Published in final edited form as:

Epilepsy Res. 2009 August ; 85(2-3): 199–205. doi:10.1016/j.eplepsyres.2009.02.020.

Different phases of afterdischarge during rapid kindling procedure in mice

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Summary

The basic mechanisms of hippocampal networks in epileptogenesis are not entirely understood. To help achieve a better understanding of these mechanisms, we studied the extra-cellular electrically-evoked responses in the hippocampi of mice during rapid kindling. Kindling protocol was achieved by stimulating the dorsal right hippocampus six times daily for four days using bipolar electrodes to produce sub-convulsive electrical discharges. Motor responses and analyzed electroencephalographic recordings showed progression from partial complex seizures to generalized seizures associated with different consecutive patterns within the afterdischarges. A spike-wave pattern appeared immediately after stimulation in combination with a poly-spike complex superimposed over the wave (AD1). AD1 was followed by a poly-spike complex (AD2), which was followed by a progressive modification of repetitive spikes (AD3). An ictal depression event was observed at the end of each AD3. Theta oscillations were observed at stage 1-2 of kindling, while beta/gamma oscillations appeared within AD2, associated with stage 4-5 from Racine's score. Benzodiazepine, a GABA (A) agonist (Diazepam) administered at non-sedative doses and only on days 3 and 4 of kindling, limited beta and gamma frequency bands and the progression of seizure severity, suggesting that the failure of GABA (A) agonism mediates the propagation or generalization of seizures. We conclude that different phases of afterdischarge occur during kindling and that high frequencies mediate generalization of seizures.

Keywords

kindling; epileptogenesis; EEG; gamma oscillations; GABA

1. Introduction

The rapid kindling model in rodents represents an elegant experimental approach for dissecting and analyzing the oscillatory components of the evoked electrical discharge related with different types of seizures. This model allows for exploration of the neuronal network that contributes to the progression of partial seizures to generalized seizures (Delgado and Sevillano, 1961; Goddard et al., 1969; Racine, 1972; Leung, 1987), without producing excessive damage to the brain, as in other experimental models of epilepsy (Morimoto et al., 2004; Pitkanen et al., 2006). The rapid kindling procedure administers repetitive, sub-

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convulsive electrical stimulation to the hippocampus (Goddard et al., 1969, Lothman et al., 1991). As a consequence of these stimulations, the clinical manifestations and the evoked electrical activity (afterdischarge, AD) observed in electroencephalographic (EEG) recordings display progressively increased complexity and duration.

Different EEG patterns in epilepsy (Penfield and Jasper, 1954) may represent a dynamic process *in vivo* – occurring within only a few seconds – among the pyramidal-interneuron network (Buzsaki et al., 1982, 1989), neuron-glia interactions (Somjen, 2001), biochemical reactions (Tu and Bazan, 2003), and gene expression (Musto and Bazan, 2006), which are all critical components in seizures. Unfortunately, the specific neural networks (associated with specific EEG patterns) that are activated have not yet been studied in detail; therefore, the relevance of EEG morphology may be underestimated. In this study, we describe three different dimensions of AD during hippocampal kindling. We observed frequencies in beta and gamma bands in kindling progression, which are related with seizure severity. Intraperitoneal administration of diazepam (the δ -amino-butyric-acid (GABA) (A) agonist) before the occurrence of the kindled state resulted in limited beta and gamma oscillations and, consequently, a reduction in severe seizures for at least one week after kindling acquisition. These observations suggest that a progressive failure of inhibition during kindling mediates seizure propagation and severity leading to a recurrent, hyperexcitable hippocampal network.

2. Materials and methods

Studies were performed on C57BL/6 adult male mice (20-25 g, Charles River Lab, Inc., MA) according to National Institutes of Health guidelines, in adherence to the recommendations from the Declaration of Helsinki, and in accordance with nationally accepted principles in the care and use of experimental animals. Protocols were approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee. Fourteen animals were used for EEG pattern analysis during kindling, while 10 mice were used to study the diazepam effect on the kindling process.

Bipolar electrode units for stimulation and recordings (Plastic One Inc., Roanoke, VA) were implanted in the dorsal right hippocampus (coordinates: 2.3 mm caudal to bregma; 1.75 mm lateral to midline; 2.00 mm ventral to dura) and a ground wire was placed on the occipital bone, guided by stereotaxis and under anesthesia induced by an intraperitoneal injection of a mixture of ketamine (200 mg/kg) and xylazine (10 mg/kg) (Vedco, MO).

Seven days after surgery, kindling acquisition was achieved by stimulating animals six times daily for four consecutive days with sub-convulsive electrical stimulations (10-s train containing 50-Hz biphasic pulses of 100- μ A amplitude) at 30-min intervals. The set up of this stimulation was based on previous results obtained of progressive increases in intensity to acquire an AD in adult C57BL/6 mice (data not shown). The animals that did not respond initially with an AD, or that displayed signs of electrode displacement, were excluded from the study. At the end of the experiments, the animals were deeply anesthetized by an intraperitoneal overdose injection mixture of ketamine (300 mg/kg) and xylazine (30 mg/kg) and then transcardially perfused with 4% paraformaldehyde (FD Neurotech, MD). Brain samples were dissected and histology techniques were conducted to verify electrode positions.

The behavioral responses were scored (Racine, 1972) by an investigator who had no information about the number of stimulations. EEG recordings were made during each AD throughout the kindling procedure using Enhanced Graphics Acquisition for Analysis (Version 3.63, RS Electronics Inc., Santa Barbara, CA). The signal was amplified 1000 times, filtered 0.5 to 40 Hz (3dB/octave) (Med Associates, VT), and digitized at a sampling rate of 200 Hz. AD duration and different morphologic components of each AD, such as spikes, sharp waves,

poly-spike waves, were analyzed using Neuroexplorer (Next Technology, Littleton, MA) software. Also, band frequencies for theta (4-8 Hz), beta (13-20 Hz) and gamma (21-40 Hz) were analyzed. Signal values were multiplied by the coefficients of Hann window and discrete fast fourier transformation of the results were calculated using formulas defined in (Press et al., 1992). Power spectrum was normalized from raw power spectral densities and represented the sum of all the spectrum values equal to the mean squared of the signal (Neuroexplorer, Next Technology, Littleton, MA). The number of spikes was analyzed as followed: The raw signal was visualized offline in time/ μ V window using Sort Client program (Rasputin software, Plexon Inc., Dallas TX.). A line above the signal before the stimulation was set up as a baseline threshold of amplitude, then the numbers of spikes above that threshold were automatically quantified selecting for peak and timestamp function.

Because the aim was to modulate inhibition during kindling progression, intraperitoneal administration of benzodiazepine (diazepam, Sigma, MO), which acts as a GABA (A) receptor ligand and facilitates inhibition, or vehicle ((2-Hydroxypropyl)- β -cyclodextrin, Sigma, MO) was injected at non-sedative doses that would not affect the locomotor activity (0.5-1 mg/kg) (Krug et al., 1998; Rosenberg et al., 1989) 30 minutes before the first stimulation on days 3 and 4 of the kindling acquisition (Fig. 1).

AD duration was examined in vehicle- and diazepam-treated groups at day 4 of kindling acquisition from the dorsal hippocampus. EEG recordings were examined using Neuroexplorer to determine AD duration. The AD's measurement (in seconds) began immediately at the termination of the 10-second sub-convulsive stimulation and ended when the EEG readings showed no spiking activity. AD2, an epileptiform pattern seen within the AD, was discerned according to the morphology of the signal (Fig. 2B). Also, AD2 was considered present if oscillations occurred above 20 Hz in vehicle- and diazepam-treated groups.

In order to verify the permanent condition of the kindled state, one week after the last stimulation, vehicle- or diazepam-treated mice were stimulated for one day using the same protocol for kindling acquisition (Fig. 1). Data were averaged and expressed as means $\pm$ S.E.M. and accompanied by the number of observations. Data comparison between the diazepam- and vehicle-treated groups was performed using the Student's t test. The probability level interpreted as statistically significant was $P < 0.05$.

3. Results

From a total of 45 EEG recordings from AD during kindling, 80% presented phases of afterdischarges as is described in Fig 2B. The duration, power of the signal, and frequencies of the AD increased from stage 1-2 to stage 4-5 (Fig. 2A). In both stages, the ADs were immediately followed by a low-energy signal representing ictal-depression (ID) activity (Fig. 2B).

Also, each stage evoked different consecutive EEG patterns, denoted by AD1, AD2 and AD3 (Fig. 2B). At stage 1-2, which represented the activation of the limbic system, spike-wave pattern bursts separated by a silent period (Fig. 2B, dot) appeared immediately after stimulation as background in combination with a poly-spike complex superimposed over the wave (AD1). AD1 was followed by a poly-spike complex (AD2), which was represented by a spike wave. AD3 consisted of spike waves that resembled a repetition of AD1. On day 4 of kindling, the repetitive stimulations evoked a consistent stage 4-5 that resembled generalized seizures. AD1 showed a burst of spikes separated by a silent period. In this stage, the number of spikes for each burst increased, and the silent period was reduced as compared with stage 1-2 (Fig. 2B). AD2 lost the wave component observed in the previous stages, and instead showed repetitive spikes followed by individual spike waves of different amplitudes (AD3) (Fig. 2B).

The oscillatory activities of AD1 and AD2 patterns were modified during kindling (Fig. 2C,D). At stage 4-5, frequencies above 20 Hz were present in 80% of the animals. Frequency bands above 10 Hz were associated with the AD2 pattern (Fig. 2D). Therefore, we studied the behavior of frequency bands (beta: 13-20 Hz; gamma: 21-40 Hz) under the effect of diazepam, a GABA A receptor agonist, at the time these frequencies occurred.

We observed that diazepam stopped the progression of kindling, keeping the animals in stage 1-2 without abolishing the AD (Figs. 3,4). Only the initial component of AD displayed high-amplitude spike waves. Also, the occurrence of the AD2 component was reduced, and the total AD duration was significantly shorter compared with vehicle-treated mice (Fig. 4A-E). Beta (13-20 Hz) and gamma (21-40 Hz) bands of AD at day 4 of kindling were limited in diazepam-treated mice (Fig. 4D). Usually one week after kindling acquisition, when using a one-day session of stimulations under the same protocol during kindling acquisition, animals show stimulation-induced locomotor responses that resemble the last days of kindling (Musto and Bazan, 2006). Here, using Racine's scoring we observed that diazepam during kindling attenuated seizure severity and limited the duration of the AD compared with vehicle-treated animals (Fig. 5).

4. Discussion

According to these experiments, we propose that different phases of afterdischarge occur during kindling and that high frequencies mediate generalization of seizures. Hippocampal response to these stimulations, or AD, becomes more complex during kindling. Most experimental designs using the kindling model focus on the duration of the AD as the main dependent variable (Racine, 1972; Goddard et al., 1969; Morimoto et al., 2004). Here, however, we concentrated on other components of the AD, like frequency and morphology of the signal, which could provide a greater understanding of the intrinsic neural network activated in kindling epileptogenesis. As we show in Fig. 2C, the frequency of the signal increases after successive electrical stimulations associated with determined patterns of oscillation. Analysis of the signal morphology led us to differentiate four successive patterns that occurred immediately after stimulation. These patterns, which we denoted as AD1, AD2, AD3, and ID, changed progressively during kindling and could easily be used to differentiate the stage 1-2 from stage 4-5, which resemble partial complex and generalized seizures in humans. This may be useful for predicting seizure generalization. According to our model, this generalization is associated with the occurrence of an increase in the power spectrum of beta and gamma waves.

Our AD analysis attempted to show three different dimensions of the signal (Fig. 2). However, there are still some questions that remain unanswered. What are the predominant neural networks that evoke each of these patterns during kindling? What are the main neurotransmitters or neuromodulators and what are the signal transductions related with each component of the ADs? Which of these patterns could tell us about the particular molecular pathway/s critical for the transition from partial to generalized seizures or epileptogenesis? Despite these lingering questions, we speculate that each of these ADs may be associated with some neural mechanism.

For example, the AD1 pattern could reveal the mechanism of the evoked response to electrical stimulation where pyramidal- and interneurons fire in concert, generating a type of complex burst of spikes mainly involving the activation of AMPA, NMDA, and GABA receptors (Morimoto et al., 2004; Ziburkus et al., 2006), which are preserved at the onset of kindling. After successive stimulations, the fast inhibition process is exhausted (Wendling et al., 2005); this could be involved in the down regulation of GABA (A) (Liefgaard et al., 2009) or GABA(B) receptors and, in turn, overcome excitatory activity (Leung et al., 2006) and evoke the AD2 pattern.

Along with the progression of kindling, modification of the spike waves and evoked frequencies above 15 Hz, observed within AD2, could reveal mechanisms of partial complex secondary generalized seizures. Assuming that the progressive loss of inhibition affects the kindling mechanism, we facilitated inhibition with the administration of the GABA (A) agonist, diazepam, which has been shown to fully suppress kindled seizures (Morimoto et al., 1986; Krug et al., 1998; Rosenberg et al., 1989). Here, however, we used anti-sedative doses in hippocampi when facilitation of inhibition was necessary (day 3 of kindling). Our results show that the increase of inhibition during kindling reduced seizure severity and limited the occurrence of frequencies above 15 Hz, which could participate in the propagation of focal seizures. In other words, we infer that the beta and gamma bands in hippocampi mediate the generalization of these seizures. Also, since extra-limbic influence could be activated (Steriade, 2005), other receptors such as dopamine receptors (D1/D2) and histamine receptor H1 could be involved in the genesis of spike waves during kindling (Bosnyakova et al., 2006).

Following the hypothesis that the interneuron-granular cell network fails to compensate for CA3-CA1 excitability during epileptogenesis, which leads to autonomous firing within the hippocampal circuit (Lothman et al., 1991), it is possible that this network failure facilitates or triggers the sharp waves observed in AD3 (Buzsaki and Eidelberg, 1982; Buzsaki, 1986; Loscher and Ebert, 1996). Therefore, this oscillatory activity with frequencies above 15 Hz could spread over the entire extra-hippocampal region (McNamara et al., 1980; Magdaleno-Madigal et al., 2002; Wada and Hamada, 1990). On the other hand, ID activity observed from the onset of kindling could reflect interactions between the glial and neuronal populations (Somjen, 2003; Cole-Edwards et al., 2006) that could contribute to the termination of the AD (Bragin et al., 1997). In addition, during ID activity, massive cellular influxes of calcium lead to the activation of phospholipase A₂ (PLA₂) (Katsura et al., 2000), and molecular signaling related to excitability is activated (Tu and Bazan, 2003).

In summary, attributing kindling generation to only one issue (i.e., AD duration) fails to take into account other contributing factors, which could impede the progress of translational research. Experimental designs using neural assembly analysis associated with the molecular pathways activated during kindling will lead to a greater understanding of the mechanisms involved in seizure generalization and propagation. Such discoveries will shed light on pathways for novel antiepileptic drugs that can correct the aberrant pathophysiology of epileptogenesis without interfering with physiological neurotransmission.

Acknowledgments

The authors would like to thank Dr. Nicolas G. Bazan, Louisiana State University Health Sciences Center, New Orleans, Neuroscience Center of Excellence, for his insightful advice and comments. This work was supported by NIH/NCRR grant P20 RR016816.

References

- Bosnyakova D, Gabova A, Kuznetsova G, Obukhov Y, Midzyanovskaya I, Salonin D, van Rijn C, Coenen A, Tuomisto L, van Luijckelaar G. Time-frequency analysis of spike-wave discharges using a modified wavelet transform. *J Neurosci Methods* 2006;154:80–88. [PubMed: 16434106]
- Bragin A, Penttonen M, Buzsáki G. Termination of epileptic afterdischarge in the hippocampus. *J Neurosci* 1997;17:2567–2579. [PubMed: 9065516]
- Buzsáki G, Leung LW, Vanderwolf CH. Cellular bases of hippocampal EEG in the behaving rat. *Brain Res* 1983;287:139–171. [PubMed: 6357356]
- Buzsáki G. Hippocampal sharp waves: their origin and significance. *Brain Res* 1986;398:242–252. [PubMed: 3026567]
- Buzsáki G, Eidelberg E. Direct afferent excitation and long-term potentiation of hippocampal interneurons. *J Neurophysiol* 1982;48:597–607. [PubMed: 6290613]

- Buzsáki G, Ponomareff GL, Bayardo F, Ruiz R, Gage FH. Neuronal activity in the subcortically denervated hippocampus: a chronic model for epilepsy. *Neuroscience* 1989;28:527–538. [PubMed: 2710328]
- Cole-Edwards KK, Musto AE, Bazan NG. c-Jun N-terminal kinase activation responses induced by hippocampal kindling are mediated by reactive astrocytes. *J Neurosci* 2006;26:8295–8304. [PubMed: 16899724]
- Delgado JMR, Sevillano M. Evolution of repeated hippocampal seizures in the cat. *Electroencephalogr Clin Neurophysiol* 1961;13:722–733.
- Dyer RS, Burden E, Hulebak K, Schultz N, Swartzwelder HS, Annau Z. Hippocampal afterdischarges and their post-ictal sequelae in rats: effects of carbon monoxide hypoxia. *Neurobehav Toxicol* 1979;1:21–25. [PubMed: 551298]
- Fox SE, Ranck JB Jr. Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. *Exp Brain Res* 1981;41:399–410. [PubMed: 7215500]
- Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969;25:295–330. [PubMed: 4981856]
- Katsura K, Rodriguez de Turco EB, Kristián T, Folbergrová J, Bazan NG, Siesjö BK. Alterations in lipid and calcium metabolism associated with seizure activity in the post ischemic brain. *J Neurochem* 2000;75:2521–2527. [PubMed: 11080205]
- Krug M, Becker A, Grecksch G, Pfeiffer A, Matthies R, Wagner M. Effects of anticonvulsive drugs on pentylenetetrazol kindling and long-term potentiation in freely moving rats. *Eur J Pharmacol* 1998;356:179–187. [PubMed: 9774247]
- Liefwaard LC, Ploeger BA, Molthoff CF, de Jong HW, Dijkstra J, van der Weerd L, Lammertsma AA, Danhof M, Voskuyl RA. Changes in GABAA receptor properties in amygdala kindled animals: in vivo studies using [¹¹C]flumazenil and positron emission tomography. *Epilepsia* 2009;50:88–98. [PubMed: 18727682]
- Leung LS, Shen B. Hippocampal partial kindling decreased hippocampal GABAB receptor efficacy and wet dog shakes in rats. *Behav Brain Res* 2006;173:274–281. [PubMed: 16887203]
- Leung LW. Hippocampal electrical activity following local tetanization. I. Afterdischarges. *Brain Res* 1987;419:173–187. [PubMed: 3676723]
- Lothman EW, Bertram EH 3rd, Stringer JL. Functional anatomy of hippocampal seizures. *Prog Neurobiol* 1991;37:1–82. [PubMed: 1947175]
- Löscher W, Ebert U. The role of the piriform cortex in kindling. *Prog Neurobiol* 1996;50:427–481. [PubMed: 9015822]
- Magdaleno-Madrigal VM, Valdés-Cruz A, Martínez-Vargas D, Martínez A, Almazán S, Fernández-Mas R, Fernández-Guardiola A. Effect of electrical stimulation of the nucleus of the solitary tract on the development of electrical amygdaloid kindling in the cat. *Epilepsia* 2002;43:964–969. [PubMed: 12199721]
- McNamara JO, Byrne MC, Dasheiff RM, Fitz JG. The kindling model of epilepsy: a review. *Prog Neurobiol* 1980;15:139–159. [PubMed: 6109361]
- Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol* 2004;73:1–60. [PubMed: 15193778]
- Morimoto K, Goddard GV. Kindling induced changes in EEG recorded during stimulation from the site of stimulation: collapse of GABA-mediated inhibition and onset of rhythmic synchronous burst. *Exp Neurol* 1986;94:571–584. [PubMed: 3780908]
- Musto A, Bazan NG. Diacylglycerol kinase epsilon modulates rapid kindling epileptogenesis. *Epilepsia* 2006;47:267–276. [PubMed: 16499750]
- Penfield, W.; Jasper, H., editors. *Epilepsy and the functional anatomy of the human brain*. Little, Brown; Boston, MA: 1954. p. 692-738.
- Pitkanen, A.; Schwartzkroin, PA.; Moshe, SL., editors. *Models of Seizure and Epilepsy*. Elsevier; Burlington, Massachusetts: 2006. p. 433-448.
- Press, WH.; Teukolsky, SA.; Vetterling, WT.; Flannery, BP., editors. *Numerical Recipes in Example Book (C)*. Vol. 2nd. Cambridge University Press; New York, New York: 1992. p. 195-221.
- Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972;32:281–294. [PubMed: 4110397]

- Rosenberg HC, Tietz EI, Chiu TH. Tolerance to anticonvulsant effects of diazepam, clonazepam, and clobazam in amygdala-kindled rats. *Epilepsia* 1989;30:276–285. [PubMed: 2721464]
- Somjen GG. Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol Rev* 2001;81:1065–1096. [PubMed: 11427692]
- Steriade M. Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends Neurosci* 2005;28:317–324. [PubMed: 15927688]
- Tu B, Bazan NG. Hippocampal kindling epileptogenesis upregulates neuronal cyclooxygenase-2 expression in neocortex. *Exp Neurol* 2003;179:167–175. [PubMed: 12618123]
- Wada JA, Hamada K. Role of the midline brainstem in feline amygdaloid kindling. *Epilepsia* 1990;40:669–676. [PubMed: 10368063]
- Wendling F, Hernandez A, Bellanger JJ, Chauvel P, Bartolomei F. Interictal to ictal transition in human temporal lobe epilepsy: insights from a computational model of intracerebral EEG. *J Clin Neurophysiol* 2005;22:343–356. [PubMed: 16357638]
- Ziburkus J, Cressman JR, Barreto E, Schiff SJ. Interneuron and pyramidal cell interplay during in vitro seizure-like events. *J Neurophysiol* 2006;95:3948–3954. [PubMed: 16554499]

Figure 1.

(A) Electrode placement for stimulation and recording of dorsal hippocampal CA1 region of the C57BL/6 mouse. (B) Seven days after electrode implantation, kindling acquisition consisted of six repetitive subconvulsive stimulations daily for four consecutive days. At days 3 and 4 of kindling acquisition, single intraperitoneal injection of diazepam was administered 30 minutes before the first stimulation. Another set of six subconvulsive stimulations was applied through the same electrode (retest kindling) one week after kindling acquisition.

Figure 2.

(A) Representative time-frequency distribution of energy (color bar: red = high energy) from hippocampal EEG recordings obtained immediately after stimulations during the time mice present stage 1-2 or stage 4-5 (Racine's score). Note increment of signal energy, frequencies, and duration of AD (*) from stage 1-2 to stage 4-5. At the end of the AD, the energy decreases and slow recovery of the normal hippocampal activity, interrupted by "wet dog shake" artifact (+), is observed at stage 1-2. (B) Representative EEG traces from hippocampal AD associated with stage 1-2 or stage 4-5. Four consecutive patterns were observed within each AD. At stage 1-2, bursts of spike wave patterns with a poly-spike complex over the wave were present immediately after the stimulation (AD1), interrupted by a period of silence. AD2 shows poly-

spike waves followed by a spike wave complex that resembles AD1. At stage 4-5, the main difference with respect to previous stages shows the following: AD1 - bursts of spikes interrupted by period of silence; AD2 - repetitive spikes with irregular amplitude and frequencies; and AD3 - sharp waves. All ADs end in ictal depression activity. (C) Representative EEG from AD showing frequencies of 20 Hz and 30 Hz only at stage 4-5.(D) Power spectral densities from AD1, AD2, AD3 and ID patterns in kindling. At stage 1-2, most of the signal intensity for each pattern is below 20 Hz, while the signal from AD2 predominates between 10 Hz and 20 Hz over the rest. At stage 4-5, the signal intensity from AD1 is below 20 Hz, whereas in the AD2 pattern the signal is above 15 Hz. Most of the signal intensity for AD3 and ID remains below 20 Hz.

Figure 3.

(A) Progression of seizures from vehicle- and diazepam-treated mice according with Racine's score observed during kindling acquisition. Mice treated with diazepam remained in stage 1 while vehicle-treated mice showed the expected progression profile during kindling, presenting stage 1-2 during day 1 and stage 4-5 at day 4. The score for each day represents the average and $\pm$ S.E.M. obtained immediately after stimulations. (B) Representative hippocampal EEG tracings from diazepam- and vehicle-treated mice at day 4 of kindling acquisition. The tracing from the diazepam-treated animal initially presents bursts of spikes that progressively decrease in amplitude and duration in contrast with the vehicle-treated mice, which present synchronous bursts of discharge resembling patterns showed in Fig. 2, stage 4-5. Note the AD2 epileptiform pattern (box). * $P < 0.0001$.

Figure 4.

(A) AD duration of treated groups from day 4 of kindling acquisition. Diazepam (red bar) treatment reduces the AD duration, compared with vehicle treatment (black bar). *P<0.01. (B) AD2 pattern identification and quantification in treated groups from day 4 of kindling acquisition. Diazepam-treated mice present reduced AD2 patterns, compared with vehicle-treated mice. (C) Representative distribution of signal power, power spectral densities (PSD) in the frequency domain measured in Hz, at day 4 of kindling acquisition. Suppression of frequencies between 15 Hz and 30 Hz are observed in diazepam-treated mice (red traced) compared with the vehicle-treated mice (black traced). (D) Diazepam administration reduced beta and gamma bands compared with vehicle administration at day 4 of kindling acquisition. *P<0.04. The red bars are diazepam-treated mice and the black bars are vehicle-treated mice.

Figure 5.

Racine's score and AD duration from treated mice one week during kindling retesting. The diazepam-treated group presents lower seizure severity, according with Racine's score (A, * $P < 0.0002$) and shorter AD than vehicle-treated group (B, * $P < 0.02$). The red bars are diazepam-treated mice and the black bars vehicle-treated mice.