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Patterns of the Up-Down State in normal and epileptic mice

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Abstract

Goal of this manuscript is to investigate whether changes that exist in epileptic brain generating spontaneous seizures are reflected in the pattern of the Up-Down state recorded from the neocortex and dentate gyrus. Experiments were carried out on naive and epileptic mice under urethane anesthesia. Local field potentials were recorded with chronically implanted microelectrodes and single unit activity was recorded with glass microelectrodes. Recorded neurons were labeled by neurobiotine and identified later as granular cells or interneurons in histological sections. The following major features differentiate the pattern of Up-Down State in epilepsy from normal. 1) The duration of Up and Down phases is significantly longer. 2) Recovery of network excitability after termination of the Up phase is longer. 3) UP-spikes occur during the Up phase, which transiently interrupt the development of the normal electrographic pattern of Up phase. Our data provide evidence that UP-spikes result from gigantic EPSPs generated in response to afferent activity. UP-spikes in the neocortex and dentate gyrus occur in close temporal relationship indicating the existence of direct or indirect pathological functional connections between these areas. Changes in the duration of UP- and Down phases as well increased time of recovery of excitability of epileptic brain after termination of Up phase suggest alterations in the homeostatic properties of neuronal network in epileptic brain. We suggest that the existence of UP-spikes in epileptic brain may be an additional electrographic pattern indicating epileptogenicity. Unraveling the neuronal substrates of UP-spikes may further improve our understanding of the mechanisms of epilepsy.

Keywords

Epilepsia; Animal model; Up-Down State; UP-spike; Interictal Spike; Neocortex; Dentate gyrus

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INTRODUCTION

Electrographic interictal epileptiform events are signatures of epileptogenicity of the brain. They are used clinically for diagnosis and for localization of the epileptogenic region in surgical candidates. Interictal spikes and pathological high frequency oscillations (pHFOs) are among the few electrographic events that characterize epileptogenicity of brain areas (Dichter and Ayala, 1987, Gotman and Wang, 1991, Schwartzkroin, 1997, Bragin et al., 1999, de Curtis and Avanzini, 2001, Staley and Dudek, 2006, Engel et al., 2009, Bragin et al., 2010). Among electrographic patterns that have not been studied in epileptic brain is the Up-Down state (UDS). This pattern occurs in situations when the flow of afferent signals to the brain, or to certain brain areas, decreases (sleep or anesthesia). In these conditions the brain generates an electrographic pattern mostly determined by the properties of internal connections of these brain areas (Buzsaki, 2006). UDS during slow wave sleep (SWS) and anesthesia was originally described by Steriade and co-workers (Steriade et al., 1993a, Steriade et al., 1993b, Timofeev et al., 2001). UDS is characterized by the occurrence of slow <1.0 Hz waves with superimposed 20–80 Hz oscillations (beta-gamma activity, BGA). During awake conditions neurons are in an active state when their membrane potentials are slightly depolarized, while during SWS neuronal membrane potentials oscillate between depolarized and hyperpolarized states (Steriade et al., 1993b, Sanchez-Vives and McCormick, 2000, Timofeev et al., 2000, 2002). The depolarized state is called the *Up phase* and the hyperpolarized phase is called the *Down phase*. An increase in BGA amplitude and an increase in neuron discharges occur during the Up phase and there is low amplitude BGA and most neurons are silent during the Down phase (Steriade et al., 1993b, Sanchez-Vives and McCormick, 2000, Timofeev et al., 2000, 2002).

It is well accepted that one of the characteristics of the epileptic brain is imbalance between excitatory and inhibitory networks and altered metabolism (Schwartzkroin, 1983, Dichter and Ayala, 1987, Chang and Lowenstein, 2003). Some features of this imbalance should be reflected as changes in the UDS pattern, because GABAA receptor-mediated inhibition is necessary for balancing persistent activity during the Up phase and activation of GABAB receptors contributes to its termination (Mann et al., 2009, Sanchez-Vives et al., 2010).

Considering that UDS is a global electrographic phenomenon and that epilepsy is a multifactorial disorder involving wide spread changes in brain functions at the molecular, neuronal and systems levels (Spencer, 2002, Rakhade and Jensen, 2009, Loscher and Brandt, 2010, Pitkanen and Lukasiuk, 2011), analysis of changes in the UDS pattern related to epilepsy may yield new insights into our understanding of the mechanisms of epilepsy. The goal of these experiments was to compare characteristics of UDS in the neocortex and dentate gyrus of normal and epileptic mice using recordings from local field potentials and identified neurons.

Experimental Procedures

All procedures described here were approved by the University of California, Los Angeles, Institutional Animal Care and Use Committee. Experiments were carried out on adult male C57B6/J mice (15–30g; Harlan, Indianapolis, IN).

Number of animals

Experiments were performed on 3 groups of mice: naive control (n=7), pilocarpine treated mice that showed status epilepticus and recurrent spontaneous seizures (stage 3–5, Racine's scale) detected during video-monitoring (epileptic after status, n=86) and pilocarpine treated mice that did not show status epilepticus and later did not develop spontaneous seizures (n=15). Due to the absence of significant differences between groups 1 and 3, these data

were merged into one group - non-epileptic mice. In six non-epileptic and 26 epileptic mice, multiple microelectrodes were implanted in the neocortex and dentate gyrus for recording UDS patterns under urethane anesthesia. Sixteen non-epileptic and sixty epileptic mice and were used for juxtacellular experiments.

Status Epilepticus induction—Status Epilepticus (SE) was induced with pilocarpine hydrochloride injection (320mg/kg, s.c.; Sigma, St. Louis, MO). Thirty min before, animals received a single injection of methyl-scopolamine bromide (1mg/kg, s.c.; Sigma) to limit pilocarpine peripheral toxic effects. Status epilepticus was interrupted after 2 hours by 10mg/kg diazepam injection.

Seizure Monitoring—Beginning one week after status epilepticus, animals were video monitored for seizure detection 16 h/day, 5 days/week.

Surgeries and experiments description: Two series of experiments were performed in this study. In the first series, animals were anesthetized with urethane (1.25g/kg, i.m.) followed 30 minutes later by one dose of ketamine+xylazine (20 and 2 mg/kg, i.m.; respectively) and fixed in a stereotaxic frame. Bipolar 50 μ m tungsten wires with 0.5 mm separation between tips were implanted in the left and right dentate gyrus (AP=−2.0; L=1.5; V=2.0) and **motor area of** neocortex (AP+2.0; L=2.0; V=0.3 for shortest microelectrode in the pair). A stimulating electrode was implanted in the ipsilateral perforant path (PP), (AP=−3.8; L=2.3; V=3.5).

Data Acquisition: Data were recorded wide-band (0.1 Hz to 3.0 kHz) and sampled at 10 kHz/channel with 12 bit precision on a Pentium PC using Run Technologies (Mission Viejo, CA) DataPac software with parallel video-monitoring of behavioral status.

A baseline electrical activity was recorded for 30 min and single pulse electrical stimulation (1/20 s) of the PP was performed to study local field potential and multiunit activity responses. The current of stimulating pulses was adjusted to evoke a response in the dentate gyrus containing a population EPSP and a single population spike and varied in different animals between 100–300 μ A.

In the second series of experiments we performed recording of extracellular activity of single neurons and their juxtacellular labeling. Animals were also anesthetized with a single dose of urethane (1.25g/kg, i.m.) followed 30 minutes later by one dose of ketamine +xylazine (20 and 2 mg/kg, i.m.; respectively) and fixed in a stereotaxic frame. A fixed bipolar microelectrode was implanted **in the motor area of** neocortex (AP+2.0; L=2.0; V=0.3 for shortest microelectrode in the pair) and holes were drilled in the skull at coordinates (AP = −2.0mm, L = 1.5–2.0 mm) and (AP = −3.0mm, L = 2.5–3.5mm) moveable glass microelectrodes intended to record single unit activity from the dentate gyrus of the anterior and posterior hippocampus. The level of anesthesia was maintained in the range when slow wave pattern and theta activity are alternating in the ratio 10/1 and the periods of theta activity were lasted no longer than 5 minutes. Supplemental doses of ketamine+xylazine were administered as needed. Body temperature was maintained with a heating pad. A stimulating electrode was implanted in the ipsilateral PP (AP=−3.8; L=2.3; V=3.5).

Electrophysiological Recordings and Juxtacellular Labeling: Extracellular unit activity was recorded with glass microelectrodes (impedance 15–25 M Ω , filled with 1.5% neurobiotin [Vector Laboratories, Burlingame, CA] in 0.5M NaCl). Local field potentials were recorded in the vicinity of recorded unit activity with a bipolar microelectrode (20 μ m

tungsten wires, with 0.5mm space separation) that was glued 500 μ m above the tip of the glass microelectrode (see Figs. 9C as an example).

After a neuron was found baseline spontaneous electrical activity was recorded simultaneously with local field potentials for 30min followed by single pulse electrical stimulation (1/20 s) of the PP (100–300 μ A) to study local field potential and unit activity responses. At the end of the experiments, the glass pipette was advanced towards the cell, which was juxtacellularly labeled by a positive current of 200 ms pulse duration for 20 min. The current intensity was slowly increased (usually 10nA) until the recorded neuron began to discharge, driven by the stimulation. In non-epileptic mice 11 identified granular cells and 3 interneurons were recorded. In epileptic mice 26 identified granular cells and 4 interneurons were recorded.

Field potentials were recorded from 0.1 Hz to 1.0 kHz and units from 0.1 Hz to 5.0 kHz (both sampled at 20kHz) using Datapac 2K2 (RunTechnology, Mission Viejo, CA) data acquisition system.

Tissue processing—Approximately 2 hours after labeling, animals received an overdose of pentobarbital and, once profoundly anesthetized, were transcardially perfused with 50 ml physiological saline (NaCl 0.9%) followed by 100 ml of 4% paraformaldehyde. Brains were removed and postfixed at 4°C overnight. They were then embedded in 3% agarose and 60 μ m coronal sections were sliced with a vibrating microtome (Leica, Heidelberg, Germany).

Immunohistochemistry: Slices were demounted from the slide and thoroughly washed in phosphate-buffered saline (PBS). Endogenous peroxidase activity was quenched by hydrogen peroxide 30% for 30min. and non-specific binding was blocked by 10% normal goat serum (NGS, Jackson ImmunoResearch Laboratories, West Grove, PA) for 2h. Neurobiotin-loaded neurons were developed using the Vectastain Elite ABC kit (Vector Laboratories) and revealed by diaminobenzidine/nickel. Sections were also counterstained with Cresyl Violet and slides were dehydrated and embedded in Permount mounting medium (Fisher Scientific, Pittsburgh, PA).

Slides were viewed and analyzed with a Zeiss microscope and AxioVision (version 4.6) software (Zeiss, G ttingen, Germany).

Data Analysis—Periods the highest amplitude of EEG signal were selected for further analysis. Raw EEG data were separated by digital filtering of electrical activity in three frequency bands: low frequency band activity <1.0 Hz; BGA – 20–80Hz and multiunit activity >600 Hz.

Because the polarity of the slow wave during UDS depends on the location of the recording electrode in relation to the cellular layer (Isomura et al., 2006) we considered an increase in amplitude of BGA to be the beginning of the Up phase. Detection of BGA we used a method described in our earlier studies (Staba et al., 2002). Each channel of wideband EEG was digitally band-passed between 10 and 80 Hz (finite impulse response filter, rolloff, –33 dB/octave). The signal was rectified and the root mean square (RMS; 100-ms sliding window) amplitude of the filtered signal was calculated (DataPac 2K2, Run Technologies, Mission Viejo, CA) (Staba et al., 2002). Successive RMS values with amplitudes >2 SDs above the mean amplitude of the RMS signal (i.e., mean amplitude calculated over the entire length of data file) longer than 200 ms in duration were detected and delimited by onset and offset boundary time markers as putative BGA. Consecutive events separated by less than 200 ms were considered as one event. Each event was visually inspected to remove events

that were contaminated by artifact, including movement, electronic, and other sources of signal noise. During development of this technique, it was determined that it was effective in detecting greater than 84% of putative oscillatory events observable with visual EEG analysis.

The following parameters of BGA were analyzed and compared between different groups of animals. 1) The frequency of BGA; 2) The duration of Up and Down phases; 3) Excitability changes after termination of UP phase; 4) The existence of UP-spikes; 5) Temporal relation between areas generating UP-spikes. 6) Temporal relation between neuronal discharges and field potentials.

Events were considered to be UP-spikes if they occurred during the Up phase and their amplitude was higher than 3 SD of the mean amplitude of the Up phase BGA.

Unit activity was detected by window discrimination and perievent histograms or event triggered averages were performed for analysis of the temporal relation between neuronal discharges and field potentials.

Statistical analysis was performed using Prism 4 (GraphPad, San Diego, CA). For samples that passed normality test, paired and unpaired t-tests, as well as one-way analysis of variance (ANOVA) followed by post hoc Bonferroni test was used where appropriate. For samples that failed the normality test, Mann-Whitney or Wilcoxon tests was applied. $P < 0.05$ was accepted as an index of statistically significant differences.

Analysis of labeled neurons: Slides were viewed and analyzed with a Zeiss microscope and AxioVision (version 4.6) software (Zeiss, G ttingen, Germany). Labeled cells were identified microscopically for their location within or outside the granule cell layer. Initially granular cells and interneurons were characterized by the shape of their soma and arborization of their axons and dendrites. The specific subtype of interneuron was determined by the location of arborization of the axon branches and immunocytochemistry. The shape of dendrites and axons was drawn using an Adobe Photoshop program. Digital images of neurons and their processes were superimposed on each other and somata as well as axons and dendrites were outlined by a brush tool.

Results

Non-epileptic mice

Spontaneous UDS—In accordance with previous publications in mice (Cossart et al., 2003, Hahn et al., 2007) and rats (Isomura et al., 2006, Wolansky et al., 2006), we found the UDS, which was characterized by slow < 1.0 Hz waves with oscillations of 20–80 Hz (beta – gamma activity, BGA) both in the neocortex and dentate gyrus (Fig.1, A, A1). The phase with increased BGA amplitude is termed the *Up phase* and the phase with decrease amplitude of BGA is termed the *Down phase*. The duration of Up phase during urethane anesthesia was 0.6 ± 0.5 s and duration of Down phase was 0.7 ± 0.6 s. There was no significant difference in the duration of UP phases recorded in the neocortex and dentate gyrus. The polarity and the amplitude of the slow wave component of Up and Down phases can change depending on the location of the recording microelectrode in relation to a cellular layer. For simplicity in presentation of our data we will display the slow wave with superimpose BGA (Up phase) always facing up.

Evoked Up-Down phases: Single electrical pulse stimulation of PP evoked in the dentate gyrus a short latency (5–20ms) initial local field potential response and a long latency secondary response (300–800ms) (Fig.2, A, A1). In the neocortex there was only a long

latency response to PP stimulation. Both, short and long latency field potential responses were accompanied by unit discharges. The shape and polarity of long latency responses were similar to the spontaneous Up phases recorded from the same electrodes (Fig. 2B, B1). These long latency responses disappeared after an overdose of anesthetic (not shown).

The probability of occurrence of evoked Up phases depended on the time interval between the electrical pulse and the termination of the previous Up phase (Fig. 3A solid line). When the electrical pulse was delivered at 3 seconds and longer after termination of the Up phase, it always evoked a short latency response followed by long latency response (Fig. 3 B1, C1, D1). The pattern of this late response was similar to the pattern that occurred spontaneously (Fig. 3, B3, C3, D3). When the electrical pulse was delivered during the Up phase or during first 500 ms after its termination, it evoked only a short latency response (Fig. 3, B2, C2, D2). An evoked Up phase occurred with different probability when electrical pulse was delivered between 500–253000 msec after termination of a spontaneous Up phase (Fig. 3A).

Epileptic mice

Compared to naive and non-epileptic mice, epileptic mice consistently showed higher resistance to urethane. The initial dose of urethane (1.25g/kg i.m.) in epileptic mice was not sufficient enough to reach the level of anesthesia when an animal does not feel a tail pinch. In these mice a 1.5–2.0 times higher dose of anesthetic was usually required to reach the level where they do not feel painful stimulation. In naive mice the level of anesthesia associated with Up-Down phases usually lasted 2–3 hours without supplemental ketamine injections. In epileptic mice additional injections were required approximately every hour to deepen the anesthesia from continuous 2–4 Hz activity, which is a sign of light anesthesia, to UDS.

Spontaneous UDS—Power spectral analysis of Up and Down phases did not reveal significant differences in the frequency of BGA between normal and epileptic groups. In both, the power of all frequencies was higher during the UP phase Fig. 1, A1, B1). The duration of the Up phase in epileptic mice both in the neocortex and dentate gyrus was longer than in non-epileptic animals (1.2 ± 0.8 s versus 0.6 ± 0.5 s; $n=2600$). Correspondingly the duration of the Down phase was also longer (1.8 ± 0.7 s versus 0.7 ± 0.6 s; $n=2600$). The rate of UDS was significantly slower in epileptic animals (14 ± 2 /min vs 24 ± 8 /min; $p < 0.01$) compared to non-epileptic animals. The field UDS pattern showed striking difference between normal and epileptic mice; instead of the gradual increases and decreases in amplitude of BGA observed in naive mice during the Up phase episodes (Fig. 1, A) a sharp increases in amplitude of single BGA waves were observed in all epileptic mice (Fig.1, B).

Evoked Up-Down phases: As in naive mice, electrical stimulation of PP also evoked a short latency response in the dentate gyrus and a long latency response both in the dentate gyrus and neocortex. Similar to long latency responses in non-epileptic animals the latter is considered to be an evoked Up phase with a pattern resembling the spontaneous events (not shown). The relative refractory period for evoking a full Up phase in epileptic mice is longer than in normal mice. In normal mice, the probability of occurrence of an evoked Up phase with electrical pulse delivered at 3 seconds after termination of previous Up phase was close to 100% (Fig. 3A solid line), in epileptic mice it was significantly lower 83% ($p < 0.01$, Fig. 3A dashed line).

UP-spikes during Up phase as a prominent sign of electrographic pathology in epileptic brain: In epileptic mice normal Up phases with gradual increments in amplitude of BGA alternated with pathological Up phases characterized by the occurrence of sudden sharp increases in amplitude of single gamma waves (Fig.1B). We termed these waves UP-spikes

and they occurred in all epileptic animals in 60–80% of all Up phases. In three mice with very frequent seizures (5–8/24 hours) UP-spikes occurred as a burst during the Up phase and some of spikes also occurred during the Down phase. The duration of UP-spikes both in neocortex and dentate gyrus varied between 10 and 30ms with an average 24.2 ± 7 ms, (2210 UP-spikes from 21 animals). Depending on the location of the recording microelectrode, the amplitude of UP-spikes varied from 0.3mV to 2.0mV (mean 1.1 ± 0.8 mV).

One to five UP-spikes occurred at the beginning, middle or end of the Up phase (Figs. 1B, 5A,C) and their occurrence was associated with suppression of BGA and unit discharges (Fig. 4 B). Discharges of individual identified granular cells were specifically time locked to either the ascending, peak or descending phases of UP-spikes (Fig. 5 black histograms) while discharges of interneurons were associated with UP-spikes, but unlike discharges of granular cells they were not time locked to a specific phase of the UP-spike but widely distributed along ascending and descending parts of the UP-spikes (Fig. 5 blue histograms).

Mechanisms of UP-spikes generation in the dentate gyrus: Due to stratified organization of the dentate gyrus this area of the brain is convenient for investigation of generation of different electrographic events because recorded local field potentials precisely reflect events that occur at the intracellular level. UP-spikes recorded in the dentate gyrus had characteristics similar to population EPSPs evoked by perforant path stimulation (Fig. 6 C). The polarity of UP-spikes recorded in the dentate gyrus during the Up phase was the same as the polarity of population EPSPs, i.e. positive in granular layer and hilus and negative in the molecular layer (data not shown). Expanding these UP-spikes (Fig. 7 A) illustrates that neuronal action potentials are superimposed on the ascending part of these events. Some of the UP-spikes are accompanied by population spikes similar to evoked population spikes (Fig. 7, B, D1–D2).

Temporal interactions between UP-spikes generated in neocortex and dentate gyrus: To exclude volume conductivity of electrical signals the UP-spikes analyzed in this experiment were recorded with a bipolar montage with 0.5mm between tips of microelectrodes.

Cross correlation analysis revealed (Table 1) that in 12 out of 26 epileptic mice, used in these experiments, closely coupled UP-spikes were recorded at least in two brain areas (in the neocortex or dentate gyrus) with a fixed time delay after the reference UP-spike (39 out of 105 recording sites (37.1%)). Examples of perievent histograms from 2 mice are presented in the Fig. 8. In one mouse (Fig. 8, A) temporal dependence of UP-spikes existed between two recording sites: dentate gyrus and ipsilateral neocortex. In the other mouse (Fig. 8, B) UP-spikes occurred in temporal dependence in 4 recording sites: both dentate gyri and ipsilateral and contralateral neocortex. The latency between UP-spikes generated in the dentate gyrus and neocortex varied between zero to 23 msec (mean 12 ± 9 msec). In the remaining 62.9% of recording sites UP-spikes occurred independently of each other. These data indicate that UP-spikes are generated by local networks and some of them are part of broad spatial networks participating in propagation of epileptiform activity.

Discussion

The main general finding of this study is that several parameters of UDS are different in epileptic animals. Although frequency of BGA is not significantly different, the duration of the Up phase and the Down phase is longer in epileptic animals. Also in these animals recovery of excitability after termination of UP phase is longer. At present little is known about the mechanisms of occurrence of the UDS as well as mechanisms of termination of UP phase. Therefore, it is difficult to speculate about mechanisms of increased duration and

increased refractory period. It is hypothesized that slow wave sleep is important for renormalization of synaptic strength to a base level that is maintainable and beneficial for processes of learning and memory (Hanlon et al., 2011, Timofeev, 2011, Vyazovskiy et al., 2011). UDS is the main pattern of slow wave sleep and one could suggest that changes in the duration of the Up and Down phases may affect the process of synaptic renormalization. The longer refractory period after termination of the Up phase in epileptic animals furthermore suggest that homeostatic mechanisms are affected in epileptic brain. Another explanation of the different pattern of UDS is that it is a result of the higher dose of anesthetic. We followed electrographic criteria to maintain similar levels of anesthesia in both groups of animals assuming that this approach will decrease individual variability. However, we cannot exclude the possibility that higher a dosage of anesthetic may affect the UDS pattern.

Based on one report (Fanselow and Connors, 2010) a “division of labor” between parvalbumin containing fast spiking cells and somatostatin expressing interneurons in layers 2/3 of neocortex may be responsible for the termination of the UP-phase. This may also explain changes in the pattern of UDS in the dentate gyrus, because some somatostatin containing hilus neurons die during the process of epileptogenesis (Buckmaster and Dudek, 1997, Buckmaster and Jongen-Relo, 1999, Buckmaster et al., 2002), and excitatory input onto remaining hilar somatostatin containing interneurons is increased in epileptic animals (Halabisky et al., 2010). So far there are no data about cellular elements participating in the maintenance of Up-Down phases in the dentate gyrus; however, considering the similarity of this pattern in all brain areas, somatostatin containing cells may be responsible for the maintenance of the synchrony of neuronal discharges during UP-phase.

The next finding is that instead of gradual alternation in amplitude of BGA during the UP phase in normal conditions we observed the appearance of UP-spikes during UP phase in epileptic brain. UP-spikes cause transient suppression of BGA and unit discharges during the UP phase (Fig. 4). Our experiments provide important characterization of UP-spikes during the UP-phase and insights into their potential mechanisms.

What UP-spikes may reflect and why they occur?

Our data indicate that UP-spikes are generated by the local network surrounding the recording electrode (spatially separated clusters) because they were observed in some recording electrodes but not in other and only in 37.1%, UP-spikes from two or more areas were generated in temporal correlation. The short delay between UP-spikes generated in the neocortex and dentate gyrus does not indicate that they propagate through multisynaptic pathways. Most likely it indicates on the existence of common drive that triggers UP-spikes in these areas.

We provide strong evidence that shape UP-spikes in the dentate gyrus is similar to the shape of population EPSPs evoked by electrical stimulation of input from the entorhinal cortex. These UP-spikes in the dentate gyrus appear to reflect the occurrence of gigantic population EPSPs in response to synchronous signals coming from entorhinal cortex. We suggest that in the neocortex they are also gigantic population EPSPs, however different mechanism of generation cannot be excluded. The occurrence of UP-spikes may reflect at least two abnormalities in the neuronal network: 1) the existence in epileptic brain of clusters of pathologically interconnected neurons (Bragin et al., 2000) that generate hypersynchronous discharges (similar to single electrical pulse stimulation) evoking population EPSPs; 2) inability of the feed forward inhibitory network to prevent the synchrony of incoming signals.

UP-spikes are generated by clusters of pathologically interconnected neurons

On the basis of analysis of mechanisms of generation of pHFOs in our previous publications (Bragin et al., 2000, Bragin et al., 2002) we hypothesized the existence in epileptic brain of clusters of pathologically interconnected neurons (PIN clusters). The main characteristic of these clusters is the existence of a high density of local excitatory connections and when one of the elements of this cluster is excited all neurons in this cluster discharge. As a result a bursts of population spikes “fast ripples” is generated, which can propagate from one cluster to another occasionally leading to seizure occurrence. Data presented in this paper indirectly suggest that UP-spikes could be another pattern generated by PIN clusters. Some of them contain population spikes in the Fast Ripple frequency (see Fig. 7 D2) which are recorded by some, but not all, microelectrodes, indicating local sources of generation.

Pathological pattern of UP-DOWN state and other epileptiform events

The relation between neuronal mechanisms of the UDS pattern and pHFOs is the subject of another study. The relationship of UP-spikes to EEG IIS is unclear. One reason for this uncertainty is the existence of several types of EEG IIS (Engel and Ackermann, 1980, Wadman et al., 1983, Kogure, 1990, Kobayashi et al., 2006, Fabo et al., 2008). On the basis of EEG-fMRI studies different types of interictal spikes may be identified, some resulting in increased while other with decreased BOLD signal (Kobayashi et al., 2006).

UP-spikes are derivatives of the abnormal development of the Up phase and may be a phenomenon similar to some EEG IISs. However, no other studies have analyzed EEG IISs in relation to the UDS pattern, because clinical EEG recordings are performed with a frequency band of 1Hz to 50–70Hz, which obliterates the slow waves of the UDS pattern. Figures in some papers (see for example Fabo, et al. 2008, Fig. 6), however, clearly suggest that some IIS in patients with temporal lobe epilepsy do occur during the Up phase. UP-spikes are an easily observed electrographic phenomenon. Our data indicate that UP-spikes in the dentate gyrus are also gigantic EPSPs. Similar mechanism may be suggested for UP-spikes generated in neocortex, they are one type of EEG IIS and their functional role in epilepsy need to be further explored.

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Highlights

- > Up-Down State pattern is different in normal and epileptic mice.
- > There is a longer recovery of excitability of the network after termination of UP phase in epileptic animals.
- > The Up phase of epileptic mice contains UP-spikes.
- > UP-spikes are gigantic EPSPs and they reflect a weak balance between inhibitory and excitatory networks in epileptic brain.

Fig. 1.

UDS patterns in the neocortex and dentate gyrus of **non-epileptic** (A) and epileptic (B) mice during urethane anesthesia. Black traces are raw data (0.1 – 1000Hz), red traces are band pass (20–80 Hz) filtered data (Dashed lines on A1 and B1). Here and in all other figures in raw data the Up phase wave is facing up. A1 and B1 correspondingly power spectrograms for Up phase (red) and Down phase (black).

Fig. 2.

Dentate gyrus identified interneuron discharges during evoked and spontaneous Up-Down waves in the neocortex and dentate gyrus **in non-epileptic** mouse. A. Up phase in the neocortex and dentate gyrus evoked by a single electrical pulse delivered to PP (white line illustrates an average of 18 responses). A1. An extended part of the early response. There is no early response in the neocortex but a DG interneuron responds with one-two spikes with the latency 5–10 ms. Arrows indicate the moment of electrical pulse delivery. B. Superimposition and average (white line) of 75 spontaneous field Up-Down phases detected in the neocortex at the level of zero crossing and unit discharges of the same neuron presented in A.. B1, Perievent histogram of unit discharges presented in B.

Fig. 3.

A. Probability of occurrence of the UP phase in response to perforant path single pulse electrical stimulation at different times after termination of the Up phase in epileptic (dashed line) and non epileptic mice (solid line). Data are presented from 3 mice in each group with at least 5 stimulations at each time point in each animal. B-D. The occurrence of an evoked Up phase in response to a single electrical pulse delivered to the PP. The left and middle columns show, a pattern of responses evoked by stimulation delivered correspondingly at 3–5 seconds and 0.8 – 1.5 seconds after termination of the Up phase. The right column shows, a spontaneous Up phase. B 1,2,3 - extracellular unit activity. C 1,2,3 – local field potentials. D 1,2,3 – perievent histograms.

Fig. 4.

Consequences of interruption of UP phase by the UP-spike. A. Normal Up phase. A1 is an example of discharges of an interneuron during a normal Up phase recorded in the vicinity of 0.5 mm. It gradually increases its frequency of discharges during the Up phase (A 2). Similar increase of discharges occurs in simultaneously recorded multiunit activity (A 3). B Pathological Up phase recorded from the same site. B1 – The UP-spike that occurs during the Up phase suppresses beta-gamma field activity as well as basket cell (B 2) and multi- (B 3) unit discharges.

Fig. 5.

Examples of identified granular cell (A) and interneuron (C) discharges outside and during UP-spike (numbers 1 and 2) in epileptic mice. B. Histograms of discharges of 9 granular cells discharges during UP-spikes. D. Histograms of discharges of 3 interneurons discharges during UP-spikes. Notice that discharges of granular cells (A 1–2) occur both on the descending phase of the UP-spike, while discharges of interneuron during the UP-spike C1 occurred on the ascending phase, while during the UP-spike C2 at the peak. The red line is the normalized shape of the UP-spike. “n” – illustrate number of UP-spikes used for collection of a histogram.

Fig. 6.

Discharges of identified interneuron during UP-spike in epileptic mouse. A. UP-spikes interrupting the development of the Up phase. A1. Raw data containing discharges of basket cell and local field potentials with UP-spike (dashed box) recorded by a glass microelectrode (see schematic in the inset). A2. Superimposed local field potentials ($n=25$) recorded by a bipolar tungsten microelectrode located $500\mu\text{m}$ from the tip of the glass microelectrode in the hilus. A3. Perievent histogram ($n=25$) of neuronal discharges before and after the occurrence of UP-spikes. B. Superimposed ($n=25$) UP-spikes recorded by the glass microelectrode (B1) and the tungsten microelectrode (B2). Notice neuronal action potentials superimposed on the UP-spikes. C. Superimposed ($n=5$) evoked potentials in response to perforant path stimulation recorded by the glass microelectrode (C1) and tungsten microelectrode (C2). Red dashed lines are superimposed UP-spikes from B1 and B2 to illustrate that the shape of the UP-spikes is similar to the shape of evoked population EPSPs. C3 – picture of the recorded interneuron reconstructed from 3 consequent sections. g.c. – granular cell layer.

Fig. 7.

Similarity of spontaneous and evoked UP-spikes **in epileptic mouse**. A. Development of spontaneous Up phase with superimposed action potentials (triangles). B. Example of evoked response to perforant path electrical shock (left dashed box) and spontaneous Up phase. The largest UP-spikes contains population spikes similar to that evoked by electrical stimulation. C. Illustration of granular cell recorded in A, showing the location of glass and tungsten recording microelectrodes. D1 and D2 extended examples of correspondingly evoked potential and spontaneous UP-spikes. D2 indicates that UP-spikes are population EPSPs and are sometimes accompanied by population spikes.

Fig. 8.

Functional connections between areas generating UP-spikes. A and B perievent histograms from 2 **epileptic** mice between different brain areas (indicated above), where UP-spikes of the left brain area are taken as a reference point - "0". In mouse "A" there is a temporal relations between UP-spikes generated in the left and right DGs and correspondingly left and right neocortex. In mouse "B" connections between the same set of electrodes are much stronger and some UP-spikes generated in the DG are correlated with the time of occurrence of UP-spikes both in ipsilateral and contralateral neocortex.

Table 1

Number of recording sites generating UP-spikes that show functional relations with each other.

number of correlated sites	0	2	3	4	5	total	%
number of mice	14	5	2	2	3	26	100
number of recording electrodes	66	10	6	8	15	105	100