Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons

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SYNCHRONIZATION of neuronal activity is fundamental in the operation of cortical networks[1]. With respect to an ongoing synchronized oscillation, the precise timing of action potentials is an attractive candidate mechanism for information coding[2–5]. Networks of inhibitory interneurons have been proposed to have a role in entraining cortical, synchronized 40-Hz activity[6,7]. Here we demonstrate that individual GABAergic interneurons[8] can effectively phase spontaneous firing and subthreshold oscillations in hippocampal pyramidal cells at θ frequencies (4–7 Hz). The efficiency of this entrainment is due to interaction of $GABA_A$-receptor-mediated hyperpolarizing synaptic events with intrinsic oscillatory mechanisms tuned to this frequency range in pyramidal cells. Moreover, this GABAergic mechanism is sufficient to synchronize the firing of pyramidal cells. Thus, owing to the divergence of each GABAergic interneuron[9,10], more than a thousand pyramidal cells may share a common temporal reference established by an individual interneuron.

Cortical oscillatory activity at the electroencephalogram (EEG) level[11] has been correlated to distinct patterns of behaviour. One form of rhythmic activity, θ oscillations (4–7 Hz), are prominent in the hippocampal EEG during exploratory behaviour[12] and have been proposed to serve as a reference for coding by 'place cells'[3]. At the cellular level, intrinsic membrane conductances support θ-frequency membrane-potential oscillations in individual CA1 pyramidal cells[13]. Moreover, intracellular recordings from pyramidal cells during θ activity have revealed rhythmic chloride-mediated conductances that originate close to the cell body[14,15], suggesting that perisomatic $GABA_A$-receptor-mediated synaptic events contribute during intracellular θ oscillatory activity. We have tested, in the absence of patterned excitatory input, whether rhythmic activation of individual GABAergic interneurons, which selectively innervate the perisomatic region of pyramidal cells, can synchronize θ-frequency oscillations in CA1 pyramidal cells in hippocampal slices.

Anatomically identified GABAergic interneurons were either basket cells ($n=8$; Fig. 1*A*) making synapses mainly on somata ($53 \pm 15\%$) and proximal dendrites ($44 \pm 15\%$), or axo-axonic cells ($n=2$) terminating on axon initial segments of pyramidal cells (100%). Both basket and axo-axonic cells elicited $GABA_A$-receptor-mediated inhibitory postsynaptic potentials (i.p.s.ps)[8] in simultaneously recorded pyramidal cells ($n=17$). Rhythmic activation of presynaptic basket and axo-axonic interneurons at 1–8 Hz instantly phase-locked firing in simultaneously recorded postsynaptic pyramidal cells ($n=7$ of 7 cells tested; Fig. 1*B*). By entraining the pyramidal cell, the interneuron could either decrease or increase the firing rate of the pyramidal cell. During entrainment, interneurons and pyramidal cells fired at alternate phases, as occurs during θ activity *in vivo*[16]. Subthreshold membrane-potential oscillations were also entrained by rhythmic activation of single basket and axo-axonic cells ($n=5$ of 5; Fig. 1*C*), indicating that interneurons can entrain pyramidal cell activities both at sub- and suprathreshold levels.

To elucidate the mechanism underlying inhibitory phasing of pyramidal neurons, unitary i.p.s.ps were evoked at different membrane potentials. At depolarized membrane potentials at which firing occurred sporadically in the pyramidal cell, single spikes or short trains of action potentials in the inhibitory interneuron produced a hyperpolarizing i.p.s.p. in the pyramidal neuron followed by 'rebound' action potentials[8,17] ($n=10$ of 13; Fig. 2*A*, *a*). These action potentials preferentially occurred in a time window corresponding to the depolarizing overshoot following i.p.s.ps at subthreshold membrane potentials (Fig. 2*A*, *b*). Brief hyperpolarizing current pulses mimicking i.p.s.ps delivered by the somatic recording electrode produced a similar depolarizing overshoot ($n=8$; Fig. 2*B*), indicating that the rebound was due to the i.p.s.p.-associated hyperpolarization interacting with

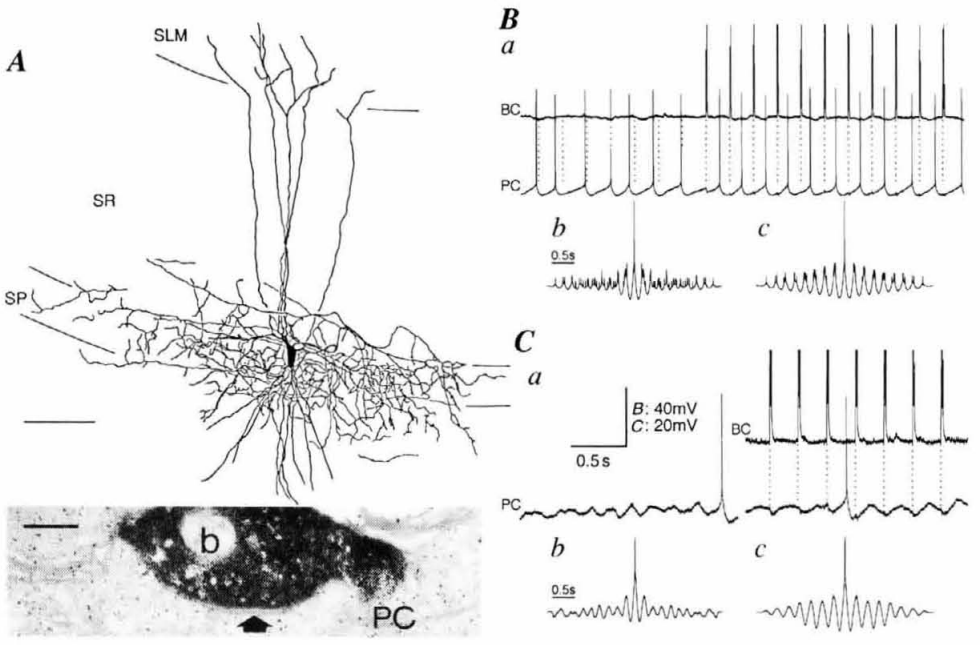

FIG. 1 Phase-locking of pyramidal cell discharge by a single presynaptic basket cell. *A*, Light-microscope-based reconstruction of a hippocampal basket cell (BC) with extensive axon mainly restricted to the cell body layer of area CA1 where it forms type II (symmetrical) synaptic contacts with somata (64%) and proximal dendrites (36%) of pyramidal cells. SP, stratum pyramidale; SR, stratum radiatum; SLM, stratum lacunosum-moleculare. Scale bar, 100 μm. Bottom: electron micrograph of a symmetrical synapse (arrow) between a terminal (b) of the labelled basket cell and an unlabelled pyramidal cell (PC) soma. Scale bar, 0.2 μm. Action potentials evoked by brief depolarizing current pulses to this basket cell produced fast i.p.s.ps in a simultaneously recorded pyramidal cell (not shown). The reversal potential was −69 mV and the average i.p.s.p. conductance was calculated to be 1.6 nS. The mean estimated reversal potential for all basket-cell-mediated i.p.s.ps was −75.5 ± 7.6 mV ($n = 7$ cell pairs), and the mean conductance was estimated to be 1.5 ± 0.7 nS ($n = 6$), as described[28]. The i.p.s.p. was blocked by bath application of 2–10 μM bicuculline ($n = 2$). *B*, *a*, Firing of a pyramidal cell (lower trace), depolarized by constant current injection, becomes instantly entrained by periodic activation (4.7 Hz) of short trains of 3–4 action potentials in the presynaptic basket cell (upper trace, action potentials truncated). Dotted lines indicate intervals of 0.21 s. In all 5 basket and 2 axo-axonic cell to pyramidal cell connections tested for entrainment, the postsynaptic pyramidal cell became instantly phase-locked to the interneuron activity, firing at opposite phase to the interneuron. By entraining the postsynaptic pyramidal cell, the interneurons could decrease or increase the mean discharge rate of the pyramidal cell. Autocorrelation of the trace before (*b*) and during (*c*) regular basket cell activity shows an increased rhythmicity of pyramidal cell firing during periodic activation of the interneuron. *C*, *a*, Effect of periodic rhythmic discharge of a single basket cell on sub-threshold membrane potential oscillations in a pyramidal cell. Dotted lines indicate intervals of 0.26 s. Autocorrelograms of the trace before (*b*) and following (*c*) entrainment show an increased rhythmicity during periodic activation of the interneuron.

METHODS. Dual intracellular recordings were made from synaptically coupled CA1 interneuron to pyramidal cell pairs from 400-μm-thick transverse adult rat hippocampal slices as described[8]. Slices were maintained at the interface between artificial cerebrospinal fluid containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 2 $MgSO_4$, 2 $CaCl_2$, 10 glucose, pH 7.2, and a humidified atmosphere of 95% O_2 and 5% CO_2, at 34–35 °C. Sharp glass microelectrodes contained 2% biocytin in 1.5 M potassium methylsulphate and were bevelled to a d.c. resistance of 60–150 MΩ. Recovered biocytin-labelled cells were analysed in the light microscope and target selectivity was determined by electron microscopy for a total of 94 basket cell and 22 axo-axonic cell synaptic contacts, ranging from 6 to 24 contacts per cell. All values are expressed as means ± s.d. The number of synaptically coupled pairs exceeds the number of interneurons because the latter could serve as the presynaptic partner for more than one recorded pyramidal cell.

intrinsic membrane conductances. Several voltage-dependent conductances have been reported to be involved in θ-frequency subthreshold membrane-potential oscillations in CA1 pyramidal cells[13], as well as other cortical neurons[18,19]. As these membrane-potential oscillations and the rebound depolarization occur in a similar voltage range, we investigated whether the entrainment observed here might be due to interaction with intrinsic oscillatory mechanisms. In pyramidal cells depolarized to fire action potentials, unitary perisomatic i.p.s.ps were repeatedly evoked. The synaptic response suppressed, and thereafter facilitated action-potential discharge in the pyramidal cell, as predicted[6]. Subsequent multiple clusters of action potentials following a single i.p.s.p. indicate that the hyperpolarizing response reset an intrinsic rhythmic state rather than simply delaying the onset of action-potential generation ($n = 10$ of 13; Fig. 2*C*). That this action of the i.p.s.ps was indeed due to resetting the phase of an intrinsic oscillatory state is apparent from the ability of i.p.s.ps to either reset or initiate subthreshold oscillations (Fig. 2*D*).

That single basket and axo-axonic cells can entrain pyramidal cell activity both at sub- and suprathreshold membrane potentials, suggests that synchronization of principal cell activity may be a fundamental role for these interneurons. We therefore tested, in the presence of blockers of excitatory amino-acid receptors, whether minimal stimulation of GABAergic interneurons could synchronize the firing of two spontaneously active pyramidal cells. Indeed, one single i.p.s.p. reset regular firing in both pyramidal cells ($n = 5$; Fig. 3*A*), whereas repeated minimal stimulation at θ-frequencies phase-locked both neurons (Fig. 3*B*). Thus, GABAergic interneurons can effectively synchronize pyramidal cell activities. The exact contribution of different subtypes of interneuron remains to be established as they may be differentially active during different behavioural states. Anatomically identified basket cells have been reported to fire phase-related to extracellularly recorded θ oscillations[16], whereas no such data are yet available for axo-axonic cells. As suggested by rhythmically occurring i.p.s.ps in basket cells during θ activity[16], the basket cells themselves may be phased by GABAergic inputs from the medial septum[20] or from other interneurons in the hippocampal network. In addition, excitation through recurrent axon collaterals from the pyramidal cells[8,21] would strengthen rhythmic basket cell discharges, suggesting a role for such a feedback loop[22] in synchronization of cortical pyramidal cells[23].

Our results suggest that post-inhibitory 'rebound' activation of cortical principal cells must be considered as a fundamental facet in the functional repertoire of GABAergic interneurons acting on $GABA_A$ receptors[24]. The interaction of the synaptic events with intrinsic conductances provides a powerful mechanism, such that one single interneuron is sufficient to synchronize a large neuronal population. Given the ubiquitous nature of perisomatic GABAergic innervation throughout cortical structures[25], GABAergic phasing may represent a general mechanism for synchronization of cortical activity[6]. Indeed, intracellular recordings *in vivo* show that, at several distinct frequencies, rhythmic oscillatory activity is associated with periodic

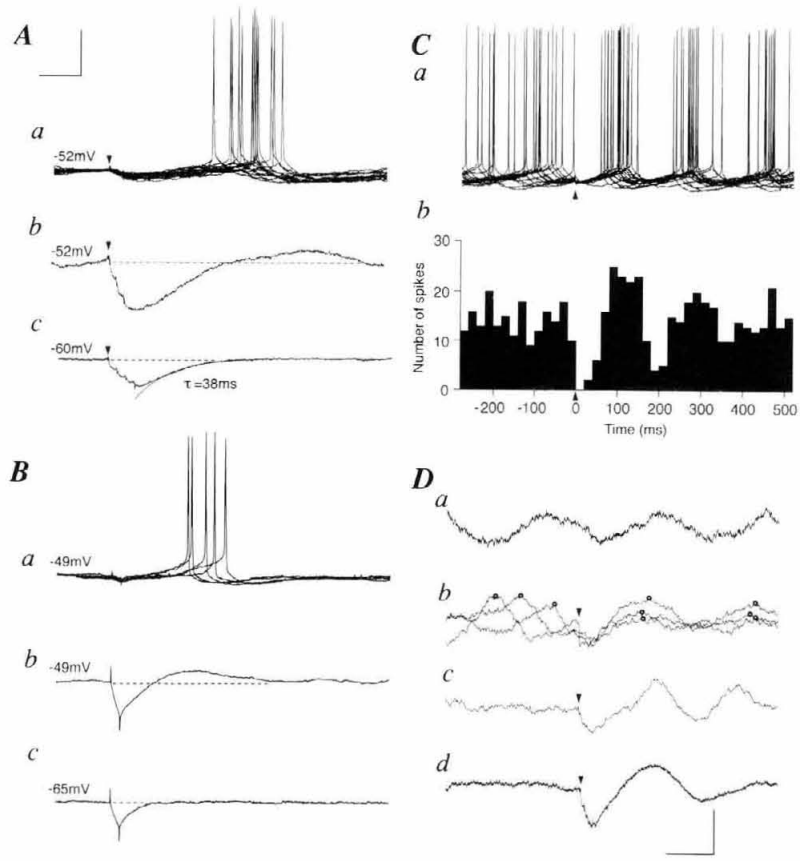

FIG. 2 Mechanism underlying phasing of pyramidal cells. *A*, Effect of a short train of 3–4 action potentials (position indicated by filled arrow) elicited in the presynaptic basket cell on a pyramidal cell at varying membrane potentials. Scale (vertical) represents 20 mV in panels *a* of *A*, *B* and *C* and 2 mV in panels *b*, *c* of *A* and *B*; horizontal, 50 ms in *A*, *B* and 100 ms in *C*. *a*, Superimposition of 12 consecutive sweeps in which the i.p.s.p. was followed by an action potential. *b*, Average of 23 sweeps showing subthreshold rebound depolarization following the i.p.s.p. Note that the action potentials shown in *a* cluster in a region temporally coinciding with the rise of the depolarizing overshoot. *c*, Postsynaptic response evoked at a more hyperpolarized membrane potential showing a monoexponential decay and no depolarizing overshoot. *B*, Effect of brief hyperpolarizing current pulses in a pyramidal cell. *a*, Superimposition of 5 single sweeps in which a small hyperpolarizing current pulse (0.05 nA) elicited a rebound action potential. *b*, Average of 333 sweeps showing subthreshold rebound depolarization following a short hyperpolarizing current pulse. *c*, Identical current pulses at more hyperpolarized membrane potentials failed to produce a depolarizing overshoot. *C*, Effect of unitary i.p.s.ps on the firing of the postsynaptic pyramidal cell. *a*, 19 consecutive sweeps aligned on the rising phase of single action potentials (trigger point indicated by filled triangle) evoked at 0.75 Hz in the presynaptic basket cell. *b*, Corresponding spike histogram for a total of 132 sweeps with 572 spikes. Note the regular occurrence of peaks in the histogram following the i.p.s.p. Bin width, 20 ms. *D*, Effect of unitary i.p.s.p. on subthreshold membrane potential oscillations of a postsynaptic pyramidal cell. *a*, Single sweep showing subthreshold membrane potential oscillations of the pyramidal cell. *b*, Three superimposed sweeps demonstrating the effect of single unitary i.p.s.ps (triangle) on subthreshold oscillations in the pyramidal cell. The positive peak of each cycle has been marked with an open circle. At whichever phase or membrane potential the i.p.s.p. occurred, the i.p.s.p. effectively reset the oscillation. *c*, Single sweep demonstrating that a unitary i.p.s.p. can initiate oscillations in the pyramidal cell when no obvious oscillatory activity was present before the i.p.s.p. *d*, Average of 15 sweeps without action potentials. Subthreshold oscillatory activity occurring at random phases resulted in a flat pre-event baseline. Following the i.p.s.p., a characteristic waveform, consistent with the i.p.s.p resetting an intrinsic oscillatory state. Scale in *D*: (vertical) *a–c*, 4 mV and *d*, 2 mV; horizontal is 100 ms for *a–d*.

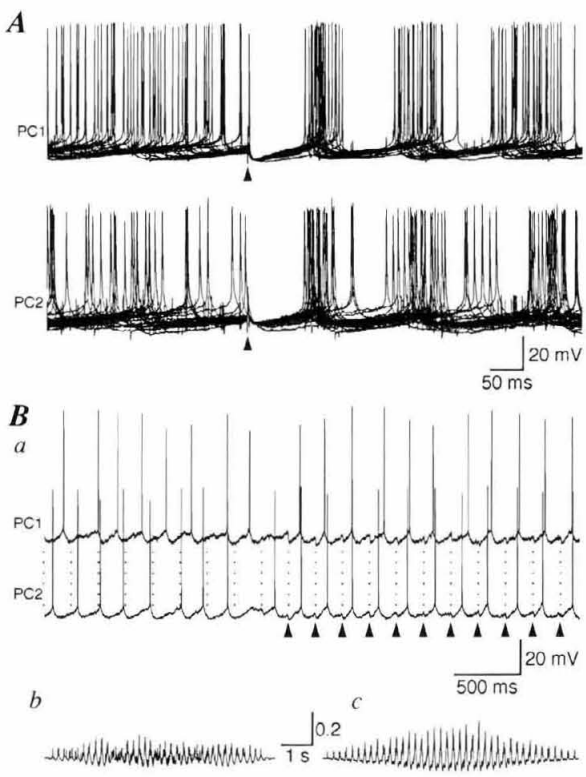

FIG. 3 Synchronization of pyramidal cell (PC) firing in the presence of ionotropic glutamate-receptor antagonists. *A*, Two simultaneously recorded pyramidal neurons were depolarized to elicit action potentials during which single i.p.s.ps (triangles), evoked at 0.2–0.5 Hz by minimal stimulation, reset the regular firing of both cells (30 consecutive sweeps; $n = 5$). The stimulation strength was adjusted to evoke an i.p.s.p. of amplitude equivalent (<3 mV) to that produced by an individual, intracellularly recorded interneuron. In addition, rebound depolarization as in Fig. 2*A*, *b* could be evoked in both pyramidal cells (data not shown). *B*, *a*, Rhythmic i.p.s.ps evoked by minimal stimulation at 5 Hz (triangles) synchronize the firing of two simultaneously recorded pyramidal neurons. Dotted lines indicate intervals of 0.2 s. *b*, Cross-correlogram for the two neurons in a 5-s period before rhythmic minimal stimulation. *c*, Corresponding cross-correlogram for 5-s period following the start of rhythmic minimal stimulation. Note more pronounced cross-correlation during entrainment.

METHODS. Dual intracellular recordings were made from unconnected pairs of pyramidal cells separated by ~100 μm. Minimal stimulation with a monopolar sharpened tungsten electrode (100 μs, 3–8 V) was made in the pyramidal cell layer at a distance of ~100 μm lateral from one of the recording electrodes in the presence of 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione and 30 μM DL-2-amino-5-phosphonopentanoic acid.

i.p.s.ps, both in the hippocampus[15,16,26] and the neocortex[27]. We therefore suggest that a subset of cortical interneurons serve a key role in synchronizing principal cell activity, thus providing the temporal reference relative to which specific information may be coded. □

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