

Spindle-Like Thalamocortical Synchronization in a Rat Brain Slice Preparation

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Tancredi, Virginia, Giuseppe Biagini, Margherita D'Antuono, Jacques Louvel, René Pumain, and Massimo Avoli. Spindle-like thalamocortical synchronization in a rat brain slice preparation. *J Neurophysiol* 84: 1093–1097, 2000. We obtained rat brain slices (550–650 μm) that contained part of the frontoparietal cortex along with a portion of the thalamic ventrobasal complex (VB) and of the reticular nucleus (RTN). Maintained reciprocal thalamocortical connectivity was demonstrated by VB stimulation, which elicited orthodromic and antidromic responses in the cortex, along with re-entry of thalamocortical firing originating in VB neurons excited by cortical output activity. In addition, orthodromic responses were recorded in VB and RTN following stimuli delivered in the cortex. Spontaneous and stimulus-induced coherent rhythmic oscillations (duration = 0.4–3.5 s; frequency = 9–16 Hz) occurred in cortex, VB, and RTN during application of medium containing low concentrations of the K^+ channel blocker 4-aminopyridine (0.5–1 μM). This activity, which resembled electroencephalograph (EEG) spindles recorded in vivo, disappeared in both cortex and thalamus during application of the excitatory amino acid receptor antagonist kynurenic acid in VB ($n = 6$). By contrast, cortical application of kynurenic acid ($n = 4$) abolished spindle-like oscillations at this site, but not those recorded in VB, where their frequency was higher than under control conditions. Our findings demonstrate the preservation of reciprocally interconnected cortical and thalamic neuron networks that generate thalamocortical spindle-like oscillations in an in vitro rat brain slice. As shown in intact animals, these oscillations originate in the thalamus where they are presumably caused by interactions between RTN and VB neurons. We propose that this preparation may help to analyze thalamocortical synchronization and to understand the physiopathogenesis of absence attacks.

INTRODUCTION

The neocortex and thalamus are able to generate brain rhythms that are observed during sleep and in some pathological conditions such as absence seizures (Avoli 1997; Steriade 1993). Mechanisms of thalamocortical synchronization have been extensively studied in vivo (cf. Steriade et al. 1997) and in vitro (cf. Huguenard et al. 1995). However, few of the in vitro studies published to date have analyzed the function of reciprocal thalamocortical connections (e.g., Golshani and

Jones 1999; Zhang et al. 1996). Most often, attention has focused on the network properties and intrinsic excitability of either cortical (Agmon and Connors 1991) or thalamic neurons (Crunelli et al. 1987; Kim and McCormick 1998).

In this study, we used most of the dissecting procedures described in the mouse by Agmon and Connors (1991) to obtain slices of the rat brain thalamus and cortex in which reciprocal thalamocorticothalamic connectivity is preserved. Here, we report that in this preparation of low concentrations of the K^+ -channel blocker 4-aminopyridine (4AP) induce rhythmic, coherent oscillatory field potentials that occur synchronously in both thalamus and cortex and resemble the electroencephalograph (EEG) spindles recorded in vivo during non-REM sleep or following barbiturate treatment (Andersen and Andersson 1968; Steriade et al. 1993).

METHODS

Wistar rats (15–28 day old) were decapitated under halothane anesthesia, and their brains were quickly removed and placed in cold, oxygenated artificial cerebrospinal fluid (ACSF). Combined coronal thalamocortical slices were obtained with a Lancer vibratome using the procedures described by Agmon and Connors (1991). We, however, used thicker slices (550–650 μm versus 400 μm) and a hybrid coronal plane forming a 45° angle with the sagittal plane. Two slices were obtained from each hemisphere at the level of the ventrobasal complex (VB). Both were then transferred to a tissue chamber where they laid in an interface between oxygenated ACSF and humidified gas (95% O_2 –5% CO_2) at 32–35°C (pH = 7.4). ACSF composition was as follows (mM): 124 NaCl, 2 KCl, 1.25 KH_2PO_4 , 0.5 MgSO_4 , 2 CaCl_2 , 26 NaHCO_3 , and 10 glucose. Chemicals were acquired from Sigma (St. Louis, MO).

Extracellular field potentials were recorded with ACSF-filled electrodes (resistance = 2–8 M Ω) positioned under visual control in cortex and thalamus [VB and/or reticular nucleus (RTN)]. Signals were fed to high-impedance amplifiers, processed through second-stage amplifiers with filtering capability, and displayed on an oscilloscope and/or on a Gould recorder. A bipolar stainless steel electrode was used to deliver extracellular stimuli (50–150 μs ; <200 μA) to selected areas of the thalamocortical slice.

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4AP (0.5–1 μ M, Sigma) was bath-applied and kynurenic acid (5 mM) was pressure-applied to restricted areas of the thalamocortical slice either as a drop delivered from a broken pipette or using a perfusion system similar to that described by Behr et al. (1998). To avoid diffusion of the drug from the area of application to distant regions, slices were positioned in the recording chamber in such a way that the treated area was downstream with respect to the flow of ACSF. Our work is based on the use of 75 slices. Measurements are expressed as mean \pm SE and n indicates the slice number. Statistical analysis of the data obtained under control conditions and during any experimental manipulation was performed with the Student's t -test. Data were considered significantly different if $P < 0.05$.

RESULTS

Single-shock stimuli delivered in VB, in the internal capsule or in the white matter, elicited in layers IV–V of the frontoparietal cortex ($n = 32$) a complex series of field potential waves that were in most cases characterized by: 1) an early, fixed peak-latency (~ 2.0 ms) negative field potential (amplitude = 0.5–2.3 mV; single arrow in Fig. 1A) that was capable of following high-frequency (>100 Hz) repetitive stimuli (Fig. 1B); and 2) a late negative field potential whose peak-latency depended on stimulus strength and showed synaptic fatigue during prolonged stimulation (double arrows in Fig. 1A). The early component, which disappeared with stimuli of lower intensity (not illustrated), likely represented the antidromic population response of cortical

neurons following the activation of corticothalamic fibers. By contrast, the late component resulted from the synaptic activation of cortical neurons in response to thalamocortical afferents.

An additional response at latency = 12–18 ms could also be observed in the frontoparietal cortex (Fig. 1A, curved arrow). This component was presumably caused by re-activation of thalamocortical afferents due to thalamocortical firing generated in response to descending corticothalamic inputs since kynurenic acid application to VB ($n = 4$) abolished it without influencing the antidromic or the initial orthodromic response (Fig. 1A). Orthodromic field potential responses were also recorded in the thalamus ($n = 18$ and 9 slices for VB and RTN, respectively) following electrical stimuli delivered in cortical layers V–VI (not illustrated).

Spontaneous synchronous field potentials were not seen in thalamocortical slices bathed in normal ACSF ($n = 29$). We reasoned that this lack of spontaneous activity was probably due to the low degree of spontaneous action potential firing observed in most *in vitro* preparations. Hence, we used low doses of the K^+ channel blocker 4AP to enhance transmitter release at both excitatory and inhibitory synapses and thus the background activity of cortical and thalamic neurons. Such an effect has been documented in the hippocampus (Perreault and Avoli 1992). Indeed, under these conditions, rhythmic oscillatory field potential activity occurred either spontaneously or following electrical stimuli delivered in either thalamic or

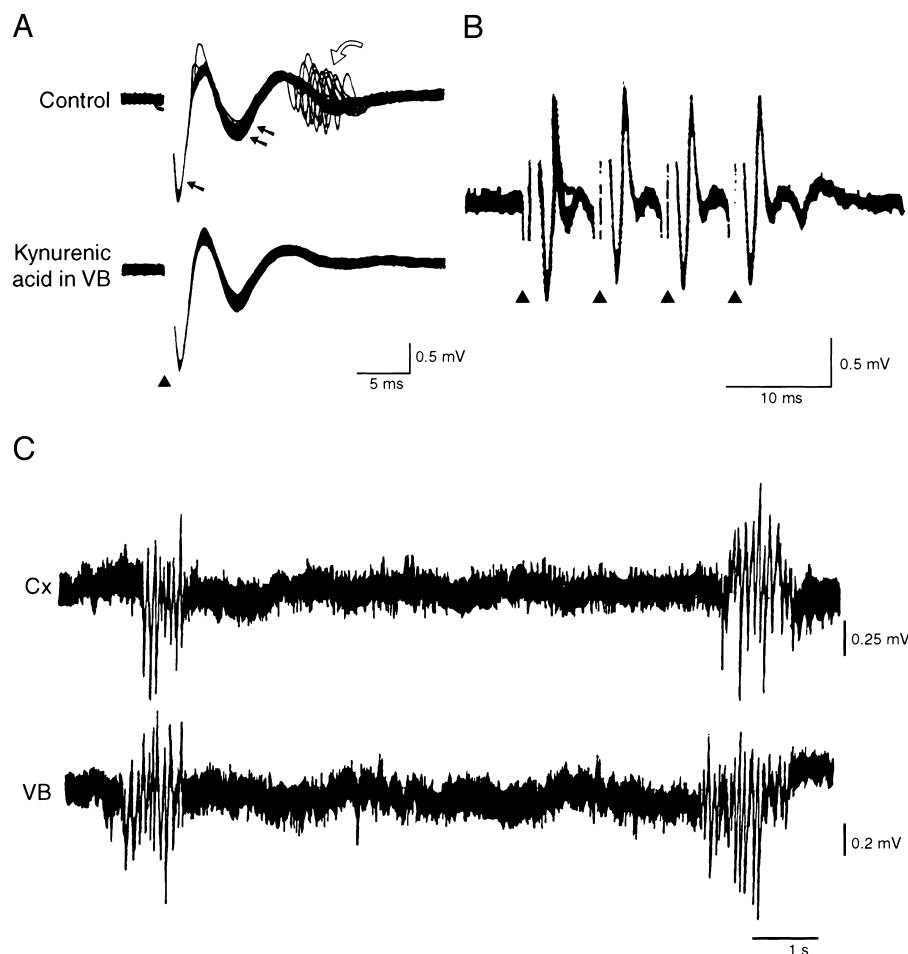


FIG. 1. A: field potential responses induced in layers IV–V of the frontoparietal cortex by internal capsule stimulation. Note the presence of an initial large-amplitude negative potential (presumptive antidromic population spike, arrow), of a late negative component (presumptive orthodromic response, double arrows), and of an additional response characterized by a single-unit discharge (curved arrow). The last response is abolished by local application of kynurenic acid in thalamic ventrobasal complex (VB). B: train of VB stimuli delivered at 300 Hz induces negative-positive field potentials that have stereotyped shape in spite of the high-frequency stimulation. C: spontaneous rhythmic field potential oscillations recorded in thalamus (VB) and cortex (Cx) during application of 4AP.

cortical areas ($n = 25$; Figs. 1C and 2). These oscillations were recorded at both cortical (500–1200 μm from the pia) and thalamic recording sites (VB or RTN) and were characterized by bursts of field potential waves of negative-positive polarity at frequencies ranging from 9 to 16 Hz. The overall duration of each of these rhythmic bursts was 0.4–3.5 s and they occurred at intervals of 6–24 s.

As shown in the experiment illustrated in Fig. 2A, the rhythmic field potential activities recorded in cortex and VB were synchronized. However, a small ($<500 \mu\text{m}$) change in the position of the recording electrode (in either cortex or thalamus) could abolish such synchronization (Fig. 2A, compare traces in *a1* and *a2* with those in *a3*). Field potential rhythmic oscillations, which could be time-locked with those seen in cortex and VB, were also recorded in RTN (not shown). Stimulus-induced oscillatory activity had features similar to those of spontaneous events and occurred with a good degree of synchronization in cortex, VB, and RTN (Fig. 2B).

Next, we used local applications of kynurenic acid in either VB or cortex to establish the contribution of thalamic and cortical networks to the 4AP-induced rhythmic oscillations.

Kynurenic acid was applied close to the site from which field potential recordings were obtained. Moreover, to limit possible activity-dependent variabilities in oscillation shape and/or duration, we used stimuli delivered every 10–30 s, and we avoided the occurrence of stimuli immediately after a spontaneous oscillatory sequence. As shown in Fig. 3A, kynurenic acid application in VB abolished ($n = 6$) the stimulus-induced rhythmic oscillatory responses recorded in both cortex and thalamus, as well as the spontaneous rhythmic oscillations ($n = 6$). Under these conditions, electrical stimuli induced in both structures a long-lasting (up to 300 ms) negative field potential that was at times followed by a late event at a latency = 200–500 ms (Fig. 3A, arrows in Cx and VB traces during kynurenic acid). In four slices, we also studied the effects induced by kynurenic acid application in the cortex. This procedure abolished the rhythmic oscillations recorded in the cortex while spontaneous and stimulus-induced oscillations continued to occur in VB (Fig. 3C). In addition, the frequency of the rhythmic oscillations elicited in VB during application of kynurenic acid in the cortex was higher than under control conditions (Fig. 3D).

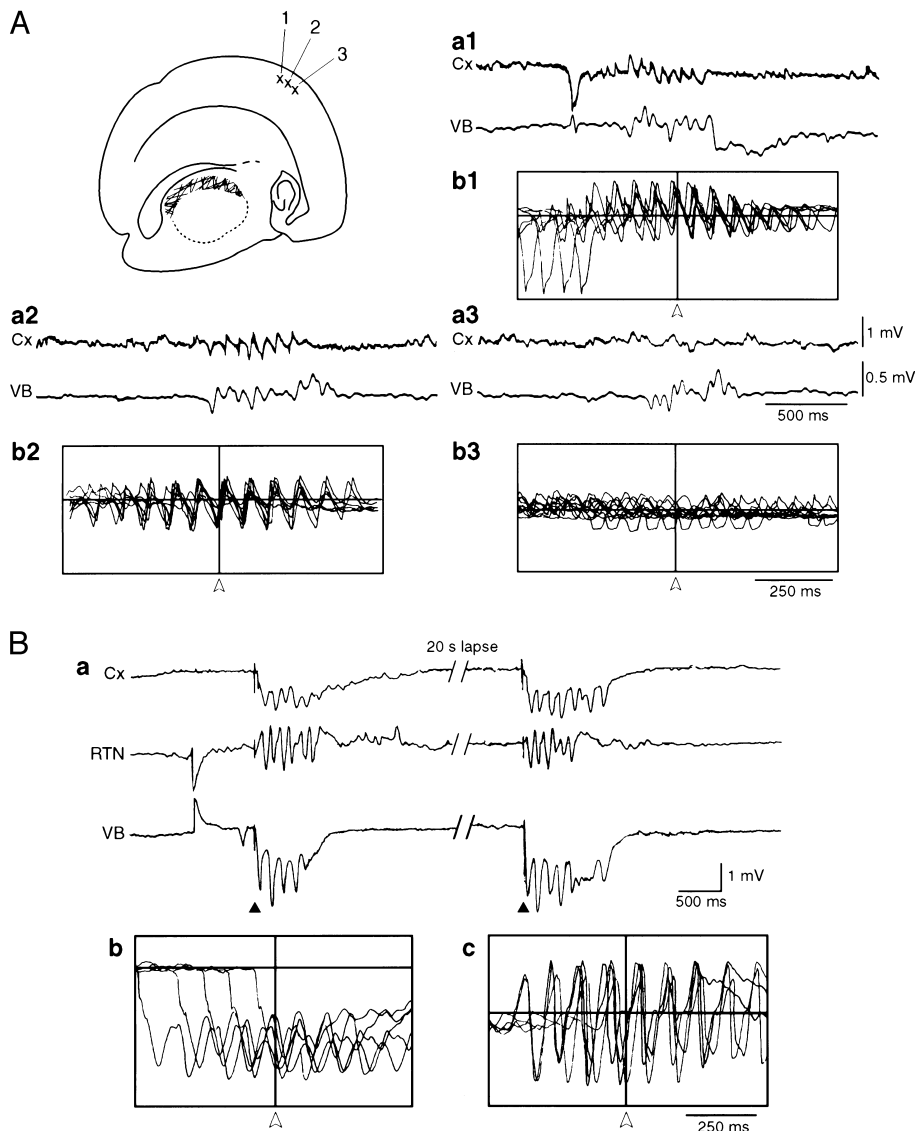


FIG. 2. A: spontaneous rhythmic oscillations recorded during 4AP application in the same VB location and in three different points of the frontoparietal cortex (Cx) as indicated in the insert are shown. In each panel, traces in *a* are actual field potential recordings while in *b* cortical field potentials are superimposed by using the negative peak of rhythmic waves recorded in VB as time 0. Note the loss of synchrony between VB and cortical oscillations when the recording electrode is moved from point 2 to 3. B: oscillatory activity induced by internal capsule stimulation and recorded simultaneously in the Cx, RTN, and VB. Field potential recordings are shown in *a*, while cortical and reticular nucleus (RTN) traces were superimposed in *b* and *c*, respectively, by using the negative peak of VB waves as time 0.

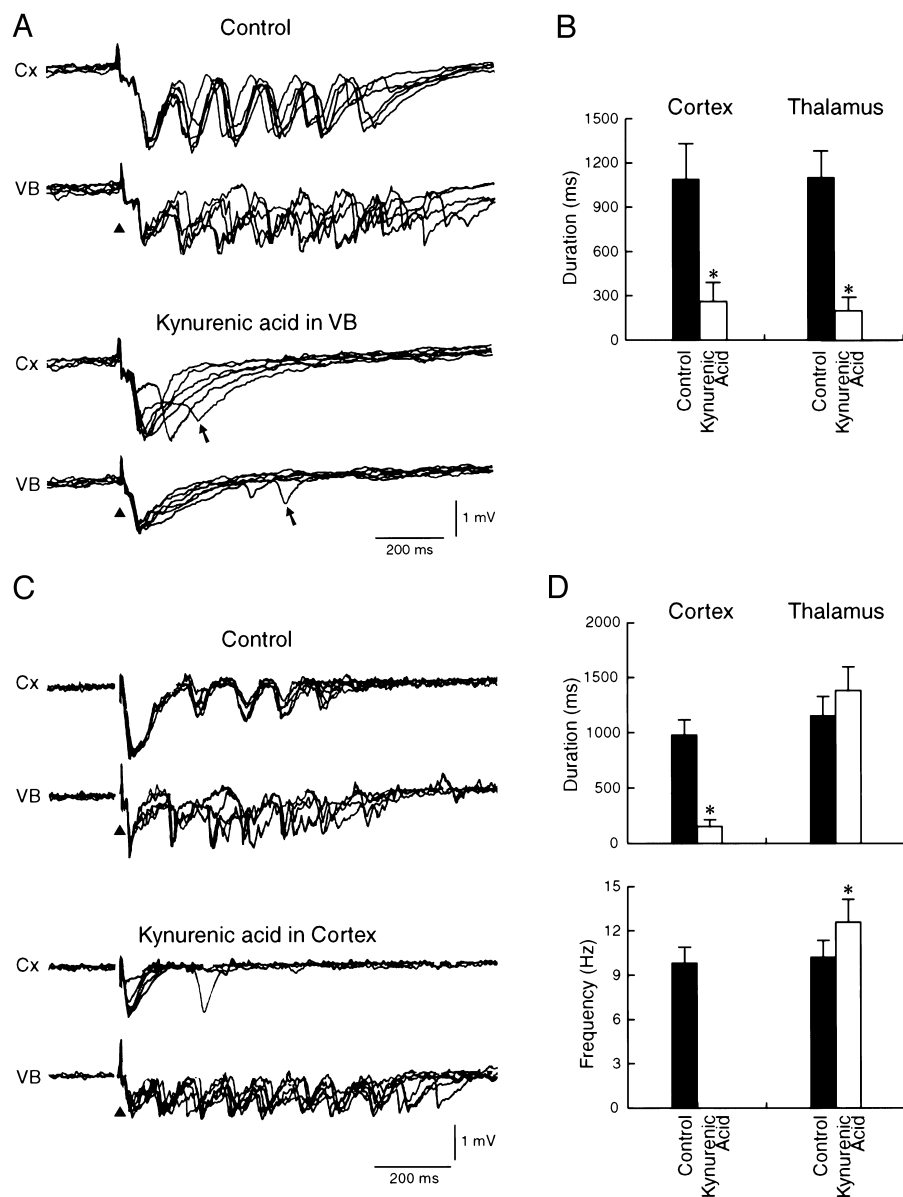


FIG. 3. Stimulus-induced field potential oscillations recorded in Cx and VB under control conditions (i.e., during bath application of 4AP) and during local application of kynurenic acid in VB (A and B) and cortex (C and D). Note that application of kynurenic acid in VB blocks the oscillatory responses in both cortex and VB. By contrast, kynurenic acid application to the cortex abolishes the rhythmic oscillations at the site of application, while rhythmic oscillations are still recorded in VB, where they have frequencies higher than under control conditions. Histograms shown in B and D were obtained from 5 and 4 experiments, respectively. Asterisks indicate statistical significance.

DISCUSSION

The field potential responses recorded in this study from the middle-deep layers of the rat frontoparietal cortex following VB stimuli bear close resemblance to those described in the mouse barrel cortex by Agmon and Connors (1991). We could identify an early negative event with fixed peak-latency that likely results from the antidromic activation of cortical neuron populations. This view is in line with its ability to follow high-frequency repetitive stimuli. In addition, we recorded a subsequent negative potential with peak-latency and amplitude that depend on VB stimulus strength and thus may reflect a postsynaptic, mainly excitatory, field potential. Hence, these data indicate that thalamocortical and corticothalamic connections are functionally operant in our slice preparation. This conclusion is supported by the presence of an additional cortical excitatory component with latency = 12–18 ms that was elicited by stimuli in VB or in the internal capsule. This cortical response is presumably due to the re-activation of thalamo-

cortical afferents caused by thalamocortical firing occurring after excitation of thalamic neurons by corticothalamic inputs. Accordingly, kynurenic acid application to VB abolished this late response without influencing the antidromic or the initial postsynaptic excitatory field potential. In addition, we have recorded orthodromic field potential responses in VB or RTN following electrical stimuli delivered in the somatosensory cortex, thus indicating that corticothalamic projections are functional in our slice preparation (Deschenes et al. 1998).

When challenged with low doses of 4AP, rhythmic oscillatory activity appeared in both cortex and thalamic areas. These field potential oscillations have characteristics different from those reported for epileptiform events induced by higher concentrations of 4AP in different cortical preparations (Perreault and Avoli 1992), including the neocortex (Golshani and Jones 1999). Indeed, they were organized in a rhythmic succession of negative or negative-positive waves that occurred at frequencies of 9–16 Hz and were reminiscent of non-REM sleep (Avoli and Gloor 1982;

Avoli et al. 1984; Steriade et al. 1993) or barbiturate (Andersen and Andersson 1968) spindles recorded by the EEG in vivo.

These in vitro oscillations appeared to be synchronized in cortex and thalamus. However, a small change in the position of the recording electrode (in either cortex or thalamus) could interfere with such synchronization, suggesting that coherent oscillatory activity between thalamic and cortical networks in rat brain slices may be restricted to very small thalamocortical sectors. This may result from the existence of reduced reciprocal connectivity between VB and RTN, as well as from a diminished corticothalamic output function secondary to lesioning of some intracortical connections (Staiger et al. 1999). It should be noted, however, that even in intact preparations, the action potential firing generated by cortical and thalamic neurons of the association system often shows a low degree of correlation during nonbarbiturate spindles (Avoli et al. 1984).

A further similarity of our findings with the EEG spindles recorded in vivo derives from the primary role played by the thalamus in generating rhythmic oscillations in vitro. Blocking synaptic excitatory transmission in VB with local application of kynurenic acid caused the rhythmic oscillations to disappear. By contrast, they continued to occur in the thalamus after application of this excitatory amino acid antagonist to the frontoparietal cortex. Experiments performed in intact animals have demonstrated that spindles can be recorded in the thalamus of decorticated cats (Avoli and Gloor 1982; Villablanca and Schlag 1968). It has also been shown that spindle oscillations are not observed in thalamic relay neurons disconnected from RTN (Steriade et al. 1985). These in vivo data further support the presence of functional connectivity between VB and RTN in our slice preparation.

We have also discovered that corticothalamic inputs are capable of modulating the rhythmic oscillations generated by VB neurons in the presence of 4AP. Depression of cortical excitability by kynurenic acid resulted in the disappearance of cortical oscillations, but at the same time caused an increase in the frequency of the oscillations recorded in the thalamus. Our data are in keeping with recent evidence obtained in slices of the ferret lateral geniculate nucleus in which corticothalamic fibers were electrically stimulated (Blumenfeld and McCormick 1999). These investigators have demonstrated that the activation pattern of corticothalamic inputs controls the frequency of the rhythmic oscillations generated by geniculate neurons.

In conclusion, our study demonstrates that rat thalamocortical slices can retain enough reciprocal connectivity to express field potential rhythmic oscillations resembling the non-REM sleep or barbiturate spindles recorded by EEG in vivo. We would like to propose that this preparation represents a powerful tool for studying thalamocortical synchronization and for understanding the physiopathogenesis of absence attacks.

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