Evoked Responses of the Dentate Gyrus During Seizures in Developing Gerbils With Inherited Epilepsy

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INTRODUCTION

Inherited epilepsy is common in domesticated Mongolian gerbils (Loskota et al. 1974). Gerbils begin having seizures when they are 1–2 mo old (Buckmaster et al. 1996; Kaplan and Miezejeski 1972). Initially, their seizures are mild, involving only a brief suspension of normal behavior, but they become more severe as gerbils mature (Loskota et al. 1974; Seto-Ohshima et al. 1992). Therefore gerbils provide an opportunity to study a process of epileptogenesis by evaluating animals at different stages of development. Another advantage of this model is that seizures can be triggered by a variety of stimuli, including placement in a novel environment (Kaplan 1975; Ludvig et al. 1991; Schonfeld and Glick 1980; Scotti et al. 1998). Although these seizures are not truly spontaneous, they occur in situations that would not trigger seizures in normal animals, seizures do not require electrical stimulation or pharmacological treatments, and their timing can be controlled by the investigator.

Based on morphological evidence (Farias et al. 1992; Paul et al. 1981; Peterson and Ribak 1987; Peterson et al. 1985) and lesion studies (Ribak and Khan 1987), the dentate gyrus has been proposed as an epileptic focus in gerbils. To test the dentate gyrus for functional abnormalities in epileptic gerbils, we previously examined field potential responses to perforant path stimulation in anesthetized adult gerbils (>3 mo old) with chronic epilepsy and juvenile gerbils (2 mo old) that had just begun having seizures. Both epileptic groups showed enhanced paired-pulse depression at short interstimulus intervals (30 ms) and enhanced paired-pulse facilitation at intermediate interstimulus intervals (70 ms) compared with age-matched controls (Buckmaster et al. 1996). However, in awake animals inhibition in the dentate gyrus is dynamically modulated (Moser 1996), so it was necessary to measure inhibition in the dentate gyrus at the onset of spontaneous seizures to test the hypothesis proposed by Peterson and Ribak (1989) that epilepsy in gerbils is caused by disinhibition of granule cells. We therefore used field potential analysis in awake, adult gerbils as they experienced seizures and found, contrary to the prediction of the disinhibition hypothesis, that population spike amplitude and paired-pulse depression did not vary significantly from baseline until after seizure onset (Buckmaster et al. 2000).

In the present study we sought to extend this approach by

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evaluating epileptic gerbils at different developmental stages and analyzing evoked field potentials as gerbils progress through the following stages of a seizure: baseline preseizure period, seizure onset, convulsive phase, postictal depression, and return to normal behavior. We addressed the following questions. 1) Unlike in chronically epileptic adult gerbils (Buckmaster et al. 2000), is there evidence for disinhibition in the dentate gyrus of juvenile gerbils when they first begin having seizures and when seizure-mechanisms might be different? 2) How do evoked responses change as gerbils develop increasingly more severe behavioral seizures? 3) Do changes in evoked responses during a seizure correlate with seizure-related changes in behavior?

METHODS

Mongolian gerbils (Meriones unguiculatus) used in these experiments came from our colony in which animals are selectively bred for the epilepsy trait. To implant electrodes, gerbils were anesthetized (pentobarbital sodium 60 mg/kg ip), placed in a stereotaxic frame, and maintained on a heating pad with feedback control. Using aseptic surgical technique, holes were drilled through the skull; a bipolar stimulating electrode (SNEX-200, Rhodes Medical Instruments) was directed toward the angular bundle, and 25-μm-diameter insulated stainless-steel recording electrodes (California Fine Wire) were directed toward the border of the hilus and the granule cell layer in the dorsal hippocampus. Electrodes were positioned at the following coordinates (relative to bregma): −3.3 mm posterior and 2.45 mm lateral for the recording electrode and −5.0 mm posterior and 4.3 mm lateral for the stimulating electrode. Electrode depths were determined by optimizing field potential responses to stimulation. For recording neocortical EEG activity, a jeweler’s screw was positioned approximately 2 mm rostral and approximately 2 mm lateral to the hole for the depth recording electrode. Jeweler’s screws for ground and reference leads were placed in the posterior cranium over the cerebellum. All leads were connected to a plug (Microtech) that was attached to the skull with cranioplastic cement (Plastics One). Gerbils recovered 3 days before recordings began. Electroencephalographic (EEG) signals were filtered (0.1–4000 Hz) and amplified (AI 402 ultralow noise differential amplifiers and CyberAmp 380, Axon Instruments), observed on-line, and stored on computer (Clampex, Axon Instruments) and on video-tape (Neuro-corder, Neuro Data Instruments) for off-line analysis. EEG recordings began while the gerbil remained in its home cage and continued as the animal was exposed to a novel environment which then triggered a seizure.

The perforant path was stimulated at 0.2 Hz with pairs of 150-μs constant current stimuli at a 15-ms interstimulus interval. Stimulus intensity was set just high enough (488 ± 43 μA; range = 50–2200 μA) so that the first response of the pair consistently evoked a population spike during the baseline period prior to novel environment exposure. Previous experiments showed that these stimulation parameters provide frequent responses for analysis and do not initiate seizures (Buckmaster et al. 2000). To minimize the effect of stimulation on spontaneous activity, a relatively low stimulus intensity was used. The goal was to monitor but not affect the excitability of the dentate gyrus. Our evoked response measurements, therefore, are likely to represent the low end of the input/output curve of the dentate gyrus.

Population spike amplitude was measured from the peak negativity to an average of the peak positivities immediately before and after the spike. The amplitude ratio, defined as the amplitude of the second spike divided by the first spike of a pair, was used as a measure of paired-pulse depression. Therefore a reduction in paired-pulse depression is reflected as an increase in the amplitude ratio. The slope of the field excitatory postsynaptic potential (fEPSP slope) was measured over approximately the first third of the rising phase of the fEPSP before the population spike, as described by Moser (1996). The slope was measured between points selected specifically for each set of responses from a recording session, instead of fixed points for all animals, because of variability in latencies and stimulus artifacts between animals. The first point was after the stimulus artifact and near the onset of the fEPSP. The second point was approximately halfway between the onset of the fEPSP and the onset of the population spike.

To analyze changes in evoked responses during seizures, results were normalized by the preseizure baseline values from the same animal and the same recording session. At least 4 min of baseline data were collected during each recording session. The baseline period ended 1 min before seizure onset. The averages of the population spike amplitude and the fEPSP slope of the first response of each pair were calculated. Those averages were used to normalize all of the population spike amplitude and fEPSP slope measurements from that recording session. For example, if the baseline averages of the population spike amplitude and fEPSP slope of the first response of each pair of responses were 5.0 mV and 4.8 mV/ms, then all of the responses recorded during that session were divided by those values.

Previous studies provide useful methods for analyzing and interpreting perforant path evoked responses of the dentate gyrus. For example, the slope of the fEPSP is related to the current generating intracellular excitatory postsynaptic potentials in granule cells (Lønborg 1971). The amplitude of the population spike is proportional to the number of granule cells discharging an action potential (Andersen et al. 1971). Paired-pulse inhibition of the population spike amplitude is due in part to feedback inhibition (Andersen et al. 1966) mediated by γ-aminobutyric acid-A (GABA A)-receptors (Sloviter 1991; Tuff et al. 1983). We used field potential responses of the dentate gyrus to perforant path stimulation as a measure of changes in tissue excitability.

After recording 1–12 seizures per gerbil, over a period lasting ≤3 mo, the electrode positions were verified anatomically. The gerbil was killed by barbiturate overdose (100 mg/kg pentobarbital ip) and perfused through the ascending aorta at 15 ml/min for 2 min with 0.9% NaCl and for 30 min with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was removed, postfixed overnight at 4°C, placed in 30% sucrose in PB until equilibrating, and sectioned coronally with a sliding microtome set at 30 μm. Serial sections were stained with 0.25% thionin.

RESULTS

In 18 gerbils (both sexes, 1.5–8.0 mo old) 84 seizures were observed and 73 were recorded and analyzed. The number of seizures recorded in 1- to 2-, 2- to 3-, and >3-mo-old gerbils was 10, 26, and 37, respectively.

Baseline responses

Baseline responses, recorded until 60 s before the onset of the electrographic seizure, were averaged and used to normalize the amplitude of the first population spike and the slope of the first fEPSP of each pair of responses. For all recordings during the baseline period, population spike amplitude was 4.6 ± 0.3 mV (mean ± SE), population spike amplitude ratio (2nd/1st) was 0.06 ± 0.01, and fEPSP slope was 4.3 ± 0.3 mV/ms. Population spike amplitude ratios increased during development. The average amplitude ratios for 1- to 2-, 2- to 3-, and >3-mo-old gerbils were 0.003, 0.057, and 0.082, respectively. The difference between 1–2 and >3 mo old is significant (P < 0.05, t-test).
Novel environment exposure

After recording responses for $\geq 4$ min in their home cages, gerbils were placed in a novel environment. Novel environment exposure triggers seizures in Mongolian gerbils (Buckmaster et al. 1996; Kaplan 1975; Ludvig et al. 1991). Novel environments included a rectangular red plastic basket, a large steel pan, and a cylindrical metal basket. After a gerbil was caught in its home cage and placed in the novel environment, the transfer was indicated on the EEG record. Novel environment exposure coincided with an increase in population spike amplitude and a decrease in fEPSP slope (Fig. 1). Changes in evoked responses slightly preceded the point when novel environment exposure was marked on the EEG record (Fig. 1B). This may be due to an effect caused by catching the gerbil in its home cage and transferring it to the novel environment and/or to the latency between placing the gerbil in the novel environment and marking the transfer on the EEG record. The effect of novel environment exposure on evoked responses decreased in older gerbils (Fig. 1B), and novel environment exposure became progressively less effective at triggering seizures. The proportion of seizures that were triggered by novel environment exposure is significantly higher in 1- to 2-mo-old gerbils (82%) than in 2-3 or >3 mo olds (28 and 13%, respectively, $P < 0.005$, $\chi^2$ test). The electrographic seizure began $9 \pm 2$ s after 1- to 2-mo-old gerbils were exposed to a novel environment. When novel environment exposure was ineffective, seizures were triggered by blowing air on the gerbil and/or shaking the cage—treatments that previously have been reported to trigger seizures in Mongolian gerbils (Frey 1987; Ludvig et al. 1991; Scotti et al. 1998).

Seizures

The behavioral and electrographic aspects of the seizures recorded in the present study are similar to those reported previously for epileptic gerbils (Loskota and Lomax 1975; Loskota et al. 1974; Majkowski and Donadio 1984). Behaviorally, seizures range from brief immobility to severe generalized tonic-clonic convulsions. At the onset of the behavioral seizure, gerbils stop exploring the environment. Convulsions begin with clonic ear flattening and neck extension before spreading to the rest of the body. Behavioral seizure onset was indicated on the EEG record when convulsions began. Behavioral seizure onset was sometimes difficult to precisely estimate, especially when seizures were mild. In gerbils $>2$ mo old, 81% of the seizures were generalized convulsions, whereas in 1- to 2-mo-old gerbils, 91% of the seizures consisted of brief immobility, sometimes with focal clonus, but without severe generalized convulsions ($P < 0.005$, $\chi^2$ test). All behavioral seizures were coincident with electrographic seizure activity recorded in the neocortex and dentate gyrus.

Electrographically, seizure onset was identified by rhythmic deflections in the neocortical and/or hippocampal potential (Fig. 2). In 94% of the seizures, the neocortical recording most clearly displayed the onset, but in $\approx 25\%$ of the seizures the hippocampal recording simultaneously displayed the seizure onset. In 90% of the seizures, onset was a positive deflection in the neocortical recording. The electrographic seizure onset preceded the onset of convulsions by $5.8 \pm 0.7$ s. The end of the electrographic seizure was identified by cessation of rhythmic deflections, which occurred virtually simultaneously in the neocortical and hippocampal potentials. Electrographic seizures lasted $35.3 \pm 2.0$ s (range = $4.2$–$85.9$ s). Mean seizure durations are slightly shorter in younger animals ($31.1$, $35.4$, and $36.3$ s in 1- to 2-, 2- to 3-, and >3-mo-old gerbils, respectively), but the differences were not significant ($t$-test).

FIG. 1. Novel environment exposure is coincident with an increase in population spike amplitude and a decrease in the field excitatory postsynaptic potential (fEPSP) slope. A: dentate gyrus evoked responses to paired-pulse stimulation of the perforant path in a 1- to 2-mo-old gerbil, 22.5 s before (A1) and 7.5 s after novel environment exposure (A2). Note the increase in population spike amplitude (arrows). A3: the rising phase of the fEPSP during the first response of the pair shown in A1 (solid line) and A2 (dashed line). fEPSP slope was measured between the arrows. B: the top graph is a plot of normalized and averaged values of population spike amplitude and fEPSP slope from gerbils of all age groups. Population spike amplitude increases and fEPSP slope decreases with novel environment exposure (time = 0). The bottom graph plots the normalized population spike amplitude averages for 1-to 2-, 2-to 3-, and >3-mo-old gerbils. The effect of novel environment exposure is greatest on 1-to 2-mo-old gerbils and least on gerbils >3 mo old. Error bars = SE.
Many seizures, especially those with generalized convulsions, were followed by a period of postictal depression, during which time the gerbil remained motionless. Postictal depression was rare in 1- to 2-mo-old gerbils and common in >2-mo-old gerbils. This is reflected by the time it took the gerbil to resume normal exploratory behavior, which occurred at 65 ± 30, 212 ± 21, and 217 ± 21 s after the onset of the electrographic seizure in 1- to 2-, 2- to 3-, and >3-mo-old gerbils, respectively. The difference between 1- to 2-mo-old versus older gerbils is significant (P < 0.0004, t-test).

**Evoked responses during seizures**

Immediately before the onset of the electrographic seizure, population spike amplitude, amplitude ratio (2nd/1st), and fEPSP slope were at or near baseline values in gerbils >3 mo old (Figs. 3, A and C, and 4C). In younger gerbils the electrographic seizure onset usually was preceded within 20 s by novel environment exposure. The latency between novel environment exposure and electrographic seizure onset was <20 s in 70, 23, and 3% of the seizures in 1- to 2-, 2- to 3-, and >3-mo-old gerbils, respectively. All of the differences between the age groups are significant (P < 0.025, χ² test). Therefore at the time of the seizure onset in younger gerbils, the amplitude of the first population spike was still above baseline in response to novel environment exposure (Figs. 3, B and D, and 4A).

In all age groups, population spike amplitude peaked around the time of seizure onset. The amplitude of the population spike peaked at 1.8 ± 0.1 times the normalized baseline value; there were no significant differences among age groups (t-test). However, the timing of the maximum population spike amplitude relative to the electrographic seizure onset varied as a function of age. It occurred after the seizure onset in 33, 79, and 89% of the 1- to 2-, 2- to 3-, and >3-mo-old gerbils, respectively. The difference between 1- to 2-mo-old versus >2-mo-old gerbils is significant (P < 0.025, χ² test). The latency of the maximum population spike amplitude relative to seizure onset was −1.9, +9.9, and +8.4 s in 1- to 2-, 2- to 3-, and >3-mo-old gerbils, respectively. The difference between 1- to 2- versus >2-mo-old gerbils is significant (P < 0.02, t-test). The fEPSP slope peaked at 1.33 ± 0.04 times the normalized baseline value 8.1 ± 1.6 s after seizure onset, and there were no significant differences between the age groups.

Shortly after the onset of the electrographic seizure, periorbital stimulation evoked multiple population spikes. Multiple population spikes were commonly observed in older but not younger gerbils. They occurred in 0, 27, and 79% of the seizures in 1- to 2-, 2- to 3-, and >3-month-old gerbils, respectively. The differences between the >3-mo-old versus younger groups were significant (P < 0.005, χ² test). Multiple population spikes began 14.4 ± 1.5 s after the seizure onset; the maximum number of spikes evoked per stimulus was 3.6 ± 0.2, and they occurred over a period lasting 13.2 ± 1.3 s.

The period of hyperexcitability, characterized by increased population spike amplitude, increased fEPSP slope, and multiple population spikes, was followed by a dramatic reduction...
in responsiveness to perforant path stimulation. This was commonly observed in older, but not younger, gerbils. It occurred in 0, 25, and 94% of the seizures in 1- to 2-, 2- to 3-, and >3-mo-old gerbils, respectively. The differences between the >3-mo-old versus younger groups were significant ($P < 0.005$, $\chi^2$ test). During this period of diminished responses, stimulation evoked no population spike and little fEPSP (Fig. 5). In many cases, responses were completely abolished (Fig. 3).
The period of diminished responses began 29 ± 2 s after seizure onset and lasted 37 ± 4 s. In 80% of the seizures that had one, the beginning of the hyporesponsive period preceded the end of the electrographic seizure. Seizure activity continued for 12 ± 2 s after the onset of the hyporesponsive period.

After the dramatic reduction in responsiveness, fEPSP slope and population spike amplitude gradually recovered. During this recovery period, fEPSP slope and population spike amplitude increased at a similar rate until approximately 150 s after the electrographic seizure onset, when they reached 50–60% of their baseline values (Fig. 6). At that point, the fEPSP slope continued to increase until it reached 100% of baseline. The population spike amplitude, on the other hand, remained at approximately 60% of baseline. Throughout this period after the convulsive phase of the seizure, the gerbil was in a state of postictal depression and was immobile. In 2- to 3-mo-old gerbils, the switch from postictal depression to resumption of exploratory behavior was abrupt and coincident with an increase in population spike amplitude back to 100% of the baseline value (Fig. 7). Simultaneously the fEPSP slope decreased slightly. In >3-mo-old gerbils the transition from postictal depression to exploratory behavior and the increase in the population spike amplitude were less abrupt and more gradual.

The population spike amplitude ratio (2nd/1st) began to increase shortly after seizure onset, especially in gerbils >3 mo old (Figs. 3, A and C, 4C, and 8). In 25, 67, and 97% of the seizures in gerbils 1- to 2-, 2- to 3-, and >3-mo-old, respectively, the population spike amplitude ratio increased to >2 SD of its baseline value (P < 0.005, χ² test). The peak amplitude ratio was 0.28, 0.98, and 1.75 in gerbils 1- to 2-, 2- to 3-, and >3-mo-old, respectively. The difference between gerbils 2–3 and >3 mo old is significant (P < 0.02, t-test). The peak amplitude ratio occurred 60 ± 5 s after seizure onset, which is after the hyporesponsive period in those seizures that had one. From seizure onset, the population spike amplitude ratio took an average of ~12 min to return to baseline after it increased dramatically during seizures in gerbils >3 mo old (Fig. 8).

DISCUSSION

In this study the development of increased seizure severity was evaluated by analyzing evoked responses of the dentate gyrus during "spontaneous" seizures in 1.5- to 8.0-mo-old gerbils with inherited epilepsy. We found little evidence of disinhibition in the dentate gyrus of juvenile gerbils when they first begin having seizures. As epileptic gerbils mature, their seizures increase in severity, while evoked responses during seizures become more abnormal and correlated with seizure-related changes in behavior.

Seizure onset

In gerbils with inherited epilepsy, mild and severe seizures begin similarly. Electrophysiologically, seizures begin with positive deflections in the EEG recorded in the neocortex and dentate gyrus. Behaviorally, seizures begin with motor arrest that may be followed with focal clonus of the head and neck. In adult gerbils, evoked responses of the dentate gyrus remain at baseline levels until after seizure onset, confirming a previous study (Buckmaster et al. 2000). These findings suggest that seizures in adult gerbils are not caused by disinhibition in the dorsal dentate gyrus, as proposed by Peterson and Ribak (1989). However, the mechanisms of seizure initiation might be different in chronically epileptic adult gerbils versus young gerbils that are just beginning to have seizures. Therefore to
further test the disinhibition hypothesis, we analyzed evoked responses in 1- to 2-mo-old gerbils but found little evidence of disinhibition preceding seizure onset. Multiple population spikes were never observed, and the population spike amplitude ratio (2nd/1st) and fEPSP slope were at or below baseline levels. Population spike amplitude was elevated; however, at this point preceding seizure onset, many young animals displayed a residual response to novel environment exposure. Previous studies have shown that the population spike amplitude transiently increases and the fEPSP slope transiently decreases when rats are exposed to a novel experience such as transitions between environments (Green et al. 1990; Moser et al. 1993).

Changes in evoked responses of the dentate gyrus during seizures

The typical sequence of changes in evoked responses during a mild and severe seizure in a juvenile and adult gerbil, respectively, is summarized in Fig. 9. To our knowledge this approach of analyzing evoked responses during spontaneous seizures has not been used previously to evaluate patients or other models of epilepsy. However, our results from spontaneous seizures are similar in some ways to results obtained during afterdischarges evoked by electrical stimulation in non-epileptic rats. In the dentate gyrus in vivo, tetanic stimulation adequate to trigger afterdischarges initially causes the popula-
tion spike amplitude to increase; then the population spike amplitude ratio (2nd/1st) increases (i.e., paired-pulse inhibition decreases), and finally multiple population spikes appear (Bur-dette et al. 1996; Emori et al. 1997; Tuff et al. 1983). This sequence of changes is similar to that seen during the first part of a severe seizure in adult epileptic gerbils, and it suggests that there are mechanisms that facilitate the amplification and spread of seizure activity. Similar changes in evoked responses can be produced by gradually blocking GABA<sub>A</sub> receptors (Buckmaster et al. 2000; Sloviter 1991), suggesting that impaired GABA<sub>A</sub> receptor-mediated inhibition might contribute to these changes, but many other alternatives exist.

After the period of dramatic hyperexcitability during severe seizures, dentate gyrus responsiveness diminishes, and the fEPSP slope and population spike amplitude decrease below 50% of baseline values for a period lasting ~1 min. In many cases, responses decrease to virtually no evoked change in the field potential. These findings suggest that seizures activate...
homeostatic mechanisms that dampen tissue excitability and help to terminate the seizure. A number of possible mechanisms might underlie seizure-induced hypoexcitability. First, less neurotransmitter might be released from activated axons. Extracellular calcium concentration decreases during seizure activity (Heinemann et al. 1977; Pumain et al. 1985; Stringer and Lothman 1989), and reducing extracellular calcium concentration impairs synaptic transmission (Dingledeine and Somjen 1981). Neurotransmitter release might be reduced by presynaptic inhibition. For example, adenosine has been proposed as an endogenous anticonvulsant (Dragunow 1988; Dundridge and Somjen 1981). Adenosine levels increase in the hippocampus during spontaneous seizures (During and Spencer 1992). Adenosine receptors are expressed in the dentate gyrus of Mongolian gerbils (Lee et al. 1986). Also, adenosine depresses fEPSPs and population spikes in the hippocampus by presynaptic and postsynaptic mechanisms (Greene and Haas 1991). In addition, neurotransmitter release might be reduced by synaptic vesicle depletion (Staley et al. 1988). Second, seizure activity might release inhibitory neurotransmitters and neuromodulators that make granule cells less responsive to synaptic activation. Finally, in our experiments we cannot exclude the possibility that during seizures perforant path axons became less responsive to electrical stimulation.

Surprisingly, electrographic seizure activity persists for an average of 12 s after the dentate gyrus stops responding to stimulation. This suggests that seizure activity continues after the possible suppression of synaptic transmission in the dentate gyrus. Seizure activity has been recorded in hippocampal slices in the absence of synaptic transmission (Jefferys and Haas 1982; Konnerth et al. 1984; Patrylo et al. 1994; Taylor and Dudek 1982). However, it is unclear whether naturally occurring seizures in vivo can or do persist in the absence of chemical synaptic transmission. An alternative explanation for our observations is that seizure activity stops in the dentate gyrus but continues in a neighboring region and is volume-conducted to the recording electrodes in the dentate gyrus.

After the seizure, while evoked responses are diminished, the animal is motionless during a period of postictal depression. The transition from postictal depression to normal exploratory behavior, especially in 2- to 3-mo-old gerbils, is abrupt and coincident with the return of population spike amplitude back to the baseline value. These findings suggest that the reduced population spike amplitude and behavioral depression might share a common neurophysiological mechanism.

During seizure-related changes in the excitability of the dentate gyrus, fEPSP slope and population spike amplitude usually change in parallel, as expected, since both measure the granule cell population response to excitatory synaptic input (Andersen et al. 1971; Lomoe 1971). Both fEPSP slope and population spike amplitude peak approximately 8 s after seizure onset, and shortly later, both decrease dramatically, suggesting that during a seizure the excitatory synaptic input and/or the excitability of the dentate gyrus synaptic input increases but then quickly decreases. However, there are several periods when fEPSP slope and population spike amplitude diverge. In young gerbils during novel environment exposure and in older gerbils at the end of the postictal period, the population spike increases while the fEPSP slope slightly decreases. Another point of divergence is during the postictal period when the fEPSP slope recovers smoothly toward its baseline value, but the population spike amplitude stalls at approximately 60% of its baseline value and stays there until the end of the postictal period. The dissociation between fEPSP slope and population spike amplitude might be due to changes in brain temperature (Moser et al. 1993), sustained depolarization of granule cells, or selective activation of inhibitory input to specific parts of the granule cell. Different subpopulations of interneurons selectively inhibit granule cell dendrites versus somata (Freund and Buzsáki 1996), and evidence suggests that inhibition at the cell body level reduces population spike amplitude, whereas inhibition at the dendritic level reduces fEPSP slope (Moser 1996). Therefore the changes in evoked responses in gerbils during novel environment exposure and at the end of postictal depression might be due to reduced inhibition of granule cell somata and simultaneous increased inhibition of their dendrites.

### Developmental aspects

As epileptic gerbils mature, their seizures and evoked responses change (summarized in Table 1). Responses obtained during the preseizure baseline period reveal that the population spike amplitude ratio (2nd/1st) increases as gerbils mature from 1 to >3 mo old. This finding is consistent with a previous study that examined evoked responses of the dentate gyrus in anesthetized gerbils (Buckmaster et al. 1996), and it suggests that paired-pulse inhibition is more effective in juvenile versus adult gerbils.

Younger gerbils respond more consistently to novel environment exposure than do older gerbils. After novel environment exposure, the population spike amplitude increases and seizures are triggered more reliably in younger versus older gerbils. To determine if the difference was due to a lack of novelty for older gerbils that had been tested repeatedly, different novel environments were tried, but they were not more effective at triggering seizures. In younger gerbils the correlation of novel environment exposure, increased population spike amplitude, and seizure onset suggests that the dentate

### Table 1. Summary of developmental differences in dentate gyrus evoked responses and seizures in gerbils with inherited epilepsy

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<th>1–2 mo</th>
<th>2–3 mo</th>
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<tr>
<td>Baseline population spike amplitude ratio (2nd/1st)</td>
<td>0.003</td>
<td>0.057</td>
<td>0.082</td>
</tr>
<tr>
<td>Seizures triggered by novel environment exposure (%)</td>
<td>82</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>EEG seizure duration (s)</td>
<td>31.1</td>
<td>35.4</td>
<td>36.3</td>
</tr>
<tr>
<td>Latency from seizure onset to resumption of exploratory behavior (s)</td>
<td>65</td>
<td>212</td>
<td>217</td>
</tr>
<tr>
<td>Population spike amplitude peaked after seizure onset (%)</td>
<td>33</td>
<td>79</td>
<td>89</td>
</tr>
<tr>
<td>Latency from seizure onset to peak population spike amplitude (s)</td>
<td>−1.9</td>
<td>+9.9</td>
<td>+8.4</td>
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<tr>
<td>Seizures with multiple population spikes evoked</td>
<td>0%</td>
<td>27%</td>
<td>79%</td>
</tr>
<tr>
<td>Seizures with hyporesponsive period</td>
<td>0%</td>
<td>25%</td>
<td>94%</td>
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<tr>
<td>Peak population spike amplitude ratio (2nd/1st) value</td>
<td>0.28</td>
<td>0.98</td>
<td>1.75</td>
</tr>
<tr>
<td>Seizures with population spike amplitude ratio (2nd/1st) exceeding 2 standard deviations (%)</td>
<td>25</td>
<td>67</td>
<td>97</td>
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See text for results of statistical analyses.
gyrus might contribute to seizure genesis. Lesioning the perforant path blocks seizures in epileptic gerbils (Ribak and Khan 1987). However, we found that seizure onset was simultaneous in the dentate gyrus and anterior neocortex, and previous studies that analyzed more recording sites found that EEG spiking frequently initiates in the posterior neocortex before generalizing (Loskota and Lomax 1975; Majkowski and Donadio 1984). The site of seizure initiation in epileptic gerbils is unclear. It is likely that the seizures recorded in the present study began outside of the dentate gyrus and then propagated through it.

Seizures become more severe as gerbils mature (Loskota et al. 1974; Seto-Oshima et al. 1992). In older gerbils, there is a tendency for electrographic seizures to be longer, the behavioral manifestations of the seizure to be more severe, and seizure-related changes in dentate gyrus excitability to be much more dramatic than in younger gerbils. The underlying causes of these changes are unclear. Rodents are maximally susceptible to seizures 2–3 wk postnatal (Moshé et al. 1996). However, in gerbils spontaneous seizures do not begin until they are 4–8 wk old, and severe generalized seizures do not begin until even later. It has been proposed that some epileptic gerbils undergo a kindling process as they mature and experience repeated seizures (Scotti et al. 1998). However, chronically epileptic gerbils do not display hilar neuron loss (Buckmaster et al. 1996) or granule cell axon reorganization (Ribak and Peterson 1991) which would be expected for kindled animals (Sutula et al. 1988; Cavazos and Sutula 1990). Another possibility is the gradual developmental expression of an inherited epileptogenic defect, or perhaps the change is due to the gradual developmental reduction of an endogenous anti-convulsant mechanism, such as the reduction in baseline paired-pulse inhibition described in this study. It may be possible in future experiments to dissociate developmental effects from the effects of repeated seizures by delaying the onset of spontaneous seizures in epileptic gerbils. This is an important question, because it may shed light on the mechanisms of seizure spread, severity, and control.

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