower trace, extracellular recording.

discharge simultaneously with the extracellular field event. This neuronal discharge consists of several short duration action potentials with an underlying slow depolarization lasting for 50–100 ms (Matsumoto & Ajmone-Marsan, 1964; Schwartzkroin & Prince, 1977). These bursts are of interest for two reasons. Firstly they can be compared with the interictal events recorded from patients with epilepsy. Secondly their investigation has provided insights into the functional organization of the cortex.

Synchronized events can also be triggered by stimulation applied to any afferent pathway. These evoked events have several distinct features. The latency is usually prolonged (30–150 ms), and is not due to afferent conduction delay, but may be explained by local integration processes. The latency is also extremely variable and is shortened when the stimulus strength is increased (Dichter & Spencer, 1969; Wong & Traub, 1983). The evoked event is followed by a long refractory period. When a stimulus is applied at intervals shorter than about once every 2 s, population responses tend to follow every other stimulus. Thus population events seem to be elicited in an all-or-none fashion. Fig. 1B shows an example of the triggered population discharge. This burst of action potentials observed in one cell is in part sustained by a synaptic depolarization revealed when the cell is hyperpolarized by intracellular current injection. Such synaptic events also underly the bursting in individual neurones during spontaneous synchronized events (Johnston & Brown, 1981).

## MEMBRANE PROPERTIES OF HIPPOCAMPAL PYRAMIDAL CELLS

Pyramidal cells in the hippocampus were originally divided on morphological grounds into CA1, CA2, CA3 and CA4 subfields (Lorente de No, 1934). Recent studies suggest that these areas are involved in different ways in the generation of synchronized events. Spontaneous events are normally initiated in the CA2 subfield (Schwartzkroin & Prince, 1978; Wong & Traub, 1983; Miles *et al.* 1984), and may occur in isolated segments of the CA3 field (Miles & Wong, 1983*a*), whereas the synchronized activity observed in the CA1 field seems to depend on projection pathways from CA2 and CA3.

Intracellular recordings also show that pyramidal cells in the CA1 and CA2–CA3 regions have different firing patterns. Neurones in CA1 usually generate repetitive single action potentials both spontaneously and in response to depolarization. Pyramidal cells in the CA2–CA3 regions, on the other hand, often generate bursts of 2–6 action potentials with an underlying depolarization (Kandel & Spencer, 1961; Wong & Prince, 1978). The characteristics of these bursts resemble those recorded during synchronized discharge, with the only difference being that in the absence of GABA blockers they do not occur simultaneously in a large population of neurones.

The burst firing of pyramidal cells was first observed in recordings made *in vivo* (Kandel & Spencer, 1961). Recent investigations have focused on whether bursts depend on intrinsic neuronal properties or might arise from synaptic interactions. It is now generally accepted that the membrane conductances possessed by the pyramidal cells can sustain burst firing (Johnston, Hablitz & Wilson, 1980; Brown & Griffith, 1983*a*,*b*; Wong & Prince, 1981). Regenerative depolarization in pyramidal cells can be mediated by both sodium and calcium conductances. During an action potential the sodium conductance produces a fast depolarization which then activates

a calcium conductance. This conductance deactivates slowly, resulting in a depolariz ing afterpotential (DAP). The DAP can reach threshold to generate another action potential and so result in a burst. It now seems that calcium entry or its intracellular accumulation during depolarization activates a potassium conductance (Hotson & Prince, 1980; Brown & Griffith, 1983*b*; Alger & Nicoll, 1980) which produces a membrane hyperpolarization lasting for up to 2 s. It is this hyperpolarization that determines the rhythm of spontaneous bursting.

Recordings from acutely isolated pyramidal cells from adult guinea pig hippocampus have directly demonstrated the intrinsic bursting capability of these cells (Fig. 2). Isolated cells have 'clean' membrane surfaces allowing intracellular recordings to be made with low resistance electrodes using the suction method (Hamill *et al.* 1981). In Fig. 2B a short depolarizing pulse applied intracellularly elicited a burst of action potentials. Furthermore isolated neurones can also generate pacemaker bursting activity (Fig. 2C).

## EXCITATORY CONNECTIONS BETWEEN PYRAMIDAL CELLS

Synaptic excitation between pyramidal cells has long been considered probable. The search for its existence began in the deafferented hippocampus (Lebovitz, Dichter & Spencer, 1971) and has been continued in studies using the hippocampal slice (MacVicar & Dudek, 1980; Knowles & Schwartzkroin, 1981). More recently monosynaptic excitatory connections between pyramidal cells have been demonstrated directly (Fig. 3). The connection between these cells was considered to be monosynaptic since each presynaptic action potential elicited an EPSP. Furthermore the latency of the EPSP was short and varied little. A crucial observation was that a barrage of EPSPs elicited by bursting in the presynaptic cell summed temporally to reach threshold and initiated a burst in the postsynaptic cell. Three factors contribute to the functional strength of this excitatory coupling. Firstly, these EPSPs facilitate at short intervals similar to those between action potentials in a presynaptic burst (Miles & Wong, 1983*b*). Secondly, the slow time course of the EPSP means that temporal summation occurs near the peak of each one of the group of synaptic events elicited by a burst of spikes. Thirdly, multiple and independent sites of burst generation are present on the soma-dendritic membrane of pyramidal cells (Wong, Prince & Basbaum, 1979). These factors ensure that activity in pyramidal cells may propagate throughout the population *via* the excitatory interconnections.

In simultaneous intracellular recordings, monosynaptically connected pairs of cells are rarely encountered, indicating that their distribution is sparse. Nevertheless, as will become apparent, this feature of local connectivity is crucially involved in shaping the synchronization.

## CELLULAR MECHANISM FOR SYNCHRONIZATION

Computer simulations of hippocampal neurone networks incorporating an intrinsic bursting capability and powerful recurrent connections will generate synchronized discharge (Traub & Wong, 1982, 1983). Our model consists of a population of 100 neurones each able to generate bursts and interconnected randomly by a sparse

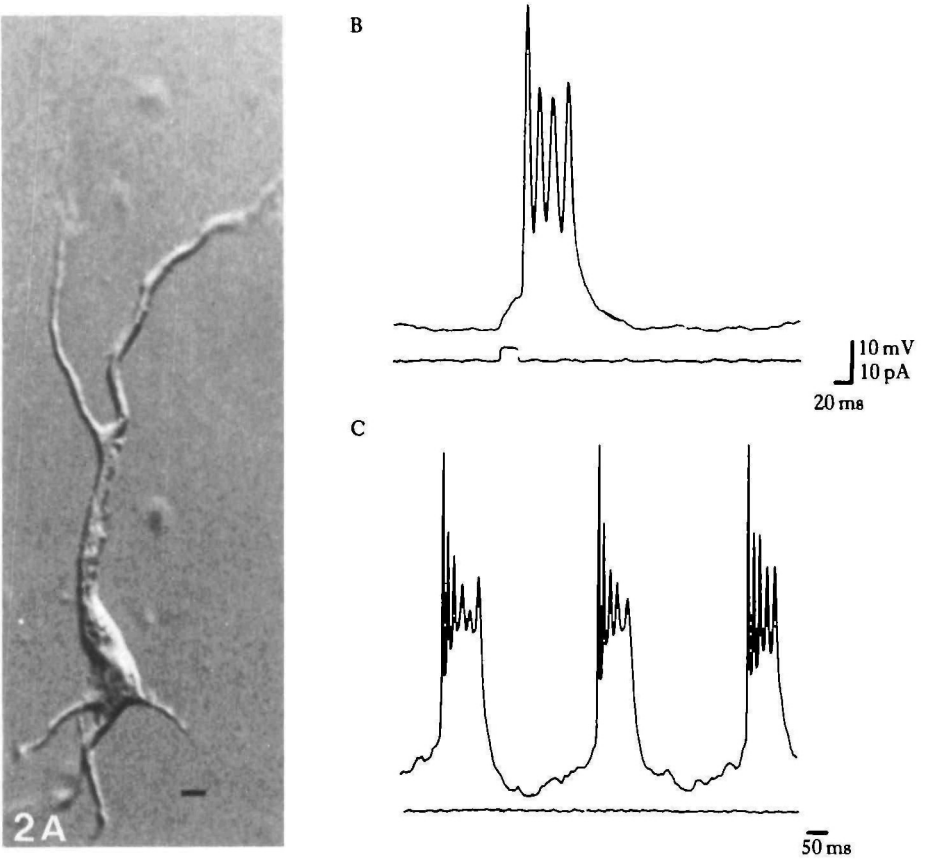


Fig. 2. Neurones may be acutely isolated from the hippocampus of adult guinea pigs. (A) Pyramidal-type cell with a long primary apical dendrite and three basilar dendrites. Calibration bar, 10 $\mu$m. (B),(C) Intracellular activity recorded from this type of neurone (upper traces). Lower traces monitor current applied intracellularly.

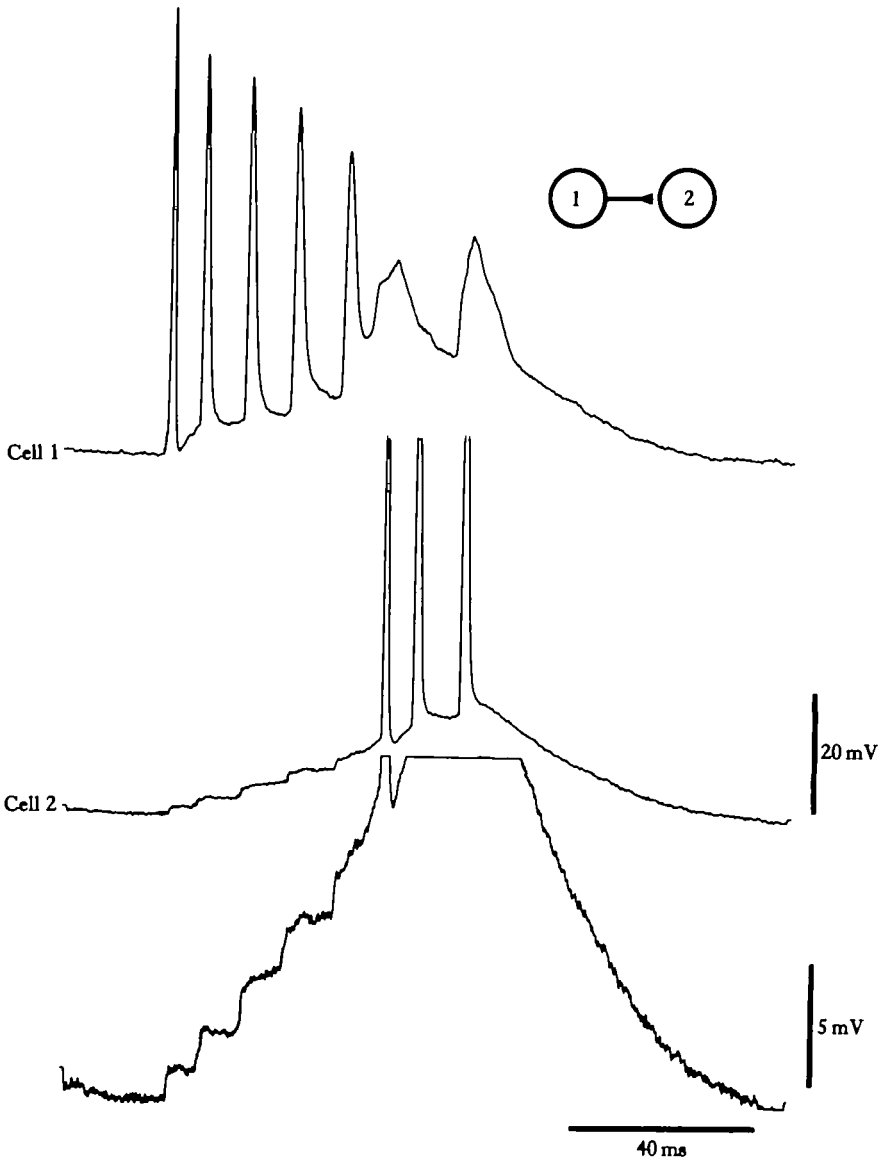


Fig. 3. Recurrent excitatory connection between pyramidal cells. Each action potential in cell 1 triggered an EPSP in cell 2 eventually leading to a burst of action potentials. The recording of cell 2 is shown at two different gains.

network of excitatory synapses (Fig. 4). There are two critical assumptions. Firstly each cell is connected to more than one follower cell and, secondly synaptic connections are sufficiently strong that a burst in one cell may evoke a burst in the follower cells. In this way a localized stimulus applied to a few cells (1–4) will excite a large population of follower cells. As this process is repeated recruitment becomes increasingly rapid leading to a synchronized population event (Fig. 5). This hypothesis for synchronization provides explanations for several experimental observations. The

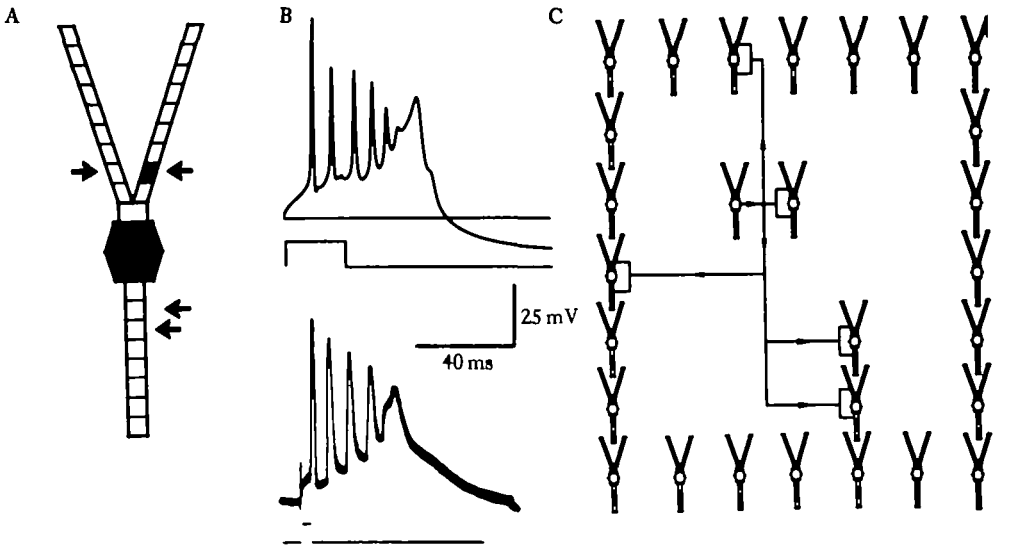


Fig. 4. Features of the model for synchronization. (A) Electrotonic structure of a single neurone divided into compartments of 0·1 space constants. Shaded compartments contain active ionic conductances. Arrows indicate location of excitatory synaptic inputs. (B) Intracellular activity elicited by a short duration depolarizing current pulse in a model neurone (upper record), and in a pyramidal cell (lower record). Calibrations: time, 40 ms; voltage, 25 mV. (C) Connectivity of the model network. Each cell excites 5 % of the other neurones in the network (Traub & Wong, 1982).

long latency of the evoked event is due to the time needed for activity to spread through the population. A few neurones burst early in the event but these should be observed only rarely experimentally and should make a negligible contribution to the extracellular field potential. They will also receive synaptic inputs from neurones bursting later in the event and thus exhibit a double burst (Fig. 5:A3, B3). The variation in latency may conceivably be caused by fluctuations in synaptic efficacy and the excitability of individual neurones.

Spontaneous rhythmic discharge can be initiated if some cells exhibit pacemaker bursting. The interval between these events may be determined in part by the intrinsic calcium-dependent potassium conductance of individual neurones. Recent studies show that other factors may contribute to regulate the rhythm. Thus, it was observed that intracellular injections of the calcium chelator EGTA blocked the hyperpolarization following bursts in individual neurones but did not prevent prolonged membrane hyperpolarization following the synchronized event (Schwartzkroin & Stafstrom, 1980; Alger, 1984). The origin of this EGTA-resistant potential remains unclear although a contribution of a GABA-dependent IPSP which is resistant to common blocking agents appears to be possible (Newberry & Nicoll, 1984).

An important, and experimentally testable, prediction derived from our simulation studies was that the stimulation of a small group of cells may be able to influence the rhythmic activity of a much larger neuronal population. This prediction has now been directly demonstrated. As shown in Fig. 6 stimulation of a single neurone by current injection can reset and entrain the rhythm of the population events. Here stimulation of one cell at a frequency higher than that of spontaneous population events caused their frequency to increase. Population events triggered

by activity in a single cell tended to occur with latency of 50–200 ms. These results clearly show that synchronized activity is initiated by local neuronal interaction. This process whereby activity in a single neurone may activate an entire neuronal

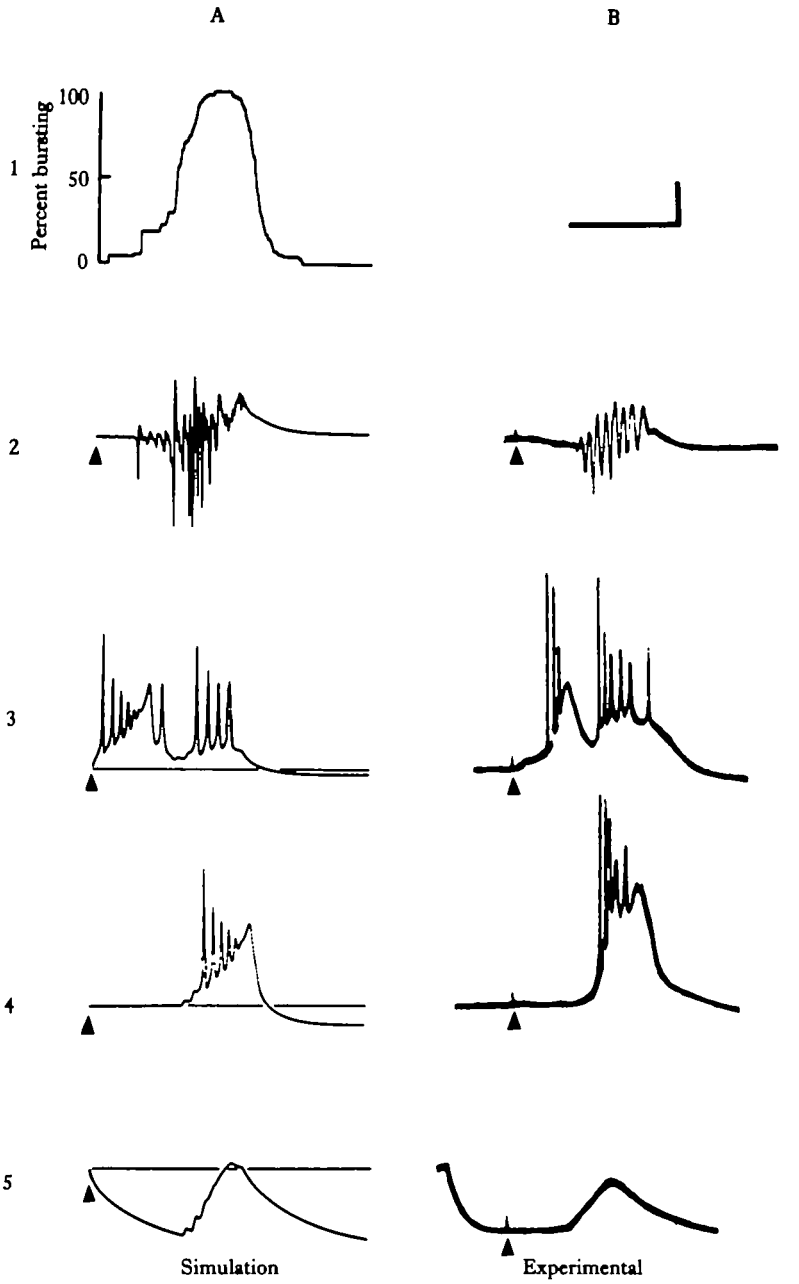


Fig. 5. Comparison of synchronized activity from simulation (A) and experimental (B) studies. (1) Percentage of neurones bursting with time during a synchronized event. (2) Extracellular activity. (3), (4), (5) Intracellular activity (Traub & Wong, 1982). Calibrations, time: 50 ms (A), 60 ms (B); amplitude: 4 mV (A2,B2), 25 mV (A3,4,5), 20 mV (B3,4,5).

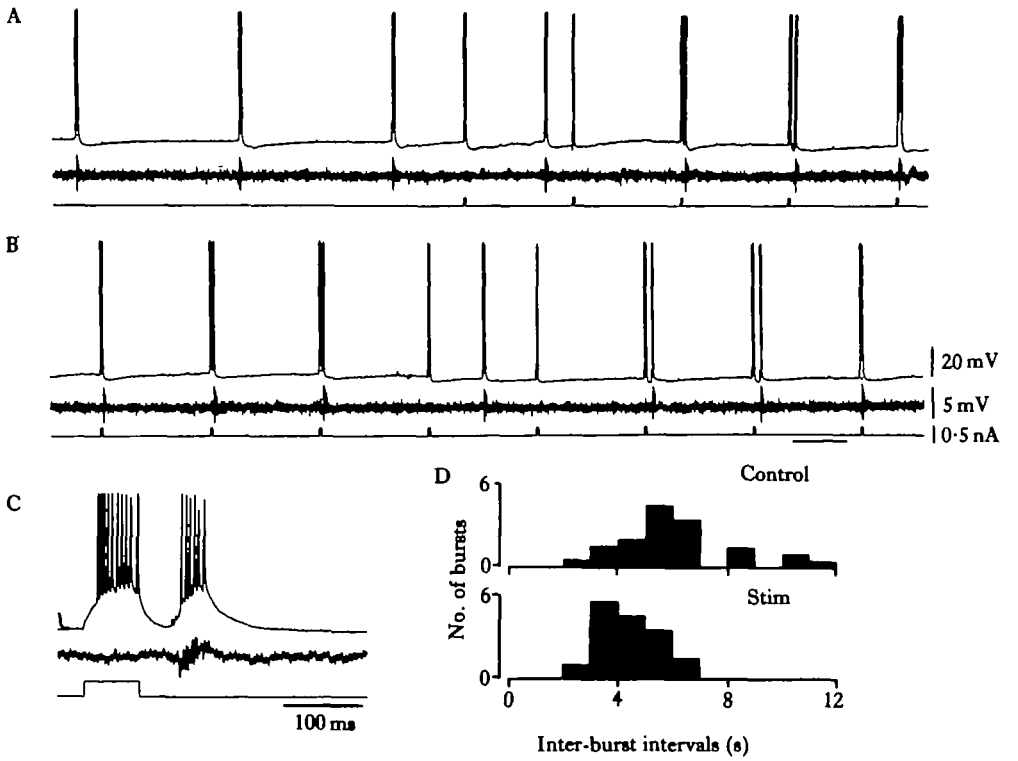


Fig. 6. Entrainment of synchronized population activity by a single neurone. (A),(B),(C) Upper traces, intracellular record; middle traces, extracellular record; lower traces, intracellular current injection. (D) Distribution of intervals between synchronized bursts preceding (control) and during stimulation (Stim). The control interval was 6·2 ± 2·2 s (mean ± S.D., $N = 36$). During stimulation at once every 4 s the interval changed to 4·3 ± 1·1 s ($N = 36$), significantly ($P > 0{\cdot}01$) lower than control (Miles & Wong, 1983*a*).

population can be viewed as an extremely powerful mechanism for signal amplification.

## SYNAPTIC INHIBITION

The synchronization of hippocampal neurones described here occurs only when GABA-dependent inhibition is blocked. How do inhibitory processes normally prevent the development of synchronization?

Inhibition in the hippocampus is mediated through both feedback (Andersen, Eccles & Loyning, 1963) and feedforward (Alger & Nicoll, 1982) circuits. In the former, axon collaterals from pyramidal cells activate the inhibitory cells, whereas in the latter, inhibitory neurones are excited directly by afferent fibres. These circuits underly the EPSP-IPSP sequence commonly observed following the activation of afferent fibres. The latency of the IPSP is such that only single action potentials are elicited in neurones which possess intrinsic bursting capability (Wong & Prince, 1979). As can be observed in Fig. 3, bursting ensures the spread of excitation between pyramidal cells which is the basis for synchronized discharge.

The way synaptic inhibition is recruited through feedback circuits by the

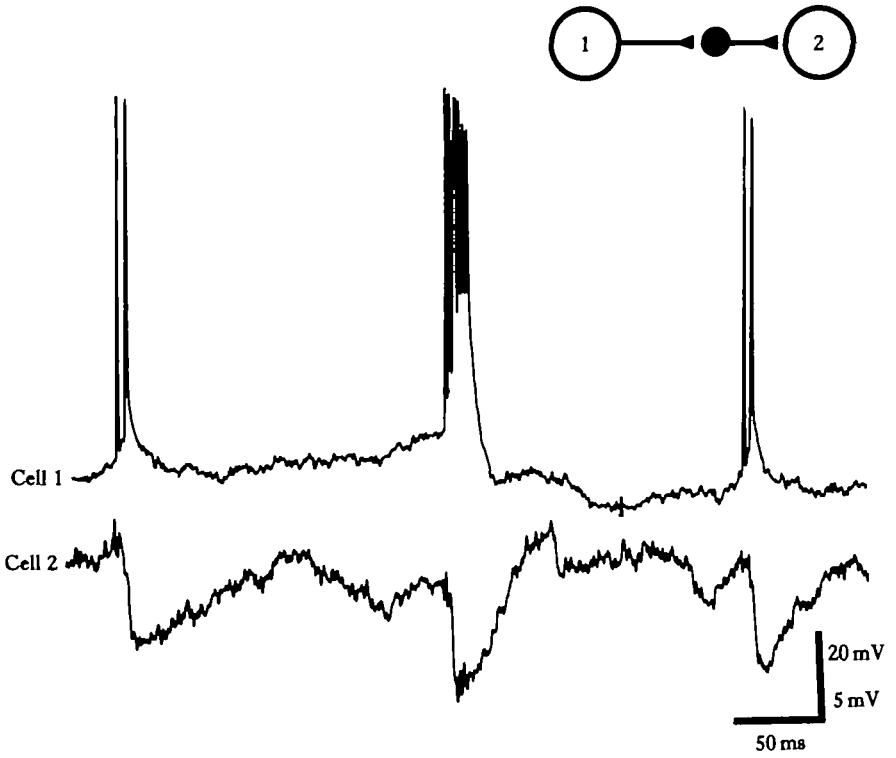


Fig. 7. Recurrent IPSPs may be initiated by activity in a single pyramidal cell. Action potentials in cell 1 elicited IPSPs in cell 2. An intercalated inhibitory neurone is assumed since inhibitory events did not follow each action potential and varied considerably in latency.

spontaneous activity of pyramidal cells is less clear. Our recent studies suggest that the influence of a pyramidal cell on inhibitory neurones in the CA3 region may be so powerful that a single pyramidal cell can activate an inhibitory neurone (Fig. 7). Inhibitory neurones appear to possess widely arborizing axonal collaterals. Thus once activated they will exert an influence over a large number of pyramidal cells lowering their excitability and tending to prevent the spread of activity through the recurrent network.

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