Increased pyramidal neuronal excitability and enhanced NMDA conductance can account for post-traumatic epileptogenesis without disinhibition: a computational model

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Abstract

Partially isolated cortical islands prepared in vivo become epileptogenic within weeks of the injury. In this model of chronic epileptogenesis, recordings from cortical slices cut through the injured area and maintained in vitro often show evoked, long- and variable-latency multiphasic epileptiform field potentials that can also occur spontaneously. These events are initiated in layer V and are synchronous with polyphasic long duration excitatory and inhibitory potentials (currents) in neurons that may last several hundred milliseconds. Stimuli that are significantly above threshold for triggering these epileptiform events evoke only a single large excitatory postsynaptic potential (EPSP) followed by an inhibitory postsynaptic potential (IPSP).

We investigated the physiological basis of these events using simulations of a layer V network consisting of 500 compartmental model neurons, including 400 principal (excitatory) and 100 inhibitory cells. Epileptiform events occurred in response to a stimulus when sufficient NMDA conductance was activated by feedback excitatory activity among pyramidal cells. In control simulations this activity was prevented by the rapid development of IPSPs. One manipulation that could give rise to epileptogenesis was an increase in the threshold of inhibitory interneurons. However, previous experimental data from layer V pyramidal neurons of these chronic epileptogenic lesions indicate: 1) up-regulation, rather than down-regulation, of inhibition; 2) alterations in the intrinsic properties of pyramidal cells that would tend to make them more excitable; and 3) sprouting of their intracortical axons and increased numbers of presumed synaptic contacts, which would increase recurrent EPSPs from one cell onto another. Consistent with this, we found that increasing the excitability of pyramidal cells and the strength of NMDA conductances, in the face of either unaltered or increased inhibition, resulted in generation of epileptiform activity that had characteristics similar to those of the experimental data. Thus, epileptogenesis such as occurs following

chronic cortical injury can result from alterations of intrinsic membrane properties of pyramidal neurons together with enhanced NMDA synaptic conductances.

Introduction

Cortical injury following brain trauma often results in epilepsy, but little is known about the mechanisms underlying epileptogenesis associated with this or other naturally occurring pathologies. It is clear that the incidence of post-traumatic seizures is related to the severity of the lesion; trauma that produces direct cortical injury, such as cortical contusion, hematoma, or penetrating cortical wounds is associated with a high incidence of late-onset epilepsy, while less severe trauma resulting in cerebral concussion carries only a small risk of subsequent seizures (Annegers et al. 1999; Salazar et al. 1985). Partially isolated neocortical islands with intact pial blood supply are a recognised in vivo model of injury-induced epileptogenesis in cat and monkey (Echlin and Battista 1963; Halpern 1972; Sharpless 1969). The injured cortex becomes increasingly hyperexcitable over a few weeks, and develops evoked prolonged ictal events and spontaneous interictal discharges (Sharpless and Halpern 1962). The histological appearance of the surgically-lesioned area resembles, in some aspects, that found in a widely-used cortical impact model in which transcortical injury leads to intracortical lesions as well as white matter cavitation that undercuts the cortex (Feeney et al. 1981).

Recent in vitro studies of chronic partial neocortical isolations have revealed a number of characteristic properties of epileptogenic slices (Prince and Tseng 1993; Hoffman et al. 1994; Prince et al. 1997): Electrical stimulation of the white matter or pial surface evokes epileptiform events lasting hundreds of milliseconds that resemble interictal EEG discharges. The intracellular correlates of these events, or similar ones that occur spontaneously, are polyphasic excitatory and inhibitory potentials, that presumably arise from feedback synaptic activity within the circuitry of the slice. Current source density analysis shows that these evoked events are initiated, after a long ($\approx$ 100 ms) and variable latency, in layer V of the cortex and then propagate to other layers (Prince and Tseng 1993; Hoffman et al. 1994).

Although a reduction in functional inhibition is often assumed to underly epileptiform activity (*e.g.*, Prince and Connors 1986), in this model evidence suggests that inhibition is relatively preserved. For example, stimuli significantly above threshold for triggering epileptiform events can block the normal evoked activities and give rise to EPSPs followed by large inhibitory events containing both the $GABA_A$ and $GABA_B$ receptor-mediated components (Prince and Tseng 1993). Recordings of inhibitory activity in these post-lesional epileptogenic slices (Prince et al. 1997) demonstrated that epileptiform discharges are associated with large-amplitude, polysynaptic inhibitory postsynaptic currents (IPSCs) in layer V pyramidal neurons, and that the frequency of spontaneous and miniature inhibitory postsynaptic currents (sIPSPs and mIPSPs), recorded using the "blind" slice-patch technique, is increased. Results of immunocytochemical experiments (D. Prince and I. Parada, unpublished data) show increases in glutamic acid decarboxylase, the synthetic enzyme for GABA, that persist for weeks after injury within the undercut cortex. Further, parvalbumin and calbindin immunoreactivity are enhanced in inhibitory interneurons and in the neuropil (Prince et al. 1997). This evidence suggests an up-regulation of GABAergic inhibition and perhaps development of new inhibitory connectivity, rather than any decrease in efficacy.

The in vitro results have also shown that there are changes in the intrinsic properties and presumed alterations in the synaptic connections of layer V pyramidal cells in the partially isolated cortex. These neurons show a 123% mean increase in input resistance and a 59% mean increase in membrane time constant relative to controls, as well as a 37% mean reduction in soma area (Prince and Tseng 1993). Similar changes in intrinsic properties also occur in identified, chronically axotomized corticospinal pyramidal neurons (Tseng and Prince 1996). Such changes would be expected to increase the intrinsic excitability of the pyramidal cells. In addition, layer V pyramidal cells from chronically injured cortex sprout additional axon collaterals, especially in the peri-somatic region, increasing total axon length by 56%, number of axon collaterals by 64% and total number of presumed boutons by

115% (Salin et al. 1995). These changes might be expected to increase recurrent excitatory interactions among pyramidal neurons.

In the studies described here we used a computer simulation of a layer V cortical circuit to determine if the cellular and network changes observed in the above experiments are sufficient to reproduce the characteristics of epileptiform behavior in this experimental model, and to determine how these changes can contribute to the epileptogenesis. The layer V neuronal network was chosen as a focus for these initial experiments because, as mentioned above, it appears to be the site of origin of interictal epileptiform discharges in this model, and information about neuronal properties and connectivity in this layer is available. Previous biophysically realistic models of epileptiform events in hippocampal circuits, notably by Traub and colleagues (Traub et al. 1993, 1994), have established a number of principles upon which this work builds, including the importance of synchronous bursts of action potentials in pyramidal cells and of EPSPs generated through reciprocal connections between these cells. The contribution of NMDA conductances to these EPSPS has also been shown to be crucial for maintaining activity over hundreds of milliseconds (Traub et al. 1993, 1994). Such studies have focused on epileptiform activities induced in the hippocampal CA3 region by convulsant drugs and ionic manipulations. Models of epileptogenesis that occur in neocortical circuits following trauma have not been examined. Our work focuses on the changes occurring in the cortical circuit that can produce epileptiform behavior under circumstances in which strong functional inhibition persists (Traub et al. 1987a,b).

Methods

A network consisting of 500 cells was simulated, including 100 intrinsically bursting (IB) cells, 300 regular firing (REG) cells and 100 fast spiking inhibitory (INHIB) cells. The latter number is based on the observation that about 20% of area 17 neurons are GABAergic (Gabbott and Somogyi 1986). The 1:3 ratio of IB to REG cells was chosen somewhat arbitrarily, based simply on the fact that fewer IB than REG cells are seen in physiological studies of layer 5 neurons (*e.g.*, Tseng and Prince 1993) although of course physiological studies cannot escape sampling biases. The exact proportions are not important to the results.

IB cells were modelled by a 9 compartment reconstruction of a layer V pyramidal cell (Bush and Sejnowski, 1993, 1994, 1996). REG cells, which are smaller with thinner and shorter apical dendrites (Chagnac-Amitai et al., 1990; Mason and Larkman, 1990; Kasper et al., 1994; but see Tseng and Prince, 1993), were modelled by an 8 compartment layer II pyramidal cell (Bush and Sejnowski, 1993, 1994), because it also possesses a short, thin apical dendrite and thus has similar geometry to layer V REG cells. (We did not have access to such a reconstructed layer V cell. The reconstruction governs only the cell geometry; cell conductances are determined, constrained by experimental values, so as to replicate physiological responses, as described below and in Results.) INHIB cells were modelled by a 7 compartment fast-spiking interneuron (Bush and Sejnowski 1996; Kawaguchi 1995). Input resistances and membrane time constants for each cell type are indicated in Table 1.

Place Table 1 about here

Each model neuron contained Hodgkin-Huxley-type active conductances at the soma implemented as described previously (Bush and Sejnowski 1994, 1996), including, in the REG cells, a calcium-dependent potassium conductance that produced adapting spike trains. Reductions in spike frequency adaptation and its underlying slow calcium-activated potassium conductance are observed in the experimental preparation (Prince and Tseng 1993). Preliminary simulations incorporating this reduction found that it was not crucial to the mechanism of interictal epileptogenesis developed here (see below), due to its relatively weak effect over the first 10-20 ms; therefore, this reduction was neglected here. The INHIB cells fired non-adapting high frequency spike trains. The IB cells, in addition to somatic spike conductances, also contained inward (gNa) and outward (gK) conductances located in the apical dendritic compartment 200μm from the soma (reversal potentials and densities: gNa 45 mV, 0.015

S/cm^2; gK -90 mV, 0.03 S/cm^2). These conductances, activated by depolarization, were responsible for the bursting action potential discharges shown by these cells. The conductance densities were assigned to produce a three-spike somatic burst in response to suprathreshold input. Other cell types had passive dendrites. Resting membrane potential for all cells was -60 mV; we have experimented with different resting potentials and found that the results do not depend on this parameter.

The absence of active conductances in dendrites, except to achieve bursting in IB cells, means that we are modeling at an intermediate level of complexity. The main effect of our cell geometry is simply to differentiate synapses by their distance from the soma: synapses on somata or proximal dendrites are more powerful than, and can shunt, synapses on distal dendrites. A lesser level of complexity would omit dendrites altogether and hence omit such distinctions between synapses; a greater level of complexity would explicitly address synaptic integration on active dendrites, a very active experimental and theoretical topic (*e.g.*, Cash and Yuste 1999; Cook and Johnston 1999; Larkum et al. 1999). We chose the present level of complexity largely for simplicity, as our focus is on circuit mechanisms of epileptogenesis. We return to this issue in the Discussion.

Synaptic conductances were implemented using the SNS software (Lytton 1996), which uses a kinetic model of receptor binding assuming that a square pulse of transmitter (of amplitude 1.0 and duration 1 ms for all conductances except $GABA_B$, for which it was 85 ms) is released with each presynaptic action potential. Note, there is only one "synapse" between a connected cell pair, so each "synapse" represents a unitary conductance rather than release at a single terminal. This model neglects stochasticity of unitary conductances. The kinetic models for each conductance packaged with the SNS software were used with parameters unchanged, yielding binding (activation) and unbinding time constants and reversal potentials for each conductance as follows: AMPA, 1 ms mM, 2 ms, 0 mV; NMDA, 0.25 ms mM, 150 ms, 0 mV; $GABA_A$, 1 ms mM, 2 ms, -70 mV; $GABA_B$, 62.5 ms mM, 213 ms, -90 mV. The GABA_B conductance inactivated with depolarization as described in Bush and Priebe (1998). All synaptic delays were 1.2$\pm$ 0.6 ms (Gaussian distribution), with a fixed minimum of 0.5 ms.

Initial (white matter) stimulation was simulated by activating 3 extrinsic synapses for 5 ms according to poisson statistics with a mean rate increasing in proportion to stimulus strength. For IB and REG cells, one extrinsic synapse was placed on each of the two basal and one oblique dendrites of each cell. For INHIB cells, all 3 extrinsic synapses were on the soma. The peak amplitude of conductances at each of these synapses was 8 nS for IB cells, 4 nS for REG cells, and 1.5 nS for INHIB cells. These values were chosen to provide realistic-sized EPSPSs in the target cells.

Within the cortical network, synapses were assigned randomly with a probability of connection between any two cells (of any type) of 0.1 (Thomson et al. 1988; Komatsu et al. 1988; Mason et al. 1991; Deuchars and Thomson 1995). The multiple synapses that may actually exist from one cell to another are here represented by at most a single equivalent synapse between any two cells. The compartment receiving the synapse was chosen randomly with equal probability from among those eligible, as described below. The network architecture is shown in Fig. 1A. Pyramidal cells (as a group) were reciprocally connected with INHIB (basket) cells, making AMPA synapses on any compartment of INHIB cell dendrites, and receiving GABA_A synapses on their own somata and proximal dendrites ("proximal" refers to the compartments adjacent to the soma). Sixteen of 100 INHIB cells made GABA_B synapses on the basal and oblique dendrites of the pyramidal cells (Solis et al. 1992; Benardo 1994). (GABA_B synapses, and oblique dendrites of excitatory cells, which branch from the apical dendrite, are omitted in Fig. 1A for clarity.) Pyramidal cells made synapses on the basal and oblique dendrites of other pyramidal cells (Kisvarday et al. 1986) that were both AMPA and NMDA mediated (Bekkers and Stevens 1989; Kim et al. 1995). NMDA-receptor mediates conductances were not included at either synapses activated by extracortical afferents, or at synapses on INHIB cells (Ling and Benardo 1995; Thomson et al. 1996).

The peak amplitudes of the individual synaptic conductances were assigned randomly according to a Gaussian distribution with a standard deviation equal to half the mean. Mean values for synaptic conductances were varied over a large range during the course of the study. Specific values are given in the Results for each instance where they differ from the "standard" values shown in Table 1 (derivation of these values described in Results, around Fig. 5; these values correspond to 1.0 in Fig. 5). The amplitudes of $GABA_B$ conductances were assigned to produce a 5-10 mV hyperpolarization following strong stimulation (Douglas and Martin 1991). Inhibitory conductances on IB cells were smaller, reflecting the reduced inhibition seen in these cells experimentally (Silva et al. 1988; Connors and Gutnick 1990; but see Salin and Prince 1996).

All simulations were run using NEURON (Hines 1984; Hines and Carnevale 1997) on a DEC ALPHA 250. We used a time step of 0.1 ms with 2nd order correct numerical integration. A simulation of 500 ms took 10 minutes of CPU time.

Results

During the epileptiform events seen in slices from chronically injured neocortex, neurons generate depolarizations lasting 100's of ms. In most instances, these discharges are eliminated by NMDA receptor antagonists and are therefore dependent, at least in part, on the activation of NMDA receptor-mediated conductances (Hoffman et al., 1994; Jacobs et al., 1996, 1999). Similar events have been included in theoretical analyses of epileptiform discharges using computer modelling (Traub et al. 1994). In these theoretical studies, the NMDA conductance is activated via recurrent collaterals between pyramidal cells (Fig. 1A). Figure 1B shows a schematic of the response of a pyramidal cell (from normal cortex) to a white matter stimulus (Huettner and Baughman 1988). The stimulus evokes monosynap-

Place Fig. 1 about here

tic EPSPs in pyramidal and basket cells. Some basket cells fire due to their relatively low threshold (McCormick et al. 1985). The resulting IPSPs truncate the EPSPs in pyramidal cells (Wong and Prince 1979; Douglas and Martin 1991), thus decreasing the probability of action potential firing and activation of significant amounts of NMDA conductance via recurrent collaterals. Any manipulation that reduces the amount of the stimulus-evoked inhibition will lead to the firing of an increased number of pyramidal cells (Douglas and Martin 1991) and consequent increased activation of NMDA conductance. Thus, the initial relative recruitment of excitatory and inhibitory cells is crucial in determining whether late depolarizing epileptiform events will occur.

A large number of preliminary simulations were done without NMDA conductances present to determine whether they are essential for producing the epileptiform behavior described here. These simulations varied AMPA and GABA conductance amplitudes over wide ranges and also varied the degree of connectivity between each cell type and the percentages of cells of each type. However, in no cases did such simulations produce neuronal activity lasting longer than a few tens of milliseconds that could also be truncated by inhibition at higher stimulus amplitudes (the characteristic of the experimental epileptiform model studied here, see Introduction). Therefore all simulations presented below relied on the activation of NMDA conductances to produce epileptiform activity as described above.

We began by setting the model parameters to reproduce normal (control) responses. Our criteria for such parameters were that the model should fit the initial EPSP/IPSP sequence, as described by: (1) production of a short (≈ 10 ms) EPSP in response to a brief stimulus; and (2) truncation of this EPSP by GABA$_A$ and GABA$_B$ IPSPs at stronger stimulus strengths, as in Fig. 1B (these model behaviors are shown in Fig. 4B). As will be described later, a range of parameters met these criteria. We choose one such set, the "standard" parameters shown in Table 1, as our starting point. How might this network be modified to reduce stimulus-evoked inhibition and thus allow epileptogenesis?

One way to reduce this inhibition is simply to increase the membrane potential of the inhibitory cells. We did this by injecting constant hyperpolarizing current into all the INHIB cells sufficient to change their membrane potential by 10 mV (Fig. 2B), while also increasing NMDA conductances. Under these conditions, in response to a white matter stimulus, few inhibitory cells are fired (Fig. 2A). The excitatory cells are then free to excite each other and activate NMDA conductance. This produces long (in this case 200 ms) depolarizations and spiking in the pyramidal neurons (Figs. 2C,D). We found that reducing the input resistance of the inhibitory cells while increasing NMDA conductances was similarly effective in producing epileptiform activity. Note that the synchronous burst occurring at the end of the 200 ms depolarization generates sufficient depolarization in the inhibitory cells to cause many of them to fire (Fig. 2A).

Place Fig. 2 about here

As in the experimental data, increasing the stimulus strength produces only a large EPSP followed by inhibition (Fig. 3). This is because the stronger stimulus (in this case 50% stronger than in Fig. 2) is able to overcome the hyperpolarization of the inhibitory cells, causing them to fire and inhibit the pyramidal cells.

Place Fig. 3 about here

In our simulations, the activation of GABA_B conductance made a strong contribution to the termination of the epileptogenic activity (*e.g.*, Domann et al. 1994; Witte 1994). This prolonged inhibitory conductance was needed to hyperpolarize pyramidal cells for a period long enough for the activated NMDA conductance to wear off. Shorter-lasting inhibition would have allowed the NMDA-driven epileptogenic activity to continue after a brief interruption by inhibition. An alternative mechanism for termination of activity is NMDA desensitization, used by (Traub et al. 1994). Although supported by experimental evidence, this mechanism is too slow to be significant for the phenomena we address: it has a time constant of 350 ms, while the epileptiform activity modelled here is often complete within about 200 ms. Thus, for simplicity no desensitization was included in our model.

As mentioned in the Introduction, electrophysiological and immunocytological evidence suggests that GABAergic inhibition may actually be enhanced in epileptogenic areas associated with chronic cortical injury (Prince et al. 1997). This casts doubt on any model, of this form of epileptogenesis that relies on a decrease in the effectiveness of postsynaptic inhibition or inability to excite inhibitory neurons, as in Figs 2-3. Thus, we did not further pursue such models, and instead turned to those more consistent with the evidence for this system.

There are no data relating to possible alterations in excitability (and consequently thresholds) of the inhibitory interneurons in epileptogenic slices, but there is substantial evidence (see Introduction) that the intrinsic excitability of the excitatory (pyramidal) cells is increased. We therefore increased the specific membrane resistance by 2.5 times and halved the specific membrane capacitance (yielding an increase of membrane time constant by 1.25 times) to mimic the experimentally observed increases in input resistance and time constant in the pyramidal cells (Prince and Tseng 1993). The somatic membrane area was also reduced to mimic the changes observed experimentally in chronically injured neurons (Tseng and Prince 1996), but this had little effect on electrical properties because the soma makes a very small contribution to total cell membrane area. The increase in the number of pyramidal axon collaterals and presumed boutons (Salin et al. 1995) found in layer V pyramidal neurons of the injured cortex is presumably associated with an increase in the number of excitatory synapses in the network. This could result in an increased density of connections within the network (McKinney et al. 1997), or an increase in the strength of unitary EPSPs between cells due to a larger number of excitatory synapses between pairs of neurons. We modelled both of these possibilities as an increase in the amplitude of synaptic conductances (Bush and Sejnowski 1996). There may be increases (or decreases) in the densities of post-synaptic receptors accompanying these changes as well (Liang and Jones 1997).

We modelled these effects by systematically varying the amplitudes of the synaptic conductances in the model (details described below) and recording the parameters that produced results compatible with the experimental data. Two criteria were used to test for compatibility between the model and previously reported epileptogenic data: 1) Brief stimuli should elicit long-lasting (on the order of 100 ms) depolarizing events associated with spiking in pyramidal neurons (Fig. 4A, 0.5T); 2) Stronger stimuli should evoke only an EPSP followed by an IPSP (Fig. 4A, 1.0T). In order to meet these criteria, we found that, in addition to the intrinsic changes in membrane properties discussed above, which act to increase the effects of excitatory inputs onto pyramidal cells, it was necessary to increase the amplitude of the NMDA receptor-mediated conductance onto these cells. Figure 4 shows the performance of the model with (Fig. 4A) or without (Fig. 4B) incorporating these changes. The increased excitability results in pyramidal firing in response to stimuli that are too small to activate significant numbers of inhibitory cells. Thus, pyramidal firing continues and enough NMDA conductance is activated to produce epileptiform late depolarizations and continued spiking. Stronger stimuli recruit enough inhibitory cells to prevent significant pyramidal neuron firing, and thus evoke only an EPSP/IPSP sequence. In the control case (Fig. 4B), inhibitory cells are activated at all stimulus strengths at which pyramidal cells receive suprathresahold input, so no significant pyramidal firing occurs.

Place Fig. 4 about here

It was not possible to reproduce the experimental epileptiform data by simply increasing the amplitude of NMDA or any other conductance. Instead, it was also necessary to introduce changes in the intrinsic membrane properties of the pyramidal cell population. Epileptiform activity could be produced simply by increasing the NMDA receptor-mediated conductance to a point where it overcame all evoked inhibition. This has been demonstrated experimentally by lowering Mg^{2+} concentrations to induce NMDA-dependent interictal bursts (Anderson et al. 1986; Neuman et al. 1989; Traub et al. 1994). However, without the change in relative excitability of the excitatory and inhibitory neuronal populations, the epileptiform activity could not be prevented by stronger stimuli, contrary to the experimental data. The change in pyramidal cell intrinsic excitability is needed so that there will be a range of stimulus strengths that activates excitatory cells without directly activating inhibitory neurons, resulting in epileptiform activity; while stronger stimuli, which directly or synaptically activate inhibitory cells, will prevent such activity. On the other hand, the changes in intrinsic membrane properties alone did not lead to epileptiform discharges unless there was a concommitant increase in NMDA receptor-mediated conductance. Thus, in this paradigm, changes are required both at the level of the synaptic network (i.e. increased postsynaptic NMDA receptor-mediated conductance, whether due to presynaptic or postsynaptic alterations) and in the membrane properties of single cells.

The amplitudes of the AMPA, NMDA and GABA$_A$ synaptic conductances on the pyramidal cells were systematically varied to determine which parameters were compatible with our criteria for control and epileptogenic conditions. Simulations were run for all possible combinations of these parameters for the values shown in Fig. 5, generating a 3 dimensional 'cube' of data. Only a narrow range of values for the excitatory conductances was compatible with epileptogenesis. In general, a value smaller than optimal produced little firing in response to stimulation, while a value larger than optimal produced epileptiform activity even at higher stimulus strengths. In contrast, the amplitude of the GABA$_A$ conductance could be varied widely, and large values were not incompatible with epileptiform behavior (Traub et al. 1987a,b). This is consistent with experimental data suggesting that epileptogenic discharges occur even in the face of inhibition in this preparation (Prince et al. 1997) and in other epilepsy models (Esclapez et al. 1997).

Place Fig. 5 about here

Paradoxically, control values were harder to constrain than epileptogenic ones, because only the initial EPSP/IPSP sequence could be used as a metric to assess goodness-of-fit (criteria described previously, and illustrated in Fig. 4B). There was some evidence that the amplitude of AMPA conductance in the control case is larger than in the epileptogenic case, because simulations in which GABA$_A$ conductance was blocked (not shown) required these larger values to produce the large and long-lasting EPSPs seen experimentally in this condition (Douglas and Martin 1991). There is stronger evidence that the amplitude of the NMDA conductance is increased in the epileptogenic case relative to controls: Control simulations, using parameters as in Fig. 4B except setting NMDA conductance large enough to allow epileptogenesis, produced EPSPs that were unrealistically large and prolonged (not shown). We also reran all simulations of Fig. 5 with the amplitude of the pyramidal-INHIB AMPA conductance decreased by 50% and then increased by 50%. No satisfactory epileptiform behavior could be produced with either of these parameter values.

Evoked epileptiform field potentials and associated synchronized activity/firing of groups of cells often show a long and variable latency following the stimulus (Prince and Tseng 1993). This feature is replicated in the model (Fig. 6) which shows that, during this latency period after the initial stimulus, REG cells are firing due to NMDA-induced depolarizations. This firing creates a positive-feedback via recurrent excitatory synapses that culminates in a synchronized discharge when the IB cells are recruited. They in turn cause enough depolarization in the INHIB cells to fire many of them and terminate the burst.

Place Fig. 6 about here

Discussion

It is important to emphasize that a large number of pathophysiological processes are potentially activated by any type of cortical injury, and that these probably vary significantly from model to model, and in various human epilepsy syndromes (see Prince, 1995, 1997, 1999 for discussion). It is obviously not possible to deal experimentally or theoretically with all potential underlying mechanisms. As a start, we have focused our attention on a model of epileptogenesis due to direct cortical trauma in which there is a reasonable amount of anatomical and electrophysiological data available.

Simulations showed that chronic epileptogenesis in the partially isolated cortical island can be understood as resulting from at least two factors: (1) An increase in the excitability of pyramidal cells, allowing these to be activated by weak stimuli that do not strongly recruit interneurons; and (2) an increase in the strength of NMDA conductances at recurrent synapses between pyramidal cells, allowing excitation of pyramidal cells to persist for 100's of milliseconds. The model produced the characteristic behavior seen in this preparation: long-lasting depolarizing events to weaker stimuli; but recruitment by stronger stimuli of strong IPSCs that blocked late polyphasic excitatory events. Our findings are consistent with observations in this preparation suggesting increased excitability of pyramidal cells (Prince and Tseng 1993; Salin et al. 1995; Tseng and Prince 1996) and undiminished or increased inhibition (Prince and Tseng 1993; Prince et al. 1997; D. Prince and I. Parada, unpublished observations), as discussed in the Introduction. In our model, we found that epileptogenesis could occur even in the presence of large increases in inhibitory efficacy.

Model Simplifications

As in any model, our work simplified certain issues to address others. A prominent simplification involves the use of neurons with passive dendrites, except for the use of active dendritic channels to achieve bursting behavior in IB cells. As discussed in the Methods, use of passive dendrites captures some aspects of synaptic integration on dendrites (relative roles of distal vs. proximal or somatic synapses) while neglecting others. Our major reason for this choice was simplicity: we were focused on testing one hypothesis for a mechanism of epileptogenesis. To also explore models of dendritic integration would combinatorially expand our task, given the lack of precise characterization of dendritic conductance dynamics, densities and locations on neocortical pyramidal cells.

Furthermore, there is little data implicating or constraining changes in dendritic integration properties in epileptogenesis. Generation of dendritic intrinsic burst discharges is known to contribute to acute disinhibitory epileptogenesis in the hippocampus (Wong and

Prince 1979). However, no data are available regarding potential alterations in properties of dendritic membranes following chronic injury. Of course, increased ability to enhance synaptic events through activation of altered local dendritic conductances, or even acquisition of burst-generation capacities in dendrites of chronically injured pyramidal cells, would be a potent mechanism to increase cortical excitability. Although such alterations may be present in axotomized spinal motoneurons (Sernagor et al. 1986), they have not been found in distantly axotomized corticospinal neurons (Tseng and Prince 1996). On the contrary, such axotomy may decrease the numbers of burst-generating corticospinal neurons (Tseng and Prince 1996).

Data on normal integration in active dendrites is also in flux and is itself an active topic of theoretical and experimental research. One possible basis for ignoring this level of complexity in our studies is the suggestion of recent work (Cash and Yuste 1999; Cook and Johnston 1999) that dendritic conductances may simple act to linearize the summation of synaptic conductances on different parts of the dendritic tree, rather than to radically alter the input/output characteristics of the cell (but see Larkum et al. (1999)). If this is true, simply setting synaptic conductances to achieve realistic EPSPs and IPSPs at the soma, as we do, might suffice to realistically model many aspects of synaptic integration on active dendrites. More generally, it is doubtful whether incorporation of such cellular mechanisms would have changed the basic insights obtained in this model that are discussed below. Similar arguments can be applied to the many other simplifications necessarily made in a model such as this, such as neglect of receptor desensitization or of frequency-dependent synaptic properties including those mediated by presynaptic inhibition. In some cases (*e.g.* NMDA desensitization) we have also made more specific arguments involving the relevant time scales of a given mechanism vs. those involved in either initiation or termination of epileptiform activity.

Epileptogenesis Without Disinhibition

Disinhibition is one of the most favored hypotheses advanced to explain the occurrence of epileptiform activity in cortical structures. This is understandable given the ease with which $GABA_A$ receptor blockers such as penicillin and bicuculline induce acute epileptogenic foci (Walker and Johnson, 1942; Matsumoto and Ajmone-Marsan, 1964; see Prince, 1978 for review), and given anatomical data from several varieties of chronic epileptogenesis in which loss of GABAergic neurons or terminals occurs (Ribak et al. 1979; Ribak 1985; Obenaus et al. 1993; Marco et al. 1996; see Prince 1999 for review). Only a small (approximately 10%) reduction in the efficacy of inhibitory synaptic activity is required to produce acute epileptogenesis in neocortical slices (Chagnac-Amitai and Connors 1989), and this small a change in inhibitory efficacy would be difficult to detect in electrophysiological studies. Inhibitory function may be reduced in some models of chronic cortical injury and hyperexcitability (Mittmann et al. 1994; Luhmann et al. 1998; Jordon and Jefferys 1992). A complete quantitative assessment of functional inhibition has not been reported in any model of chronic epileptogenesis. A reduction of GABAergic inhibition associated with network hyperexcitability has recently been reported in deep-lying pyramidal cells following acute cuts that separated superficial from deeper cortical layers of in vitro neocortical slices (Yang and Benardo 1997), while an increase in $GABA_A$ receptor function appears to follow acute trauma that amputates distal dendrites of dentate granule cells in hippocampal slices (Soltesz and Mody 1995).

The present results, along with those of previous experiments, suggest that something other than disinhibition can be responsible for chronic epileptogenesis in the partially isolated cortical island (see also Hoffman et al. 1994). Epileptiform activity is also generated in the face of intact or even enhanced inhibition in other preparations. For example, application of 4-aminopyridine (4-AP) to hippocampal slices induces acute epileptogenesis in which synaptic inhibition (as measured by evoked IPSCs) is actually increased. In 4-AP-treated slices, $GABA_A$ receptor-mediated depolarizations appear to play a role somewhat analogous to that played by NMDA receptor-activated depolarizations in the present study (Rutecki et al. 1987; Traub et al. 1995). Tetanus toxin produces a chronic model of focal epileptogenesis in which synaptic inhibition is suppressed acutely, but not chronically, even though epileptogenesis persists (Empson and Jefferys 1993; Whittington and Jefferys 1994).

Mechanisms of Epileptogenesis

Our simulations support the idea that the occurrence of epileptiform activity, manifest as long latency depolarizing potentials and repetitive spike discharge, is critically dependent on the relative activation of excitatory and inhibitory neurons in response to the *initial* stimulus. If the duration of excitatory activity is not rapidly truncated by inhibition, then activation of NMDA conductances via recurrent pyramidal collaterals can lead to late depolarizations and repetitive action potentials in pyramidal neurons. Thus, the critical parameters are the relative shift in the stimulus thresholds for activating excitatory vs. inhibitory cells, as well as the strength of the NMDA conductance evoked when excitation is activated without significant inhibition.

Increasing (hyperpolarizing) the resting membrane potential of inhibitory cells, which is equivalent to increasing the amount of excitation required to generate an inhibitory input, while increasing NMDA conductances at recurrent pyramidal/pyramidal connections, provided one means of producing epileptiform activity like that observed in the partially isolated cortical island. Decreased polysynaptic inhibition due to decreased excitatory drive onto inhibitory interneurons has been suggested as a potential mechanism for epileptogenesis in the injured hippocampus (Sloviter 1991, 1994; Bekenstein and Lothman 1993; but see Mangan et al. 1995; Buckmaster and Dudek 1997; Esclapez et al. 1997). While this mechanism appears unlikely in chronically isolated cortex (Prince and Tseng 1993; Prince et al. 1997), as mentioned in the Introduction, conclusions regarding interneuron thresholds for activation and input/output relationships will have to await data from interneuronal recordings.

We found that increased intrinsic excitability of pyramidal (excitatory) neurons, in the form of increased input resistances and time constants, along with increased NMDA conductances at recurrent pyramidal/pyramidal connections, could also produce the epileptiform activity characteristic of this preparation. Simulations incorporating these changes replicated the experimental data (Figs. 4,5). An increase in post-synaptic NMDA conductance has also been proposed in some chronic models of temporal lobe epilepsy (Mody and Heinemann 1987; Isokawa and Mello 1991; Mody et al. 1992; Kohr et al. 1993; Lothman et al. 1995). We implemented the increase in NMDA conductance as a simple increase in the amplitude of the synaptic conductance. A plausible alternative is some modification of the NMDA receptor or the conductance it opens, such as a reduction of the sensitivity of the NMDA channel to Mg^{++} in pyramidal neurons of the epileptogenic cortex (Zhang et al. 1996). Such a change could also increase the magnitude of the NMDA conductance and lead to epileptiform activity just as in our simulations.

Alternative mechanisms cannot be ruled out by our studies. For example, with less dense connectivity or a larger model network, a weak stimulus that activates a small number of pyramidal cells might lead to sparse activation that "percolates" among pyramidal cells without recruiting sufficient inhibitory interneurons to quench the activity; while a strong stimulus would simultaneously stimulate many principal cells, thereby recruiting large numbers of inhibitory interneurons and shutting off activity. However, such sparse activation of pyramidal cells does not seem obviously consistent with the synchronous cell discharges and field potentials observed experimentally in the partially isolated cortex.

Experimental Implications and Predictions

With the exception of the partially isolated cerebral cortex preparation, alterations in intrinsic membrane properties have not been emphasized as a potentially important factor in cortical epileptogenesis in various experimental models (for review, see Prince 1999). However, our results show that this could be a key element in the epileptogenesis that follows chronic injury produced by direct cortical trauma. Increase in excitability of pyramidal cells can play a role in some ways comparable to that of reduced inhibition in other model systems, as discussed above. The key experimental contribution of our model is to show that the observed epileptogenesis can arise due to the changes in intrinsic properties, along with increases in NMDA conductances and either normal or increased levels of inhibition.

This contribution can be broken down into three components: (1) The model provides an existence proof for this previously unproposed mechanism of epileptogenesis, which is compatible with existing results from the partially isolated cortical preparation. (2) The requirement of an increase in NMDA conductances constitutes a strong prediction, as this has not been studied, and was not a prior assumption of the model. (3) The demonstration of the model's insensitivity to increased inhibition provides an additional piece of evidence that unchanged or increased inhibitory efficacy is compatible with the observed physiological behavior.

The results of this modeling study, together with those from previous cellular electrophysiological experiments, lead to several predictions that can be tested in future slice experiments. First, if inhibitory efficacy is indeed unchanged or increased, then direct recordings from interneurons should show no significant decreases in input-output (I-O) slopes for monosynaptic EPSCs onto control interneurons versus those in epileptogenic slices. If anything, larger EPSCs or steeper I-O slopes might be seen, reflecting increased innervation of interneurons by sprouting pyramidal cell axons. A second prediction deals with connectivity between pyramidal cells. Recordings from synaptically connected pairs should show either unitary EPSCs that have larger conductances, or an increased "hit rate" for obtaining such paired recordings, or both. There should be a larger NMDA-receptor mediated component to the evoked EPSCs in such cells in epileptogenic slices, either because of a larger number of synaptic contacts and receptors, or fundamental changes in the properties of NMDA receptors such as alterations in sensitivity to Mg^{++} block following injury (Zhang et al. 1996). Additional immunocytochemical studies also might be expected to show increased or altered patterns of expression of NMDA receptor subunits on pyramidal neurons in the epileptogenic tissue. Since there is a delay from the time of injury to the onset of epileptogenesis in this model, the above findings should coincide temporally with development of stimulus-evoked epileptiform events.

Additional, more specific predictions can be derived. For example, EPSCs should be evoked at relatively lower stimulus intensities than IPSCs in pyramidal neurons of epileptogenic versus control cortex. However, such a result could also occur if excitability of inhibitory neurons were reduced in epileptogenic cortex, so this prediction is not unique to our model. A second prediction concerns the effect of incrementally reducing NMDA conductance with blockers such as APV: Because of the narrow window of NMDA conductance amplitude effective in producing epileptiform behavior (Fig. 5), blockers might have an all-or-none effect, so that epileptiform activity either would continue unabated or be abolished entirely as the concentration of blocker was increased. Also, because the termination of the epileptiform activity in our model is strongly dependent on the activation of $GABA_B$ receptors, blockade of these receptors should give rise to an increased duration of epileptiform responses. Note however, that one can imagine alternative mechanisms of response termination; this model prediction is separable from predictions relating to the initiation of epileptiform activity.

Functional Implications

Interestingly, similar patterns of long latency, all-or-none polysynaptic activities in response to a brief stimulus are present in unmodified juvenile (postnatal days 11-20) rat cortex, but not in neonatal or adult cortex (Luhmann and Prince 1990). The polysynaptic activity in juvenile rat cortex shows the same dependence on stimulus strength and NMDA receptor activation as that found in the partial isolation model (Luhmann and Prince 1990). There is, in addition, an immaturity of intrinsic membrane properties, which are characterized by a high input resistance and prolonged time constant (Kriegstein et al. 1987; McCormick and Prince 1987; Hamill et al. 1991). The similarities, of both neuronal intrinsic properties and circuit behaviors, in the partial isolation model and juvenile cortex raises the interesting possibility that the epileptogenic alterations following cortical trauma represent a reversion to an earlier developmental state.

We speculate that the changes in intrinsic neuronal properties and increased relative efficacy of NMDA receptor-mediated synaptic excitation modeled here might play a significant role in the establishment of new intracortical connections after injury, through mechanisms similar to those that may be operant during normal developmental processes (*e.g.*, Constantine-Paton et al. 1990). The increased neuronal excitability and loss of spike frequency adaptation (Prince and Tseng 1993) might be regarded as mechanisms that compensate for the loss of subcortical and intracortical excitatory afferents onto pyramidal neurons. Increased excitability may play a similar role in juvenile cortex, compensating for initially weak connectivity. In both systems, enhanced NMDA receptor-mediated currents could serve to stabilize connections made by newly sprouted axons. Such processes would be adaptive to the extent that they reshaped the capacity of injured cortex to process information normally, and maladaptive if excessive recurrent excitation led to epileptogenesis.

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	Intrinsic Bursting	Regular Firing	Inhibitory
Number of Cells	100	300	100
Input Resistance (MΩ)	45	110	164
Membrane Time Constant (ms)	20	20	15
Resting Potential (mV)	-60	-60	-60
Synaptic Delay (ms)	1.2 ± 0.6	1.2 ± 0.6	1.2 ± 0.6
Extrinsic Input Synaptic Conductance (nS)	8	4	1.5
Mean Recurrent Input Synaptic Conductance (nS):			
AMPA	2	1	1
NMDA	1	0.5	
GABA_A	20	4	
GABA_B	0.5	0.2	

Table 1: Summary of Control Model Cell and Synaptic Parameters. Synaptic conductances are the peak amplitude of each time-dependent conductance. For simulations of epileptogenesis, input resistances and time constants of excitatory neurons were increased and recurrent input conductances were varied as indicated in text; the recurrent conductance values listed here are the "standard" values, corresponding to "1.0" in Figure 5.

Figure Legends

FIG. 1. A: Model circuit diagram. Excitatory synapses are represented by arrows, $GABA_A$ synapses by filled circles. WM = white matter (extrinsic input). B: Schematic of control pyramidal cell membrane potential response to WM stimulus. Initial EPSP is truncated by rapidly developing IPSP.

FIG. 2. Epileptiform response of network to WM stimulus at 50 ms, with all inhibitory cells receiving hyperpolarizing current that lowers their resting potential by 10 mV (introduction of this hyperpolarization is noted in B). A: Raster plot of total network activity, with each point representing one action potential. Cells #0-399 are pyramidal or excitatory (E), #s 0-99 are IB and #s 100-399 are REG. #s 400-499 are inhibitory (I). B-D: representative membrane potential traces for each of the 3 cell types in the network; one cell of each type was chosen at random from model network. Mean NMDA conductance 0.7 nS onto REG cells, 1.4 nS onto IB cells. Threshold stimulus is lowest stimulus strength that produces spikes in approximately 10% of pyramidal cells.

FIG. 3. 50% stronger white matter stimulus than that shown in Fig. 2, delivered to same network as in Fig. 2, produces only a large EPSP followed by inhibition. A: Raster plot of total network activity. B-D: membrane potential traces for the same cells as shown in Fig. 2. Inhibitory cells are hyperpolarized and format and parameters are the same as in Fig. 2.

FIG. 4. Lowering thresholds of pyramidal cells and doubling amplitude of NMDA conductance onto them produces epileptiform activity. Lower 3 traces (B) show control responses to stimuli of increasing amplitude. A "pure" EPSP is evoked by 0.5T stimulus (T=control

threshold stimulus). IPSPs evoked by more intense stimuli (2.0T, 3.0T) truncate EPSPs. Top 3 traces (A) show response of pyramidal cell in epileptogenic cortex. Firing and late depolarizations occur at 0.5T, while a stronger stimulus produces a large EPSP followed by strong inhibition (1.0T). Control cell has parameters as in Table 1; epileptogenic cell has increased resistance and time constant, as described in text, and has mean NMDA conductances: onto REG cells 1 nS; onto IB cells, 2 nS.

FIG. 5. Range of amplitudes of synaptic conductance onto pyramidal cells that produce control or epileptogenic responses. Amplitudes shown in left of each column are given as multiples of standard values specified in Table 1 (standard values represented by 1.0 in this figure). For AMPA and NMDA the scale is linear from 0-2 and then linear from 2-4. All possible combinations of parameter values shown were simulated. Conductances onto inhibitory cells remain at standard values. Clear and shaded boxes delimit the range of values giving correct responses for the control and epileptogenic cases respectively. The term ‘correct’ corresponds to satisfying either the control or epileptogenic criteria specified in the text. Points labelled as O gave correct responses when other parameters were also at O’s; Y’s gave correct responses only in a very small number of cases; X’s never gave correct responses. Boxes were subjectively estimated from these points.

FIG. 6. Initial latency period can vary widely. Three trials are shown as raster plots (as Fig. 2). Each trial shows a different latency (50-200 ms) from initial firing to first synchronized burst. Each trial used a different value for the GABA_A synaptic conductance, respectively 1.5, 2 and 2.5 times the standard GABA_A conductance amplitude, but there was no overall correlation of latency with GABA_A conductance amplitude. Mean NMDA conductance onto REG cells 1 nS; onto IB cells, 2 nS.

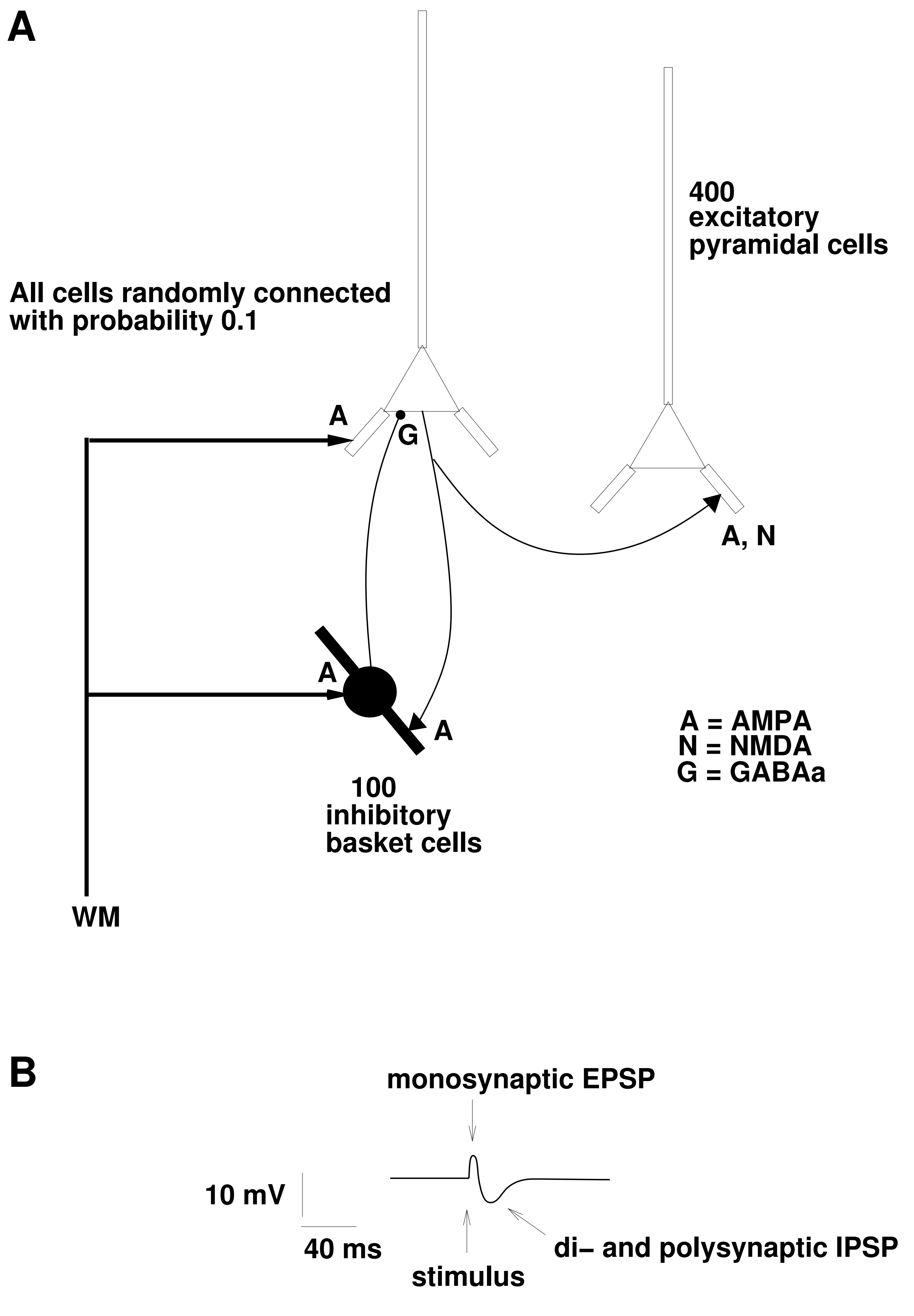

A
400
excitatory
pyramidal cells
All cells randomly connected
with probability 0.1
A
G
A, N
A
A
A = AMPA
N = NMDA
G = GABAa
100
inhibitory
basket cells
WM
B
monosynaptic EPSP
10 mV
40 ms
stimulus
di– and polysynaptic IPSP

Figure 1:

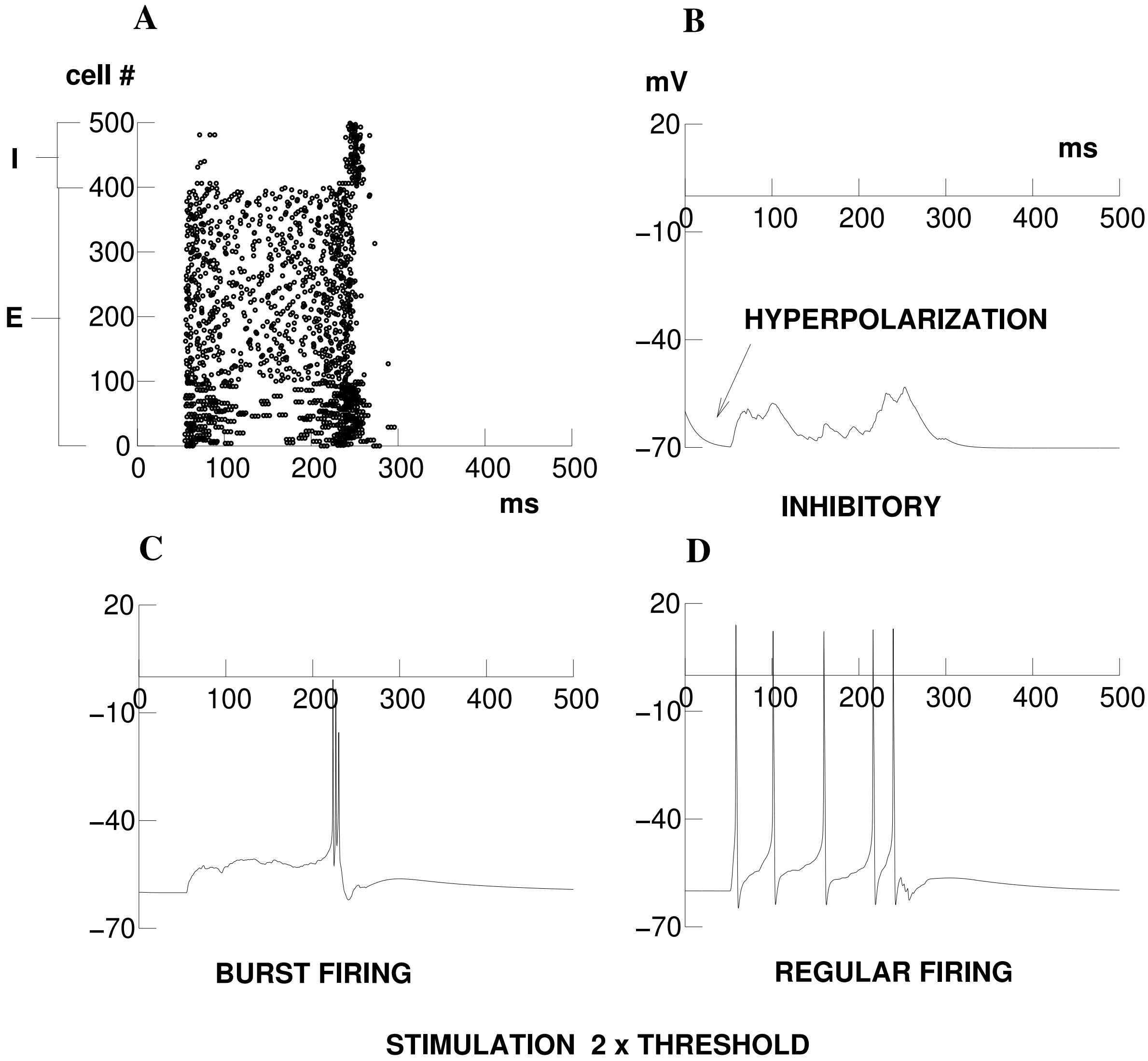

A
cell #
I
E
500
400
300
200
100
0
0
100
200
300
400
500
ms
B
mV
20
ms
0
100
200
300
400
500
−10
HYPERPOLARIZATION
−40
−70
INHIBITORY
C
20
−10
−40
−70
BURST FIRING
D
20
−10
−40
−70
REGULAR FIRING
STIMULATION 2 x THRESHOLD

Figure 2:

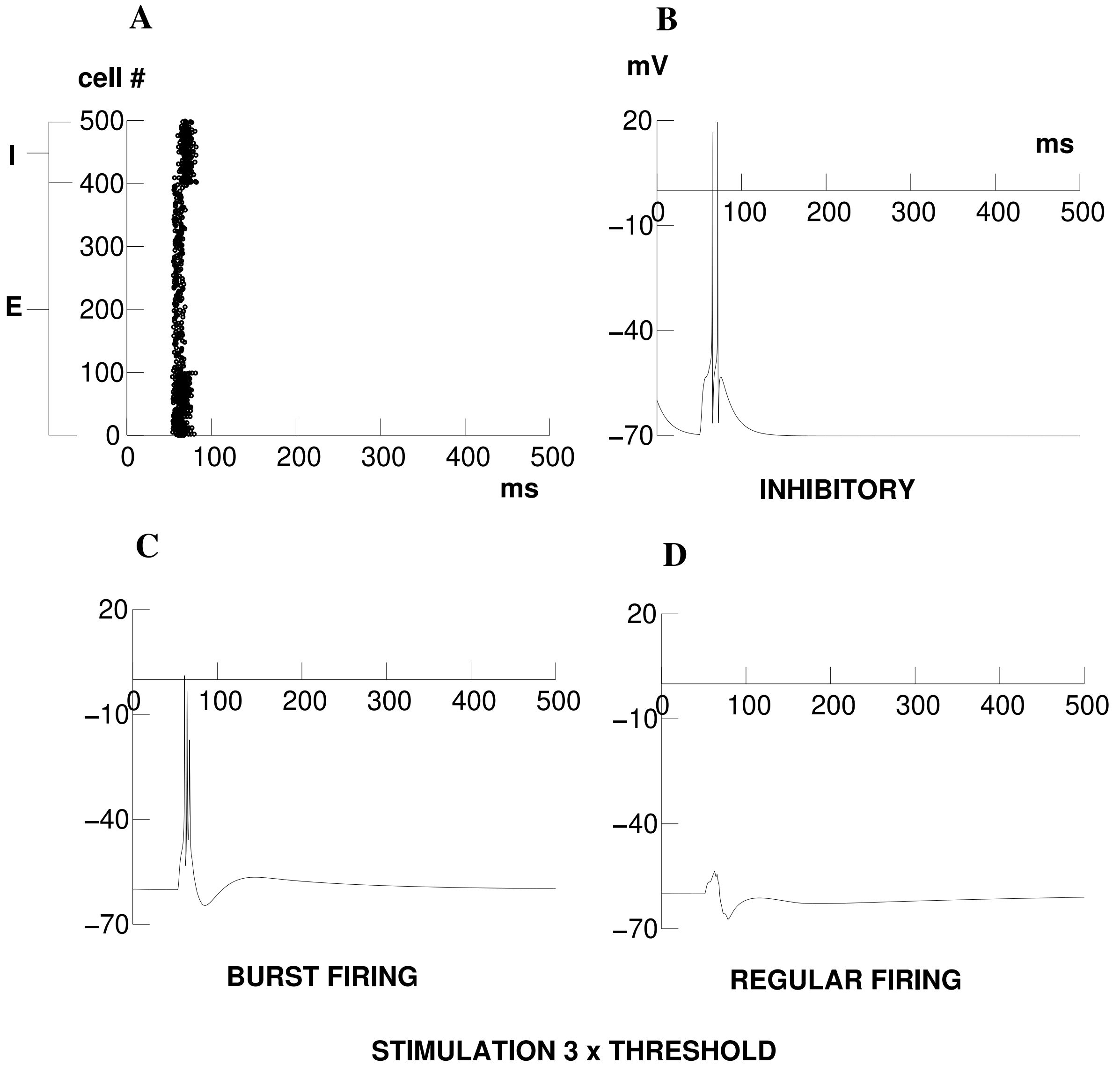

A
cell #
500
400
300
200
100
0
I
E
0
100
200
300
400
500
ms
B
mV
20
-10
-40
-70
ms
0
100
200
300
400
500
INHIBITORY
C
20
-10
-40
-70
0
100
200
300
400
500
BURST FIRING
D
20
-10
-40
-70
0
100
200
300
400
500
REGULAR FIRING
STIMULATION 3 x THRESHOLD

Figure 3:

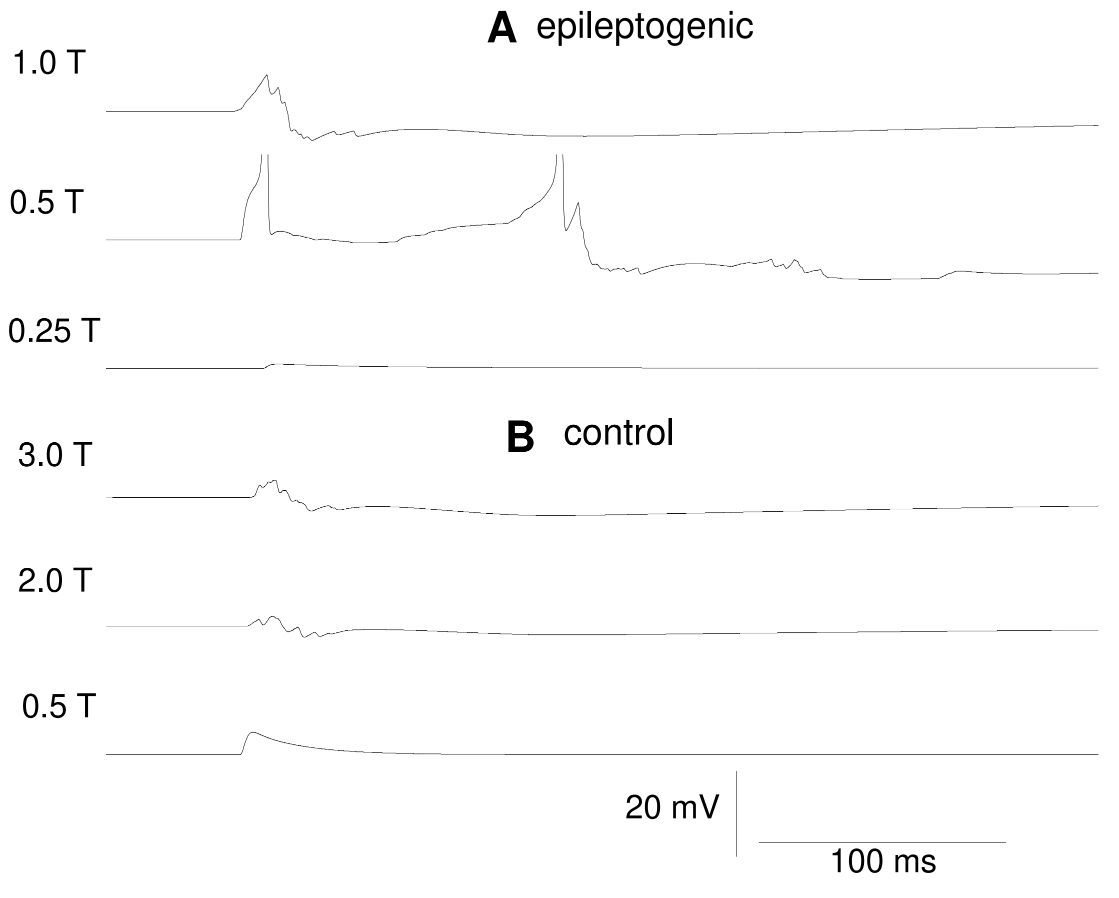
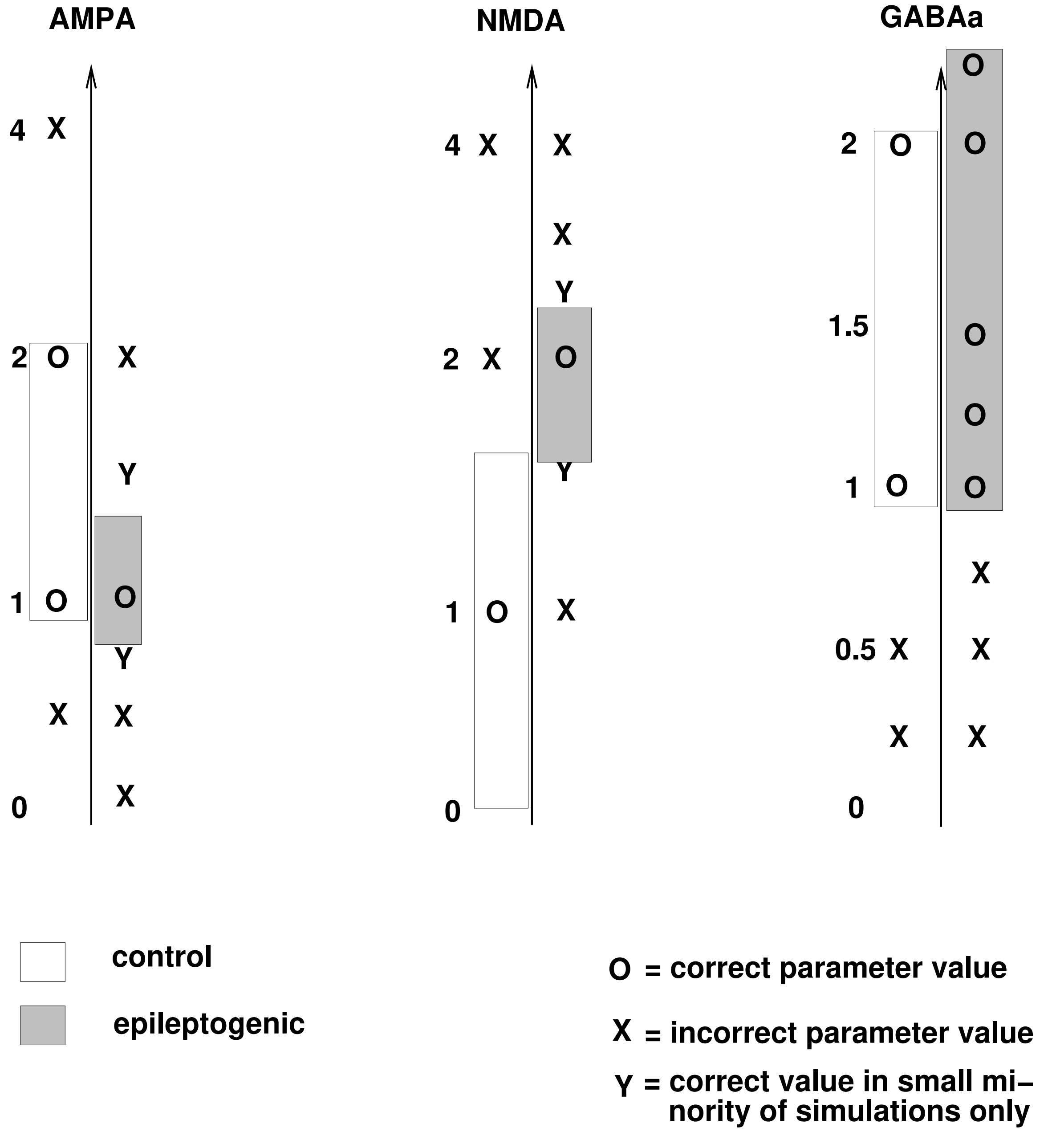

A epileptogenic
1.0 T
0.5 T
0.25 T
B control
3.0 T
2.0 T
0.5 T
20 mV
100 ms

Figure 4:



AMPA
NMDA
GABAa
4
2
1
0
1.5
0.5
X
O
Y
control
epileptogenic
O = correct parameter value
X = incorrect parameter value
Y = correct value in small minority of simulations only

Figure 5:

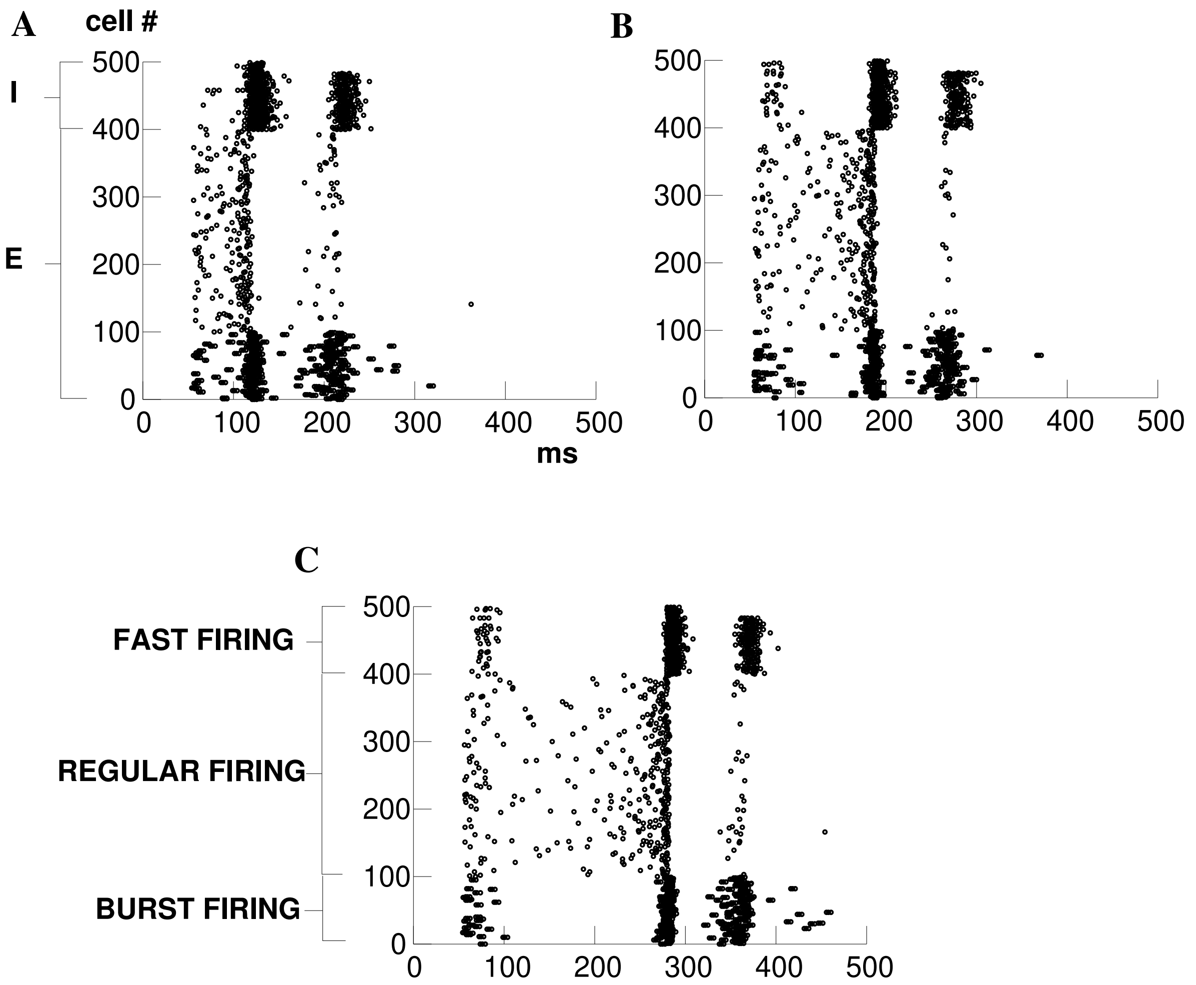

A
cell #
500
400
300
200
100
0
I
E
0
100
200
300
400
500
ms
B
500
400
300
200
100
0
0
100
200
300
400
500
C
FAST FIRING
REGULAR FIRING
BURST FIRING
500
400
300
200
100
0
0
100
200
300
400
500

Figure 6: