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Clonazepam suppresses oscillations in rat thalamic slices

Vikaas S. Sohal, John R. Huguenard*

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305-5122, USA

Abstract

Hypersynchrony of spindle-like thalamic oscillations may contribute to thalamic dysrhythmias including generalized absence epilepsy. Here we show that, as predicted by previous modeling studies, enhancement of inhibition between thalamic reticular (RE) cells by clonazepam suppresses evoked oscillations in rat thalamic slices. We also observed spontaneous oscillations, which have a variable and limited spatial extent. Clonazepam reduces the frequency of occurrence and the spatial extent of these spontaneous oscillations. These results suggest ways in which the enhancement of intra-RE inhibition by clonazepam can prevent hypersynchronous oscillatory activity in the thalamus, and produce therapeutic effects. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Spindle rhythm; $GABA_A$ receptors; Synchrony; Thalamus

1. Introduction

The thalamus participates in a wide range of thalamocortical oscillations and may be particularly important for the genesis of 7–14 Hz sleep spindles [9]. Hypersynchrony of spindle-like oscillations may contribute to generalized absence seizures [4] and other conditions comprising the neuropsychiatric syndrome of thalamocortical dysrhythmia [7]. While spindle-like oscillations are sustained by reciprocal connections between thalamic reticular (RE) and thalamocortical (TC) cells [10], recent in vitro [5] and modeling [8] studies suggest that inhibitory synapses between RE cells desynchronize and shorten these oscillations.

The anti-absence drug clonazepam (CZP) enhances $GABA_A$ receptor-mediated currents associated with intra-RE synapses, but has less of an effect on currents

* Corresponding author. Tel.: + 1-650-723-5522; fax: + 1-650-723-1080.
E-mail addresses: vikaas@stanford.edu (V.S. Sohal), john.huguenard@stanford.edu (J.R. Huguenard).

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associated with synapses from RE to TC cells [1]. By selectively enhancing intra-RE inhibition, clonazepam is thought to reduce inhibitory output from RE to TC cells [3]. Thus, to evaluate the proposed roles of intra-RE inhibition, we studied the effects of clonazepam on spindle-like oscillations in rat thalamic slices.

2. Methods

Slice experiments were performed as previously described [2]. Briefly, 11–13 day old rat pups were used to make 400 μm horizontal slices. The in vitro recording chamber was maintained at $34 \pm 1^{\circ}C$. The extracellular superfusion solution consisted of (in mM) 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1.2 MgCl, 2 CaCl, and 10 glucose.

Multiunit activity was recorded using arrays of 500 kΩ tungsten electrodes with an inter-electrode spacing of approximately 100 μm. We evoked thalamic oscillations with constant voltage extracellular stimuli (40–70 V, 20–40 μs) delivered to internal capsule through a pair of 500 kΩ tungsten electrodes. Spikes were detected by an algorithm that searched for sufficiently steep negative deflections followed by sufficiently steep positive deflections in the extracellular voltage. Analyses of spike trains excluded the burst of spikes immediately following delivery of the extracellular stimulus.

3. Results

3.1. Clonazepam shortens evoked oscillations in rat thalamic slices

Fig. 1 shows evoked oscillations from a single slice under control conditions (top), following application of 100 nm clonazepam (middle), and after washing out clonazepam for 20 min (bottom). Each panel shows simultaneous multiunit recordings from five electrodes, three of which recorded TC cell activity while the other two recorded RE cell activity. Evoked oscillatory activity of both RE and TC cells is reversibly suppressed by clonazepam. Both in control conditions and following clonazepam washout, activity in several electrodes persists for the duration of the recording (5 s). However, immediately following clonazepam application, in only one electrode does activity persist for this duration.

Recordings from 11 slices yielded a total of 41 electrodes exhibiting prominent oscillatory activity. We measured activity before, during, and following clonazepam application by counting the number of spikes in each electrode. In 34 electrodes, clonazepam application suppressed the spike count by an average of 20% (in the remaining 7 electrodes, the spike count increased by an average of 5%). In 28 of these electrodes, washout of clonazepam for 15 min resulted in a reversal of this suppression (in these 28 electrodes, the spike count during the washout actually increased to 128% of the control value).

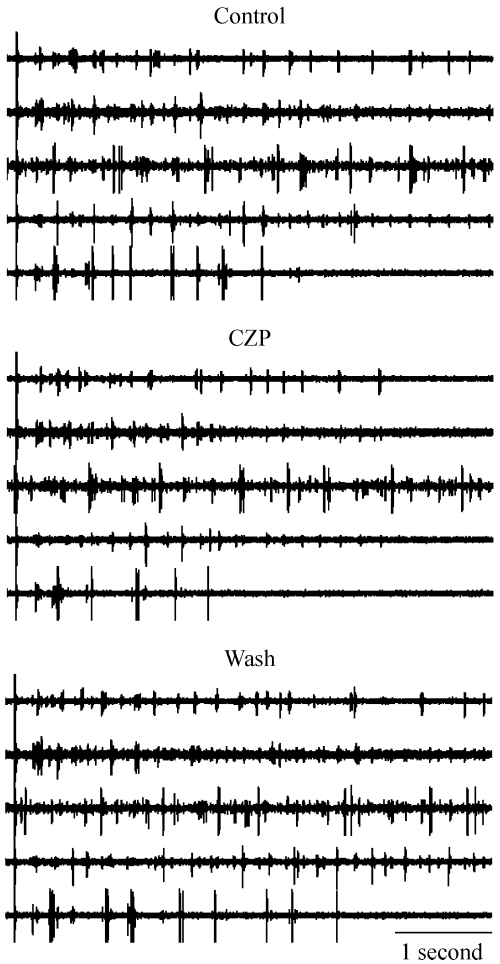

Fig. 1. Evoked oscillations in a thalamic slice under control conditions (top), immediately following CZP application (middle), and after washing CZP out for 20 min (bottom). Oscillations were evoked by electrical stimulation of internal capsule, and the stimulus artifact can be seen at the left of each recording. Each panel shows simultaneous multiunit recordings from five electrodes. The two electrodes at the top of each panel recorded activity from RE cells, whereas the three at the bottom of each panel recorded activity from TC cells. Note that for almost every electrode, activity under control conditions and after CZP washout outlasts activity immediately following CZP application.

3.2. Spontaneous spindle oscillations have a variable and limited extent

In addition to evoking oscillations, we occasionally recorded spontaneous spindle oscillations in rat thalamic slices. Fig. 2 shows two sets of recordings, made at different times from the same electrodes in the same slice. In both cases, spindle oscillations arise spontaneously and propagate across RE and TC cells. However, note that in each case, some electrodes fail to exhibit spindle activity. Furthermore, different

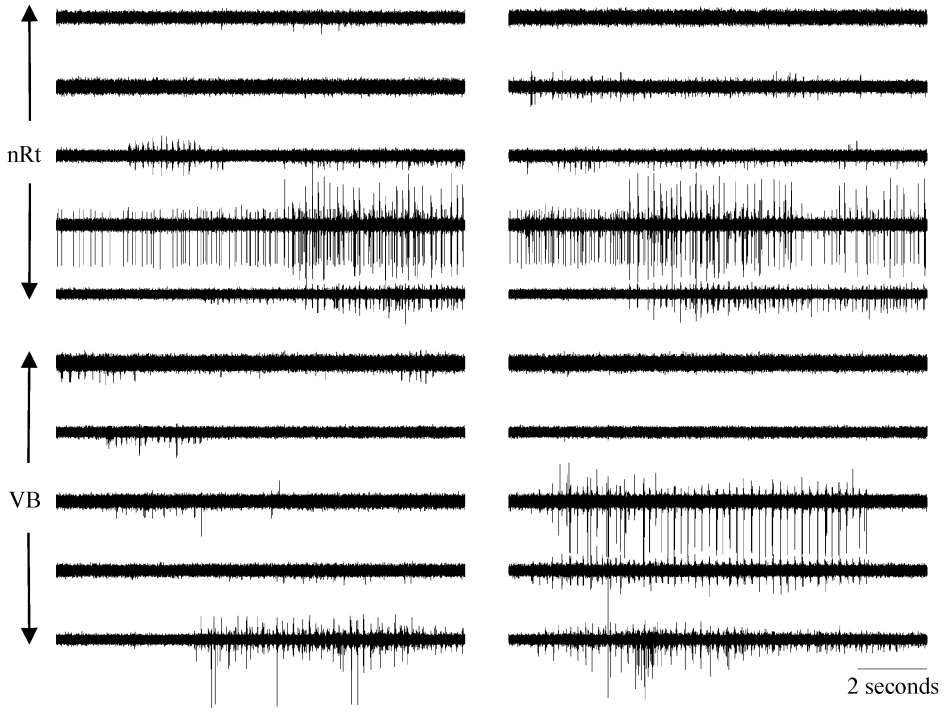

Fig. 2. Spontaneous spindles have variable extent. The left and right panels show spontaneous spindle oscillations recorded at different times from the same electrodes in the same slice. Each panel depicts simultaneous recordings from 10 electrodes. These were configured as two linear five-electrode arrays. One array, located in the reticular nucleus (nRt), recorded the activity of RE cells, while the other, located in the ventrobasal complex (VB), recorded the activity of TC cells. In both arrays, the spacing between adjacent electrodes was 100 μm. Note that in both panels, spindle activity propagates across both nRt and VB. However, some electrodes which exhibit oscillatory activity in the left panel are silent in the right panel. Furthermore, even within one electrode, the set of neurons which participate in the oscillation depicted on the left is different from that participating in the oscillation depicted on the right (these different neurons can be distinguished by their spike amplitudes).

electrodes are active in the two cases, and even when the same electrode is active, different neurons (distinguishable by spike amplitude) may be active. These results are consistent with earlier work in ferrets, which found propagating oscillations that were occasionally limited in their spatial extent [6].

3.3. Clonazepam reduces the occurrence and extent of spontaneous spindles

Fig. 3 shows the effect of CZP application on activity in the same electrodes shown in Fig. 2. Although a spontaneous spindle still occurs, its spatial extent and duration are both far more limited than in control conditions. To quantify these differences, we detected spikes in each multiunit recording, and Fourier transformed the resulting time series. Fig. 4 shows total spectral power in the spindle range (5–14 Hz), for each

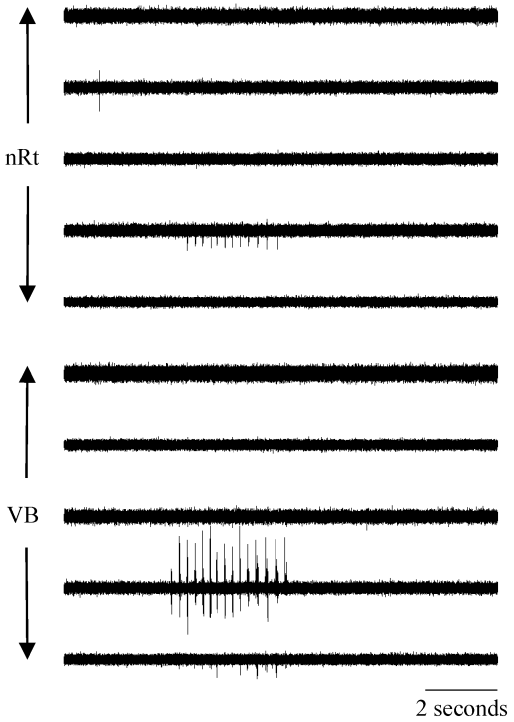

Fig. 3. CZP attenuates spontaneous spindles. A recording from the same electrodes depicted in Fig. 2 shows a spontaneous spindle during CZP application. Note that this spindle is much shorter in duration than those shown in Fig. 2. Furthermore, whereas the spindles in Fig. 2 propagate across both nRt and VB, this spindle remains relatively localized.

electrode as a function of time. Thus, each dark bar corresponds to the occurrence of a spindle. This figure shows that both the frequency of occurrence and the spatial extent of spindles decreases during CZP application and recovers during washout. Note that spindles cease to occur during application of picrotoxin, a $GABA_A$ antagonist.

4. Conclusions

We found that clonazepam, which preferentially enhances $GABA_A$ receptor mediated currents on RE cells [1], suppresses evoked and spontaneous oscillatory activity in rat thalamic slices. Earlier modeling work has shown that enhancing intra-RE inhibition could produce this suppression by shortening the duration of RE cell bursts and shunting excitatory input to RE cells [8]. We also found that in one slice, when spontaneous spindles occurred, clonazepam reduced their frequency of occurrence and spatial extent. The latter observation is also consistent with predictions from modeling [8]. In those simulations, intra-RE inhibition shunted excitation to some RE cells, preventing recruitment of those RE cells, such that spindle-like oscillations remained focal.

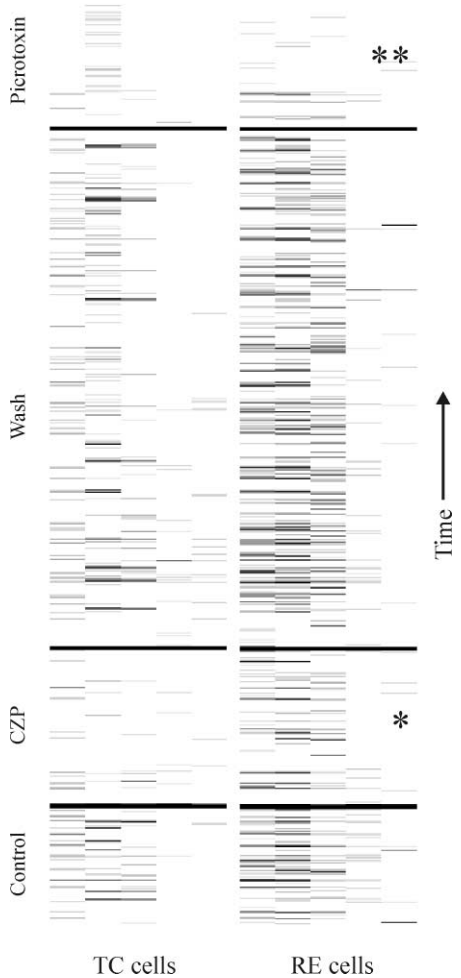

Fig. 4. CZP reduces the frequency and extent of spontaneous spindles. This figure summarizes spindle activity in the 10 electrodes shown in Figs. 2 and 3 over the course of the entire experiment. Time runs vertically, such that each column represents spindle activity in one electrode. We detected spikes in the multiunit recording from each electrode, and Fourier transformed the resulting spike trains. The darkness of each bar is proportional to the total spectral power in the spindle range (5–14 Hz; computed over an 800 ms window) for one electrode. Thus, each dark bar represents the occurrence of a spindle oscillation in one electrode at one point in time. Heavy horizontal black lines separate the control condition, CZP application, CZP washout, and picrotoxin application. Comparison of activity during CZP application (*) with that during control or wash conditions, shows that in CZP the frequency of spindle occurrence declines. Furthermore, during CZP application, when spindles do occur, spindle activity is seen in fewer electrodes. Note that application of picrotoxin (**), a $GABA_A$ receptor antagonist, abolishes spindle oscillations.

These results suggest that intra-RE inhibition may restrict both the amount of activity at a particular location and the spatial extent of activity during thalamic oscillations. These mechanisms may explain how, by enhancing intra-RE inhibition,

clonazepam can prevent the hypersynchronous thalamic activation characteristic of absence epilepsy.

Acknowledgements

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