# Deficit of quantal release of GABA in experimental models of temporal lobe epilepsy

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Because GABA (γ-aminobutyric acid) receptor-mediated inhibition controls the excitability of principal neurons in the brain, deficits in GABAergic inhibition have long been favored to explain seizures. In an experimental model of temporal lobe epilepsy, we have identified a deficit of inhibition in presynaptic GABAergic terminals characterized by decreased GABA quantal activity associated with reduced synaptic vesicle density. This decrease in vesicle number primarily seems to affect the reserve pool, rather than the docked or the readily releasable pool.

Modes of inhibition are physiologically and anatomically heterogenous[1,2]. Perisomatic inhibition limits action potential firing, whereas dendritic inhibition primarily controls synaptic integration. Here we focus on perisomatic inhibition mediated by miniature inhibitory postsynaptic currents (mIPSCs) in epileptic neurons. In dentate gyrus granule cells[3] and in CA1 pyramidal cells (J.C. Hirsch *et al., Soc. Neurosci. Abstr.* 24, 362.1, 1998), mIPSCs resulting from the action-potential-independent release of single GABA-containing vesicles are restricted to the perisomatic region and regulate neuronal output[4].

We examined GABAergic inhibition in the hippocampal CA1 region of rats that developed spontaneous recurrent seizures following kainate or pilocarpine treatment[5,6], two models of human temporal lobe epilepsy. To record $GABA_A$-receptor-mediated IPSCs, we used whole-cell recording in pyramidal cell somata voltage clamped at +10 mV[7]. All recorded neurons were filled with biocytin and morphologically identified *post hoc*[7]. In the presence of tetrodotoxin (TTX; 1 μM) to block action-potential-dependent synaptic activity, mIPSC frequency was 62% lower in epileptic than in control animals (epileptic, 6.3 ± 1.6 Hz, $n$ = 10 slices from 4 kainate- and 4 pilocarpine-treated animals; control, 14.8 ± 3.6 Hz, $n$ = 11 slices from 7 animals; Fig. 1a and b, $p < 0.0001$, unpaired student´s $t$-test). In addition, average mIPSC amplitude was decreased by 12% (control, 15.1 ± 0.2 pA, $n$ = 11; experimental, 13.3 ± 0.2 pA, $n$ = 10, $p < 0.0001$, Kolmogorov-Smirnov test; Fig. 1c), lending support to a report of 50% reduction in currents following GABA application in epileptic tissue[8]. The discrepancy between these results and our own may have occurred because we characterized synaptic events specifically, whereas the previous report[8] characterized both synaptic and extrasynaptic $GABA_A$-receptor-mediated responses.

A purely postsynaptic origin for the 60% decrease of mIPSC frequency in epileptic animals can be excluded because a 12% decrease of mIPSC amplitude in control events would only reduce their frequency by 10%. Failure to detect mIPSCs is unlikely, as our detection algorithm[9] picked up even small-amplitude synaptic events (~4 pA, Fig. 1c; currents below 3 pA correspond to the opening of single channels rather than quantal release[4]). This strongly suggests that the decreased mIPSC frequency in epileptic animals results from a presynaptic alteration of GABAergic inhibition via a decreased number of perisomatic GABAergic synapses and/or an alteration of GABA quantal release.

To test for a possible morphological correlate of deficits in GABAergic inhibition, we three-dimensionally reconstructed the somata of CA1 pyramidal neurons and associated GABAergic terminals from electron microscopic serial sections of slices used for electrophysiology. Among hundreds of randomly sampled perisomatic terminals, nine from control and eight from epileptic animals showed symmetric synapses with clear active zones and distinct immunogold labeling for GABA. There was no significant difference between control and epileptic animals either in the total number of perisomatic terminals making symmetrical synapses (control, 94 ± 3, $n$ = 5 somata from 3 animals; pilocarpine treated, 95 ± 3, $n$ = 5 somata from 3 animals; Fig. 2a) or in the size of these boutons (control cross-sectional area, 0.6 ± 0.1 $\mu m^2$, $n$ = 9 GABA immunostained terminals from 3 animals; pilocarpine treated, 0.9 ± 0.1 $\mu m^2$, $n$ = 8 from 3 ani-

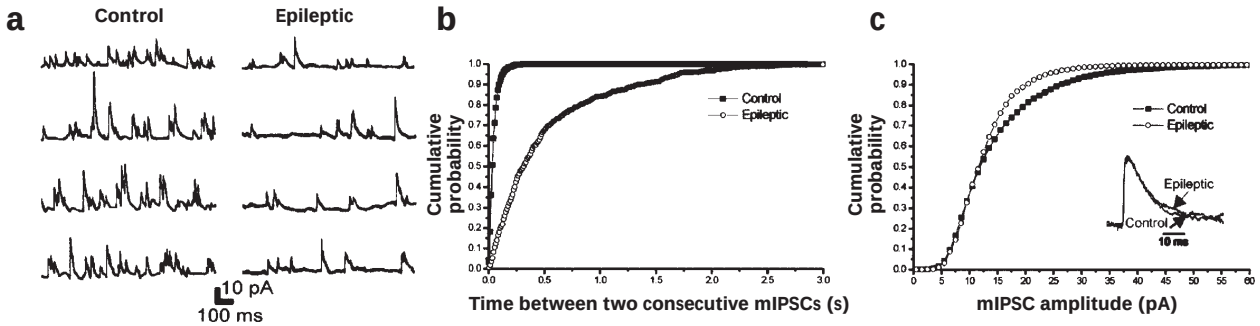


**Fig. 1.** Deficit of GABAergic miniature activity in epileptic animals. **(a)** Miniature inhibitory postsynaptic currents recorded in the presence of the $Na^+$ channel blocker TTX (1 μM) at the reversal potential of glutamatergic events in pyramidal cell somata in the CA1 area of the hippocampus in control and epileptic animals. Continuous four-second recordings are displayed. The frequency of miniature events was considerably lower in epileptic animals. **(b)** Cumulative probability plot of the intervals between two consecutive events, showing that the frequency is decreased in epileptic animals. **(c)** The cumulative probability plot of the amplitudes of miniature events shows a left shift, indicating a decreased frequency of amplitudes larger than 15 pA in epileptic animals. The kinetics of miniature events (normalized averages) does not seem modified (inset); rise time 0.99 and 0.98 ms, decay time 9 and 10 ms in control and epileptic animals, respectively.

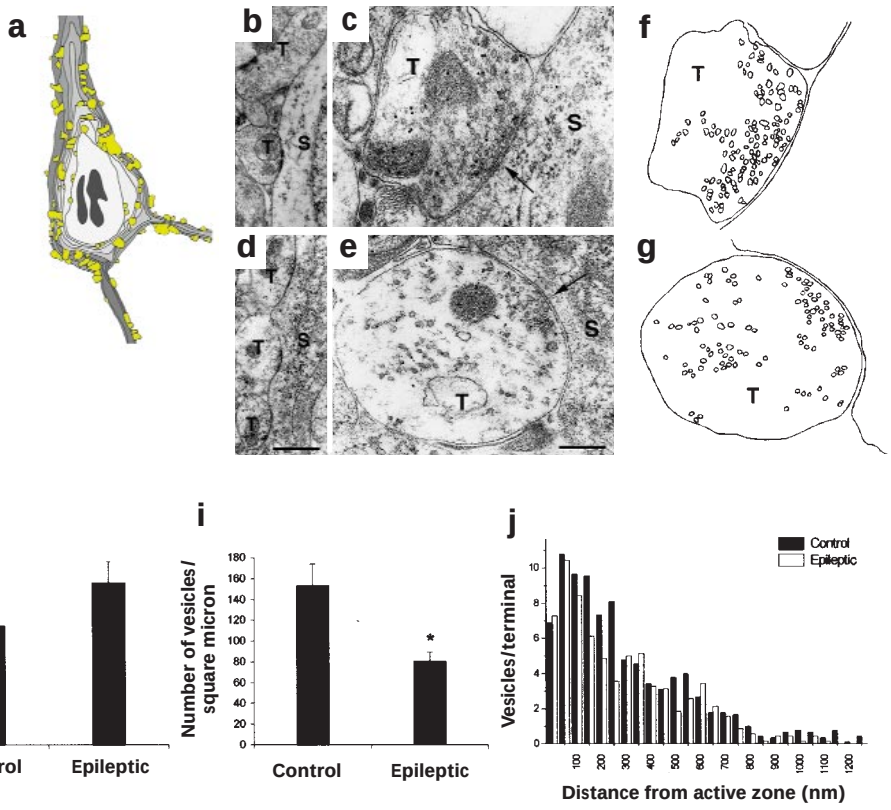


**Fig. 2.** Perisomatic GABAergic terminals in control and epileptic animals. **(a)** Scheme of the three-dimensional reconstruction of the soma of a CA1 pyramidal neuron and associated GABAergic terminals (yellow) in a pilocarpine-treated rat with chronic spontaneous seizures. **(b–e)** Electron photomicrographs of axon terminals (T) immunoreactive for GABA (postembedding immunogold) **(c, e)**, making symmetrical synapses (arrows) on the soma (S) of a CA1 pyramidal neuron **(b, d)**, in a control **(b, c)** and a pilocarpine **(d, e)** animal. **(f, g)** Drawings of the axon terminals in **(c)** and **(e)**, showing the reduced vesicle density in perisomatic GABAergic terminals from epileptic animals. Scale bars, 0.77 μm for **(b, d)**; 0.23 μm for **(c, e)**. **(h)** Average cross-sectional area of synaptic vesicles per synaptic terminal in control ($n = 9$) and pilocarpine-treated ($n = 8$) animals. **(i)** Synaptic vesicle density expressed as the number of vesicles per square micron. **(j)** Spatial distribution of synaptic vesicles around the active zone in control (solid bars) and pilocarpine-treated (open bars) animals. Abscissa, distance from the vesicles to the presynaptic membrane specialization at intervals of 50 nm. Ordinate, average number of vesicles per terminal.



mals; Fig. 2h). In contrast, the density of synaptic vesicles (mean number per $\mu m^2$) was reduced in the terminals from epileptic animals, 80 ± 9 ($n = 8$), as compared to control, 153 ± 21 ($n = 9$, Fig. 2b–g and i, $p < 0.01$, unpaired student´s $t$-test).

Because the spatial relationship of synaptic vesicles with the synaptic active zone is considered to be closely connected with exocytosis[10], we examined the distribution of vesicles in terminals from control and pilocarpine-treated animals. Histograms of the number of synaptic vesicles present in concentric shells around the synaptic active zone[11,12] showed comparable numbers within 50 nm from the active zone (docked vesicles[12]) in control and pilocarpine-treated animals (Fig. 2j) but a 48% decrease in the 100–300 nm range in terminals from pilocarpine-treated animals. Thus, the reserve or depot pool of synaptic vesicles rather than the immediately releasable pool seems to be affected.

The unchanged number of GABAergic boutons in epileptic animals suggests that the reduction of mIPSC activity is due to a decreased probability of GABA quantal release in all or a subpopulation of perisomatic synapses. The docked or readily releasable pool of vesicles does not seem affected in epileptic animals. This is consistent with the presence of action-potential-dependent inhibition, directly proportional to the number of releasable vesicles[13] in these chronic models of temporal lobe epilepsy[7]. In contrast, the reserve pool (between 100 and 300 nm) is depleted by 50%. The mechanism of miniature activity is still unknown, but our results raise the possibility that it is somehow linked to the size of the reserve pool. Further studies are necessary to determine whether the loss of mIPSCs in chronic experimental temporal lobe epilepsy is restricted to GABAergic pathways, and whether it is a direct effect of seizures or of the lesion induced by pilocarpine or kainate treatments[9]. Our observations suggest that the synaptic vesicle release machinery[14] is impaired[11] in experimental models of epilepsy. The findings that this neurological disorder affects the reserve pool and quantal release of GABA-containing vesicles suggest new targets for antiepileptic drugs.

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1. Miles, R., Toth, K., Gulyás, A. I., Hajos, N. & Freund, T. F. *Neuron* 16, 815–823 (1996).
2. Freund, T. F. & Buzsáki, G. *Hippocampus* 6, 347–470 (1996).
3. Soltesz, I., Smetters, D. K. & Mody, I. *Neuron* 14, 1273–1283 (1995).
4. Otis, T. S., Staley, K. J. & Mody, I. *Brain Res.* 545, 142–150 (1991).
5. Nadler, J. *Life Sci.* 29, 2031–2042 (1981).
6. Turski, W. A., Cavalheiro, E. A., Schwarz, M., Czuczwar, S. J. & Kleinrok, Z. *Behav. Brain Res.* 9, 315–335 (1983).
7. Esclapez, M., Hirsch, J. C., Khazipov, R., Ben-Ari, Y. & Bernard, C. *Proc. Natl. Acad. Sci. USA* 94, 12151–12156 (1997).
8. Gibbs, J. W., Shumate, M. D. & Coulter, D. A. *J. Neurophysiol.* 77, 1924–1938 (1997).
9. Esclapez, M., Hirsch, J. C., Ben-Ari, Y. & Bernard, C. *J. Comp. Neurol.* 408, 449–460 (1999).
10. Rusakov, D. A., Skibo, G. G. & Vasilenko, D. A. *Neurosci. Lett.* 131, 156–158 (1991).
11. Rosahl, T. W. *et al. Nature* 375, 488–493 (1995).
12. Hess, S. D., Doroshenko, P. A. & Augustine, G. J. *Science* 259, 1169–1172 (1993).
13. Murthy, V. N., Sejnowski, T. J. & Stevens, C. F. *Neuron* 18, 599–612 (1997).
14. Wu, M. N. & Bellen, H. J. *Curr. Opin. Neurobiol.* 7, 624–630 (1997).