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IN the thalamus, paradoxical changes in response to augmentation of inhibition can occur as a result of either cellular or network effects. Clonazepam, a GABA_A agonist, produces a paradoxical reduction in evoked thalamocortical neuron inhibitory postsynaptic potential (IPSP) in thalamic slice. This has been hypothesized to be a result of augmentation in inhibitory to inhibitory connections. In a computer model, orthodromic stimulation produced an increase in initial IPSP, a result contrary to that found experimentally. This failure was traced to the inability of orthodromic activation to produce fast enough recurrent inhibition to alter initial reticularis neuron firing. Simulated antidromic stimulation was able to reduce this initial spike train and reproduced the experimental finding.

Key words: Absence epilepsy; Clonazepam; Computer simulation; Inhibition; Oscillation

Computer model of clonazepam's effect in thalamic slice

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Introduction

Thirty years ago, Andersen and colleagues created a computer model of the thalamus¹ that embodied their central hypothesis: the role of inhibitory phasing in producing synchronization of thalamocortical neurons. 'Inhibitory phasing' and 'post-anodal exaltation' were their early terms for the function and form of what came to be called the low-threshold spike, a self-regenerative intrinsic excitation following hyperpolarization. This represented a new mechanism whereby inhibition could facilitate firing through intrinsic cellular properties. An additional mechanism that also effects inhibitory facilitation is due to interactions at the network level: inhibition of an inhibitory cell (double negation) leads to facilitation.

Reciprocal connections between excitatory thalamocortical (TC) neurons and inhibitory thalamic reticular (RE) neurons is a central interaction in the slow oscillations that underlie absence seizures and slow-wave sleep.^{2,3} The low-threshold spike has been described in both cell types,^{4,5} while thalamic connectivity includes RE→RE connections which give the double negation effect. The presence of multiple intrinsic and network sources of paradoxical effects can yield complex situations. One example, studied physiologically, is a reduction in TC neuron IPSPs in the presence of clonazepam, despite the direct IPSP augmenting effect of this GABA_A agonist.⁵ This particular paradoxical effect attracted attention because, in distinction to other GABA_A agonists that aggravate absence seizures,^{7,8} clonazepam is actually used to treat absence epilepsy.⁹ Huguenard and

Prince⁵ suggested that the effect that they found might be due to a relatively greater augmentation of GABA_A responses at the RE→RE site than at the RE→TC site. This seemed most likely since the decreased IPSP is observed immediately, before activity had time to cycle through the network and accumulate the effects of intrinsic facilitation. The rapidity of the effect also suggests a critical dependence on the relative timing of excitatory and inhibitory signals. Therefore, we used a computer model to evaluate this hypothesis.

Materials and Methods

Simulations were run on a Sun Sparcstation 10 using the NEURON simulator.¹⁰ Each neuron was represented by a single compartment with an area of 1000 μm². Voltage-sensitive channels included were similar to those used in previous studies.^{4,11} The TC neuron had the following voltage-sensitive channels and typical maximal conductances (in mS/cm²): fast sodium 0.03, delayed rectifier 0.002, T channel 2.4×10^{-4} , and H 1.2×10^{-4} . In the RE neuron, fast sodium 0.1, delayed rectifier 0.01, T channel 1.75×10^{-3} , slow calcium-dependent potassium channel (I_{AHP}) 0.005, non-specific calcium-sensitive cation channel (I_{CAN}) 3.5×10^{-4} . Calcium removal in both neuron types was effected by a first-order calcium pump.

The synaptic projection from the TC neuron to the RE neuron involved activation of simulated AMPA receptors. The connection from RE neuron to TC neuron utilized GABA_A and GABA_B synapse components. All synapses were parameterized using the two-state model in which a channel activates in

response to a square-wave transmitter pulse of duration $Cdur$.^{12,13} Channel opening occurs at rate α while transmitter is present; channel closing occurs at rate β . Parameters were as follows: AMPA: $\alpha = 1.1/\text{ms}/\text{mM}$, $\beta = 0.19/\text{ms}$, $Cdur = 1.1 \text{ ms}$, $E_{rev} = 0 \text{ mV}$, $\bar{g} = 0.5 \text{ nS}$. GABA_A: $\alpha = 0.53/\text{ms}/\text{mM}$, $\beta = 0.18/\text{ms}$, $Cdur = 1.0 \text{ ms}$, $E_{rev} = -80 - -90 \text{ mV}$, $\bar{g}_{RE \rightarrow TC} = 0.5 \text{ nS}$, $\bar{g}_{RE \rightarrow RE} = 0.14 \pm 0.4 \text{ nS}$. GABA_B: $\alpha = 0.01/\text{ms}/\text{mM}$, $\beta = 0.005/\text{ms}$, $Cdur = 150 \text{ ms}$, $E_{rev} = -95 \text{ mV}$, $\bar{g} = 0.06 \text{ nS}$.

GABA_B responses show sensitivity to intensity of stimulation which could be explained by cooperativity in the intervening second messenger system producing large increments in response depending on the duration of the presynaptic burst.¹⁴ We used a simple model that treats each spike of a burst as if it were a separate synaptic projection to the same location. Although this produces a response that differs in detail from that of a true cooperative model, overall synaptic strength can be adjusted to give responses that are comparable to those of the more complex models.

Orthodromic stimulation was simulated with AMPAergic excitation to the RE neuron ($\bar{g} = 0.5 \text{ nS}$). Since we did not model axons, antidromic stimulation was simulated with a brief, large current injection that produced a single spike (5 nA for 0.2 ms). Network architecture was that used in a previous paper.³ Each of nine columns was headed by a single RE neuron which controlled 5 TC neurons. This architecture was suggested by anatomical studies that have shown a restricted zone of axonal connectivity for an individual RE neuron.^{15,16} Within a column, reciprocal connectivity was complete. Between columns, connectivity probability for TC neuron to RE neuron and RE neuron to TC neuron was 0.67, 0.34 and 0.15 for consecutive neighboring columns in either direction with wrap-around. RE neurons were fully connected to immediately neighboring RE neurons and were connected with an 0.5 probability to RE neurons two columns distant. All synaptic delays were taken to be 0.5 ms.

One second of simulated time took 5.6 minutes to run on a Sun Sparcstation 10.

Results

As in many parts of the brain, the IPSP from TC neuron to RE neuron is made up of two parts, a fast component mediated by GABA_A receptors and a slow component mediated by GABA_B receptors. Benzodiazepines, such as clonazepam, exclusively affect the fast GABA_A receptor component. The direct effect of clonazepam is an augmentation of the duration of the single GABA_A channel open time, rather than an augmentation of the channel

conductance.¹⁷ This effect may indirectly produce an augmentation of the macroscopic current, due to the summation of currents through a large number of channels, more of which are open at any given time.¹⁸ Since each of these many channels is open for a longer time, there will be relatively more channels open at various times during the PSP, resulting in a larger peak as well as greater total current. Both the increase in duration and the increase in magnitude can be readily reproduced by a simple two-state model of the GABA_A response (Fig. 1).

While the synaptic connection from RE neuron to TC neuron is clearly a mixed GABA_A/GABA_B receptor response, the inhibitory connections within thalamic reticular nucleus appear to be mostly GABA_A receptor mediated.¹⁹ The apparent differential clonazepam effect on RE neuron and TC neuron could be due to different GABA_A-receptor subtypes in the two locations. Alternatively, it might simply be a consequence of GABA_A receptors playing a greater role in the inter-RE connections where GABA_B is absent. Therefore, we tested augmentation of GABA_A response at both locations as well as augmentation at the RE neuron site alone. These initial efforts to replicate the experimental results were unavailing. When simulated clonazepam effect was restricted to the thalamic reticular nucleus alone, little effect was seen. With a clonazepam effect on all GABA_A synapses, an augmentation of the initial IPSP was seen with the secondary IPSP being larger and earlier (Fig. 2). These changes were opposite to those observed experimentally.

The reduction in TC neuron IPSP demonstrated experimentally required that the duration of the RE neuron spike train associated with the initial

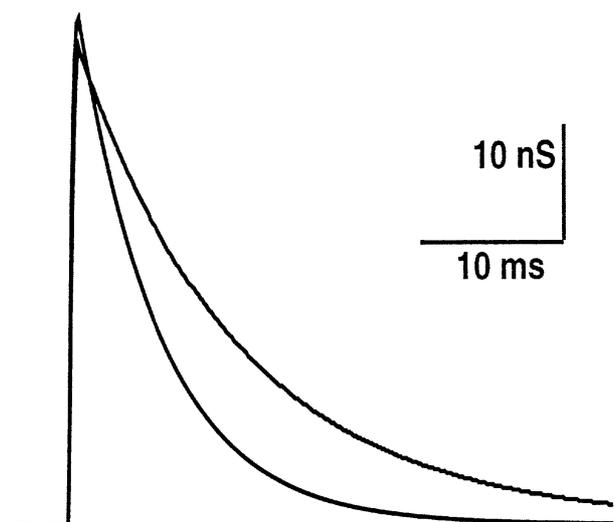


FIG. 1. Synaptic model of clonazepam's augmentation of GABA_A response. Parameters were taken from Otis and Mody.²⁴

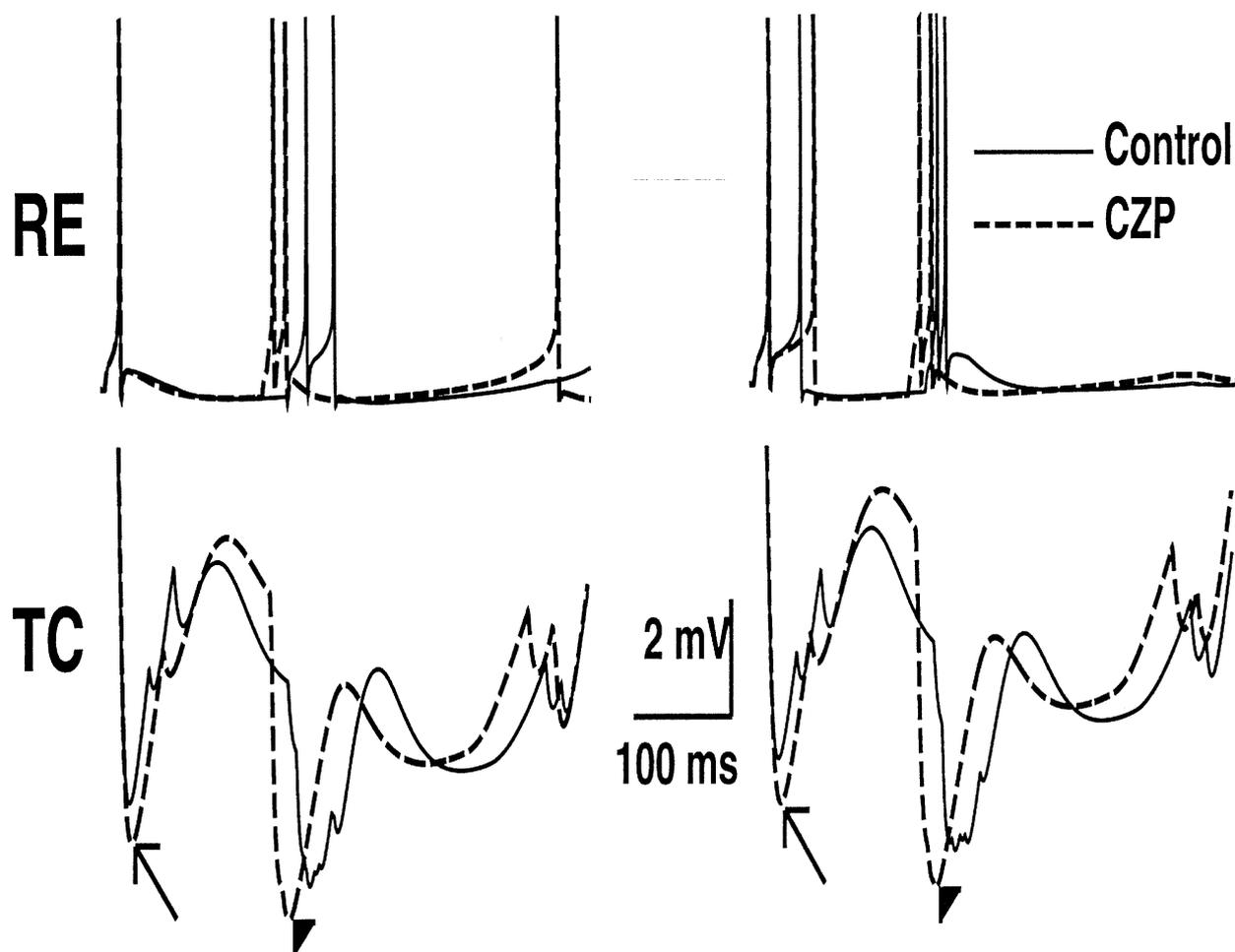


FIG. 2. Simulation of clonazepam effect at both RE→RE and RE→TC locations with orthodromic stimulation. Voltage traces of two representative examples for each cell type are shown (out of five REs and 45 TCs). Primary TC IPSPs (arrows) and secondary TC IPSPs (arrowheads) are increased relative to controls. These changes are opposite to those seen physiologically. (Voltage scale applies to lower traces only. Thin dashed horizontal line between upper traces shows resting potential for TCs.)

stimulation be reduced. In Fig. 2, although the RE neuron spike times are different, the same number of spikes occur both with and without clonazepam effect. We hypothesized that more powerful and more rapid stimulation of the RE neurons might allow inter-RE inhibition to take effect more quickly, thereby producing the internal inhibitory effect that would reduce the TC neuron IPSP. Therefore, rather than simulating orthodromic corticoreticular input, we simulated antidromic stimulation of the RE neurons.

Using simulated antidromic stimulation, we were able to alter the initial RE neuron spike train with the clonazepam-mediated GABA_A augmentation. A two-spike initial burst in RE neurons was reduced to a single spike in the presence of clonazepam (Fig. 3). TC neurons that received projections from the RE neurons with fewer spikes demonstrated a reduction in IPSP comparable to that recorded experimentally (Fig. 4, arrows). Those receiving

projections from unaffected RE neurons did not. The secondary IPSP was sometimes reduced in amplitude as observed experimentally (Fig. 4, trace 2; arrowhead). More generally, this secondary IPSP was shortened without amplitude alteration (Fig. 4, other arrowheads). Except in a few cases, this secondary IPSP occurred earlier, rather than being delayed as seen experimentally.

Recent studies have determined that the reversal potential for inter-RE GABA_A projections is considerably less hyperpolarized than it is for RE→TC projections.²⁰ The values used in the parameter explorations above were therefore reasonably accurate for the projection onto TC cells, but were too hyperpolarized for the projections between RE cells, which would tend to be largely shunting. The simulations were, therefore, repeated with inter-RE GABA_A reversal potential set to the level of the resting membrane potential. These simulations reproduced the major findings: reduction in RE

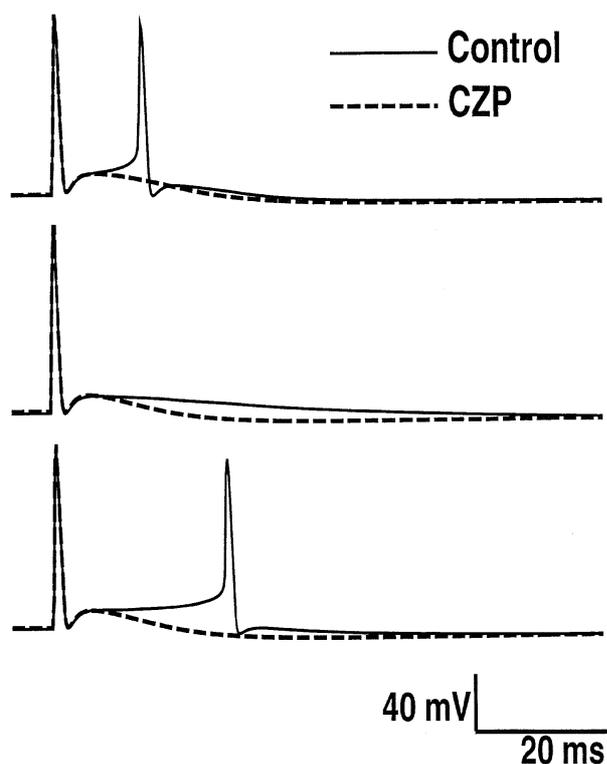


FIG. 3. Simulation of clonazepam effect at RE→RE location with antidromic stimulation of REs: RE cells. Number of spikes in most REs is reduced from 2 to 1 (traces start at resting membrane potential for the cell).

firing, reduction in the primary TC IPSP and a small reduction in amplitude or size of the secondary IPSP.

Discussion

Computer modeling of neural systems is a process where the gradual refinement of a model permits it to capture progressively more of the physiology. In this case, the original model could not replicate the diminished TC neuron IPSP, but provided a direct clue that permitted its refinement. Examination of Fig. 2 demonstrated no change in RE neuron activity despite augmentation of inter-RE inhibition. Sparse RE neuron firing in the control condition resulted in a 'floor' effect where pharmacological augmentation of the inhibitory synapse onto the RE neuron had no significant consequences. Additionally, the timing of the excitatory and inhibitory inputs reduced the efficacy of the latter.

Clearly, changes in inter-RE inhibition would only be observable in the initial IPSP in the TC neuron if the initial RE neuron response was altered. Altering RE neuron activity required coordination of the timing of internal inhibition and the excitatory

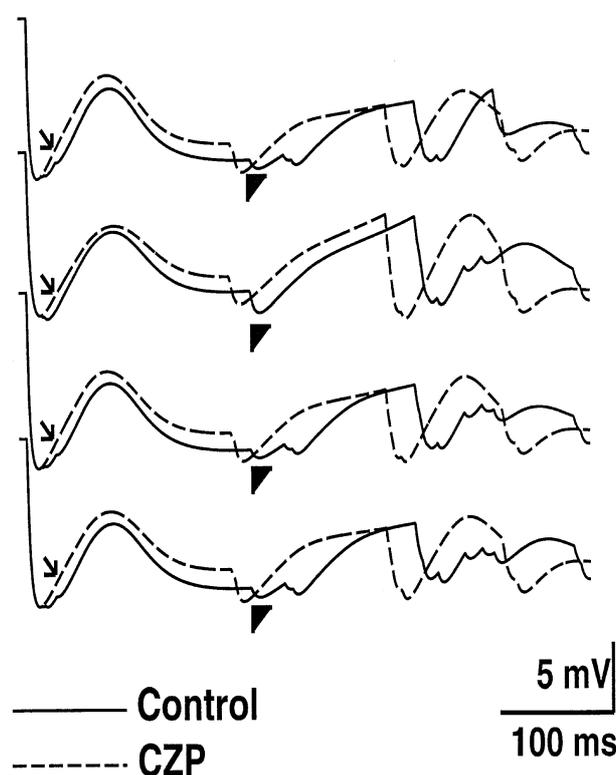


FIG. 4. Simulation of clonazepam effect at RE→RE location with antidromic stimulation of REs: TC cells. Primary TC IPSPs (arrows) and secondary TC IPSPs (arrowheads) are both decreased relative to controls, as seen physiologically (traces start at resting membrane potential for the cell).

response to permit the IPSP to truncate the initial spike train. Since the initial 'train' was only one or two spikes in length, the internal inhibition would have to occur very quickly in order to reduce spike number. Using simulated orthodromic stimulation, the onset of excitation was slow. Consequently, there was overlap between the excitation and the subsequent inhibition that rendered the latter less effective. Antidromic activation had the advantage of being more coherent and briefer. This coherence caused all of the RE neurons to spike simultaneously, leading to more coherence in the subsequent convergent feedback inhibition. The inhibition was also more effective since it did not have to summate with ongoing excitation. The predicted importance of antidromic stimulation could be tested *in vitro* by repeating the experiments in the presence of glutamatergic blocking agents.

Alterations of the model to permit a more prolonged initial RE neuron spike train would be expected to make the alteration in inter-RE inhibition more apparent. This could be achieved by reworking the RE neuron model to make it respond more vigorously or by altering the stimulation paradigm to allow prolonged stimulation, with a

combination of the antidromic activation with long-lasting orthodromic NMDA activation. However, the duration of the initial TC neuron IPSP makes it likely that the initial RE neuron spike train is brief. The dramatic GABA_B non-linearity described by Destexhe and Sejnowski¹⁴ will tend to amplify even minor alterations in RE cell spiking pattern.

The secondary IPSP seen in the model was generally reduced in size with the clonazepam effect, as found experimentally. However, in most cases, this potential was shifted to earlier, rather than later, time of occurrence. This shortening of the interval between IPSPs in the model was due to the following sequence: reduction of the initial TC neuron IPSP→earlier burst in the TC neuron→earlier burst in the RE neuron→earlier secondary IPSP back in the TC neuron. What then might cause the lengthening of the inter-IPSP interval seen experimentally? Two factors could be important: paradoxical delay in onset of the initial TC neuron burst caused by reduced T channel deinactivation, and lengthening of the interburst interval in the RE neurons due to the increased inter-RE inhibition.

Connectivity in this model was based on a columnar organization. In other recent network models, a uniform line of RE neurons has been connected to a parallel line of TC neurons with divergence from each neuron given by a 'footprint' function describing the connections onto the opposing line.²¹ The connectivity used in this study can be readily translated into these terms, by noting that the projection of an RE neuron onto its column and neighboring columns defines a relatively large footprint while the reciprocal connection defines a much smaller footprint. The main difference with these other network studies is that the other studies always used equal numbers of RE and TC neurons, while there are much fewer RE neurons than TC neurons here. This assumption seems reasonable given the large disparity in the volume of the thalamic reticulat nucleus compared with that of principal thalamic nuclei.

Conclusion

The computer model supports the hypothesis of Huguenard and Prince⁵ that the paradoxical decrease in IPSP size would be due to a primary effect of this

drug at the inhibitory connections between RE neurons. However, the model suggests that the specific form of this response may be dependent on antidromic stimulation. These predictions could be tested physiologically with differential placement of stimulating electrodes or by using glutamatergic blockade. Although antidromic activation has been suggested to play a role in some forms of epilepsy,^{22,23} orthodromic activation of this circuit may be expected to prevail under the specific pathophysiological conditions of absence seizures. If a different response to orthodromic activation were confirmed physiologically, this would tend to cast doubt on the direct relevance of the reduced primary TC neuron IPSP as a part of the causal chain of clonazepam's antiepileptic effect. Reduction of IPSPs in subsequent cycles of the aberrant oscillation could still be part of the causal chain, however.

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