Abolition of spindle oscillations and 3-Hz absence seizure-like activity in the thalamus by using high-frequency stimulation: potential mechanism of action

KENDALL H. LEE, M.D., PH.D., FREDERICK L. HITTI, MARK H. SHALINSKY, PH.D., UHNHOH KIM, PH.D., JAMES C. LEITER, M.D., AND DAVID W. ROBERTS, M.D.

Section of Neurosurgery and Department of Physiology, Dartmouth Medical School, Lebanon, New Hampshire; and Department of Biomedical Sciences, Interdepartmental Neuroscience Program, Iowa State University, Ames, Iowa

Object. The mechanism of action whereby high-frequency stimulation (HFS) in the thalamus ameliorates tremor and epilepsy is unknown. The authors studied the effects of HFS on thalamocortical relay neurons in a ferret in vitro slice preparation to test the hypothesis that HFS abolishes synchronized oscillations by neurotransmitter release.

Methods. Intracellular and extracellular electrophysiological recordings were made in thalamic slices. The neurons in the thalamic slice spontaneously generated spindle oscillations, and treatment with picrotoxin, a γ-aminobutyric acid A receptor antagonist, resulted in 3- to 4-Hz absence seizure-like activity. High-frequency stimulation (stimulation parameters: 10-1000-μA amplitude; 100-μsec pulse width; 100-Hz frequency; 1–60 seconds) was applied using a concentric bipolar stimulating electrode placed adjacent to the recording electrodes.

High-frequency stimulation within the thalamus generated inhibitory and excitatory postsynaptic potentials, membrane depolarization, an increase in action potential firing during the stimulation period, and abolished the spindle oscillations in the thalamocortical relay neurons. High-frequency stimulation applied to 20-μM picrotoxin-treated slices eliminated the 3- to 4-Hz absence seizure-like activity.

Conclusions. High-frequency stimulation eliminates spontaneous spindle oscillations and picrotoxin-induced absence seizure-like activity in thalamic slices by synaptic neurotransmitter release; thus, HFS may abolish synchronous oscillatory activities such as those that generate tremor and seizures. Paradoxically, HFS, which is excitatory, and surgical lesions of the ventrointermedius thalamus, which are presumably inhibitory, both suppress tremors. This paradox is resolved by recognizing that HFS-mediated neurotransmitter release and thalamic surgery both disrupt the circuit generating tremor or seizure, albeit by different mechanisms.

KEY WORDS • high-frequency stimulation • deep brain stimulation • thalamus • absence epilepsy • tremor • oscillation • ferret

High-frequency DBS applied to the thalamus is an effective treatment for epilepsy and drug-resistant tremor due to Parkinson disease, essential tremor, and multiple sclerosis; however, the precise mechanism of action for the therapeutic effect of HFS is unknown. Intraoperative recordings obtained in patients have shown that “tremor” neurons in the thalamus discharge rhythmically, either prior to or in synchrony with the 3- to 6-Hz oscillatory muscular tremor. Similarly, absence seizures are associated with 3-Hz spike and wave oscillations on the electroencephalography and probably represent a perverse form of thalamocortical activity related to the normal generation of spindle waves.

Abbreviations used in this paper: DBS = deep brain stimulation; EPSP = excitatory postsynaptic potential; GABA = γ-aminobutyric acid; HFS = high-frequency stimulation; IPSP = inhibitory postsynaptic potential; LGN = lateral geniculate nucleus; PGN = perigeniculate nucleus.

It was previously demonstrated that HFS of the subthalamic nucleus and the thalamus in vitro results in the release of neurotransmitters during the stimulation period. In a recent study in the in vitro rat thalamus model, Anderson, et al. showed that the effects of HFS were reversibly eliminated by the Na⁺ channel blocker tetrodotoxin, glutamate receptor antagonists (kynurenate or APV-DNQX), and the Ca⁺⁺ channel antagonist cadmium, suggesting that the mechanism of HFS action was due to synaptic activation. In addition, they suggested that HFS disrupts local synaptic function; however, they were not able to test this hypothesis directly because the rat thalamic brain slice does not contain an intact neural network capable of generating spontaneous network oscillations.

In the present study, we used ferret thalamic slices, in which there is an intact neural network that manifests spontaneous network oscillations. The intracellular effects of HFS on thalamic neurons were examined to test the hypothesis that HFS abolishes synchronized oscillations, such as...
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Spindle waves and 3-Hz absence seizure-like discharges, by neurotransmitter release.

Materials and Methods

Slice Preparation

For the preparation of slices, male or female ferrets (Mustela putorius furo; Marshall Farms, North Rose, NY), 2 to 4 months old, were deeply anesthetized with sodium pentobarbital (30–40 mg/kg) and killed by decapitation. The forebrain was rapidly removed, and the hemispheres were separated using a midline incision. Four hundred-micron-thick slices were cut using a vibratome (Leica Microsystems, Nussloch, Germany) in the sagittal plane. A modification of the technique developed by Aghajanian and Rasmussen was used to increase tissue viability. During preparation of the slices, the tissue was placed in a solution (~5°C) in which NaCl solution was replaced with succrose while maintaining an osmolarity of approximately 307 mOsm. After preparation, the slices were placed in an interface-style recording chamber (Fine Science Tools, Inc., Forster City, CA), maintained at 36 ± 1°C, and allowed to recover for at least 2 hours. The bathing medium contained: NaCl, 126 mM; KCl, 2.5 mM; MgSO₄, 1.2 mM; NaH₂PO₄, 1.25 mM; CaCl₂, 2 mM; NaHCO₃, 26 mm; dextrose, 10 mM; and was equilibrated with 95% O₂, 5% CO₂ to a final pH of 7.4. For the first 10 minutes, the thalamic slices were placed in the recording chamber and perfused with an equal mixture of the normal NaCl and the sucrose-substituted solutions. Subsequently, the slices were perfused only with the normal NaCl solution.

Electrophysiological Activity

Intracellular recording electrodes were formed on a P-87 micropipette puller from medium-walled borosilicate capillaries (1B100F; WPI, Sarasota, FL). Micropipettes were filled with 2 M K⁺ acetate and 2% biocytin for intracellular labeling of recorded neurons within 50 to 100 MΩ. Biocytin-filled intracellular electrodes were visualized through standard avidin-biotin-horseradish peroxidase reaction with diaminobenzidine. Only those neurons exhibiting a stable resting membrane potential of less than ~55 mV were included for analysis. A concentric bipolar stimulating electrode was connected to a current isolator (ISO-Flex; AMPI, Jerusalem, Israel) and was used for extracellular stimulation. Intracellular recordings from dorsal LGN thalamocortical relay neurons were visualized through the standard avidin-biotin-horseradish peroxidase reaction with diaminobenzidine. The location of the PGN, A, A1, and C laminae were easily visualized under the microscope at 36 to 72 magnification. In addition, the PGN was confirmed through immunocytochemical staining for GABA, as previously reported. The data were analyzed using eDAQ Chart software (eDAQ Pty Ltd, Denistone East, Australia) on a Pentium-style computer. Figures were drawn using CorelDRAW software (Corel Corp., Ottawa, Ontario, Canada).

Results

Spindle Wave Generation

Simultaneous extra- and intracellular recordings were obtained from the thalamocortical relay neurons in lamina A1 of the dorsal LGN in 21 ferret thalamic slices maintained in vitro that showed spontaneous spindle wave generation (Fig. 1a). Spindle oscillations have been described as 1- to 3-second epochs of synchronized 7- to 14-Hz oscillations that are generated as a result of interactions between thalamocortical relay and thalamic reticular/perigeniculate neurons. During the occurrence of spindle waves, intracellular recordings from dorsal LGN thalamocortical relay neurons received barrage of IPSPs at a frequency of 7 to 14 Hz, and these IPSPs resulted in the generation of rebound low-threshold Ca²⁺ spikes (this phenomenon is described in more detail in the Discussion). The thalamocortical relay neurons were visualized through the standard avidin-biotin-horseradish peroxidase reaction with diaminobenzidine (Fig. 1b).

High-Frequency Stimulation During Spindle Wave

Spindle activity was recorded from a population of neurons (extracellular electrode), and the electrophysiological activity associated with the spindle activity was recorded from single neurons within that population (intracellular electrode; Fig. 2). The intracellular recording revealed synchronized Ca²⁺ bursts (Fig. 2 lower trace) concurrent with the population spindles (Fig. 2 upper trace). High-frequency stimulation was applied by a stimulating electrode positioned within approximately 100 μm of the intra- and extracellular recording electrodes in 12 slices. It was not possible to observe the effect of HFS on spindle activity during HFS because of the stimulation artifact in the extracellular trace. Intracellularly, the stimulation artifact did not prevent the observation of an initial IPSP followed by a prolonged EPSP, membrane depolarization, action potential generation, depolarization block, and further action potential generation (Fig. 2f). In the immediate poststimulation period, neuronal activity was absent. The activity returned gradually in approximately 10 to 30 seconds while spindle waves returned in 30 to 60 seconds (Fig. 2), indicating that the neurons were not lesioned or damaged.

High-Frequency Stimulation During Slowed Oscillations

As previously reported, when GABA_A receptors were blocked by 20 μM picrotoxin, the spindle waves in five slices were transformed into events that resembled absence seizure-like activity (Fig. 3a). In picrotoxin-treated slices, HFS applied to thalamocortical relay neurons eliminated the 3- to 4-Hz seizure-like activity in five slices, as observed when using extracellular recording electrodes (Fig. 3). Intracellular recordings from picrotoxin-treated thalamocortical relay neurons during HFS in five cells revealed EPSPs, membrane depolarization, action potential generation, and depolarization block followed by further action potential generation. The initial IPSPs seen in the current clamp in the absence of picrotoxin were not seen in the picrotoxin-treated cells (Fig. 4a). In one cell, brief HFS (100-msec duration) elicited a slow oscillation (Fig. 4b). High-frequency stimulation during the oscillation resulted in EPSPs, membrane depolarization, action potential generation, and abolition of the slowed oscillation.

Discussion

The results of the present study support the hypothesis that HFS causes the release of neurotransmitters in the thalamus. High-frequency stimulation of the thalamus in ferret slice preparations resulted in EPSPs and IPSPs, depolarization, action potential generation, depolarization block, and further action potential generation in the thalamocortical relay neurons. These effects of HFS are similar to those documented during HFS of the subthalamic nucleus and suggest that neurotransmitter release is a general response to HFS. This neurotransmitter release potently affects thalamic oscillatory activity, as evidenced by the ability of HFS to influence the generation of rebound low-threshold Ca²⁺ spikes.
to abolish both spindle oscillations and 3-Hz absence seiz-
urelike activity.

Effects of HFS on the Spindle Wave Activity and
Thalamocortical Relay Neurons

Spindle waves are 1- to 3-second epochs of synchronized
7- to 14-Hz oscillations that are generated as a result of
interactions between thalamocortical relay and thalamic
reticular/perigeniculate neurons. The generation of a
burst of action potentials in the GABAergic neurons of the
thalamic reticular/perigeniculate nuclei results in an IPSP in
the thalamocortical relay neurons that is characterized by a
2- to 10-mV amplitude hyperpolarization. A subset of tha-
lamocortical neurons generate rebound low-threshold Ca
spikes and a burst of action potentials, which then return as
a barrage of EPSPs to the thalamic reticular/perigeniculate
neurons. This barrage of EPSPs activates a low-threshold
Ca spike in the perigeniculate cells and initiates the next
cycle of the spindle wave. Spindle waves “wax” or general-
ize through the progressive recruitment of neurons into this
oscillation, presumably due to axonal interconnections be-
tween the thalamocortical and PGN neurons.

High-frequency stimulation near the recording electrode
transiently abolished the spindle waves in the thalamic slice.
During the stimulation period, we observed an initial IPSP
and a subsequent prolonged EPSP, membrane depolariza-
tion, action potential generation, depolarization block, and
further action potential generation. The inhibitory GABA-
ergic inputs into the thalamus are believed to be from the
nucleus reticularis thalami and local GABAergic interneu-
rons. The excitatory glutamatergic inputs into the thalamus
are thought to originate from the cerebral cortex and from
specific sensory systems—for example, retinal ganglion
cells in the case of the visual thalamus. The presence of
IPSPs and EPSPs during HFS in the thalamus indicates that
both excitatory and inhibitory systems were activated in a
nonspecific manner. Furthermore, Anderson and associates
recently demonstrated that DBS in the ventrolateral thalamus
evoked depolarization that was reversibly eliminated by the
Na channel blocker tetrodotoxin, glutamate receptor an-
tagont (kynureate or APV-DNQX), and the Ca channel
antagonist cadmium, indicating that HFS causes synaptic ac-
tivation. The presence of IPSPs and EPSPs and the effect
of neurotransmitter receptor antagonists indicate that neuro-
transmitters are released during HFS; however, it has yet to
be determined by direct measurement that neurotransmitters

Fig. 1. a: Simultaneous intra- and extracellular recordings from thalamic neurons in lamina A1 revealed spontaneous
spindle wave generation. Intracellular recordings from thalamocortical neurons in lamina A1 revealed spontaneous spin-
dle wave activity. During the generation of spindle waves, dorsal LGN thalamocortical relay neurons received barrages of
IPSPs at a frequency of 7 to 14 Hz, and these IPSPs resulted in the generation of rebound low-threshold Ca spikes. b:
Photomicrograph of a biocytin-filled thalamocortical relay neuron. Avidin-biotin-horseradish peroxidase reaction with
diaminobenzidine. Bar = 50 μm.
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are released by HFS. Nonetheless, analysis of these results also suggests that other synaptic inputs into the thalamus, such as those from the reticular activating system (acetylcholine from the parapontine tegmental nuclei, norepinephrine from the locus coeruleus, serotonin from the raphe nucleus, and histamine from the tuberomammillary nucleus of the hypothalamus) may also be provoked, although this requires further experimental investigation.

The effect of DBS in patients with Parkinson disease and tremor is usually similar to that of a surgically created lesion in the same area, indicating that DBS may act by silencing the neurons of the stimulated structure. In agreement with this hypothesis, previous work in the subthalamic nucleus reported by Beurrier, et al., showed that HFS blocked action potential generation by inhibiting voltage-sensitive Na⁺ channels. In contrast to this mechanism, our intracellular recordings of thalamocortical relay neurons indicate that the most potent effect of HFS was mediated by neurotransmitter release. Our finding that the EPSPs were greater than the initial IPSPs indicates that the excitatory neurotransmission outweighs the inhibitory inputs. Functionally, however, excitation may block the formation of action potentials due to a depolarization block (Fig. 2f), although this must be confirmed by further experimental investigation. Anderson and

Fig. 2. Simultaneous recordings of spindle oscillations were made with an extracellular and an intracellular electrode in lamina A1 of the ferret LGN. a: The intracellular recording revealed synchronized Ca⁺⁺ bursts (lower trace) concurrent with the population spindles (upper trace). b: High-frequency stimulation was applied through an electrode positioned within 100 μm of the intra- and extracellular recording electrodes, resulting in stimulation artifact. During the HFS stimulation period, it was not possible to observe the effect of HFS because of the stimulation artifact in the extracellular trace. Intracellularly, however, HFS resulted in an initial IPSP followed by a prolonged EPSP, membrane depolarization, action potential generation, depolarization block, and further action potential generation. c: In the immediate poststimulation period, there was cessation of all activity in both the extra- and intracellular recordings. d: The spindle wave returned, indicating that the neurons were not lesioned or damaged. e: Enlargement of a section of the intracellular recording seen in Fig. 2a displaying Ca⁺⁺ spikes and rebound bursts of action potentials. f: Enlargement of a section of the intracellular recording in Fig. 2b with the stimulation artifacts manually removed using CorelDRAW. An initial IPSP followed by several EPSPs, action potential generation, depolarization block, and further generation of action potential activity can be seen.
colleagues failed to observe IPSPs in rat thalamic slices. Why we observed IPSPs while they did not may be due to the fact that GABAergic inputs remain intact in the ferret thalamic brain slice, as demonstrated by the spontaneous generation of spindles. Furthermore, these initial IPSPs were not seen when picrotoxin (a GABA<sub>A</sub> antagonist) was added to the bath solution, suggesting that these initial IPSPs are mediated by GABA<sub>A</sub> receptor activation.
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During normal spindle waves, the IPSPs in thalamocortical cells elicited by activation of GABA\textsubscript{A} receptors last approximately 100 msec. When GABA\textsubscript{A} receptors were blocked, the duration of the IPSPs increased to approximately 300 msec due to activation of GABA\textsubscript{B}, and the oscillations slowed from 7 to 14 Hz to 3 to 4 Hz. Because the intrinsic harmonics of the thalamocortical cells (which oscillate preferentially at ~ 3 Hz) match those of the thalamocortical–PGN loop (which also oscillates preferentially at ~ 3 Hz), these 3- to 4-Hz bursts became very strong and generated a massive synchronized discharge at approximately 3 to 4 Hz. In this manner, normal spindle waves in vitro can be “perverted” into absence seizure-like events.

Both absence seizures and tremor appear to involve abnormal oscillatory activity in the thalamus—3-Hz oscillations for absence seizures and 3- to 6-Hz oscillations for tremor.\textsuperscript{23} Interestingly, HFS applied to the tremor cell-containing area leads to immediate tremor arrest, but tremor rapidly returns when stimulation ceases.\textsuperscript{6} The depolarization of thalamocortical neurons is likely capable of abolishing spindle wave, tremor, and 3-Hz absence seizure-like oscillations because of the inhibition of rebound responses required to drive nucleus reticularis thalami PGN neurons.
to discharge in synchrony. Evaluation of our previous results supports this hypothesis in that both application of specific neurotransmitter agonist drugs block spindle wave generation\(^{20-21}\) and HFS, as demonstrated in this study, resulted in a marked depolarization of thalamocortical neurons and abolished oscillatory activity. In this manner, HFS-induced neurotransmitter release in the thalamus may abolish normal oscillations, such as spindle waves, and abnormal oscillations, such as tremor and absence epilepsy.

**Ionic Mechanisms Underlying Abolition of Thalamic Oscillations by HFS**

Activation of muscarinic, \(\alpha_1\)-adrenoceptors, \(H_1\)-histaminergic, and glutamate metabotropic receptors on thalamocortical cells results in a reduction of \(I_{\text{h}}\), resulting in a membrane depolarization of 5 to 20 mV.\(^{26,30,31,32}\) This reduction of \(I_{\text{h}}\) results from the activation of a nonpermeant toxin sensi-

The current finding that HFS can abolish rhythmic burst firing and the promotion of single spike activity in single thalamocortical neurons.\(^{33,34}\) Based on this and similar findings, it has been hypothesized that the neuromodulation of \(I_{\text{h}}\) may underlie the transition from rhythmic, synchronized oscillations associated with slow wave sleep, tremor, and seizures to the tonic, single spike activity associated with waking. Furthermore, the reduction of \(I_{\text{h}}\) has been shown to block the generation of spindle oscillations through depolarization of the membrane potential, resulting in inactivation of the low-

Anderson and associates\(^1\) have demonstrated that glutamate was the neurotransmitter most likely released by HFS and thus the primary effect was depolarization. Excitatory synaptic inputs in the thalamus are provided predominantly by corticothalamic glutamatergic fibers.\(^{15}\) Although we have not measured the neurotransmitters released directly, we believe that various neurotransmitters are involved in the complex pattern of activity.\(^{35}\) Our work indicates that inhibitory GABAergic inputs also contribute to the generation of oscillatory behavior within the slice.

In thalamic relay neurons, hyperpolarization of the membrane potential results in a depolarizing sag back toward a resting condition due to the presence of the hyperpolarization-activated cation current, or \(I_{\text{h}}\).\(^{20}\) Application of a variety of putative neurotransmitters results in alterations in the voltage dependency of \(I_{\text{h}}\).\(^{20}\) We have previously shown that enhancement of \(I_{\text{h}}\) alone is capable of abolishing spindle waves, which depend critically on the generation of rebound low-threshold \(Ca^{2+}\) spikes in thalamocortical cells.\(^{20}\) We have found in the present study that HFS applied to thalamocortical relay neurons eliminates the generation of spindle waves and 3-Hz seizure-like activity. The finding of IPSPs, EPSPs, and slow membrane depolarization in the present study suggests that the mechanism of action of DBS involves the release of neurotransmitters, similar to that observed in the STN\(^{19,22}\) and the thalamus.\(^2\) Crucial to the hypothesis that an increase in brain neurotransmitter levels is responsible for the efficacy of HFS of the thalamus, however, is the demonstration that neurotransmitter levels actually increase during HFS. The electrochemical technique of amperometry can be used to measure directly the change in stimulation-induced neurotransmitter level.\(^{30,31,32}\) We are currently in the process of measuring glutamate release during HFS in our ferret thalamic slice preparation.

**Clinical Implications**

The clinical benefits of DBS mirror those associated with surgical lesioning,\(^34\) which suggests a similar mechanism of action.\(^22\) Schuman, et al.\(^{34}\) have found that thalamic stimulation and thalamotomy were equally effective in suppressing drug-resistant tremor but that the former had fewer adverse effects and resulted in greater functional improvement. Previously reported side effects of thalamic stimulation include dysarthria, paresthesias, dystonia, balance disturbance, ataxia, and limb weakness.\(^34\) Despite these side effects, many patients leave the stimulation on all the time; this tendency indicates that for many patients, the benefit of being able to control one’s tremors outweighs the side effects of the treatment.\(^34\) The current finding that HFS can block both normal and abnormal oscillations, however, underscores that thalamic DBS abolishes oscillation nonselectively. The physiological purpose of spindle oscillations is currently unknown, but adverse effects may result from chronically blocking the spindles during thalamic DBS. Thus, it is conceivable that there may be potential clinical benefit from timing DBS to permit the occurrence of normal thalamic oscillatory behavior when the therapeutic effect of HFS is less important.

**Conclusions**

High-frequency stimulation abolished synchronous spontaneous oscillations in the ferret thalamic slice preparation. High-frequency stimulation appears to disrupt oscillatory activity by releasing inhibitory and excitatory neurotransmitters. It disrupts the thalamic circuitry that generates oscillatory activity underlying tremor and absence seizure-like activity. Paradoxically, both HFS (excitatory) and surgical lesioning of the ventrointermedius thalamus (presumably inhibitory) suppress tremor. Both HFS-mediated neurotransmitter release and thalamic surgery disrupt the circuit generating tremor or seizure, albeit by different mechanisms.

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**References**


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