PHASE RESETTING ANALYSIS OF HIGH POTASSIUM EPILEPTIFORM ACTIVITY IN CA3 REGION OF THE RAT HIPPOCAMPUS

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The theory of phase resetting can reveal important information about the dynamic behavior of a periodic system when a single brief stimulus is applied to that system at the appropriate time. Phase resetting studies have revealed the existence in some biological systems of a vulnerable stimulus window generating desynchronization and suppression of the activity. The objective of this study was to test the hypothesis that a “singular” stimulus could annihilate this activity. Perfusion with the high-K solution produced synchronous, quasi-periodic population bursts with inter-burst interval of $\sim 0.8–1.5$ seconds. A single 0.1 ms duration anodic pulse of programmable delay and magnitude was applied to the somatic layer of the CA3 pyramidal cells. Three types of phase-resetting behavior were observed: (1) Weak resetting with little or no effect on the timing of the subsequent burst, (2) Strong resetting where the applied current pulse delayed the next event by one time period, (3) Singular behavior where the applied pulse partially or completely suppressed the subsequent bursting. The singular stimulus parameter window, however, was very narrow making it difficult to generate the singular behavior reliably. Nevertheless, the results indicate that singularities exist for high potassium neural activity and that a well timed pulse applied with the right amplitude can suppress this activity. This study suggests that phase resetting of a population of neurons is possible for quasi-periodic interictal activity and similar techniques could be applied to the control of epileptic seizures.

Keywords: Hippocampus; CA3; epileptiform; phase-resetting; unit-latency.

1. Introduction

Epilepsy is a disorder of the nervous system with severe consequences. About 3 million people have been diagnosed with epilepsy in the US with about 200,000 new cases each year. Antiepileptic drugs (AEDs) are effective at controlling seizures in a large number of patients but many do not receive any benefit from drug therapy. An effective alternative therapeutic approach is surgical resection. However, this procedure cannot be applied in many patients with multiple foci or with foci located in areas that cannot be removed without significant side-effects. Therefore, novel therapeutic approaches, such as electrical stimulation, have been developed for the treatment of epilepsy.

Externally applied currents such as DC and AC currents or electric fields can modulate the frequency of spontaneous firing in the rat. $1^-4$ Low frequency and high frequency currents pulses can also suppress abnormal neural activity with mechanisms that are still unclear.$5^-7$ Techniques based on the dynamic properties of the neural network such as chaos control, desynchronization and phase resetting hold some promise$8^-11$ but no effective method of seizure control has emerged clinically. Yet phase resetting has been shown to be quite effective at
inducing fibrillation\(^\text{12}\) (Winfree, 1987) it is used clinically routinely in clinical practice. The method relies on a property of dynamic systems called bistability.

For some biological periodic systems, stimuli applied within a small window of the period have been shown to reset the periodicity and sometimes to disrupt the oscillatory behavior.\(^\text{12}\) Phase resetting theory predicts the response of a rhythmic system to an applied stimulus. If the stimulus is weak, it will not affect the timing of the subsequent firing significantly. If the stimulus is sufficiently strong it can shift the next event by one time period of the system. Winfree (1987)\(^\text{12}\) showed that systems that display strong resetting must also present a singularity in the perturbation response. That is, if a stimulus of a critical magnitude and delay is applied, the latency of the next event is unpredictable. In systems capable of displaying both oscillations and quiescent behaviors (bistability), singular stimulus completely or partially annihilate the oscillations by switching the system to a quiescent behavior. Bistability has been observed in several models of neurons\(^\text{13}\) and neural networks.\(^\text{14}\) Phase-resetting analysis has been applied to the squid axon repetitive activity\(^\text{15}\) (Guttmann et al., 1980), respiratory rhythms\(^\text{16,17}\) and cardiac activity.\(^\text{18−22}\) However, very little research has been done to apply phase-resetting techniques to suppress abnormal epileptiform activity. Recently, Osorio and Frei (2009)\(^\text{23}\) showed that a DC pulse 100 ms long could decrease the number of seizures and that this effect could be attributed to phase resetting. However, the effect of the DC current could also directly affect the neuronal activity (Kayyal and Durand, 1991). Durand and Warman (1994)\(^\text{24}\) applied the theory of phase-resetting to the epileptiform activity in the CA3 region of the rat hippocampus generated by orthodromic stimulation in the presence of penicillin. Application of a single well-timed stimulus could desynchronize the firing pattern of individual neurons thereby annihilating the extracellular response.

The singular stimulus-induced de-synchronization of the neuronal activity took place on a short time scale on the order of 100 ms.\(^\text{24,25}\) Yet it might be possible to control neural activity on a much longer time scale. Therefore, the hypothesis that a singular pulse capable of annihilating the interictal activity generated an in-vitro model of epilepsy was tested. The high potassium model because it is capable of inducing repetitive and quasi-periodic interictal-like activity\(^\text{26,27}\) suitable for phase resetting studies. A single pulse with variable intensity and delay was applied extracellularly and its effect on the spontaneous firing pattern of the CA3 cells was studied. It was found that the CA3 neural network displayed weak and strong phase-resetting behaviors for different combinations of the intensity and delay of the applied pulse. It was also found that for a specific magnitude and delay of the applied pulse, the firing of the neurons was partially or completely suppressed. The parameters of the singular stimulus were such that the neuronal firing displayed both weak and strong types of resetting before being partially annihilated.

2. Methods

2.1. Preparation of slices

Adult male Sprague-Dawley rats weighing 150–200 g were anesthetized with ether and decapitated. The hippocampus was quickly dissected out and transversely cut into 400 µm slices. These slices were then incubated for one hour at 25°C in oxygenated (95% O\(_2\), 5% CO\(_2\)) artificial cerebral spinal fluid containing the following (in mM): 124 NaCl, 3.75 KCl, 1.25 KH\(_2\)PO\(_4\), 2.0 MgSO\(_4\), 2.0 CaCl\(_2\), 26 NaHCO\(_3\), and 10 Dextrose. After one hour at room temperature, individual slices were placed in the recording chamber, maintained at 34°C and were perfused with an oxygenated ACSF solution containing (in mM): 124 NaCl, 8.75 KCl, 1.25 KH\(_2\)PO\(_4\), 1.5 MgSO\(_4\), 1.5 CaCl\(_2\), 10 Dextrose, and 24 NaHCO\(_3\). The elevated potassium concentration in this solution produced spontaneous epileptiform bursts.

2.2. Electrical recording and data analysis

Extracellular recordings were obtained with microelectrodes filled with 2 mM NaCl (3–5 MΩ) and placed in the somatic region of the CA3 cells (Fig. 1(a)). The signals were amplified (Axoprobe 1A) and AC-filtered. A threshold detector circuit was used to detect an event and to externally trigger a stimulator (A.M.P.I. Master-8-cp) programmed to generate a 100 µs anodic or cathodic current pulse with a preset magnitude and delay upon detection of a burst. The pulse was applied to the tissue with
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2.3. Definitions of type 0 and type 1 resetting

Type 0 resetting occurs when the applied stimulus resets the subsequent burst by one time period (equal to the average inter-burst interval before stimulation) of the system. Type 1 resetting occurs when the applied stimulus has no or little effect on the next burst.

2.4. Stimulation protocol

The polarity of the stimulation in all experiments reported here was anodic. Anodic stimulation was also tested and produced similar results but with higher amplitudes (data not included). Two different experimental protocols were used. The range of coupling intervals and current amplitudes studied were ~0.1 to 0.9 (normalized with respect to \( \tau \)) and 40 uA to 500 uA respectively:

- **Phase-Response Analysis.** The magnitude of the current was set at a given level and a 100 \( \mu \)s anodic current pulse applied at increasing coupling intervals in steps of either 50 ms or 100 ms. At each coupling interval the stimulation was repeated 3 to 4 times after 2–3 seconds. This procedure was then repeated for increasing amplitudes of current.

- **Detection of Singularity.** This protocol was performed to detect the singularity lying in the region between weak (Type 1) and strong (Type 0) resetting behaviors. The coupling interval was set equal to a value of 50 ms less than the mean value of the natural time period and the intensity of the current was adjusted at amplitude producing either weak or strong phase-resetting. The stimulation was repeated after 2–3 seconds, 5–15 times for the same magnitude of the current. The same procedure was then repeated for consecutively smaller coupling intervals. This protocol was also tested using cathodic pulses.

3. Results

3.1. Statistical analysis of high-potassium neural activity

Results are presented from 39 slices obtained from 34 rats. Spontaneous inter-ictal-like epileptiform activity was generated in the rat hippocampal slices as a result of an elevated concentration of potassium in the artificial cerebrospinal fluid (Fig. 2(a)). The 39 slices selected for the study produced interictal spikes activity of 0.6 mV to about 4 mV peak to peak. Figure 2(b) shows the variation of the inter-burst interval for a single slice. The activity was regular.
Fig. 2. High potassium induced epileptiform activity. (a) Periodic spontaneous bursts induced in the CA3 region of the hippocampal slices due to an elevated potassium concentration in the ACSF. (b) Variation of the interburst interval from one event to another for a single slice. The interval varies from 1.1 Hz to 1.4 Hz. The mean interburst interval for this slice was 1.09 seconds and standard deviation is 0.088 seconds. (c) Mean interburst interval and standard deviation plotted for 23 different slices.

with the inter-burst interval ranging from 1.1 Hz to about 1.4 Hz. The mean inter-burst interval \( \tau \) for this slice was 1.09 s and the standard deviation \( \Delta \tau \) was 0.088 s. Figure 2(c) shows the variation of the mean inter-burst interval for 23 different slices. The inter-burst interval \( \tau \) of the neuronal activity ranged from about 0.4 s to about 1.4 s and \( \Delta \tau \) ranged from 53.3 ms to 570 ms.

3.2. Weak and strong resetting

The theory of phase-resetting predicts the response of a rhythmic system to external perturbations or stimuli. If the stimulus is weak, it has only a small effect on the next burst producing Type 1 or weak resetting. An example of Type 1 resetting is shown in Fig. 3(a) where an anodic pulse of 100 \( \mu \)s duration and an intensity of 75 \( \mu \)A (see arrow), applied at a normalized coupling interval \( \phi \) of 0.28, does not affect significantly the timing to the next burst. However, if a sufficiently strong anodic stimulus of 213.7 \( \mu \)A is applied (Fig. 3(b)) between two spontaneous events, a burst occurs coincidently with the stimulus and the next burst is delayed by one time period. This behavior is referred to as Type 0 or strong resetting. For Type 0 resetting, the stimulation can trigger a burst. Since it is then difficult to differentiate between the stimulus artifact and the burst occurrence, we refer to the interval following that burst as the latency \( \theta \). By varying the coupling interval at which the pulse is applied, a relationship between coupling interval \( \phi \) and latency \( \theta \) (Figs. 3(c) and 3(d)) is obtained. For Type 1 (weak) resetting (Fig. 3(c)), as the coupling interval \( \phi \) is increased from 0 to 1, the latency decreases \( \theta \) from 1 to 0 thus indicating that the timing of the next event is not affected by the pulse. For the Type 0 (strong) resetting (Fig. 3(d)), the stimulus of 213.7 \( \mu \)A produced strong resetting at all coupling intervals indicating that the application of the current pulse at any chosen coupling interval delays the next burst by one time period. Phase response curves obtained for the same slice as in Fig. 3, are shown in Fig. 4 when the stimulus intensity is increased from 88 \( \mu \)A to 197.8 \( \mu \)A. Figure 4(a) shows that, as the magnitude of the current is increased to 88 \( \mu \)A, Type 0 behavior occurs at a coupling interval of around 0.9. Thus for a stimulus intensity of 88 \( \mu \)A and a coupling interval of 0.9 the slice displays either weak or strong resetting behavior on repetitive stimulation. As the intensity is increased from 88 \( \mu \)A to 197.8 \( \mu \)A, the coupling interval at which Type 0 resetting occurs decreases until an intensity of 213.7 \( \mu \)A is reached (Fig. 3(d)) and the slice displays strong behavior at all coupling intervals. The phase-resetting data of Figs. 3
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\[ \theta = 0.77 \]

\[ \phi = 0.28 \]

1 mV

\[ \phi = 0.28 \]

\[ \theta = 1.12 \]

Stimulus

1 sec

(a)

(b)

Fig. 3. Phase-Resetting Analysis (a) Type 1 resetting. Application of a 0.1 ms, 75 µA anodic pulse does not affect the timing of the next burst. (b) Type 0 resetting. Application of a 0.1 ms, 214 µA pulse delays the next burst by one interburst interval. The coupling intervals and latencies have been normalized to the mean interval of 1.09 seconds. (c) Stimulus intensity of 75 µA produced weak resetting at all coupling intervals. (d) Stimulus intensity of 214 µA produced strong resetting at all coupling intervals.

and 4 are presented using isochronal maps in Fig. 5. The associated latencies for each coupling interval from the repetitive stimulations have been averaged to generate this map. The resolution of the coupling interval \( \phi \) is equal to 100 ms. Each isochronal map shows the combination of current intensity and coupling interval producing the same latency. This map was derived from Fig. 4 on the following basis: every combination of \( \phi \) and intensity which produced both behaviors: either strong resetting or weak resetting on repetitive stimulations was specified as unknown and mapped into a white region of Fig. 5. All other combinations have one single value of latency that either corresponds to a weak or strong resetting. The white region on the map is referred to as the region of unpredictable latencies. It is clear from Fig. 5 that for every coupling interval \( \phi \) there is a singular stimulus window, \( \Delta I \), which corresponds to the height of the white region at that value of \( \phi \) and for every value of \( I \) there is a singular \( \phi \) window, \( \Delta \phi \), which corresponds to the width of the white region on the map for that value of \( I \). The two windows \( \Delta I \) and \( \Delta \phi \) for this map are approximate since in order to generate a contour map, latencies have been averaged and \( \Delta \phi \) is limited by the resolution in coupling intervals (100 ms for these experiments). All combinations of stimulus intensities and coupling intervals above the region produce strong resetting and all combinations below the region produce weak resetting behavior. Similar results were observed in all slices studied using the
Fig. 4. Phase Response curves for increasing amplitudes of the stimulus: (a) 88 \mu A (b) 101 \mu A (c) 114 \mu A (d) 127 \mu A (e) 153 \mu A (f) 166 \mu A (g) 181.9 \mu A (h) 197.8 \mu A. Normalized coupling intervals and normalized latencies are plotted on the y-axis and x-axis respectively. As the current amplitude is increased from 88 \mu A to 197.8 \mu A, the coupling interval producing the strong resetting behavior decreases.
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Region of unpredictable latencies

\[ \Delta I \Delta \phi \]

\( \phi \) (Normalized)

Current (\( \propto A \))

Unit Latency Line

Fig. 5. Isochronal map of phase-resetting data of the spontaneous epileptiform activity presented. Each contour line shows the combination of stimulus amplitudes and coupling intervals that resets the event to the same latency. An approximate unit latency line is drawn through the combination of stimulus intensities and coupling intervals that produce either of the weak or strong resetting behaviors on repeated stimulations. All combinations of stimulus intensities and coupling intervals above the line produce strong resetting and below the line produce weak resetting.

phase-response analysis protocol. However, the corresponding procedure is very time consuming and complete maps were generated only in few slices.

3.3. Singularity

Once the full or partial region of unpredictable latencies for a given slice is mapped; a stimulus is applied in that region. Out of the 39 slices tested, 9 slices displayed 22 instances of longer than normal latency (defined as twice the mean period of the activity) were counted as singularities. Two types of singular behavior were observed: short time annihilation (\( n \approx 20 \)) where the stimulus would suppress one or more spontaneous bursts following its application and long term annihilation (\( n = 2 \)) where the activity was suppressed for at least 20 seconds.

(a) Short-Term Annihilation

The amplitude and the coupling of a stimulus capable of generating a response with an unpredictable latency (singular anodic stimulus) were first determined by choosing a coupling interval and finding the amplitude capable of generating either type 0 or type 1 Phase resetting response. The pulse was applied to the interictal activity generated by high potassium solutions. The activity was very regular with a mean inter-burst interval of 0.98 seconds and a relatively small standard deviation of 0.11 s (Fig. 6(a)). Figure 6(b) shows an example of a short term latency increase resulting from the application of a singular stimulus of amplitude 372.25 \( \mu \)A applied at a normalized coupling interval \( \phi \) of 0.37. The stimulation generated an event with a latency of 1.86 (nearly twice the mean value of baseline). For the same slice, at a different coupling interval of 0.26, a current intensity of 388 \( \mu \)A produces a value of \( \theta \) equal to 1.98 (Fig. 6(c)). A t-test was used to compare the increased latency of the singular event to the mean value of the interpulse interval and showed that the singular events had a significantly longer latency (\( N = 22, p < 0.001 \)). Figure 6(d) shows the unpredictable latency region for the slice whose short-term annihilation behaviors are shown in Figs. 6(b) and 6(c) as indicated by stimulation parameters producing phase 0 or phase 1 resetting behavior. Parameters producing singular behavior with increased latency for that slice are also included. This plot indicates that the singular stimulus amplitudes and the values of \( \phi \) that produced singular behaviors are similar to the ones that produced alternatively weak and strong resetting on repetitive stimulations. This map could be determined completely only for slices in which the detection of singularity stimulation protocol could be carried out at all values of \( \phi \).

(b) Long-Term Annihilation

The procedure described above also produced suppression of activity for longer periods of time (defined as greater than three times the mean value of the period) in 9 out of 22 instances of singularities somewhat uniformly distributed from 39 slices obtained from 35 rats. Figure 7(a) shows an example of such a long-term event. The mean inter-burst interval for this slice was about 1.02s with standard deviation of 0.08s. Application of a 100 \( \mu \)s, 344 \( \mu \)A stimulus at a coupling interval of 0.29 annihilated the bursting for about 18 seconds which then returned by itself without any stimulation. However, following the singular event, the activity was more aperiodic than before the singular event. The mean inter-burst interval increased to 1.4s with standard deviation increasing to 0.23s. A t-test comparing the distribution of the interburst intervals before and after a singular
Fig. 6. Short-term singular responses (a) Histogram of the baseline activity. (b)–(c) Examples of short-term singular responses in two different slices as indicated by an increase in latency of the event following the singular stimulus. (d) The combination of stimulus intensity and coupling interval which produced the increased latencies are located on the unit-latency line of the phase-resetting plot for that slice.

episode confirms that the two distributions are statistically different with a value of $p < 4 \times 10^{-13}$. Analysis of the intensity/coupling interval map showed that the parameters of the singular stimulus are such that they produce weak and strong resetting before annihilation.

Another example is shown in Fig. 7(b) where a stimulus with an intensity of 335 $\mu$A was applied several times at a coupling interval of 0.234. The stimulus could generate a long-term annihilation event with duration of 42s. A train of stimuli was applied 42s later in order to restore the spontaneous activity and to demonstrate that the slice was still capable of generating epileptiform activity. A single burst of activity returned and with further stimulation the activity was completely restored (not shown).

Figure 7(c) shows the distribution of coupling interval and latency for the 22 singular behaviors observed in 9 slices. The average normalized latency for a singular event is 4.2 indicating that for an average inter-burst interval of 1 Hz, 4 consecutive bursts following the application of a singular stimulus were suppressed. The coupling intervals for which the singular behaviors were observed are spread throughout the inter-burst interval. The current intensity required to suppress the firing ranged from about 47.14 $\mu$A to about 430 $\mu$A, with the amplitude increasing for decreasing coupling intervals.

4. Discussion

The results presented above show that the application of the theory of phase-resetting analysis to the periodic activity in the CA3 pyramidal cell layer of the rat hippocampus in elevated potassium can produce singular behaviors. The only other
Fig. 7. Long-term annihilation. (a) Example of long-term annihilation at a coupling interval of 0.29. The baseline activity is periodic with a mean interburst interval equal to 2.13 seconds and standard deviation equal to 0.22 seconds. A current pulse of 344 $\mu$A is repeatedly applied at a coupling interval of 0.29 and is able to suppress the activity for 18 s after which the activity returned spontaneously. (b) A second example is shown with a stimulus applied at a coupling interval of 0.23. A pulse with a stimulus amplitude of 335 $\mu$A was applied and could generate annihilation of the activity. After a period of 42 s, a short train of pulses was applied to determine if the activity could be restored. The activity did come back but with an irregular pattern. The singular stimulus parameters for the long term annihilation were also located in the unit-latency region (not shown). (c) Singular behavior statistics. The average duration for the singular events was about 4.2 seconds. The coupling intervals were spread throughout the inter-burst interval. The current intensity required to produce the singular event ranged from about 47.14 $\mu$A to 430 $\mu$A.

earlier study that has involved the application of the phase-resetting theory to epileptiform activity was by Durand and Warman (1994). \(^{24}\) They showed that the application of a single well-timed anodic pulse to the CA1 cell layer was able to suppress the evoked potentials within a single burst. Intracellular recordings indicated that individual cells were still firing but were desynchronized. The suppression of the extracellular activity was limited by the duration of the penicillin-induced event which itself is less than 40 ms long. However, for a phase resetting analysis system to be useful at controlling seizure activity, longer duration of annihilation must be obtained. To our knowledge, the present study is the first study to analyze the phase-resetting behavior of epileptiform activity over a longer time period in the range of 50 s and to demonstrate the existence of singular parameters for resetting the epileptiform activity in the CA3 cell layer.

The determination of a singular stimulus requires that the activity be somewhat periodic in order to reliably search the parameter space. Therefore, to test the ability of phase resetting to induce suppression over a longer period of time, a model of epilepsy with regular bursts and long burst periods produced by the potassium solutions was used. Immersing the hippocampal slices in 10 mM $[K^+]_o$ produced spontaneous discharges resembling interictal spiking in the CA3 region of the rat hippocampus. These bursts were similar to those previously reported (Traynelis and Dingledine 1988). \(^{26}\) The epileptiform activity ranged from 0.8 mV to about 4 mV in burst amplitude with the mean inter-burst interval ranging from $\sim$ 0.8 to 2.5 seconds. The activity was periodic with
70% of the slices having a standard deviation equal to less than 0.2 of the mean inter-burst interval. Phase-resetting until now has been applied to respiratory rhythms (Eldridge et al., 1989) and cardiac cells (Antzelevitch, 1985; Guevara and Jongma, 1990; Guevara and Shrier, 1987). These systems display a weak resetting behavior and a strong resetting behavior. The results obtained above showing that the singular stimulus had amplitudes intermediate between those required for completely weak or strong resetting is in agreement with the above studies. The behavior that was thoroughly investigated in these previous studies is the relationship between the stimulus intensity, coupling interval and resetting behavior for stimulus intensities intermediate between those producing either completely weak or strong resetting. Figure 5 shows that for a stimulus below 75 \( \mu \text{A} \), weak resetting is produced at all coupling intervals and for stimulus intensities above 231 \( \mu \text{A} \) strong resetting was observed at all coupling intervals. The resetting pattern between these two intensities is strongly dependent upon the coupling interval. As the stimulus intensity is increased the coupling interval at which either of weak or strong resetting behavior is produced decreases (Fig. 4). The behavior observed for stimulus intensities between 75 \( \mu \text{A} \) and 231 \( \mu \text{A} \) suggests the presence of a discontinuity between the resetting behavior for a single intensity when the coupling interval is 0.8 or when it is 0.9. This discontinuity shifts in time as the stimulus intensity is increased. These discontinuities in phase space behavior can be plotted to produce isochronal maps such as the one in Fig. 5 revealing the singular stimulus parameters region lying in the white region. At all other points in the map the equal latency isochrons are continuous in time.

One of the differences between our findings and the other phase-resetting studies (Eldridge et al., 1989; Paydarfar et al., 1996; Reiner and Antzelevitch, 1985; Guevara and Jongma, 1990) is that singular responses for the various slices are observed throughout the inter-burst interval. In other studies, a singular response was usually observed around a coupling interval of 0.5 and the singular response at other coupling intervals was either not observed or not investigated. The phase-resetting studies in cardiac cell rhythms show that a depolarizing stimulus applied early during the phase delays the next time event and applied later during the phase accelerates the next time event so that singular behavior is reported only when the depolarizing stimulus is applied early during the cycle. This study indicates that for spontaneous interictal-like activity in the CA3 cells, singular behavior exists for all coupling intervals. Phase resetting studies have shown that an intracellular hyperpolarizing stimulus would cause a singular behavior if applied later in the cycle. Our results using a cathodic polarity produced similar results to those produced by anodic but with different thresholds as expected from known properties of neural stimulation (results not shown).

The phase resetting of the CA3 cell network can be explained by the limit cycle process (Elridge et al., 1989) which is involved in a variety of biological rhythmic systems. A limit cycle is the periodic solution of a differential equation that attracts in the limit \( t \rightarrow \infty \) from all points in the neighborhood of the cycle. An isochron (see Fig. 5) is the locus of all points having the same latency. The isochrons come close together near a stationary (equilibrium) point and also in the vicinity of any singular trajectory leading to the singular point. Along such a stationary point, a small displacement may displace the oscillator across many isochrons to a different latency. In the results presented above, the stationary point was found to lie along the unit latency line (strong resetting). Since the isochrons are very close together on the unit latency line applying a critical stimulus could produce a Type 1 or Type 0 resetting or complete annihilation. Since out of the 22 instances of singular behavior observed for the CA3 cells in the rat hippocampus long-lasting annihilation could be generated in only 2 slices with no return to spontaneous bursting, the annihilation point is an unstable point (a compact “phaseless set”). These results also indicate that the CA3 region of the hippocampus producing interictal activity is bistable with a stable periodic behavior and an unstable quiescent behavior.

The theory of chaos control proposes to maintain rhythmic processes in a system by decreasing the degree of chaos in that system. There is a fundamental difference between the approach proposed here and the chaos control presented by (Schiff et al., 1994). They have shown that the firing pattern of the CA3 cells in a high-K solution follows a chaotic pattern and if a critically-timed pulse is applied to divert the system away from the stable direction, it
could move the system to further chaos and this could produce annihilation of the activity. Phase-resetting theory is applicable to periodic systems having a stable limit cycle as opposed to chaotic systems. An important feature of the CA3 activity is that without any stimulation the firing is nearly periodic. When a singular stimulus produced short term or long term annihilation, the periodicity of the returning signal was disrupted as indicated by an increased in the variability of the interburst intervals. Thus these data suggest that the application of the singular pulse induced a bifurcation away from a limit cycle.

This study shows that the spontaneous high-potassium periodic epileptiform activity generated in-vitro in the rat hippocampus is vulnerable to the phase of the applied single pulse anodic stimulus. Moreover, the results indicate that the CA3 region of the brain exposed to high potassium solutions can be bistable. If the timing and amplitude of a singular stimulus is chosen for either Type 1 or Type 0 resetting, activity can be suppressed for short or long periods of time. However, the range of the singular stimulus parameters that produce the singular behavior is very narrow and the effect of the stimulus is not reliable since the same pulse can induce three possible behaviors: weak resetting, strong resetting and suppression. These results suggest that even in this highly periodic system, the quiescent state is either not always present or the system is too noisy to switch reliably between periodic and the quiescent. The variable duration of the suppression period suggests that the quiescent state is unstable.

It would be important to know how the width of the window is related to various experimental conditions governing the generation of the epileptiform activity such as the different ionic concentrations, temperature etc. By controlling the experimental conditions such as potassium concentration and temperature it might be possible to increase the width of this window in order to obtain a more reliable way to control the suppression period. Soft phase resetting approaches to first synchronize the activity and then produce suppression with an applied singular stimulus.\(^{32}\) Our data also suggest that the use of phase resetting to control seizure might be somewhat limited since suppression of activity was difficult to achieve in a well-controlled periodic model of epilepsy generated by high potassium solution. Clinical seizures are not periodic and cannot be predicted (See reviews by Iasemidis, 2003 and 2009).\(^{31}\) Therefore the determination of the singular behavior in clinical studies would be quite difficult to investigate. However, suppression of frequent and periodic interictal activity could be studied through phase resetting techniques and could lead to seizure suppression with the assumption that interictal activity progressively leads to seizures.

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