Synaptic excitation of principal cells in the cat's lateral geniculate nucleus during focal epileptic seizures in the visual cortex

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Abstract. Principal cells of the lateral geniculate nucleus are intensely activated during focal seizures in the visual cortex. The intracellular recordings technique from geniculate cells was used to show that this activity is induced by a strong excitatory synaptic input from discharging cortico-geniculate neurones in layer 6 of the cortex.

Vigorous activation of thalamic neurones during epileptic activity in the cerebral cortex has been observed in many studies (for ref. see Prince 1978, Snead *1995)*. Two mechanisms have been proposed for this activation, synaptic excitation *via* cortico-thalamic fibres or anti-dromic driving of thalamo-cortical cells from an ectopic firing zone in the epileptic cortex (Fig. 1). In this short contribution we intend to show that intracellular recordings from a system with known connectivity may be used for direct differentiation between these possibilities. Most previous studies have concerned the somatosensory and motor parts of thalamus where the cortico-thalamic relations are still poorly understood. Here we present observations from principal cells in the lateral geniculate nucleus (LGN) of the cat which are known to receive monosynaptic excitation from cortico-geniculate pyramidal neurones in layer 6 of the primary visual cortex (Gilbert and Kelly *1975,* Ahlsén et al. 1982; Fig. 1). The layer 6 cells in turn receive monosynaptic excitation from LGN principal cells (Ferster and Lindström 1983) and thus form one limb in a two neuronal excitatory feed-back system between the cortex and
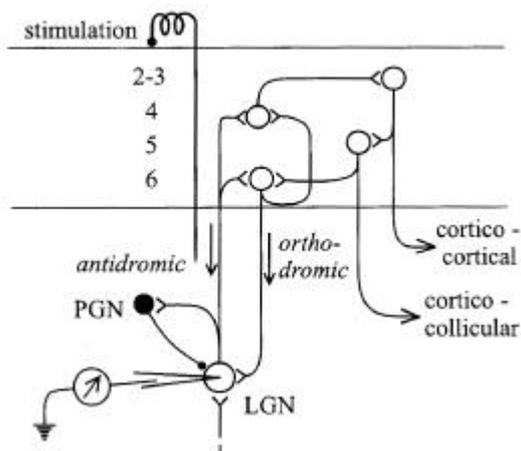

Fig. I. Simplified diagram of excitatory feedback pathways of the visual system and experimental setup. The paper discuss which of the two branches of the thalamo-cortico-thalamic loop (antidromic or orthodromic) is responsible for activation of the geniculate neurons during cortical epileptic discharges. Note that all major cortical efferent systems participate in the intracortical loop. PGN, perigeniculate inhibitory interneurones responsible for prominent IPSP in response to first cortical stimulation (see Fig. 2). Further details in the text.

the LGN. They also project *via* intracortical axon collaterals to layer 4 simple cells, the primary cortical targets of geniculate afferents.

Thereby they close a polyneuronal intracortical excitatory loop (Ferster and Lindström *1985a,b)*. We have previously described that this circuitry is directly involved in focal epileptic seizures of the cortex (Hedström and Lindström 1987).

Such focal epileptic seizures may be initiated by electrical stimulation. Brief trains of cathodal pulses at 10-20 Hz were applied to unipolar stimulation electrodes in the white matter just below the visual cortex (Fig. 1). A nearby surface electrode was used to monitor the induced epileptic discharge (not shown). To have an effect on a particular LGN cell the stimulation electrode had to be in approximate retinotopic register with the recording site. Receptive field mapping was used to align the electrodes. If stimulation and recording electrodes were misaligned by more than a principal cell receptive field center a good focal seizure in the cortex was typically without effect on the LGN cell. This indicated that the seizures evoked in LGN were limited to the region which was interconnected with the cortical locus around the stimulation electrode.

Intracellular recordings of LGN principal cells were obtained from normal adult cats with the brain stem transected at a prepontine level (low cerveau isolé). A brief acting anaesthetic (Saffan, Glaxovet) was used during the surgery. After the brain stem transection the animals remained, without further anaesthesia, in a comatose state with slow wave EEG activity. For recordings they were paralyzed with gallamine triethiodide (Flaxedil, May and Baker) and artificially ventilated to end-expiratory CO_2 of *3.5%*. A pneumothorax was also performed and the animals suspended by clamps on two vertebrae. Body temperature was kept at 38^0C and blood pressure above 110 mm Hg. Glass micropipettes filled with 3 M sodium acetate were used as recording electrodes. The thalamo-cortical principal cells were identified by their location in lamina A and Al of the LGN, by their receptive fields properties and differentiated from intragenicul ate interneurones by antidromie activation from the cortex.

All principal cells in register with an active epileptic focus discharged bursts of spikes in synchrony with the cortical surface response. At threshold intensity the seizures lasted 7 to 20 s and typically were localized within few cortical hypercolumns. The frequency of bursts was quite high (13-24 Hz) during the 'tonic' phase of seizure immediately after the stimulation
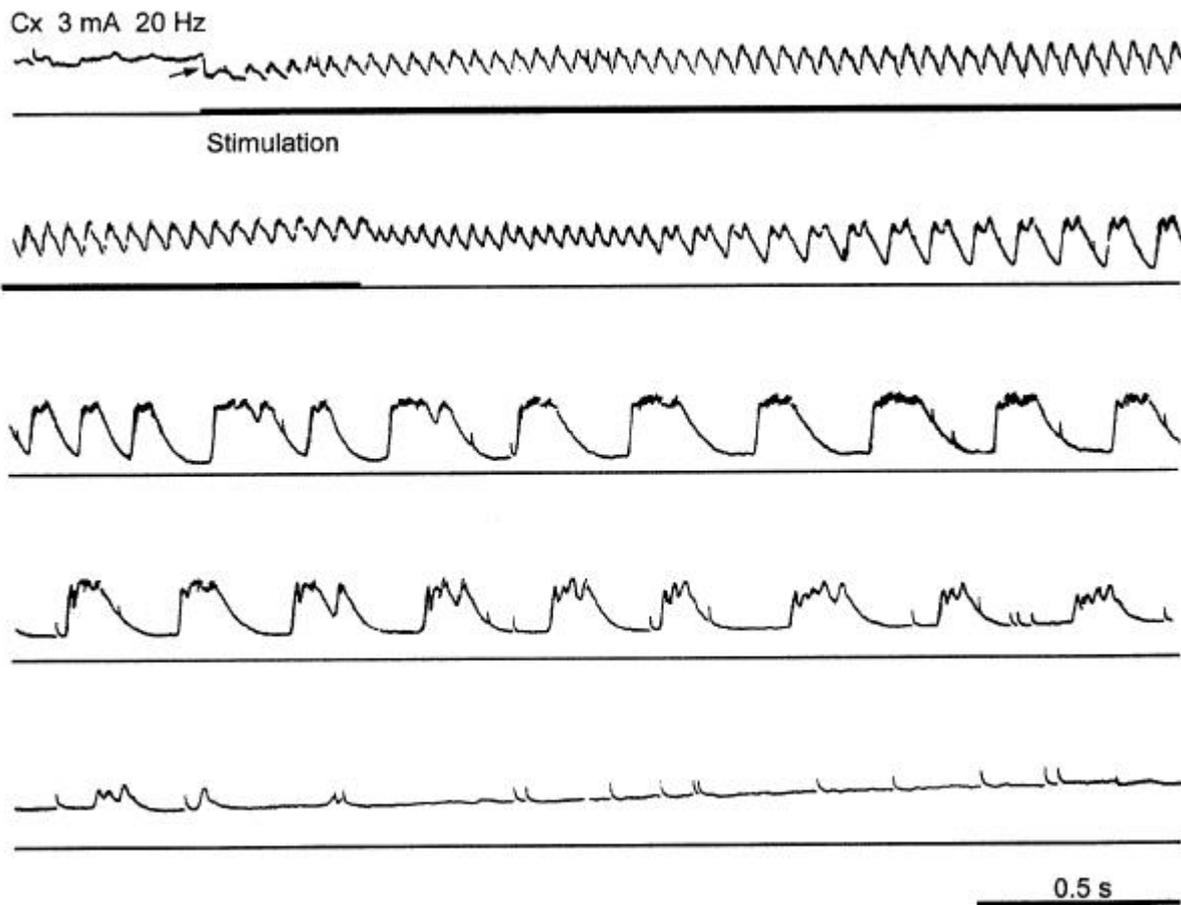
Cx 3 mA 20 Hz

Stimulation

0.5 s

Fig. 2. Intracellular recording of epileptic potentials in a principal cell of the LGN. A focal seizure was induced in the visual cortex by intracortical electrical stimulation at 3 mA, 20 Hz during a period indicated by a thick line. The first stimulus evoked the recurrent IPSP (arrow) *via* synaptic activation of antidromically excited principal cells axons (comp. Fig. 1). The large EPSPs during the early phase of the seizure were truncated by induced spike activity, not resolved by this high gain, low speed recording. Brief, irregularly occurring depolarizations are unitary EPSPs from a spontaneously active retinal ganglion cell. The recorded cell was hyperpolarized by a steady current injection of 1 nA *via* the recording electrode. DC recording with an upwards deflection represents membrane depolarization. PDS, paroxysmal depolarization shifts, in the indicated two examples (i.e., Figs. 2 and 3) are truncated by spike activity.

(Fig. 2). At the end of tonic phase or after weaker stimulation (Fig. 3) this bursts tended to cluster in pairs. Such an activity abruptly reverted to a lower *(3-5* Hz) rate during the following "clonic" phase. This stage was characterized by longer bursts of discharges, often composed of short spike clusters at a frequency similar to that during the tonic phase.

With intracellular recordings it was clear that the spike activity was due to synaptic excitation. A typical response of a principal cell to epileptic activity in the cortex is shown in Fig. 2. The cell was slightly hyperpo larized by current injection

to reveal the underlying synaptic potentials. The seizure was initiated by electrical stimulation and the first stimulus of the 20 Hz train elicited a recurrent IPSP in the principal cell. Later stimuli were followed by a large frequency-potentiating EPSP from cortico-geniculate fibres (Lindström and Wróbel 1990). Similar potentials occurred spontaneously after the stimulation, first individually but later grouped into large PDS (paroxysmal depolarization shifts) like potentials. The parallel recording from the cortex revealed a seizure with a tonic phase having the
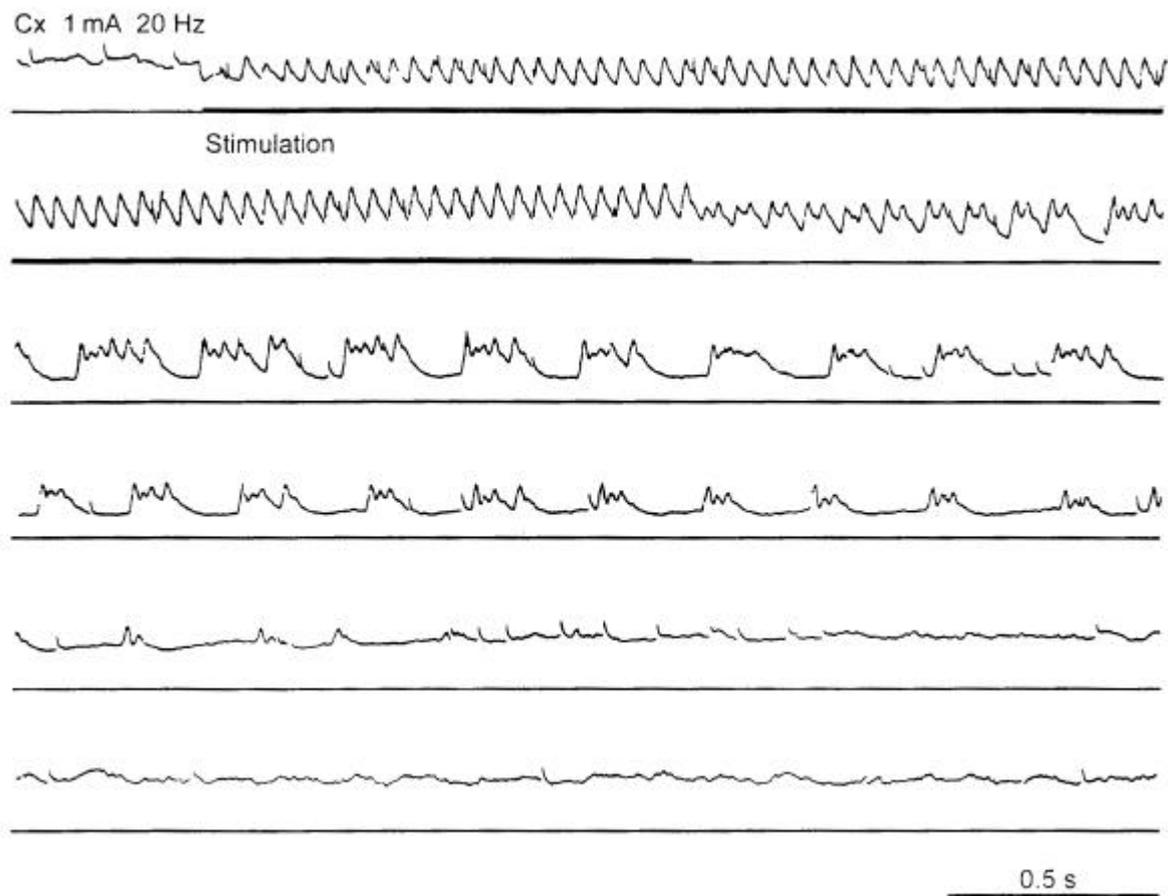
Fig. 3. Enhanced epileptic EPSPs following weaker stimulation (1 mA). Other explanation like in Fig. 1.

same frequency as the early post-stimulus depolarizations and a later clonic phase with the same rhythm as the grouped potentials (Hedström and Lindström 1987).

The rhythmic potentials in the principal cells were clearly prolonged EPSPs evoked by the epileptic burst discharges in a number of layer 6 cortico-geniculate cells converging onto the recorded unit. The compound nature of these potentials was justified by graded amplitude of their decreased responses as can be seen towards the end of the episode in Fig. 3. Weaker stimuli, producing more restricted seizures, gave smaller plateau potentials (Fig. 3) while stronger stimuli, activating a larger part of the cortical network, produced larger potentials (Fig. 2). Even with the cell artificially hyperpolarized they reached the spike threshold level, which explains their flat tops in Fig. 2. The plateau potentials were associated with large conductance increases in the cells and they

changed in amplitude with hyper- or depolarization as classical EPSPs. No action potentials were evoked unless the EPSPs were allowed to cross the spike threshold level. In other words, we did not observe antidromic spikes that could have originated from an ectopic spike generation zone near the cortical terminals of the cells. Thus, ectopic spike generation does not seem to be a prerequisite for focal seizures in the visual cortex.

The EPSP frequency during the tonic phase of the seizure differed from the stimulation frequency, implying that the EPSPs were not directly driven by the stimulus. In fact the overall frequency of the different phases were the same irrespective of the intensity or frequency of the stimulus (cf. Figs. 2 and 3), provided both parameters were suprathreshold. It follows that the different rhythms of the seizure are determined by the intrinsic properties of the active network and that the
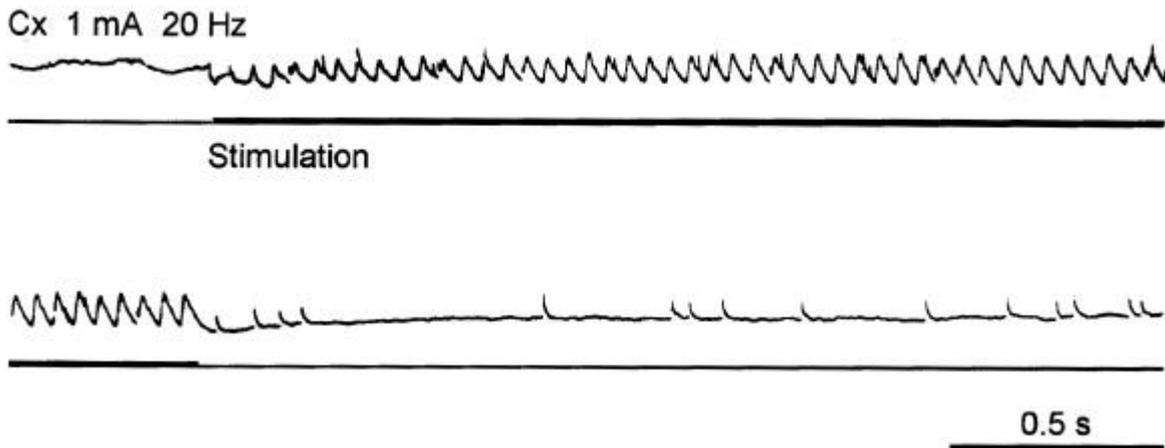
Cx 1 mA 20 Hz

Stimulation

0.5 s

Fig. 4. Lack of epileptic activity following a short stimulation train. Stimulus parameters as in Fig. 2.

stimulation merely serves to unbalance the circuit. Similarly a perturbation of involved inhibitory interneurones or any of the modulatory systems influencing cortico-thalamic circuitry may result in pathological epileptic activity (Snead 1995, Steriade and Contreras *1995)*. It is currently debated whether characteristic frequencies of such oscillatory phenomena may indicate the activation of specific mechanisms used within the brain in order to reach different physiological states such as arousal or attention (Bekisz and Wróbel 1993, Steriade et al. 1993).

By intracellular recordings from principal cells it was easy to monitor the layer 6 cell involvement in seizure generation. No epileptic activity could be induced in normal untreated animals unless the stimulus intensity was suprathreshold for cortico-geniculate fibres. This intensity was often well above thresholds for principal cells as judged by their antidromic activation and the size of their recurrent IPSPs. The stimulation frequency had to be high enough (7-10 Hz) to induce a sizeable frequency potentiation of the cortico-geniculate EPSPs (Lindström and Wróbel *1985;* cf. above).

The duration of the train was also critical. For strong stimuli at 50 Hz a 0.5 s train could be enough while at threshold intensity and 10Hz it was necessary to prolong the train to 6-7 s. A too short train was completely ineffective, it did not induce the slightest trace of a seizure. This is illustrated in Fig. 4 by a similar stimulation sequence as in Fig. 2. The ineffective train was 2.7 s compared to 3.8 s for the effective one. Up to the end of the shorter train the two responses were quite similar with the same amount of frequency potentiation of the cortico-geniculate

EPSPs. So what happened during the re maining 1.2 s of the longer train? There was no remarkable increase in EPSP amplitudes but a close scrutiny of the recording revealed a gradual depolarizing shift of the base line membrane potential or rather the starting point of each evoked EPSP. A similar DC shift occurred at an earlier time with stronger stimuli (Fig. 3). This depolarization is presumably caused by an asynchronous synaptic activation of layer 6 cortico-geniculate neurones *via* the intracortical loop (Hedström and Lindström 1985). Such an orthodromic firing signals a functional closure of the recurrent excitatory ioops that seems to be required for seizure development in the visual cortex. The exact mechanisms triggering higher oscillatory frequencies within cortico-thalamic loop should be further studied. Future experiment should also determine the role played by the geniculate afferent inflow to layer 6 and 4 cells in shaping the ongoing epileptic discharge.

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