Convulsant and Anticonvulsant Effects on Spontaneous CA3 Population Bursts

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Yee, Audrey S., J. Mark Longacher, and Kevin J. Staley. Convulsant and anticonvulsant effects on spontaneous CA3 population bursts. J Neurophysiol 89: 427–441, 2003; 10.1152/jn.00594.2002. This paper analyzes the effects of a convulsant and an anticonvulsant manipulation on spontaneous bursts in CA3 pyramidal cells in the in vitro slice preparation under conditions of low (3.3 mM [K\(^+\)]\(_{o}\)) and high (8.5 mM [K\(^+\)]\(_{o}\)) burst probability. When burst probability was low, the anticonvulsant, pentobarbital, produced the anticipated effects: the burst duration decreased and interburst interval increased. However, when burst probability was high, both anticonvulsant and convulsant manipulations decreased the interburst interval and the burst duration. To reconcile these findings, we utilized a model in which CA3 burst duration is limited by activity-dependent depression of CA3 excitatory recurrent collateral synapses and the interburst interval is determined by the time required to recover from this depression. We defined the burst end threshold as the level of synaptic depression at which bursts terminate, and the burst start threshold as the level of synaptic depression at which burst initiation is possible. Synapses were considered to oscillate between these thresholds. When average burst duration and interburst interval data were fit using this model, the paradoxically similar effects of the convulsant and anticonvulsant manipulations could be quantitatively interpreted. The convulsant maneuver decreased both the burst start and end thresholds. The start threshold decreased more than the end threshold, so that the thresholds were closer together. This decreased the time needed to transition from one threshold to the other, i.e., the interburst interval and burst duration. The anticonvulsant manipulation primarily increased the burst end threshold. This also decreased the difference between thresholds, decreasing both interburst interval and burst duration. This model resolves the paradoxical proconvulsant effects of pentobarbital in the CA3 preparation and provides insights into the effects of anticonvulsants on epileptiform discharges in the human EEG.

INTRODUCTION

The periodic discharge of the hippocampal CA3 pyramidal cell network is a well-studied model of pathological network synchronization (Jefferys 1993). A number of experimental manipulations that induce seizures in vivo also produce episodic depolarizations and bursts of action potentials of CA3 pyramidal cells in the in vitro slice preparation (Buzsaki 1986; Jefferys 1994; Traub and Miles 1991; Traub and Wong 1982). This synchronized bursting occurs across the entire CA3 population and closely resembles interictal epileptiform activity recorded in vivo (Buzsaki 1986; Matsumoto and Ajmone Marsan 1964) that underlies interictal spikes on the human electroencephalogram (DeCurtis and Avanzini 2001; Gloor 1991; Sundaram et al. 1999).

Because numerous manipulations of the CA3 in vitro slice preparation produce robust epileptiform activity, and rapid, precise changes in drug concentrations are possible, this network is a useful preparation in which to study convulsant and anticonvulsant mechanisms (Anderson et al. 1987; Duong and Chang 1998; Rose et al. 1986; Scharfman and Schwartzkroin 1990; Schwartzkroin and Prince 1978; Stasheff et al. 1985; Swartzwelder and Wilson 1987; Swartzwelder et al. 1986). Intuitively, a convulsant should increase the probability of bursting so that bursts become longer and/or more frequent, whereas an anticonvulsant should decrease the probability of bursting so that bursts are shorter and/or less frequent.

For example, elevating extracellular potassium ([K\(^+\)]\(_{o}\)) (Korn et al. 1987; Rutecki et al. 1985) depolarizes the pyramidal cell resting membrane potential (RMP), increasing CA3 burst probability proportionately to the increased [K\(^+\)]\(_{o}\) (Staley et al. 1998). Raising [K\(^+\)]\(_{o}\) from 5 to 10 mM, a convulsant manipulation, decreased both the interburst interval and burst duration (Korn et al. 1987; Rutecki et al. 1985; Staley et al. 1998). Perplexingly, anesthetic concentrations of the anticonvulsant pentobarbital also decreased both the interburst interval and burst duration (Korn et al. 1987; Rutecki et al. 1985; Staley et al. 1998). Thus when burst probability is high (8.5 mM [K\(^+\)]\(_{o}\)), both a convulsant and an anticonvulsant manipulation produced qualitatively similar effects in the periodically discharging CA3 network. However, when CA3 burst probability is low, pentobarbital produces the predicted changes: the interburst interval lengthens and burst duration decreases (Swartzwelder and Wilson 1987).

These findings raise the following questions: why does a convulsant and an anticonvulsant manipulation produce qualitatively similar effects on the interburst interval and burst duration when burst probability is high? Why is pentobarbital ineffective when burst probability is high? What is the mechanism governing the impact of burst probability on convulsant effects?

To address these questions, we extended a model of CA3 bursting in which the burst duration is limited by activity-dependent depression of the CA3 recurrent excitatory synapses, and the interburst interval is determined by the time

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required to recover from this depression (Staley et al. 1998). We define the “burst end threshold” as the level of synaptic depression at which bursts terminate. The “burst start threshold” is the level of synaptic depression at which burst initiation is possible. The cycling of CA3 between bursting and interburst quiescence depends on the time required to transition between these two thresholds. Next, we examine the effect of a convulsant and an anticonvulsant manipulation on these bursting thresholds. The burst start and end thresholds were calculated from experimentally measured mean interburst interval and mean burst duration. The convulsant and anticonvulsant manipulations have predictable and opposing effects on the bursting thresholds, and these effects are influenced by the baseline burst probability.

METHODS

Slice preparation

Wistar rats of age 4–7 wk were used for all experiments. Housing and treatment of all animals were in accordance with animal welfare protocols approved by the Institutional Animal Care and Use Committee. After pentobarbital anesthesia (60 mg/kg ip), the rats were decapitated and hemi-brain slices were cut in the coronal plane. The slices were incubated in artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 2.5 KCl, 2.0 CaCl₂, 1.2 NaH₂PO₄, 1 glucose, and 26 NaHCO₃. ACSF was saturated with 95% O₂–5% CO₂. Slices were incubated at 31°C–2 hr before experimentation. Slices were recorded at 33°C and superfused continuously with ACSF at a rate of 1–2 ml/min.

Recordings

Extracellular recordings were made using glass pipettes pulled on a Narishige electrode puller (Tokyo) and filled with 150 mM NaCl. For recording spontaneous bursts, a bipolar stimulating electrode and the recording electrode were placed under visual guidance in the stratum pyramidale of the CA3 region (Fig. 1A1). Electrodes were adjusted so that large-amplitude population spikes were elicited as an indication of slice viability and proper electrode positioning. The placement of the recording electrode remained unchanged for the duration of the experiment. Recordings using Axoclamp 2B amplifiers were digitized at 2 kHz on a PCI-DAS 1602/16 Board (Measurement, Middleboro, MA) using routines written in Visual Basic 6.0 (Microsoft, Seattle, WA).

Burst induction

Bursting in CA3 (Fig. 1A1) was induced by one of two methods: a single tetanic stimulation to the CA3 pyramidal cell layer (Bains et al. 1999; Stasheff et al. 1985) or increasing extracellular potassium (Korn et al. 1987), the depth of the recording electrode, the conductivity of the slice (Traub and Miles 1991), or the location from which the slice was obtained in the septal-temporal axis (Staley et al. 1998). This causes inter-slice variability of the burst duration measurements. However, in any one slice and recording electrode position, burst duration measurements are stable throughout the control and experimental manipulations (Fig. 1B). We adjusted for the inter-slice variability of the burst duration by using each slice as its own control and reporting changes as “percentage change from control.”

Rationale for dose of pentobarbital and co-application of pentobarbital and acetazolamide

To increase gamma-aminobutyric acid A (GABA_A) receptor-mediated inhibition, we applied 60 μM pentobarbital to CA3. Pentobarbital (PB) has been extensively studied in vitro and produces anesthesia at CSF levels from 50 to 100 μM (MacDonald 1984). GABA_A receptors are the principal mediators of synaptic inhibition, yet when intensely activated (as may occur during spontaneous population bursts), dendritic GABA_A receptors excite rather than inhibit neurons (Alger and Nicoll 1982b; Andersen et al. 1980; Barker and Ransom 1978). PB prolongs the average open time of the GABA receptor and but also increases the depolarizing GABA response in cell culture (Alger and Nicoll 1982a; Thalman 1988) and the hippocampal slice preparation (Alger and Nicoll 1982a; Perreault and Avoli 1988; Wong and Watkins 1982). To exclude the possibility that PB was causing proconvulsant effects by depolarizing the dendrites during bursts, acetazolamide was co-applied with PB (reviewed in Staley 2002). This acetazolamide effect has been experimentally verified in vivo (Archer et al. 2001; Sato et al. 1981).

Data analysis: burst duration

Extracellular burst intensity is measured using a variety of parameters including the coastline bursting index (CBI), area under a burst, and burst duration. The CBI is the line integral of the extracellular waveform (Korn et al. 1987). We expect the CBI to increase with increases in neuronal synchrony, firing frequency, number of neurons participating in the burst, or the size or duration of the depolarizing wave within the burst. The CBI, however, does not distinguish among these possibilities and is sensitive to action potential synchronization between CA3 neurons as well as electrode placement. The “burst area” represents the area under the burst waveform. For intracellular recordings of membrane potential, burst area is an unambiguous measure of the intensity and duration of membrane depolarization. However, the area under the curve of extracellular recordings is increased by current flowing out of the somatic membrane and excitatory currents flowing into the dendritic membrane. Both inhibitory and excitatory currents therefore increase burst area. Because of this ambiguity, we chose to use burst duration to measure burst intensity. The burst duration was calculated as the time during which the absolute value of the burst was above a threshold value, generally three times the baseline noise (Fig. 1A2) (Staley et al. 1998).
Summary of experimental design

We performed three experiments in the CA3 in vitro preparation as delineated in the flow diagram of Fig. 1C. Using these three experiments, we addressed the three questions posed in the introduction. First, we examined the effects of a convulsant and an anticonvulsant manipulation under conditions of high burst probability. Second, we addressed why PB appears ineffective when burst probability is high. Third, we examined the role of the baseline burst probability on PB effects on burst duration and interburst interval.

Data analysis: interburst interval

The time between bursts is generally described in terms of burst frequency in units of Hertz. When burst frequency is less than once per second, we expressed the burst frequency in terms of the period as
The interburst interval was calculated as the point from the beginning of a burst to the beginning of the next burst (Bains et al. 1999; Staley et al. 1998).

Statistical analysis

CA3 burst duration and interburst intervals vary between slices (Bragdon et al. 1986; Korn et al. 1987; Mueller and Dunwiddie 1983; Scharfman 1994; Swartzwelder and Wilson 1987; Staley et al. 1998; Whittington and Jefferys 1994). Because of this variability, we calculated a "control value" for each slice, defined as the mean value in the control situation once the recordings were stable. Mean values were also calculated after stabilization of the interburst interval and burst duration for each drug application and normalized to the control mean. The mean ± SE of each of these "normalized experimental values" was then calculated.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>High Burst Probability</th>
<th>Low Burst Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 8.5 mM [K+]o</td>
<td>10.5 mM [K+]o</td>
</tr>
<tr>
<td>Interburst interval, s</td>
<td>3.6 ± 0.5</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Burst duration, ms</td>
<td>120 ± 17</td>
<td>90 ± 9</td>
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</table>

The convulsant manipulation (experiment 1, 1st 3 columns) was performed in the presence of picrotoxin. n = 7 and 10 slices, respectively, for convulsant and anticonvulsant manipulation. Values are means ± SE. All levels of significance obtained by 2-tailed t-test. PB, pentobarbital; ACTZ, acetazolamide. * Significance accepted at P ≤ 0.05.

“interburst interval” rather than as fractions of Hertz. The interburst interval was calculated as the point from the beginning of a burst to the beginning of the next burst (Bains et al. 1999; Staley et al. 1998).

![A1](image1.png) 8.5 mM [K+]o 10.5 mM [K+]o

![A2](image2.png) 8.5 mM [K+]o 10.5 mM [K+]o

**B1**

![B1](image3.png) 8.5 mM [K+]o 10.5 mM [K+]o

**B2**

![B2](image4.png) 8.5 mM [K+]o 10.5 mM [K+]o

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**Statistical analysis**

CA3 burst duration and interburst intervals vary between slices (Bragdon et al. 1986; Korn et al. 1987; Mueller and Dunwiddie 1983; Scharfman 1994; Swartzwelder and Wilson 1987; Staley et al. 1998; Whittington and Jefferys 1994). Because of this variability, we calculated a “control value” for each slice, defined as the mean value in the control situation once the recordings were stable. Mean values were also calculated after stabilization of the interburst interval and burst duration for each drug application and normalized to the control mean. The mean ± SE of each of these “normalized experimental values” was then calculated. Addition-
ally, a percentage change from control was calculated as $\frac{100 \times (\text{drug} - \text{control})}{\text{control}}$. We report the effect of the drugs as a “percentage change”.

We performed all statistical analyses using Microsoft Excel or Prism GraphPad software. Significant differences between normalized interburst interval and burst duration, and convulsant or anticonvulsant maneuvers were assessed using 2-tailed t-test. Statistical significance was accepted at $P \leq 0.05$.

Using the experimentally derived mean interburst interval and mean burst duration (see Appendix for derivations), we calculated values for the bursting thresholds. Differences between control start and end thresholds were assessed using 2-tailed t-test. Formulas for calculating the bursting thresholds are available on request in Microsoft Excel format.

**RESULTS**

**Baseline stability of slice preparation and burst probability**

We assessed CA3 network behavior by measuring the burst duration and interburst interval (Fig. 1B) (Bains et al. 1999; Staley et al. 1998). When bursts were induced by 8.5 mM $[K^+]_o$, the interburst interval ranged from 2.1 to 4.5 s (high burst probability; see also Table 1, 1st column). When bursts were induced in 3.3 mM $[K^+]_o$, the intervals ranged from 5.3 to 24.8 s (low burst probability; see also Table 1, 7th column). For each individual slice, the interburst interval (Fig. 1B1) and burst duration (Fig. 1B2) were stable.

**When burst probability is high, a convulsant manipulation decreases both burst interval and duration**

Figure 2 displays the effect of a convulsant manipulation on interburst interval (Fig. 2A1) and burst duration (Fig. 2A2) when burst probability was high. Figure 2A, 1 and 2, depicts representative slices. Figure 2B, 1 and 2, reflects pooled data. The convulsant manipulation consisted of raising $[K^+]_o$ from 8.5 to 10.5 mM, which further increased burst probability. PTX (100 μM) was applied throughout this experiment to avoid confounding effects due to decreased GABA release and altered Cl⁻ gradients (see METHODS). The interburst interval decreased by 32 ± 9% and burst duration decreased by 19 ± 8% compared with controls. Table 1, first three columns, shows pooled interburst interval and burst length data during control and experimental conditions.

**When burst probability is high, an anticonvulsant manipulation also decreases both burst interval and burst duration**

Figure 3 illustrates the effect of an anticonvulsant manipulation on CA3 bursting when burst probability is high. Figure 3A shows a representative slice, whereas Fig. 3B depicts pooled data. Table 1, middle three columns, displays pooled...
control and experimental interburst intervals and burst durations. Spontaneous bursting was induced in 8.5 mM [K⁺]o. No PTX was used in this experiment because we were interested in examining GABAergic anticonvulsant effects. To increase GABA_A receptor-mediated inhibition, we applied 60 μM PB, which augments the average open time of the GABA_A receptor but also increases GABA_A receptor-mediated dendritic depolarization (Alger and Nicoll 1982), and 10 μM ACTZ to inhibit the depolarization due to intense activation of dendritic GABA_A receptors (Archer et al. 2001; Staley et al. 1995). This manipulation decreased the interburst interval by 9 ± 5% and decreased the burst duration by 16 ± 5% (Fig. 3B and Table 1, middle 3 columns).

When burst probability is low, an anticonvulsant manipulation increases the interburst interval and decrease burst duration

Next, we repeated these experiments under conditions of low burst probability in 3.3 mM [K⁺]o modified ACSF (Stasheff et al. 1989). Figure 4A shows a representative slice and B illustrates pooled data. Tetanic stimulation of the CA3 pyramidal cell layer produced spontaneous bursting (Bains et al. 1999; Stasheff et al. 1985). Table 1, last three columns, shows summary control and experimental interburst intervals and burst durations. Addition of 60 μM PB + 10 μM ACTZ increased the interburst interval from control by 136 ± 41% and decreased the burst duration from control by 44 ± 3% (Fig. 4B, Table 1, last 3 columns). Similar to Swartzwelder and Wilson (1987), we found that when burst probability is low, anticonvulsant effects on interburst interval and burst duration are more intuitive: burst duration decreased while interburst interval increased.

Model

In high burst probability, both proconvulsant (elevating [K⁺]o) and anticonvulsant (increasing GABA_A mediated postsynaptic inhibition with PB + ACTZ) manipulations decreased the burst duration and interburst interval (Figs. 2 and 3, Table 1, 1st 3 columns and middle 3 columns). These qualitatively similar results can be reconciled using a model of CA3 bursting where cycling between the bursts and interburst quiescence is determined by the onset of and recovery from activity-dependent synaptic depression.

Traub and Miles (1991) and Senn et al. (1996) have shown that below a critical level of synaptic strength, synchronous network activity ceases. Thus activity-dependent depression is a reasonable mechanism for burst termination (Staley et al. 1998). As described in the APPENDIX, the average synaptic strength of the CA3 network oscillates between burst start and burst end thresholds during a bursting cycle. We defined the “burst start threshold” as the level of average network synaptic strength needed for burst initiation and the “burst end thresh-

![Fig. 4. Low burst probability: an anticonvulsant increases the interburst interval and decreases the burst duration. Representative traces of the effects of PB + ACTZ (A, 1 and 2) are shown when bursting is induced in low burst probability (3.3 mM [K⁺]o modified ACSF + tetanic stimulation). When burst probability is low, addition of PB 60 μM and ACTZ 10 μM produces the anticipated results. The interburst interval increased by 136 ± 41% (B1) and burst duration decreased by 44 ± 3% compared with control (B2, n = 10, Table 1, last 3 columns).](/jn.physiology.org/lookup/figure/10.1152/jn.00553.2002/-/10.220.33.3/May302017/4.17.17)
old” as the level at which synaptic strength is too low to support burst activity (Fig. 5A). During a burst, synapses depress to the level of the burst end threshold and in between bursts, synapses recover from synaptic depression to the level of the burst start threshold.

The burst duration reflects the time required for CA3 recur-
A1 20% Change in Burst Start Threshold

B1 20% Change in Burst End Threshold

C1 20% Change in Both Thresholds

A2 Burst Interval (y axis, s)

A3 Burst Duration (y axis, ms)

B2

B3

C2

C3
The interburst interval to decrease while burst duration increases. The greatest effect on burst interval occurs when the burst start thresholds and duration compared with control (A, 2 compared with control). Alternatively, decreasing the burst start threshold by 20% decreases both the interburst interval and burst duration (dotted line; increase 20%: thick black line; decrease 20%: thin black line) increases both the interburst interval and burst duration.

The rates of synaptic depression and recovery are not linear but exponential with time constants $\tau_{\text{depress}}$ and $\tau_{\text{recover}}$ (APPENDIX, Fig. 5A) (Staley et al. 1998, 2001; Tsodyks and Markram 1997). The behavior of our model is not critically dependent on the values of these time constants, so we have used previously published estimates for $\tau_{\text{depress}}$ and $\tau_{\text{recover}}$ (Dobrunz and Stevens 1997; Liu and Tsien 1995; Staley et al. 1998, 2001). We assumed that these time constants remained constant throughout the experiment. In the APPENDIX, we derive the equations that relate the mean interburst interval and burst duration to the bursting thresholds.

**Effects of a convulsant and an anticonvulsant in the model**

When burst probability was high (Fig. 2), a convulsant manipulation increased the burst probability. In our model, this would be interpreted as lowering the burst start threshold so that bursting becomes possible before full recovery from synaptic depression (Fig. 5B1). This decreases the recovery time, which determines the interburst interval. Bursts also had a shorter duration because synaptic depression was more extensive at the start of the burst, so that less time was required to reach the level of synaptic depression where bursting fails.

Figure 5B2 depicts the action of a hypothetical anticonvulsant when burst probability was high. The anticonvulsant terminated bursts before the CA3 network reached the baseline level of synaptic depression by raising the burst end threshold. The burst duration diminished because bursting ended before all involved synapses depressed to the baseline level of activity-dependent synaptic depression. Furthermore, the interburst interval of subsequent bursts was also shorter because recovery from synaptic depression began at a higher level of synaptic strength.

Thus in conditions of high burst probability, either convulsant or anticonvulsant manipulations shorten burst duration and interburst interval. Analysis using our paradigm explains these seemingly paradoxical results. While the convulsant manipulation lowers the burst start threshold, the anticonvulsant manipulation raises the burst end threshold. In both cases, the bursting thresholds move closer together, resulting in shortened interburst interval and burst duration. In summary, the convulsant lowers the burst start and/or end thresholds and anticonvulsant raises either burst start and/or end thresholds (Fig. 5B3).

Because convulsants and anticonvulsants alter the bursting thresholds, we can predict associated changes in burst duration and interburst interval. Figure 6A1 illustrates a hypothetical increase (heavy line) and decrease (fine line) in the burst start threshold while holding the burst end threshold constant. We used the experiment performed in low burst probability (3.3 mM $[K^+]_o$ data) for our control. Figure 6A, 2 and 3, shows changes in the interburst interval and burst duration. Figure 6B depicts a 20% change in the burst end threshold while holding the burst start threshold constant. Figure 6C illustrates the additive effect of changes in both thresholds. The consequences of altering either or both thresholds and the subsequent effects on interburst interval and burst duration (Figs. 2–4) are not easily ascertained a priori.
CONVULSANT MANIPULATION (ELEVATION OF [K\(^+\)]\(_{\text{in}}\) FROM 8.5 TO 10.0 MM IN THE PRESENCE OF PTX, 100 MM). Changes in the bursting thresholds under conditions of high burst probability are displayed in Table 2, first three columns. When we applied a convulsant manipulation to CA3 by elevating the [K\(^+\)]\(_{\text{in}}\), from 8.5 to 10.5 mM, the burst start threshold decreased by 16 ± 6% and the burst end threshold to decrease by 2 ± 7% (Fig. 7A1). In this experiment, the bursting thresholds moved closer together. The difference between the burst start and end thresholds decreased by 28 ± 8% (Table 3, 1st 3 columns).

ANTICONVULSANT MANIPULATION (SPONTANEOUS BURSTING IN 8.5 MM [K\(^+\)]\(_{\text{in}}\), ADDITION OF PB + ACTZ, NO PTX). Addition of PB + ACTZ to slices bursting under high burst probability (8.5 mM [K\(^+\)]\(_{\text{in}}\)) did not change the burst start threshold but increased the burst end threshold by 8 ± 3% (Fig. 7B1, Table 2, middle 3 columns). In this experiment, the bursting thresholds also moved closer together. The difference between the burst start and end thresholds decreased significantly by 14 ± 3% (Fig. 7B2, Table 3, middle 3 columns). Note that even though the change in interburst interval did not reach significance under these conditions (Fig. 7B1, Table 1, middle 3 columns), the thresholds, which are based on both the burst interval and burst duration, can change significantly.

Thus when burst probability is high, both anticonvulsant and convulsant manipulations diminish the difference between the start and end thresholds. This results in an accompanying decrease of the interburst interval and burst duration.

Fitting the experimental data: changes in bursting thresholds when burst probability is low

Under conditions of low burst probability (spontaneous bursting after tetanic stimulation of CA3, no PTX added), addition of PB + ACTZ increased the start threshold by 17 ± 3% and increased the burst end threshold by 66 ± 7% (Fig. 7C1, Table 2, last 3 columns). While the bursting thresholds both increased, the difference between the burst start and burst end thresholds decreased by 24 ± 4% compared with control (Fig. 7C2, Table 3, last 3 columns). This decreased the burst duration, but unlike the high burst probability experiments, the interburst interval lengthened (Fig. 4) (Swartzwelder and Wilson 1987). This occurs because the threshold shift caused the synapses to oscillate in a region of the synaptic recovery curve where the rate of recovery was much slower, which increased the interburst interval. Figure 8 illustrates this effect.

**TABLE 3.** Changes in the difference between burst start and burst end thresholds in response to convulsant and anticonvulsant manipulations under high and low burst probability

| Control 8.5 mM [K\(^+\)]\(_{\text{in}}\) | 10.5 mM [K\(^+\)]\(_{\text{in}}\) | Percent change | Control 8.5 mM [K\(^+\)]\(_{\text{in}}\) | PB + ACTZ | Percent change | Control 3.3 mM [K\(^+\)]\(_{\text{in}}\) | PB + ACTZ | Percent change |
|---|---|---|---|---|---|---|---|---|---|
| Diff between thresholds: 0.24 ± .03 | 0.16 ± .02 | **-28 ± 8** (P < 0.01) | 0.18 ± .01 | **0.15 ± .01** | **-14 ± 4** (P < 0.01) | 0.43 ± .03 | **0.33 ± .03** | **-24 ± 4** (P < 0.001) |

*Significance accepted at P ≤ 0.05.*
FIG. 8. Effects of changing burst probability when the inter-threshold difference is fixed. When burst probability is low (curves a and b), synaptic recovery is nearly complete, and the rate of synaptic recovery is slow. A small change in thresholds will cause a marked lengthening of the interburst interval. The burst duration is not significantly affected in either high or low burst probability because the time constant for depression ($\tau_{\text{depress}}$) is much shorter than the time constant for recovery ($\tau_{\text{recover}}$). Thus the rate of depression is not very different for these threshold values and consequently the burst duration is also similar.

DISCUSSION

We have analyzed the effects of a convulsant and an anticonvulsant manipulation on the bursting CA3 network by transforming the mean burst duration and interburst interval data into thresholds for burst initiation and termination. Three related observations are difficult to decipher without this translation: the tendency of the burst interval and burst duration to change in the same direction when burst probability is high, the qualitatively similar effects of convulsants and anticonvulsants on interburst interval and burst duration when burst probability is high, and the pronounced impact of the baseline burst probability on the effects of anticonvulsants.

Changes in the interburst interval and burst duration when burst probability is high

Increasing [K$^+$]o from 8.5 to 10.0 mM, a convulsant manipulation, decreased the interburst interval and the burst duration (Fig. 2). When we transform these values into the burst start and end thresholds, the reason for the shortened burst duration becomes clear. Convulsants increase the burst probability by lowering the burst start threshold. Bursting becomes possible before synaptic recovery is complete, so that synapses are already relatively depressed at the beginning of the burst. Under these conditions, synapses also reach the burst end threshold more quickly, which decreases the burst duration (Fig. 5B1, Table 2, 1st and 3rd columns). Thus convulsants cause the network to oscillate between two thresholds that are now closer together due to a decrease in the burst start threshold that exceeded the decrease in the burst end threshold.

Similar effects of a convulsant and an anticonvulsant when burst probability is high

An anticonvulsant results in burst termination before synapses are fully depressed by raising the burst end threshold. Because the burst start and end thresholds are now closer together, the transitions between thresholds occur more quickly (Fig. 5B2). This produces an effect similar to a convulsant that predominately decreased the burst start threshold as described in the preceding paragraph. Convulsants and anticonvulsants have opposing actions on the bursting thresholds. However, when burst probability is high, their net effect on CA3 network activity is qualitatively similar: a decrease in both the burst duration and the interburst interval (Fig. 7, A and B; Table 1, 3rd and 6th columns). This occurs because the bursting thresholds are now closer together.

Impact of burst probability

Using the synaptic depression model of CA3 bursting, we examined the impact of initial burst probability on interburst interval and burst duration. When burst probability is high (8.5 mM [K$^+$]o), bursting can occur before the recovery from synaptic depression is complete, and the rate of synaptic recovery is relatively fast. A small change in the thresholds may not be appreciable in terms of changes in the interburst interval. However, when the burst probability is low (3.3 mM [K$^+$]o), synaptic recovery is nearly complete and the rate of synaptic recovery is slow. A small change in the bursting thresholds will cause a marked lengthening of the interburst interval (Fig. 8). In high burst probability, anticonvulsant effects are dominated by decreased depression and shortened time to recovery. In low burst probability, anticonvulsant effects are dominated by additional time required for more complete recovery from depression.

Assumptions underlying this analysis

The transformation of the burst duration and interburst interval into periods during which CA3 synapses depress to the burst end threshold or recover to the burst start threshold is based on three assumptions. First, we have assumed that depression and recovery are mono-exponential processes. Although this is a reasonable assumption (Dobrunz 1997; Liu and Tsien 1995; Staley et al. 1998, 2001; Tsodyks and Markram 1997) (APPENDIX), it is not proven. For instance, the rate of depression during a spontaneous burst has not been measured experimentally, although it has been quantified during high-frequency stimulation (Selig et al. 1999). Second, we have assumed that inter-synapse variation in rates of depression and recovery (Stevens and Wesseling 1998) can be neglected, at least to a first approximation. Third, we have assumed that a shorter burst produces less synaptic depression; this is true for evoked release (Debanne et al. 1996) but is not proven for spontaneous bursts. Pacemaker currents and de-inactivation of a voltage-dependent depolarizing membrane conductance are alternative timing mechanisms for CA3 bursts (discussed in Staley et al. 2001). We favor the synaptic recovery model described in this paper because measured rates of depression and recovery are consistent with the burst durations and interburst intervals, whereas conductances with the appropriate activation/inactivation/de-inactivation kinetics have not been described in this preparation.

Clinical implications

PERIODIC LATERALIZED EPILEPTIFORM DISCHARGES. This work provides some insight into the limited utility of anticonvulsants...
in the treatment of periodic lateralized epileptiform discharges (PLEDs), a condition associated with acute forebrain lesions due to a variety of catastrophic conditions such as stroke, tumor, or infection (Chartrian 1964; Pohlmann-Eden et al. 1996). The frequency and duration of discharges found in PLEDs are similar to those seen the CA3 in vitro preparation when bursting is induced by elevated 8.5 mM [K+]o. Despite treatment of patients with PLEDs with anticonvulsants, there may be little alteration in the pattern of PLEDs (Lawn et al. 2000), mimicking the diminished efficacy of anticonvulsants in 8.5 mM [K+]o (Fig. 2) (Korn et al. 1987; Swartzwelder et al. 1986). We propose that this high-[K+]o model of synchronous epileptiform activity may be an appropriate in vitro model in which to study anticonvulsant therapy for PLEDs.

**Anticonvulsant Effects on Interictal Spike Frequency.** Interictal spikes on human electroencephalograms are observed in the setting of an increased probability for spontaneous seizures in temporal lobe epilepsy (Gloor 1991; Sundaram et al. 1999). Interictal spikes have a long interburst interval compared with the bursts seen in PLEDs. Figure 8 predicts that barbiturates will produce a more significant effect on the interburst interval when the burst probability is low. This is corroborated clinically in patients with generalized seizures (Buchthal et al. 1968) and suggested in those with partial onset seizures (Kellaway et al. 1978). However, the depression of the level of consciousness by barbiturates may also increase the frequency of interictal spikes (Hellier and Dudek 1999). Our model might allow us to directly measure the effects of anticonvulsants on epileptic networks independently of the effects on loss of consciousness.

**Future directions**

There are multiple cellular sites for targeting new anticonvulsant compounds (Dichter 1997). When we interpret anticonvulsant actions in the context of burst start and end thresholds, the CA3 in vitro preparation becomes very useful for evaluating anticonvulsant mechanisms. Because an anticonvulsant can raise either the burst start and/or burst end threshold, combination of anticonvulsants that act differently may prove to be more effective than choosing anticonvulsants that alter the same threshold. For instance, an anticonvulsant that enhanced the initial afterhyperpolarization should raise the burst end threshold. In contrast, an anticonvulsant that depolarized interneurons and increased spontaneous GABA release might only change the burst start threshold. Isolating the locus of action of an anticonvulsant in this system may focus subsequent research on the cellular basis of the anticonvulsant mechanism.

**Appendix**

In contrast to more detailed modeling of CA3 network behavior (Traub and Miles 1991), we have developed a simple quantitative description of synaptic depression and recovery in CA3 to assess convulsant and anticonvulsant effects on two parameters: the mean burst duration and the mean interburst interval. We analyzed the mean versus variance of the interburst interval in a separate paper (Staley et al. 2001). Due to extensive excitatory collateral connections, CA3 exists in two stable states, full synchronous activation (burst) or minimal activity (interburst quiescence). The degree of synaptic depression at recurrent excitatory synapses connecting CA3 neurons (Staley et al. 1998) determines the transitions between these states. (Alternatively, the degree of recovery from inactivation of a voltage-dependent conductance could also determine this transition, although we could find no conductances with the appropriate time courses of inactivation and recovery.) As shown in Fig. A1, the synaptic strength of CA3 can vary between a theoretical maximum (1) and a theoretical minimum (0). The maximum synaptic strength corresponds to the level at which all recurrent excitatory synapses in CA3 are completely recovered from depression, while the minimum synaptic strength corresponds to the level of absolute activity-induced depression.

If CA3 network synaptic strength declines continuously from the theoretical maximum beginning at $t = 0$ (Fig. A1, dotted line), then the burst start threshold ($S$) may be expressed as a point along this mono-exponential decay curve by

$$S = e^{-t/t_{\text{ depress}}},$$  \hspace{1cm} (A1)

Similarly, the burst end threshold ($E$) may be expressed as a point along the curve by

$$E = e^{-t/t_{\text{ depress}}},$$  \hspace{1cm} (A2)

The burst duration ($bdur$) is the time required to transition from the burst start threshold to the burst end threshold and can be expressed as

$$bdur = t_E - t_S.$$  \hspace{1cm} (A3)

Using Eqs. A1 and A2, we solve for $t_S$ and $t_E$

$$\log S = -t_S/t_{\text{ depress}},$$  \hspace{1cm} (A4)

$$t_S = -t_{\text{ depress}} \cdot \log S,$$  \hspace{1cm} (A5)

$$\log E = -t_E/t_{\text{ depress}},$$  \hspace{1cm} (A6)

$$t_E = -t_{\text{ depress}} \cdot \log E.$$  \hspace{1cm} (A7)

**Definition of Terms**

- $S$: burst start threshold
- $E$: burst end threshold
- $t_S$: time to decline to the burst start threshold
- $t_E$: time to decline from the burst end threshold
- $\tau_{\text{ depress}}$: time constant for initiation of short term synaptic depression
- $bdur$: burst duration

**FIG. A1.**
Using curve by expressed as a point along the theoretical mono-exponential growth et al. (2001) as shown in Fig. A2. The burst start threshold can be mono-exponential growth curve (Stevens and Wesseling 1998, Staley term synaptic depression of the CA3 network can be described using Substituting \( t_{S} \) and \( t_{E} \) (Eqs. A5 and A7) into Eq. A3, we solve for the burst duration in terms of the start and end thresholds

\[
\text{bdur} = t_{E} - t_{S} = -\tau_{\text{depress}} \cdot \log E - (-\tau_{\text{depress}} \cdot \log S) = \tau_{\text{depress}} \cdot [\log S - \log E]
\]

\[
\text{bdur} = \tau_{\text{depress}} \cdot \log (S/E)
\]

(A8)

During the period of interburst quiescence, recovery from short-term synaptic depression of the CA3 network can be described using mono-exponential growth curve (Stevens and Wesseling 1998, Staley et al. 2001) as shown in Fig. A2. The burst start threshold can be expressed as a point along the theoretical mono-exponential growth curve by

\[
S = 1 - e^{-t_{S}/\tau_{\text{recover}}}
\]

Similarly, the burst end threshold can be expressed as

\[
E = 1 - e^{-t_{E}/\tau_{\text{recover}}}
\]

The interburst interval can be expressed as

\[
\text{ibi} = t_{S} - t_{E}
\]

(A11)

Using Eqs. A9 and A10, we solve \( t_{S} \) and \( t_{E} \)

\[
\log(1 - S) = -t_{S}/\tau_{\text{recover}}
\]

\[
t_{S} = -\tau_{\text{recover}} \cdot \log(1 - S)
\]

(A13)

\[
\log(1 - E) = -t_{E}/\tau_{\text{recover}}
\]

\[
t_{E} = -\tau_{\text{recover}} \cdot \log(1 - E)
\]

(A15)

Substituting Eqs. A13 and A15 into Eq. A11, we solve for the interburst interval in terms of the start and end thresholds

\[
\text{ibi} = t_{S} - t_{E} = -\tau_{\text{recover}} \cdot \log(1 - S) - \tau_{\text{recover}} \cdot \log(1 - E)
\]

\[
= \tau_{\text{recover}} \cdot [\log(1 - E) - \log(1 - S)]
\]

\[
\text{ibi} = \tau_{\text{recover}} \cdot \log[(1 - S)(1 - E)]
\]

(A16)

We have solved for the burst duration (A8) and interburst interval (A16) in terms of the burst start threshold and burst end threshold. Now we express the bursting thresholds in terms of the burst duration and interburst interval so that changes in these thresholds due to experimental manipulation may be calculated from the experimentally obtained burst duration and interburst intervals.

Using Eq. A8, we solve for the burst start threshold and burst end threshold in terms of the burst duration. Rearranging and simplifying

\[
\text{bdur} = \tau_{\text{depress}} \cdot \log (S/E)
\]

\[
e^{\text{bdur}/\tau_{\text{depress}}} = \frac{S}{E}
\]

\[
E = S \cdot e^{\text{bdur}/\tau_{\text{depress}}}
\]

\[
S = E \cdot e^{-\text{bdur}/\tau_{\text{depress}}}
\]

Similarly, using Eq. A16, we solve for the burst start threshold and burst end threshold in terms of the interburst interval

\[
\text{ibi} = \tau_{\text{recover}} \cdot \log\left(\frac{1 - E}{1 - S}\right)
\]

\[
\text{ibi} = \frac{\tau_{\text{recover}}}{\log(1 - S)}
\]

\[
= \frac{1 - E}{1 - S}
\]

\[
e^{\text{ibi}/\tau_{\text{recover}}} = \frac{1 - E}{1 - S}
\]

\[
(1 - S) \cdot e^{\text{ibi}/\tau_{\text{recover}}} = (1 - E)
\]

\[
(1 - S) = \frac{(1 - E)}{e^{\text{ibi}/\tau_{\text{recover}}}}
\]

\[
S = 1 - \frac{(1 - E)}{e^{\text{ibi}/\tau_{\text{recover}}}}
\]

(A25)

Substituting Eq. A19 for burst end threshold (E) in Eq. A25, we solve for both burst start and end thresholds in terms of interburst interval and burst length

\[
S = 1 - \frac{(1 - E)}{e^{\text{ibi}/\tau_{\text{recover}}}} = 1 - \frac{(1 - S) \cdot e^{-\text{bdur}/\tau_{\text{depress}}}}{e^{\text{ibi}/\tau_{\text{recover}}}}
\]

(A26)

Simplifying

\[
S \cdot e^{\text{ibi}/\tau_{\text{recover}}} = e^{\text{ibi}/\tau_{\text{recover}}} - [1 - S \cdot e^{-\text{bdur}/\tau_{\text{depress}}}]\]

\[
S \cdot e^{\text{ibi}/\tau_{\text{recover}}} - S \cdot e^{-\text{bdur}/\tau_{\text{depress}}} = e^{\text{ibi}/\tau_{\text{recover}}} - 1
\]

\[
S(e^{\text{ibi}/\tau_{\text{recover}}} - e^{-\text{bdur}/\tau_{\text{depress}}}) = e^{\text{ibi}/\tau_{\text{recover}}} - 1
\]

\[
S = \frac{e^{\text{ibi}/\tau_{\text{recover}}} - 1}{e^{\text{ibi}/\tau_{\text{recover}}} - e^{-\text{bdur}/\tau_{\text{depress}}}}
\]

(A30)

Similarly, substituting Eq. A30 for the burst start threshold (S) into Eq. A19, we obtain an equation for the burst end threshold (E) in terms of interburst interval and burst duration.

\[
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