Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation

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PARTIALLY synchronous 40-Hz oscillations of cortical neurons have been implicated in cognitive function. Specifically, coherence of these oscillations between different parts of the cortex may provide conjunctive properties[1,2] to solve the 'binding problem': associating features detected by the cortex into unified perceived objects. Here we report an emergent 40-Hz oscillation in networks of inhibitory neurons connected by synapses using $GABA_A$ (γ-aminobutyric acid) receptors in slices of rat hippocampus and neocortex. These network inhibitory postsynaptic potential oscillations occur in response to the activation of metabotropic glutamate receptors. The oscillations can entrain pyramidal cell discharges. The oscillation frequency is determined both by the net excitation of interneurons and by the kinetics of the inhibitory postsynaptic potentials between them. We propose that interneuron network oscillations, in conjunction with intrinsic membrane resonances and long-loop (such as thalamocortical) interactions, contribute to 40-Hz rhythms *in vivo*.

Oscillations at ~40 Hz occur in CA1 pyramidal cells in hippocampal slices following the excitatory response to afferent stimulation applied 2 s after a conditioning train (which activates metabotropic glutamate receptors[3] and erodes slow inhibition[4]; Fig. 1*a*). The oscillation frequency is independent of membrane potential in the impaled pyramidal cell over the range −40 to −90 mV (data not shown). Application of the $GABA_B$-receptor antagonist 2-hydroxysaclofen greatly prolongs the oscillations (Fig. 1*b*), which have power spectra peaking at ~40 Hz (Fig. 1*d*). Although the components of the oscillation appear to be depolarizing, they are fast inhibitory postsynaptic potentials (i.p.s.ps), reversed by the effects of the conditioning train[5]: they are blocked by the $GABA_A$-receptor antagonist bicuculline (Fig. 1*c*), and they survive the block of ionotropic glutamate receptors and $GABA_B$ receptors, a treatment that isolates fast i.p.s.ps[4,6] (Fig. 2). Power spectra of responses evoked in CA1 by glutamate application under these conditions reveal a strong peak close to 40 Hz and a small peak at double that frequency (Fig. 2*b*). Rhythmic i.p.s.ps occur synchronously in pairs of pyramidal cells separated by ~1 mm (Fig. 2*a*), with cross-correlations revealing strong interactions with phase lags of 1.0–3.5 ms (Fig. 2*c*). When pyramidal cells are held close to threshold by current injection, the i.p.s.p. oscillation elicited by glutamate modulates their discharge into a collective 40-Hz rhythm synchronized between cells ~1 mm apart (Fig. 2*e*).

The 40-Hz oscillations are a collective behaviour of the network of interneurons in the hippocampus. First, the inhibitory postsynaptic currents (i.p.s.cs) are large, up to 10 times those expected for the activity of a single interneuron[7]. Second, individual components often have a complex waveform (Fig. 2*d*). Third, they are synchronized between pyramidal cells separated by 1 mm (Fig. 2*a*). Fourth, they are evoked by stimuli that do not elicit monosynaptic i.p.s.cs (see Fig. 4*C*).

The necessary features for the inhibitory network oscillation were examined using computer simulations of a network of 128 realistic hippocampal interneurons (Fig. 3*a*). The simulated network oscillates at ~40 Hz as long as the interneurons are interconnected by $GABA_A$-ergic synapses and receive tonic excitation, which is provided in the experiments by glutamate or the metabotropic glutamate receptor agonist (1*S*, 3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S, 3R)ACPD). Gap junctions[8] are not required, but, if present, enhance synchronization. In contrast, gap junctions appear central to a model of one form of synchronization induced by 4-aminopyridine in pharmacologically isolated hippocampal interneurons[9] (simulations: R.D.T., unpublished).

The key role of mutual inhibition in determining the frequency of the 40-Hz i.p.s.c. oscillation is shown by the effect of modify-

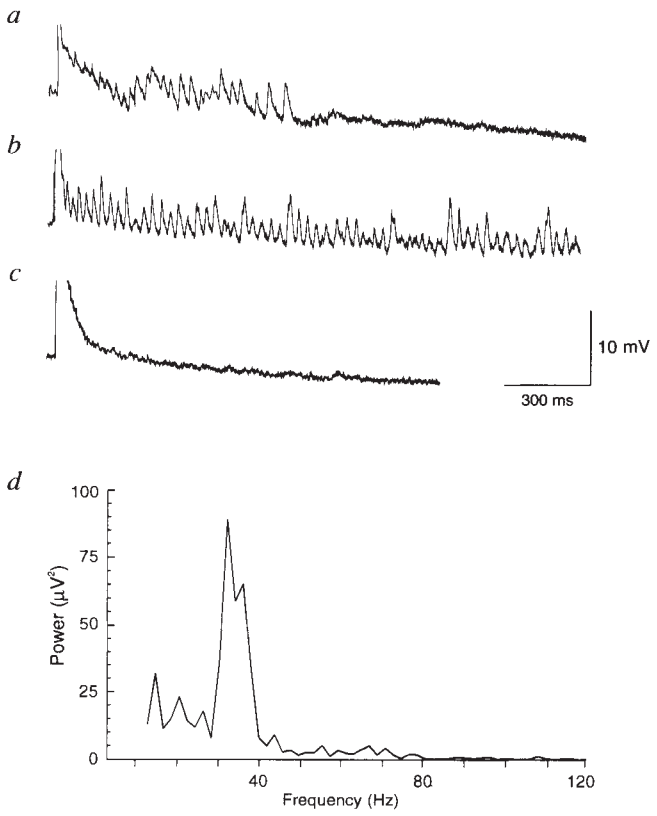

FIG. 1 40-Hz oscillations in intracellular recordings from CA1 pyramidal cells, evoked by afferent stimulation 2 s after a weak conditioning train in the absence of any drugs (*a*), are prolonged by the presence of the $GABA_B$ antagonist 3-amino-2-(4-chlorophenyl)-2-hydroxypropylsulphonic acid (2-OH-saclofen) (*b*), and abolished by the $GABA_A$ antagonist bicuculline (*c*). The response to afferent stimulation without the conditioning train is an e.p.s.p. as seen in *c*, followed by fast and slow i.p.s.ps. The power spectrum of the last 1.8 s of the response in *b* reveals a sharp peak at 33.4 Hz (*d*).
METHODS. Intracellular recordings from CA1 pyramidal cells from 400-μm-thick slices of dorsal hippocampus maintained at 36 °C and perfused with artificial cerebrospinal fluid (ACSF, in mM: NaCl, 135; $NaHCO_3$, 16; $CaCl_2$, 2; KCl, 3; NaH_2PO_4, 1.25; $MgCl_2$, 1; glucose, 10) under warm, wet, 95% O_2, 5% CO_2. In *b*, ACSF also contained 0.2 mM 2-OH-saclofen and in *c*, 10 μM bicuculline methiodide. The conditioning train consisted of stimuli at half the strength needed to evoke an action potential, repeated at 100 Hz for 1 s. Sharp glass micropipettes were filled with 2 M potassium methylsulphate (resistance 30–60 MΩ). Cross-correlation and power spectrum analyses used SPIKE2 (Cambridge Electronic Design, UK).

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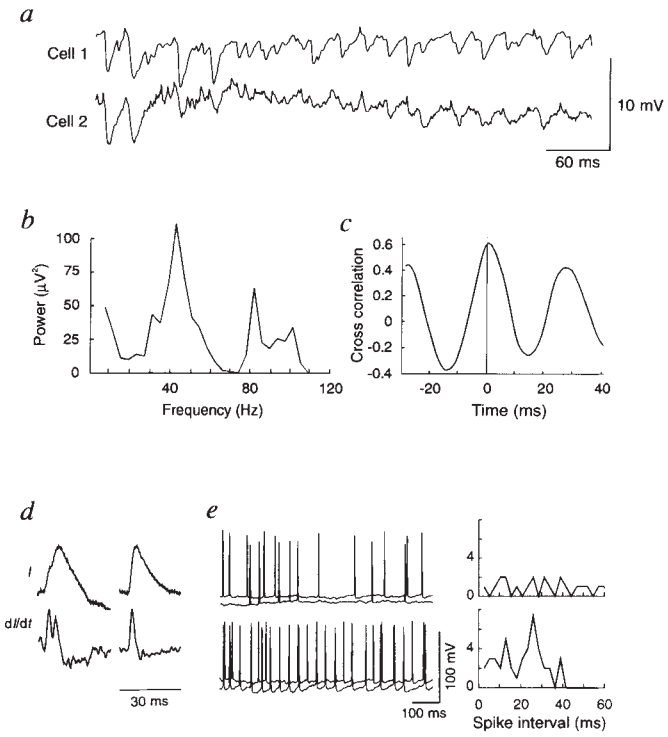
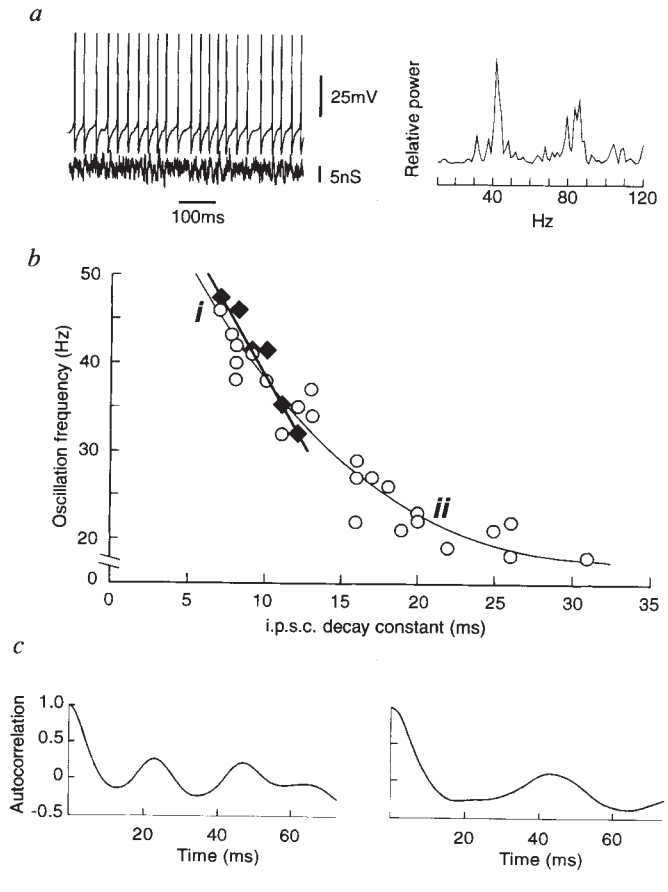


FIG. 2 40-Hz oscillations in CA1 pyramidal cells with blockade of ionotropic excitatory postsynaptic potentials (e.p.s.ps) and slow i.p.s.ps. *a*, A pair of pyramidal cells, 1 mm apart, were depolarized to −40 mV by current injection in bridge mode. Pressure ejection of glutamate into stratum pyramidale generated trains of i.p.s.ps synchronously in the two cells. *b*, Power spectrum of a train of i.p.s.ps in a single pyramidal cell indicating a strong peak at 42 Hz along with a harmonic at 82 Hz. *c*, Cross-correlation of the trains of i.p.s.ps in *a* revealed a strong correlation between the cells, with cell 2 lagging by 1.5 ms, and a 37-Hz rhythm (27-ms period). *d*, Voltage-clamp records of individual i.p.s.cs in a 40-Hz train as in *a* often had complex rise and decay kinetics, markedly different from the simple kinetics more typical of unitary i.p.s.cs. *e*, top, Current injection (0.6 nA) into a pair of pyramidal cells (resting potentials −67 and −69 mV) elicited action potentials independently in each cell, and accommodation in both (not shown). Frequency distribution of interspike intervals for the combined cells was flat. Bottom, Current injection concurrently with pressure ejection of glutamate produced: less accommodation, partial synchrony of the action potentials and combined output from the 2 cells having a modal interspike interval of 26 ms (38 Hz) during the plateau phase of depolarization.

METHODS. Intracellular recordings from CA1 pyramidal cells from 400-μm-thick slices in the presence of drugs to block AMPA and NMDA receptor-mediated e.p.s.ps and slow i.p.s.ps (20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 100 μM D(−)-2-amino-phosphonovaleric acid (D-APV) or 50 μM ketamine, and 2-OH-saclofen. Glutamate, 100 μM, was applied by pressure ejection through a blunt micropipette in stratum pyramidale (0.6 kg cm^{-2}, 100 ms). Recordings of trains of i.p.s.ps at −40 mV were done with electrodes containing an additional 50 mM QX314. *b*, *c*, *e*, Cross-correlation and power spectrum analyses used SPIKE2 (Cambridge Electrode Design, UK). The i.p.s.cs were recorded using switched single-electrode voltage clamp (Axoclamp).

FIG. 3 Frequency of inhibitory neuronal network oscillations is a function of i.p.s.c. kinetics in individual inhibitory interneurons. *a*, Simulation of a network of 128 interneurons interconnected by $GABA_A$ synapses. Left, Somatic potential (spikes truncated) and total synaptic conductance to the same neuron (a measure of population activity). Right, Power spectrum of conductance signal of the same neuron. Note peaks at ~40 and 80 Hz. *b*, Responses were recorded from electrophysiologically identified inhibitory neurons evoked by glutamate application (Fig. 2) during the wash-in of 2 μM pentobarbital. These reveal the relationship of the principal oscillation frequency, from autocorrelation analyses, versus the decay constant (τ_D) of individual i.p.s.cs within a train (derived from single exponential curve fit; ○). Note the close match with the relationship between spectral peak and τ_{GABA} predicted by computer simulation (thick line, ◆). The nonlinearity of the experimental data at large τ_D is a function of the dual effects of pentobarbital in increasing both τ_{GABA} and i.p.s.c. amplitude. *c*, left, Autocorrelations of i.p.s.c. trains in control solution reveal a period of 22.7 ms (44 Hz); τ_D was 9.1 ± 0.4 ms (n = 20 epochs before pentobarbital; *b*: i). Right, During equilibration with pentobarbital the period slowed to 44.5 ms (22 Hz) at ii in *b*; τ_D reached >30 ms (n = 30 epochs).

METHODS. Simulations: individual neurons: each model neuron has 46 somadendritic (SD) and 5 axon initial segment compartments, with SD τ_m = 37.5 ms and somatic R_{input} = 96.5 MΩ. In response to tonic depolarizing current injection, the cells generate repetitive trains of action potentials, with rapid after hyperpolarizations (AHPs) and little adaptation. The proximal and middle dendrites contain voltage-dependent sodium conductance in order to replicate the data of ref. 28 that a single dendritic release site can evoke a short-latency action potential (R.D.T. and R. Miles, manuscript in preparation). Network interactions: we simulated 128 neurons interconnected by '$GABA_A$' synapses, with 20 connections per neuron, and no other types of interaction (such as $GABA_B$, electrical synapses). Synapses are located on 7 different proximal dendritic compartments, with one release site per connection, and reversal potential of −15 mV from resting potential. Unitary conductance time course consists of a sudden rise to 2.0 nS, followed by exponential decay with τ_{GABA}, a parameter from 6 to 12 ms. The cells receive a tonic somatic bias current of 0.225 to 0.255 nA, different for each cell. Simulations were done on an IBM SP1 parallel computer using 16 nodes. A 1,000-ms simulation took about 85 min. Experimental: inhibitory neurons impaled in stratum oriens of CA1 were identified by their brief action potential (<3 ms at base) and lack of accommodation. The decay phase of i.p.s.cs clearly separated from their neighbours



were fitted by a single exponential (EPC package, Cambridge Electronic Design Ltd); control τ_D was in the range 8–15 ms, faster than that for pyramidal cells (22.4 ± 0.8 ms). After equilibration with 2 μM pentobarbital the decay of i.p.s.cs was too slow for reliable measurements.

ing i.p.s.c. duration. Computer simulations of an inhibitory network in which i.p.s.c. decay time course varies from 6 to 12 ms predict a close relationship with frequency (Fig. 3*b*, thick line). This is confirmed by recordings of the response of interneurons to glutamate during the wash-in of 2 μM pentobarbital (Fig. 3*b*, thin curve). The change in slope at decay constants >12 ms probably reflects the effect of higher doses of pentobarbital on i.p.s.c. amplitude, which is not included in the simulation.

Single electric stimuli evoke i.p.s.cs oscillating at 40 Hz when ionotropic glutamate receptors and $GABA_B$ receptors are blocked (Fig. 4*A*). Such stimuli were effective whether delivered close to the recording, or whether far enough away not to produce a monosynaptic i.p.s.c. (Fig. 4*B*, *C*). Tetrodotoxin (TTX) abolishes the oscillation, leaving either a monosynaptic i.p.s.c. or no response[6].

Drugs in the bath blocked ionotropic glutamate receptors, so that metabotropic glutamate receptors are candidates to drive the oscillation of the inhibitory network. The metabotropic receptor antagonist (+)-α-methylcarboxyphenylglycine (MCPG) blocks i.p.s.c. oscillations evoked electrically, but has no effect on monosynaptic i.p.s.cs (Fig. 4*D*). The agonist, (1*S*, 3*R*)-ACPD, elicits 40-Hz i.p.s.c. oscillations in CA1 pyramidal cells very similar to those evoked electrically, which are also blocked by MCPG (Fig. 4*E*, *F*). (Oscillating i.p.s.ps in response to metabotropic agonists have been described before, but attributed to rhythmic activity in single interneurons[3].) Tetanic stimulation of the kind required for long-term potentiation (LTP) provides the necessary conditions for these 40-Hz oscillations[10].

Application of (1*S*, 3*R*)-ACPD to neocortical slices results in prolonged i.p.s.c. oscillations at ~40 Hz, which are most prominent in superficial laminae (Fig. 4*G*). They have properties very similar to those studied in detail in the hippocampus and probably reflect a similar mechanism.

Cortical oscillations at 30–50 Hz became a central issue for cortical mechanisms of perception when they were described in discharges of groups of neurons and in local field potentials in cat visual cortex[11,12]. Their synchrony over wide distances, and between hemispheres[13–15], provides a potential solution for the problem of grouping, or 'binding' features detected at widely separate cortical regions into perceived objects. They also provide means for scene segmentation to distinguish which features belong to which objects[16]. In man, 40-Hz reverberating activity occurs between thalamus and cortex[17,18]. Similar frequencies occur in other parts of the brain, including the hippocampus during theta activity[19] and the olfactory system, where they were first described[20,21].

Several theories have been proposed for the cellular mechanism of 40-Hz oscillations[2]. Intrinsic ~40-Hz oscillations occur in sparsely spiny stellate[1] and pyramidal cells[1,22] in neocortex,

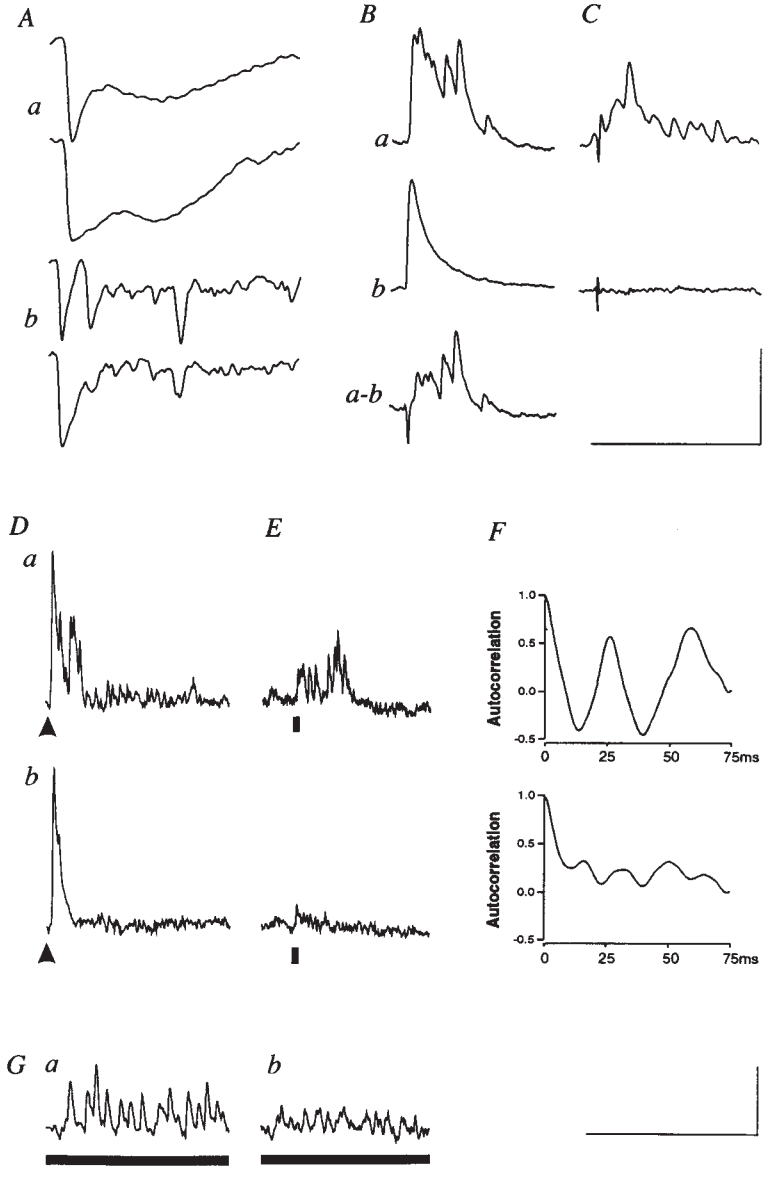


FIG. 4 Activation of metabotropic glutamate receptors in the absence of $GABA_B$-receptor-mediated inhibition is required for sustained interneuronal network oscillations. *Aa*, Simultaneous recordings of biphasic i.p.s.ps in CA1 pyramidal cells evoked by proximal stimulation in the presence of 20 μM CNQX and 50 μM D-APV. Responses were evoked from depolarized membrane potentials of −42 and −40 mV for the two cells and show an initial fast $GABA_A$, followed by a slow $GABA_B$, receptor mediated potential. *Ab*, Simultaneous recordings from the same cells after the addition of 0.2 mM 2-OH-saclofen to block $GABA_B$ receptors reveal stimulus-evoked synchronous trains of i.p.s.ps. *Ba*, Voltage-clamp records of i.p.s.c. trains evoked by proximal stimulation as in *Ab* (holding potential −40 mV). *Bb*, After the addition of 2 μM tetrodotoxin to block action potentials i.p.s.cs evoked in the same cell were monophasic[4], suggesting that the i.p.s.c. train (subtraction *a* − *b*) depended on an intact network. *Ca*, Distal stimulation (CA3c) demonstrating that synaptic excitation from remote sites can elicit i.p.s.c. oscillations in area CA1 without an initial large monosynaptic component. *Cb*, Addition of 2 μM TTX blocked all evoked activity. *D*, Metabotropic glutamate receptors are necessary for i.p.s.c. oscillations. *Da*, Proximal stimulation of stratum pyramidale (▲) generated a maximal i.p.s.c. in a CA1 pyramidal cell followed by oscillations. *Db*, Bath application of 0.2 mM (+)-α-methylcarboxyphenylglycine (MCPG) did not affect the initial i.p.s.c. but almost completely abolished oscillations. *Ea*, i.p.s.c. oscillations lasting 200 ms were elicited by pressure ejection of (1S, 3*R*)-ACPD (100 μM 1.2 kg cm^{-2}, 10 ms) (▮). *Eb*, Bath application of MCPG abolished oscillations induced by (1S, 3*R*)-ACPD. *Fa*, Autocorrelation analyses of responses to pressure ejection of (1S, 3*R*)-ACPD in *E* revealed a period of 27 ms (37 Hz). *Fb*, No coherent oscillations were seen in the presence of 0.2 mM MCPG. G, Pressure ejection of (1S, 3*R*)-ACPD into coronal neocortical slices cut in planes either including the rostral or the caudal extremities of the hippocampus also elicited oscillating i.p.s.cs in the presence of APV, CNQX and 2-OH-saclofen as described above. Those in superficial laminae (*Ga*; layer II, 37 Hz) were more prominent than those in deeper laminae (*Ab*; layers IV/V, 35 Hz). Scale bars *A*, 15 mV, 200 ms; *B*, *C*, 2 nA, 200 ms; *D*, *E*, *G*, 1 nA, 500 ms.

and in thalamocortical projection neurons[23]. Two hypotheses[21,24] were proposed for the population ~40-Hz rhythms in the olfactory cortex; EEG power spectra, and phase relations with unit recordings, supported negative feedback between populations of excitatory and inhibitory neurons over the coupling of intrinsically oscillatory neurons[21,24].

The data presented here suggest a new model. We propose that networks of inhibitory neurons can generate 40-Hz oscillations in pyramidal cells that can entrain their firing[25,26]. Emergence of network oscillations would be assisted by the intrinsic properties of interneurons[1]. Entrainment will be particularly robust if the excitatory cells are themselves intrinsic oscillators[1,22]. Longer-range synchrony could occur through coupling of the inhibitory oscillator by intercortical interconnections or, in the case of the neocortex, by resonating with the thalamocortical loop[23] (but see ref. 2). The inhibitory network oscillator is under synaptic control: it is blocked by slow, $GABA_B$-receptor-mediated i.p.s.ps, and driven by tonic activation of inhibitory neurons, here by metabotropic glutamate receptors, which have potent, excitatory effects on interneurons[3,27]. These synaptic inputs provide the means to determine which sets of interneurons generate synchronized 40-Hz i.p.s.ps *in vivo*, and hence which populations of pyramidal cells will have their activity entrained. □

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The E-selectin-ligand ESL-1 is a variant of a receptor for fibroblast growth factor

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E-SELECTIN is an inducible cell-adhesion molecule on endothelial cells, which mediates the binding of neutrophils and functions as a Ca^{2+}-dependent lectin[1–3]. We have recently identified a 150K glycoprotein as the major ligand for E-selectin on myeloid cells, using a recombinant antibody-like form of mouse E-selectin as an affinity probe[4,5]. Here we report the isolation of a mouse complementary DNA for this E-selectin ligand (ESL-1). The predicted amino-acid sequence of ESL-1 is 94% identical (over 1,078 amino acids) to the recently identified chicken cysteine-rich fibroblast growth-factor receptor[6], except for a unique 70-amino-acid amino-terminal domain of mature ESL-1. Fucosylation of ESL-1 is imperative for affinity isolation with E-selectin–IgG. A fucosylated, recombinant antibody-like form of ESL-1, but not of L-selectin, supports adhesion of E-selectin-transfected Chinese hamster ovary cells. Antibodies against ESL-1 block the binding of mouse myeloid cells to E-selectin. ESL-1, with a structure essentially identical to that of a receptor, thus functions as a cell adhesion ligand of E-selectin.

To obtain sequence information about the 150K E-selectin ligand, we purified the ligand from the mouse neutrophilic progenitor 32Dcl3. Peptides were generated and microsequencing of four of them revealed strong homology to the recently identified chicken cysteine-rich fibroblast growth-factor (FGF) receptor (CFR)[6], with homologies of 83, 92, 100 and 100%. CFR is completely unrelated to any of the other known FGF receptors[7]. A reverse-transcribed polymerase chain reaction (RT-PCR) fragment was used to screen a cDNA library generated from poly(A)$^+$ RNA of 32Dcl3 cells. The cDNA clone with the largest open reading frame covered the sequence depicted in Fig. 1. The complete amino-acid sequence of the mature ESL-1 protein was 94% identical to the chicken CFR except for a 70-amino-acid domain at the N terminus, replacing the first 35 amino acids of the N terminus of CFR. This was confirmed by a second cDNA clone which covered the first 507 base pairs (bp) (coding for the first 167 amino acids) of the depicted sequence and which was isolated from another, specifically primed cDNA library. A signal sequence of 27 amino acids is predicted, leaving a mature polypeptide of 1,148 amino acids. This sequence predicts a transmembrane protein with a relative molecular mass (M_r) of 131K, consisting of a 1,114, amino-acid extracellular domain, a 21-amino-acid transmembrane region and a short, charged 13-amino-acid cytoplasmic tail. The five putative *N*-glycosylation sites and the calculated M_r of the mature, unglycosylated protein backbone correspond well with the determined M_r of the purified E-selectin ligand before (150K) and after (135K) treatment with endoglycosidase F[4,5]. These data suggest that the ligand is only weakly, if at all, modified by *O*-linked carbohydrate side chains.

Chinese hamster ovary (CHO) cells were transfected with the ESL-1 cDNA alone or in combination with a cDNA coding for the human fucosyl transferase III (FT-III)[8,9] and analysed in immunoprecipitations with rabbit antiserum against the purified E-selectin ligand from 32Dcl3 cells. The serum recognized a protein of 150K already in mock-transfected as well as in FT-III-transfected CHO cells (Fig. 2*a*). In ESL-1-transfected, as well as in double-transfected CHO cells, this immunoprecipitation signal was strongly enhanced. As shown in sequential immunoprecipitations (Fig. 2*b*), the endogenously expressed 150K protein of CHO cells did not bind to E-selectin IgG, but became competent to bind to E-selectin if FT-III was expressed in these

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