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RESULTS from a computer model of a thalamic network predict that agents augmenting GABA_A-mediated inhibition in the reticular thalamic (RE) nucleus will be antiepileptic or desynchronizing. This provides support for the hypothesis that antiepileptics like benzodiazepines may exert their effects through an isolated increase of inhibition in the RE nucleus. When desynchronized, the model thalamocortical neurons showed a decreased probability of firing a low threshold spike, a decreased secondary inhibitory postsynaptic potential and a higher frequency of oscillations. The transition to desynchrony was also accompanied by an increased frequency in the firing of the model RE neurons.

Key words: Absence epilepsy; GABA_A; Synchrony; Thalamus

Computer model of antiepileptic effects mediated by alterations in GABA_A-mediated inhibition

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Introduction

Inhibition is known to play a critical role in the generation and maintenance of the synchronous, rhythmic firing in the thalamocortical system that characterizes absence epilepsy.^{1,2} Various GABA mimetics, including the GABA_A receptor agonist muscimol, aggravate spike and wave discharges (SWD) in the GAERS (Genetic Absence Epilepsy Rat from Strasbourg) model of absence epilepsy.¹ The ability of the thalamus to generate rhythmic activity depends on the generation of the low threshold spike (LTS).² This sort of bursting activity requires hyperpolarization.^{3–5} It is therefore puzzling that benzodiazepines, which act by augmenting inhibition, can act as antiepileptic agents. To explain the antiepileptic effects of benzodiazepines it was proposed that the augmentation of inhibition may be taking place in a localized manner in a particular anatomical structure. It was proposed that this structure was the reticular thalamic nucleus.^{6,7}

The purpose of our project was to test whether such an isolated augmentation of GABA_A-mediated inhibition in the RE nucleus was capable of exerting a desynchronizing effect. All tests were carried out on a computer model of thalamic network. The results of this test were compared with the result of augmenting GABA_A-mediated

inhibition in the thalamocortical (TC) neurons. The consequences to single cell activity of these changes was also studied.

Materials and Methods

Construction of the model Simulations were run on a Sun Sparcstation 10 using the NEURON simulator.⁸ The simulated network consisted of six RE neurons and 18 TC neurons arranged in six columns. Each column consisted of one RE neuron reciprocally connected to three TC neurons. Each RE neuron also received input from the RE and TC neurons in the column immediately adjacent to it. Steps were taken to ensure that the connections for each TC neuron were not exactly the same. This ensured that synchrony did not arise as the result of a false uniformity in the properties of the neurons. The TC neurons of the second row received connections from the RE neuron on the immediate right column while the TC neurons of the third row received connections from the RE neuron in the left column.

Each neuron was represented by a single compartment with an area of 1000 μm². The equations describing the neurons and the voltage sensitive channels included were similar to those used in previous

papers.⁹⁻¹¹ The change in membrane potential with time was described with the following equations:

$$C_m \dot{V}_{TC} = -I_T - I_b - I_{Na} - I_K - I_l - I_{GABA_A} - I_{GABA_B} \quad (1)$$

$$C_m \dot{V}_{RE} = -I_T - I_{Na} - I_K - I_{AHP} - I_{CAN} - I_l - I_{GABA_A} - I_{GABA_B} - I_{AMPA} \quad (2)$$

Intrinsic as well as synaptic currents could be described by the following general equation:

$$I = \bar{g} \times g \times (V - E_{rev}) \quad (3)$$

where \bar{g} is the maximum conductance of the current, g is a factor < 1 that describes the current condition of the synaptic or intrinsic channel and E_{rev} is the reversal potential of the current.

As described in equation 1, the TC neuron had the following voltage sensitive channels and typical maximum conductances (in mS/cm²): the fast sodium I_{Na} , 30.0; delayed rectifier I_K , 2.0; the low threshold calcium I_T , 0.24; and the hyperpolarization activated I_b , 0.12. In the RE neuron: fast sodium I_{Na} , 100.0; delayed rectifier I_K , 10.0; the low threshold calcium I_T , 1.75; slow calcium dependent potassium channel I_{AHP} , 5.0; and the non-specific calcium-sensitive cation channel I_{CAN} , 0.35. Calcium removal in both neuron types was affected by a previously described calcium pump.^{9,10}

All synapses were parametrized using the two-state model described previously.¹² The synaptic projection from the TC neuron to the RE neuron involved an AMPA component. The connection from RE neuron to TC neuron utilized both GABA_A and GABA_B components. Inhibition between the RE neurons was only mediated by GABA_A synapses. An investigation of the inhibition between the RE neurons by Ulrich *et al.*¹³ demonstrated that only a small component of it was mediated by GABA_B. The parameters used for the two-state synaptic model were as follows: AMPA: $\alpha = 1.1 \text{ ms}^{-1} \text{ mM}^{-1}$, $\beta = 0.19 \text{ ms}^{-1}$, $C_{dur} = 1.1 \text{ ms}$, $E_{rev} = 0 \text{ mV}$, $\bar{g} = 0.005 \text{ } \mu\text{S}$. GABA_A: $\alpha = 0.53 \text{ ms}^{-1} \text{ mM}^{-1}$, $\beta = 0.18 \text{ ms}^{-1}$, $C_{dur} = 1.0 \text{ ms}$, $E_{rev} = -90 \text{ mV}$. The value of E_{rev} for GABA_A was based on some experimental values in rat thalamic cells reported by Destexhe *et al.*¹⁴ The maximum conductance \bar{g} for GABA_A-mediated inhibition was varied for both the TC and RE neurons. The values utilized in the various trials are specified in the Results section. The parameters for GABA_B were $\alpha = 0.01 \text{ ms}^{-1} \text{ mM}^{-1}$, $\beta = 0.005 \text{ ms}^{-1}$, $C_{dur} = 150 \text{ ms}$, $E_{rev} = -95 \text{ mV}$, $\bar{g} = 0.005 \text{ } \mu\text{S}$.

Augmentation of GABA_A-mediated inhibition: The augmentation of GABA_A-mediated inhibition in the model was implemented by increasing the maximum conductance \bar{g} of the synapse. This led to an increase

in the amplitude as well as decay time of the inhibitory postsynaptic potentials (ipsp). Such alterations in GABA_A ipsp has been reported by Mody *et al.*¹⁵ following the treatment of brain slices with the benzodiazepine zolpidem.

Evaluating population activity: Rastergrams were used to observe the network activity (Fig. 1a-d). The y -axis of the graphs represented the neurons of the network numbered 0-23, while the x -axis represented time. The neurons of the RE nucleus are numbered 18-23 while the TC neurons are numbered 0-17. For each neuron, a black dot indicates that the membrane potential had exceeded the threshold membrane potential of 0 mV.

A more quantitative method was also used to characterize the activity of the network. A discretized version of the synchrony index used by Golomb *et al.*¹⁶ was used to analyze the data presented in the rastergrams. We utilized a time bin of size 15 ms. A test case of perfect synchrony was found to give a synchrony index (SI) of 1.0 using this modified method. The value of SI was obtained using the following equations:

$$x_i = 1 \quad V_i > 0 \text{ mV} \\ = 0 \quad V_i < 0 \text{ mV} \quad (4)$$

$$\bar{x} = 1/M \sum_{j=0}^M x_j \quad (5)$$

$$X(M) = 1/N \sum_{i=0}^N x_i \quad (6)$$

$$\bar{X} = 1/M \sum_{j=0}^M X_j \quad (7)$$

$$SI^2 = \frac{1/M \sum_{j=0}^M (X_j - \bar{X})^2}{1/N \sum_{i=0}^N 1/M \sum_{j=0}^M (x_j - \bar{x}(i))} \quad (8)$$

Where x is a discrete measure describing whether or not the membrane potential of the neuron has reached threshold. The activity of the population is described by X . Both \bar{x} and \bar{X} describe the time-averaged activities of the single neuron and population, respectively. The variable M is the total number of time bins for which the degree of synchrony was analyzed and N is the total number of neurons. The index i is used to denote each neuron while j is used to denote a time bin.

Results

Effects of increasing the amplitude of $GABA_A$ -mediated inhibition in the RE nucleus: We investigated the effects of augmenting $GABA_A$ -mediated inhibition in the RE nucleus. As the values of \bar{g}_{GABAA} in the RE nucleus were progressively increased, the \bar{g}_{GABAA} of the inhibition in the TC neurons was kept constant at $0.002 \mu S$. Each simulation was begun with a short pulse to the RE nucleus. The population activity was monitored graphically as well as with the use of a synchrony measure (see Materials and Methods).

The \bar{g}_{GABAA} was varied along the range 0.002 – $0.016 \mu S$. At a value of $0.002 \mu S$, the initial stimulus to the RE nucleus gave rise to two cycles of synchronized activity (Fig. 1a). Increasing \bar{g}_{GABAA} to $0.0025 \mu S$ led to a vast improvement in synchrony. The number of cycles of synchronized activity here increased to 6 (Fig. 1b). The frequency of these oscillations was ~ 3 Hz. Such low frequencies are characteristic of absence epilepsy.

All further increments in \bar{g}_{GABAA} led to lower levels of synchrony than that obtained at $0.0025 \mu S$. By the time \bar{g}_{GABAA} had reached $0.004 \mu S$, no cycles of TC neurons activity were elicited following the initial stimulus to the RE nucleus (Fig. 1d). As \bar{g}_{GABAA} was increased beyond $0.004 \mu S$, the system did not return to the high level of synchrony that was obtained at $0.0025 \mu S$ (Fig. 2). The results from the model support the hypothesis that an isolated augmentation of inhibition in the RE nucleus is capable of desynchronizing a network of synchronized neurons.

In comparing the activity of the RE neurons in the synchronized (Fig. 1b) with desynchronized states (Fig. 1c,d), it is evident that the RE neurons fire at a higher frequency and appear more excitable in the desynchronized state.

Changes in single neuron activity with augmented inhibition: A comparison was made of the activity of the TC neurons both in the case of high network synchrony (\bar{g}_{GABAA} in RE nucleus = $0.0025 \mu S$) and in the case of desynchronized activity (\bar{g}_{GABAA} in RE nucleus = $0.004 \mu S$). Many TC neurons that had previously fired a low threshold spike (LTS) failed to do so in the desynchronized network (Fig. 3).

Some TC neurons in the model did not undergo an LTS following the initial stimulus. What was observed instead was a change in the pattern of inhibition displayed in the synchronized *vs* desynchronized states (Fig. 4). The first ipsp in the TC neurons following a stimulus to the RE nucleus is called the primary ipsp. The ipsp following this is called the secondary ipsp. We observed that while the primary inhibition in both cases was the same,

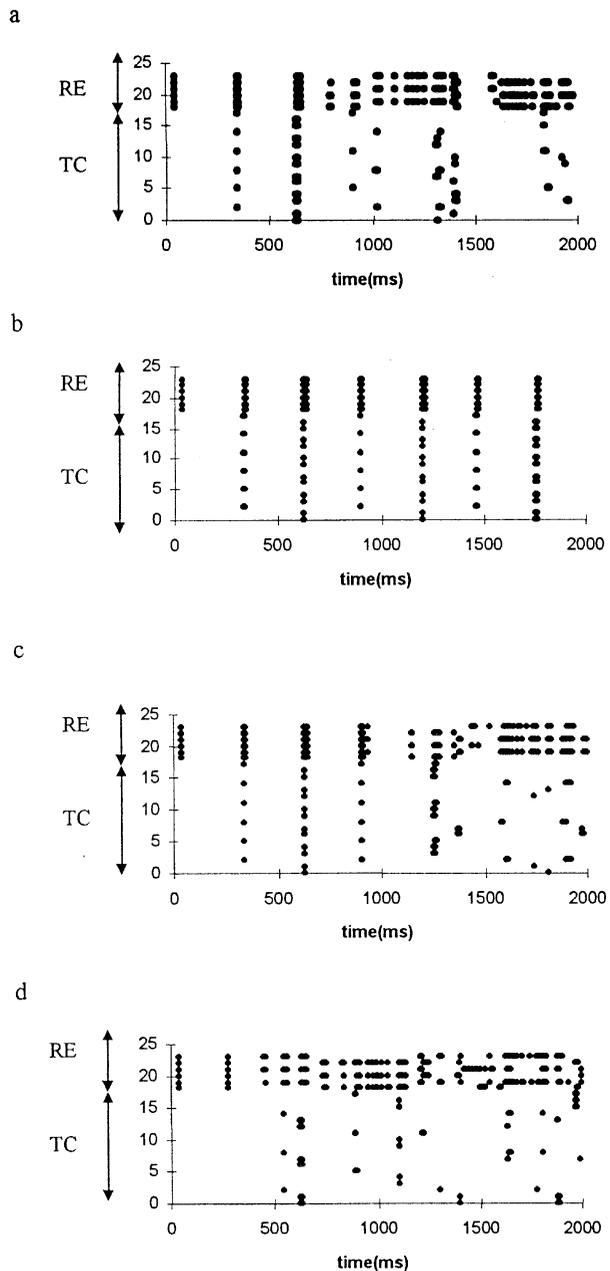


FIG 1. Network activity at different values of \bar{g}_{GABAA} in the RE nucleus while \bar{g}_{GABAA} in the TC neurons is kept constant. A) \bar{g}_{GABAA} in the RE nucleus is $0.0020 \mu S$. B) \bar{g}_{GABAA} in the RE nucleus is $0.0025 \mu S$. C) \bar{g}_{GABAA} in the RE nucleus is $0.0035 \mu S$. D) \bar{g}_{GABAA} in the RE nucleus is $0.0040 \mu S$.

the secondary inhibition had decreased in the desynchronized case.

Augmentation of $GABA_A$ mediated inhibition in TC neurons: Tests were also made to investigate whether the augmentation of $GABA_A$ -mediated inhibition at the TC neurons was able to desynchronize. The value of \bar{g}_{GABAA} between the RE neurons was kept constant at $0.0025 \mu S$ while \bar{g}_{GABAA} from the RE neurons to the TC neurons was varied. The values

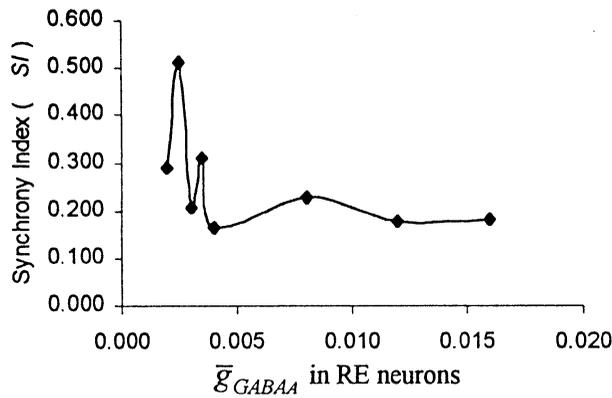


FIG. 2. Changes in the Synchrony Index (see Methods) of network activity as \bar{g} of GABA_A mediated inhibition between the RE neurons is varied.

— \bar{g}_{GABAA} in RE nucleus = 0.0025 μS
 — \bar{g}_{GABAA} in RE nucleus = 0.0040 μS

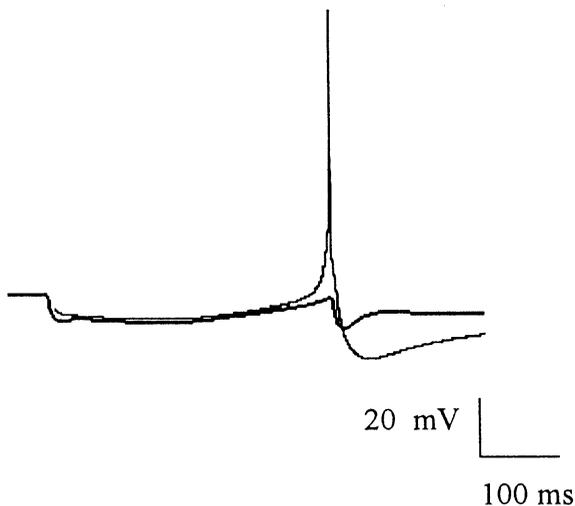


FIG. 3. Comparison of one TC neuron activity when the network was synchronized with the desynchronized case. The value \bar{g}_{GABAA} in RE nucleus is 0.0025 μS in the synchronized state while it is 0.0040 μS in the desynchronized case. Other TC neurons in the network displayed the kind of activity displayed in Fig. 3.

of \bar{g}_{GABAA} from the RE neurons to the TC neurons was varied over the range 0–0.006 μS . A very synchronized network remained synchronized (Table 1). The rastergrams in each case revealed six cycles of synchronized activity very similar to that shown in Fig. 1b. The results from this test indicate that the isolated augmentation of GABA_A-mediated inhibition to the TC neuron does not serve to desynchronize the network.

— \bar{g}_{GABAA} in RE nucleus = 0.0025 μS
 — \bar{g}_{GABAA} in RE nucleus = 0.0040 μS

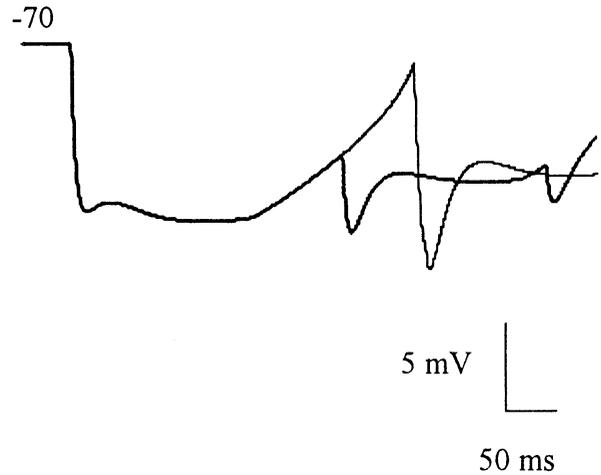


FIG. 4. Comparison of one TC neuron activity when the network was synchronized with the desynchronized case. The value \bar{g}_{GABAA} in RE nucleus is 0.0025 μS in the synchronized state while it is 0.0040 μS in the desynchronized case.

Simultaneous augmentation of GABA_A-mediated inhibition in TC and RE neurons: We examined the effects on synchrony of making simultaneous changes to \bar{g}_{GABAA} for the RE as well as TC neurons. The value of \bar{g}_{GABAA} was varied over the range 0.0025–0.004 μS . The network moved from a highly synchronized state at a value of 0.0025 μS to a desynchronized state at 0.004 μS (Fig. 5). The results are, therefore, in keeping with previous investigations of thalamic models in which the decrease of GABA_A-mediated inhibition was found to improve synchrony in the model.^{16,17}

Discussion

The results of our model support the hypothesis that an isolated increase in the GABA_A-mediated inhibition in the RE nucleus is capable of desynchronizing a network. A very high state of synchrony was obtained at the \bar{g}_{GABAA} value of 0.0025 μS in the RE nucleus (Fig. 1b). All subsequent increases in the amplitude of this inhibition, led to a lower level of synchrony than this. The increment of \bar{g}_{GABAA} to the TC neurons, however, did not lead to the loss of synchrony. The results of the model are, therefore, in keeping with results observed experimentally. Liu *et al.*⁶ observed that SWDs were suppressed with the injection of agents that augment GABA_A-mediated inhibition (γ -vinyl GABA and muscimol) in the RE

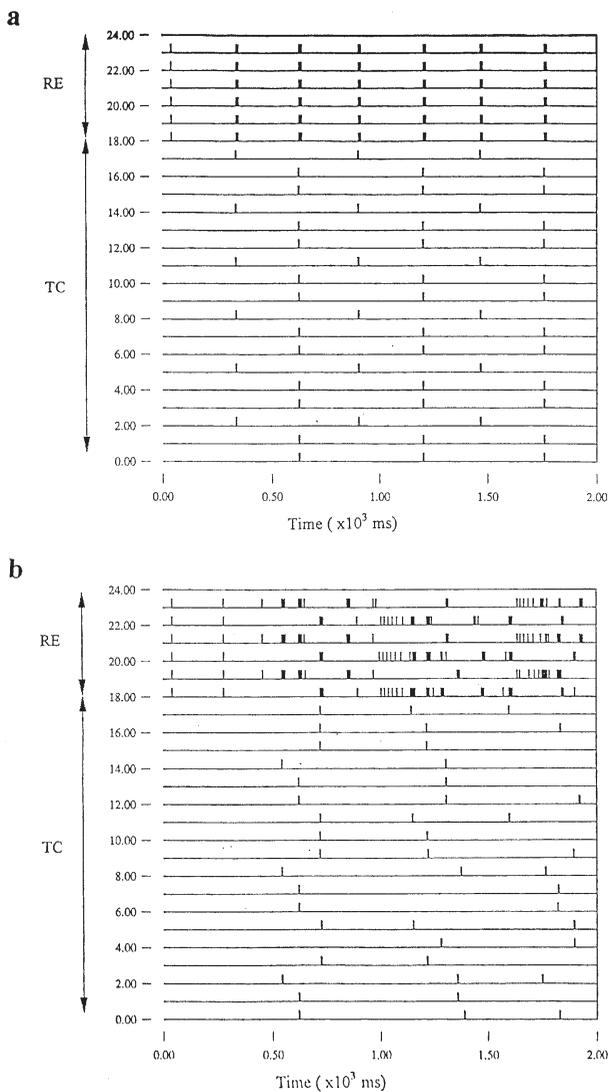


FIG 5. Results from simultaneous changes in \bar{g}_{GABAA} for both the RE as well as TC neurons. a) \bar{g}_{GABAA} in the network is 0.0025 μS . b) \bar{g}_{GABAA} in the network is 0.004 μS .

Table 1. Changes in the Synchrony Index (see Materials and Methods) of network activity as \bar{g} of GABA_A mediated inhibition in the TC neurons is varied.

\bar{g}_{GABAA} in TC neurons (μS)	Synchrony Index
0.002	0.516
0.0025	0.532
0.003	0.513
0.0035	0.490
0.004	0.442
0.0045	0.418
0.005	0.427
0.0055	0.451
0.06	0.458

nucleus of the GAERS rat. Similar injections in the relay nucleus failed to suppress the SWD. The reason for the different results in the RE and TC neurons may be the difference in the nature of inhibition for

the RE and the TC neurons. While inhibition in the TC neurons is both GABA_A- and GABA_B-mediated, inhibition in the model RE neurons is only GABA_A-mediated. The lack of a GABA_B-mediated I_{PSP} in the model RE neurons was based on experimental observations by Ulrich and Huguenard¹³ in slices of the rat thalamus.

The role of GABA_A-mediated inhibition in synchrony has also been studied in previous investigations of models of thalamic networks.^{16,17} They demonstrated that a decrease in total GABA_A-mediated inhibition led to improvements in synchrony. While our study dealt with the results of isolated increments in GABA_A-mediated inhibition, the overall change to synchrony with the changes in \bar{g}_{GABAA} are in keeping with the results of previous studies: the increase in GABA_A-mediated inhibition led to the loss of synchrony.

Even though such a high state of synchrony was not achieved at any values of $\bar{g}_{GABAA} > 0.0025 \mu S$, there were some local improvements in synchrony as \bar{g}_{GABAA} was increased to 0.0160 μS (Fig. 2). This indicates that the increase in GABA_A in the RE nucleus affects factors that are both facilitatory as well as detrimental to the occurrence of synchrony. The increase in inhibition is beneficial to the occurrence of an LTS and rhythmicity. However, by causing a delay in the firing of the RE neurons, it also subjects the RE neurons to the desynchronizing effects of a heterogeneous TC neuron population which has not been similarly delayed. The same reasons may explain the initial improvement in synchrony at $\bar{g}_{GABAA} = 0.0025 \mu S$ between the RE neurons.

It is evident from comparing Fig. 1b, where the network is highly synchronized, with Fig. 1d, where the network has become desynchronized, that the RE neurons fire with a higher frequency in the desynchronized state. This increased activity accompanying the desynchronized state may seem rather surprising, since an increased activity may imply an increased inhibition in the TC neurons and consequently an increased synchrony. It has also been observed that the RE neurons paradoxically fire at a higher frequency during the wake state, a desynchronized state.¹⁸ It is evident from the model, however, that although the transition to desynchrony is accompanied by a higher frequency in the firing of the RE neurons, the lack of synchrony among them leads to lowered inhibition at the TC neurons (Fig. 4). This therefore leads to a lowered tendency for rhythmic firing of the TC neurons. An increase in ipsp's as the result of synchronous firing and summation has also been observed by Mody *et al.*¹⁵

The transition to desynchrony in the model was observed to occur with a lowered probability of firing an LTS as well as a decreased secondary ipsp. Such

a lowered probability of firing an LTS as well as a decrement in the secondary ipsp was also observed by Huguenard and Prince⁷ following the application of the benzodiazepine, clonazepam (CZP) to the rat thalamic slice. In contrast to their observations however, the transition to desynchrony in the model was not accompanied by any changes to the primary ipsp, and resulted in an increase rather than a decrease in the firing frequency of the RE neurons. These differences may be due to the fact that when CZP was applied to the rat thalamic slice, there may have been some minor augmentation of the GABA_A mediated in the TC neurons as well as between the RE neurons. The model however was used to address questions regarding the effects of isolated increases in GABA_A-mediated inhibition. Another possible reason for the lack of change in primary ipsp may be due to the low number of spikes in the initial burst of the RE neurons. It is clear from the rastergrams shown in Fig. 5 that the first cycle of synchronized activity in the RE neurons involves fewer of spikes than subsequent cycles.

Conclusion

Results from our computer model of a thalamic network support the hypothesis that an isolated increase of GABA_A-mediated inhibition in the RE nucleus of the thalamus can desynchronize the network. Agents that bring about this effect may therefore act as antiepileptic agents.

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