bathing solution contained 110 m*M* potassium aspartate, 20 m*M* KCl, 2 m*M* EGTA, 2 m*M* $MgCl_2$, 20 m*M* glucose, 5 m*M* Hepes (*p*H 7.4 with tris), and the patch pipette contained 90 m*M* $BaCl_2$, 10 m*M* glucose, 10 m*M* Hepes (*p*H 7.4 with tris). L(−)-Isoprenaline (ISO) (Sigma) and GTPγS (tetralithium salt) (Boehringer Mannheim) were dissolved in distilled water as 10 m*M* stock solutions. All drugs and nucleotides were added either to the patch pipette solution or bath solution to obtain the final desired concentrations. External solutions were perfused through the chamber at 2 ml/min by gravity flow. Unitary currents were filtered with a 4-pole Bessel filter at 2 kHz, digitized at 5 kHz, and stored on a PDP 11/73 computer [H. D. Lux and A. M. Brown, *J. Gen. Physiol.* **83**, 727 (1984)]. Analyses of transitions were done on records filtered subsequently with a zero phase four-pole nonringing digital filter.

23. Bovine cardiac sarcolemmal vesicles were prepared and stored at −70°C [R. S. Slaughter, J. L. Sutko, J. P. Reeves, *J. Biol. Chem.* **258**, 3183 (1983); L. R. Jones, S. W. Maddock, H. R. Beach, *ibid.* **255**, 9771 (1980)]. Experiments were carried out at room temperature (20° to 22°C) in lipid bilayers formed from decane solutions of equimolar brain phosphatidylserine and phosphatidylethanolamine (Avanti Polar Lipid, Birmingham, AL). The cis chamber (500 μl) contained 50 m*M* NaCl, 100 m*M* $BaCl_2$, 2 m*M* $MgCl_2$, 10 m*M* Hepes (*p*H 7.4 with NaOH). The trans chamber (500 μl) contained the same solution as the cis chamber without $BaCl_2$. Bay K 8644 (1 μ*M*) was present on both sides. Vesicles were added to the cis chamber to a final concentration of 5 to 10 μg protein per milliliter. Incorporation occurred as for conventional right-side-out vesicles and depolarizing pulses opened channels more frequently. The cis chamber was connected to ground, and potentials were applied to the trans chamber. Thus, the trans chamber represents the intracellular side. The current conventions were those used in whole-cell recordings, and inward current (cis to trans) gave downward deflections. Current traces were recorded with a List EPC7 amplifier, filtered at 300 Hz (four-pole Bessel, low pass), and stored in a videocassette recorder. Data were digitized at sampling rates of 1 to 3 kHz and analyzed with a PDP 11/73 computer (*22*).
24. A. M. Brown, D. L. Kunze, A. Yatani, *Nature (London)* **311**, 570 (1984); P. Hess, J. B. Lansman, R. W. Tsien, *ibid.*, p. 538; S. Kokubun and H. Reuter, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4824 (1984).
25. Supported by NIH grants HL-31154, HL-37044, HL-36930, DK 19318, and AHA 851159.

4 May 1987; accepted 21 August 1987

Technical Comments

Epilepsy Hypothesis

The report "Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy" by Robert S. Sloviter (*1*) demonstrates a loss of somatostatin-containing hilar neurons ipsilateral to perforant path stimulation. However, the report contains incomplete immunocytochemical results for γ-aminobutyric acid (GABA) neurons in the hilus of the dentate gyrus. The author does not appear to have replicated the findings of many investigators (*2–4*) who have shown large numbers of GABAergic hilar neurons. In fact, two of these studies (*3*) have shown that many somatostatin-containing neurons in the hilus are GABAergic. This finding was expected because many GABAergic hilar neurons resemble the morphology of somatostatin neurons in the hilus of the rat, and it is now clear that both GABAergic (*4*) and somatostatin-containing hilar neurons in the rat have commissural and associational projections. Therefore, the loss of somatostatin hilar neurons indicates that significant numbers of GABAergic hilar neurons are also degenerating.

It is possible that Sloviter's immunocytochemical results for GABAergic neurons in the hilus are related to the fixation protocol, in which a low concentration of glutaraldehyde (0.01%) was used. Although this fixative provides good staining for peptide-containing neurons, the antiserum to GABA is usually more effective with preparations that are fixed with higher concentrations of glutaraldehyde (*2, 3*). In order to use these same preparations to localize GABAergic neurons, it might be better to use an antiserum to glutamate decarboxylase (the synthesizing enzyme for GABA) that does not require glutaraldehyde in the fixative.

Sloviter interprets his results as indicating that GABAergic hilar neurons are not lost. Because he did not stain the normally large population of GABAergic neurons in the hilus, it is not known whether a significant change occurred in that population after stimulation of the perforant path. It is possible that such a change did occur, especially in light of the numerous degenerating hilar neurons on the stimulated side. Thus Sloviter's first conclusion, that the GABA-containing hilar neurons are impervious to the stimulation, could be incorrect. Since GABA and somatostatin are colocalized in many hilar neurons in the rat and cat (*3*), Sloviter's second and final conclusions also could be incorrect because the population of somatostatin-containing neurons that appears to be lost in this study would include many GABAergic neurons. Therefore, the proposed novel epilepsy hypothesis, which states that the loss of GABAergic neuron activation by hilar neurons on the stimulated side is the basis for the physiological loss of inhibition, is questionable.

CHARLES E. RIBAK
Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717

REFERENCES

1. R. S. Sloviter, *Science* **235**, 73 (1987).
2. C. E. Ribak, J. E. Vaughn, K. Saito, *Brain Res.* **140**, 315 (1978); L. Seress and C. E. Ribak, *Exp. Brain Res.* **50**, 173 (1983); O. P. Ottersen and J. Storm-Mathisen, *Neuroscience* **16**, 589 (1985); K. Lubbers, J. R. Wolff, M. Frotscher, *Neurosci. Lett.* **62**, 317 (1985); T. Kosaka *et al.*, *J. Comp. Neurol.* **239**, 420 (1985); E. Mugnaini and W. H. Oertel, in *Handbook of Chemical Neuroanatomy*, A. Bjorklund and T. Hökfelt, Eds. (Elsevier, Amsterdam, 1985), vol. 4, pp. 436–608; D. G. Amaral and J. Kurz, *Neurosci. Lett.* **59**, 33 (1985); K. J. Anderson, B. E. Maley, S. W. Scheff, *ibid.* **69**, 7 (1986).
3. P. Somogyi *et al.*, *J. Neurosci.* **4**, 2590 (1984); D. E. Schmechel *et al.*, *Neurosci. Lett.* **47**, 227 (1984).
4. C. E. Ribak *et al.*, *J. Neurosci.* **6**, 3492 (1986); C. Léránth and M. Frotscher, *J. Comp. Neurol.* **261**, 33 (1987).

20 January 1987; accepted 14 April 1987

Response: Ribak asserts that I have not replicated the results of other investigators who have shown large numbers of hilar γ-aminobutyric acid (GABA)–containing neurons, and he cites an impressive number of studies to support his statement. In fact, the studies he cites do not support his assertion. Indeed, four of the citations say nothing whatever about the proportion of hilar neurons that are GABA- or glutamic acid decarboxylase (GAD)–positive and show few photomicrographs of the hilus (*1, 2*). Our results in the hippocampus with antiserum to GABA (*3, 4*) are identical to those of Ottersen and Storm-Mathisen (*5*), who used a different antiserum to GABA, and to those of Anderson and his colleagues (*2*), who used the same antiserum to GABA we used. Our results are also similar to those of Mugnaini and Oertel (*6*), who used antiserum to GAD. Our results differ significantly only from those of Seress and Ribak, who concluded that at least 60% of the cells of the dentate hilus are GABA neurons (*7*). Excluded from their analysis were the GABA- and GAD-positive basket cells within or subjacent to the granule cell layer. Immunocytochemical experiments conducted in this laboratory with antiserum to GABA, with the use of the high glutaraldehyde fixation Ribak suggests, show numerous hilar GABA neurons (*4*), but contradict Seress and Ribak's conclusion that a majority of hilar neurons are GABA neurons.

Ribak's second point is that other studies have shown that many hilar somatostatin-positive neurons are GABAergic and that therefore my finding that hilar somatostatin neurons have degenerated means that a loss of GABA neurons must have occurred. Only one study, by Schmechel and colleagues (*8*),

has addressed the colocalization of somatostatin and GAD in the hippocampus of the rat (*8*), the species used in our studies (*3, 4*); and Ribak appears to have misinterpreted their results. They found that, whereas there was a high degree of coexistence of somatostatin and GAD in most hippocampal subregions, it was clearly lower in the dentate hilus. This has been confirmed by the author (*9*).

The question Ribak raises about precisely which and how many hilar neurons contain GABA is a valid one that remains to be answered definitively. It is conceivable that some GABAergic cells synthesize high concentrations of GABA in their terminals but little in their cell bodies, thereby appearing to be GABA-negative when an antiserum to GABA is used. His suggestion about using antiserum to GAD is valid, and this question is currently under study in several laboratories. However, whereas Ribak focuses on this subtlety of hippocampal structure, he ignores the main point of my report. Most investigators, including Ribak (*10*), agree that the nonhilar GABA-positive basket cells mediate recurrent inhibition in the dentate granule cells (*11*). My report demonstrated that, weeks after granule cell seizure activity was induced, recurrent inhibition was greatly decreased and hilar mossy cells and somatostatin neurons had degenerated (*3*). Despite this severe damage in the hilus, silver staining for degenerating cells showed that no damage to GABA-positive basket cells or their terminals was apparent, and this was corroborated by GABA immunocytochemistry. In addition, despite a loss of inhibition and degeneration of hilar cells, there was no apparent change in the GABA-immunoreactive axosomatic plexus that surrounds and inhibits the granule cells (*3*). Even if some of the damaged somatostatin-positive hilar neurons did contain GAD, their loss did not remove the GABA-positive inhibitory terminal plexus around the granule cells.

Ribak states incorrectly that I concluded that "the GABA-containing hilar neurons are impervious to the stimulation." What I actually wrote was that "the GABA-containing *basket* cells [not hilar cells] predicted to be most sensitive to the effects of seizure activity are, in fact, *relatively* impervious to the excitatory input they receive from the granule cells" (italics added). I stand by that statement as it was written.

Therefore, the intuitively appealing hypothesis that a seizure-associated loss of inhibition is due simply to a loss of GABAergic inhibitory neurons (*12*) was not supported by experiments specifically designed to test the hypothesis (*3*). Conversely, the hypothesis to which Ribak objects suggests that seizure activity causes a long-lasting decrease in inhibition by irreversibly damaging hilar cells that normally excite the surviving inhibitory basket cells (*3*). Therefore, despite their survival, the GABA-containing basket cells do not exert their normal inhibitory influence because they have lost much of their afferent excitatory input. This hypothesis was formulated to account for the unexpected experimental results that were obtained.

Finally, I do not take issue with Ribak's concluding point that this novel hypothesis is questionable. That is, after all, the nature and purpose of hypotheses.

ROBERT S. SLOVITER
Neurology Research Center, Helen Hayes Hospital, New York State Department of Health, West Haverstraw, NY 10993, and *Departments of Pharmacology* and *Neurology, Columbia University, New York, NY 10032*

REFERENCES

1. D. G. Amaral and J. Kurz, *Neurosci. Lett.* **59**, 33 (1985); T. Kosaka *et al.*, *J. Comp. Neurol.* **239**, 420 (1985); K. Lubbers, J. R. Wolff, M. Frotscher, *Neurosci. Lett.* **62**, 317 (1985).
2. K. J. Anderson, B. E. Maley, S. W. Scheff, *Neurosci. Lett.* **69**, 7 (1986).
3. R. S. Sloviter, *Science* **235**, 73 (1987).
4. ——— and G. Nilaver, *J. Comp. Neurol.* **256**, 42 (1987).
5. O. P. Ottersen and J. Storm-Mathisen, *Neuroscience* **16**, 589 (1985).
6. E. Mugnaini and W. H. Oertel, in *Handbook of Chemical Neuroanatomy*, A. Bjorklund and T. Hokfelt, Eds. (Elsevier, Amsterdam, 1985), vol. 4, pp. 436–608.
7. L. Seress and C. E. Ribak, *Exp. Brain Res.* **50**, 173 (1983).
8. D. E. Schmechel *et al.*, *Neurosci. Lett.* **47**, 227 (1984).
9. D. E. Schmechel, personal communication.
10. C. E. Ribak and L. Anderson, *J. Comp. Neurol.* **192**, 903 (1980).
11. P. Andersen, B. Holmqvist, P. E. Voorhoeve, *Acta Physiol Scand.* **66**, 448 (1966).
12. C. E. Ribak, A. B. Harris, J. E. Vaughn, E. Roberts, *Science* **205**, 211 (1979); C. E. Ribak, R. M. Bradburne, A. B. Harris, *J. Neurosci.* **2**, 1725 (1982); C. E. Ribak, in *Recent Advances in Epilepsy*, T. A. Pedley and B. S. Meldrum, Eds. (Churchill Livingstone, Edinburgh, 1986), No. 3, pp. 1–20.

13 October 1987; accepted 22 October 1987