restored in the retinotectal system (*17*) and in other regions of the CNS (*18*) of anamiotes after injury. Synaptic interactions have also been shown in mammals between grafted fetal or neonatal neurons and nerve cells in various regions of the host CNS including the tectum (*19*). We have now shown that adult mammalian CNS neurons have the capability to form functioning synapses with neurons in distant CNS regions after regeneration of their cut axons. Whether or not such synapses can be formed in sufficient density and with appropriate specificity to subserve behavioral function can now be explored.

REFERENCES AND NOTES

1. A. J. Aguayo, in *Synaptic Plasticity*, C. W. Cotman, Ed. (Guilford, New York, 1985), pp. 457–484.
2. M. Vidal-Sanz, G. M. Bray, M. P. Villegas-Pérez, S. Thanos, A. J. Aguayo, *J. Neurosci.* **7**, 2894 (1987).
3. D. A. Carter, G. M. Bray, A. J. Aguayo, *J. Neurosci.* **9**, 4042 (1989).
4. S. A. Keirstead *et al.*, *Brain Res.* **359**, 402 (1985).
5. In nine 90- to 120-day-old hamsters (*Mesocricetus aureatus*), the right optic nerve was transected intraorbitally, and one end of a segment of autologous peroneal nerve approximately 2 cm long was anastomosed to the ocular stump (*2, 3*). After 7 to 8 weeks, the right (ipsilateral) SC was exposed by aspiration of some of the overlying occipital lobe and the distal end of the graft was inserted into the lateral aspect of the SC (*3*). Two to three weeks later, the left optic nerve was transected in an attempt to vacate synaptic sites on neurons in the right SC at the time when the regenerating RGC axons reached the SC (*2, 3*). All of these surgical procedures were done under pentobarbital anesthesia (35 mg/kg, intraperitoneal). For the terminal electrophysiological experiments, animals were anesthetized with urethane (1.25 g/kg, intraperitoneal) and the right SC was reexposed by aspiration of overlying cerebral cortex and scar tissue. The graft was exposed as it emerged from the orbit and desheathed to permit electrical stimulation. The animal's head was fixed to an animal frame by an aluminum bar attached to the occipital bone with low-melting-point dental wax (Moyco, Philadelphia). This arrangement avoided any obstruction of the visual field of the grafted eye. The right pupil was dilated with topical atropine and the cornea protected with a noncorrective contact lens. The animal's temperature was maintained at 37°C.
6. M. Armstrong-James and J. Millar, *J. Neurosci. Methods* **1**, 279 (1980). The exposed carbon filament was electrolytically etched with 2*M* potassium nitrate to a length of 20 to 30 μm and a tip diameter of <1 μm.
7. Microelectrode penetrations were made systematically through the superficial layers of the SC to a depth of 600 μm (inferred from the reading on the hydraulic micromanipulator) at 50- to 200-μm increments in the anteroposterior and mediolateral planes. In animals in which the insertion site of the graft could be seen, recordings were begun in this region.
8. C. J. Shatz and P. A. Kirkwood, *J. Neurosci.* **4**, 1378 (1984).
9. Erratic responses to single electrical stimuli could reflect intermittent failure of impulse transmission at terminal branch points of regenerated RGC axons. There may be some relief of such terminal branch point failure after tetanization of nerve, as reflected in phenomena such as the enhancement of motor neuron excitatory postsynaptic potential amplitude after tetanization of Ia afferents [H. R. Luscher, P. Ruenzel, E. Henneman, *J. Neurophysiol.* **49**, 269 (1983)]. However it is unlikely, in the absence of tetanization, that a second of two closely spaced impulses in an axon could give rise to consistent transmission of two or more impulses past a low safety branch point at which conduction had just failed, with reduction of jitter from several tens of milliseconds to <0.5 ms, as shown in Fig. 1.
10. Our criteria for identification of SC neurons are criteria of exclusion, SC neurons being identified as units that are not RGC axons. These criteria could fail to identify postsynaptic SC neurons that responded consistently and at fixed latency to electrical stimulation of the graft.
11. Electrical stimulation of PN grafts inserted into the rat retina gives rise to inhibition of RGC discharge by antidromic invasion of the retina, but this inhibition lasts no more than 30 ms (S. A. Keirstead and M. Rasminsky, unpublished results).
12. At the end of each experiment, the microelectrode was moved a measured distance medial to the last recording site, lowered into the brain, and broken off to serve as a positional reference. The animal was perfused through the heart with 4% paraformaldehyde, the brain was removed, and coronal sections were cut on a cryostat. Because the axons in the grafts were not labeled, we were not able to identify the extent of penetration or arborization of RGC axons within the SC. However, we estimate that all recordings from light-responsive SC neurons were made within 1 mm of the graft.
13. L. M. Chalupa and R. W. Rhoades, *J. Comp. Neurol.* **186**, 561 (1979); G. M. Sachs and G. E. Schneider, *ibid.* **230**, 155 (1984).
14. Y.-C. Tiao and C. Blakemore, *ibid.* **168**, 483 (1976); B. L. Finlay, S. E. Schneps, K. G. Wilson, G. E. Schneider, *Brain Res.* **142**, 223 (1978); R. D. Mooney, B. G. Klein, R. W. Rhoades, *J. Neurosci.* **5**, 2989 (1985).
15. B. E. Stein, E. Labos, L. Kruger, *J. Neurophysiol.* **36**, 680 (1973); B. E. Stein, *Annu. Rev. Neurosci.* **7**, 95 (1984).
16. D. A. Carter and T. Zwimpfer, unpublished data.
17. S. C. Sharma and M. Romeskie, in *Comparative Neurology of the Optic Tectum*, H. Vanegas, Ed. (Plenum, New York, 1984), pp. 163–183; R. Levine, *ibid.*, pp. 417–467; B. Grafstein, in *The Retina*, R. Adler and D. Farber, Eds. (Academic Press, Orlando, FL, 1986), pp. 275–335.
18. D. W. Y. Sah and E. Frank, *J. Neurosci.* **4**, 2784 (1984); S. A. Mackler and M. E. Selzer, *Science* **229**, 774 (1985).
19. L. Olson and B. Hoffer, in *Neural Grafting in the Mammalian CNS*, A. Björklund and U. Stenevi, Eds. (Elsevier, Amsterdam, 1985), pp. 15–21; M. Segal, A. Björklund, F. H. Gage, *Brain Res.* **336**, 302 (1985); D. J. Simons and R. D. Lund, *Dev. Brain Res.* **21**, 156 (1985); H. Klassen and R. D. Lund, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6958 (1987); T. Hamasaki *et al.*, *Brain Res.* **422**, 172 (1987); R. Gardette, R. M. Alvarado-Mallart, F. Crepel, C. Sotelo, *Neuroscience* **24**, 777 (1988); H. Klassen and R. D. Lund, *Exp. Neurol.* **102**, 102 (1988).

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Protection of Dentate Hilar Cells from Prolonged Stimulation by Intracellular Calcium Chelation

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Prolonged afferent stimulation of the rat dentate gyrus in vivo leads to degeneration only of those cells that lack immunoreactivity for the calcium binding proteins parvalbumin and calbindin. In order to test the hypothesis that calcium binding proteins protect against the effects of prolonged stimulation, intracellular recordings were made in hippocampal slices from cells that lack immunoreactivity for calcium binding proteins. Calcium binding protein–negative cells showed electrophysiological signs of deterioration during prolonged stimulation; cells containing calcium binding protein did not. When neurons without calcium binding proteins were impaled with microelectrodes containing the calcium chelator BAPTA, and BAPTA was allowed to diffuse into the cells, these cells showed no deterioration. These results indicate that, in a complex tissue of the central nervous system, an activity-induced increase in intracellular calcium can trigger processes leading to cell deterioration, and that increasing the calcium binding capacity of a cell decreases its vulnerability to damage.

CALCIUM-MEDIATED PROCESSES INside neurons are critical to a variety of cell functions (*1*). Intracellular free Ca^{2+} is normally maintained at a low level, but the level may increase significantly by events that release intracellular stores of bound Ca^{2+} or that allow Ca^{2+} flux across the membrane. Large increases of intracellular Ca^{2+} are thought to occur during periods of excessive neuronal excitation and trigger processes that lead to cell death (*2*). Specific cell types appear to be especially vulnerable to damage under conditions that facilitate Ca^{2+} influx via voltage- or neurotransmitter-gated channels. Intracellular Ca^{2+} binding proteins are important in regulating free Ca^{2+} (*3*), and their capacity to buffer intracellular Ca^{2+} may be important in determining cell vulnerability under conditions associated with Ca^{2+} influx.

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In in vivo experiments on rat dentate gyrus, prolonged stimulation of the major afferent pathway to dentate granule cells (the perforant path) was shown to lead to the histological degeneration of specific cell populations in the adjacent hilar region, without damage to the granule cells (*4*). The hilar cells that degenerated were the large spiny cells called mossy cells and a subpopulation of aspiny local circuit neurons (interneurons) that are somatostatin immunoreactive (*4, 5*). The mechanisms underlying the selective vulnerability of the mossy cells and the somatostatin-immunoreactive interneurons are unknown. However, immunocytochemical studies have shown that the dentate neurons that survive the period of stimulation (granule cells and some interneurons) contain significant levels of a Ca^{2+} binding protein (CaBP)—either parvalbumin or calbindin (D_{28K}) (*5*). In contrast, the vulnerable hilar cells—the mossy cells and other interneurons—contain neither CaBP (*5*). Does low Ca^{2+} binding capacity underlie the vulnerability of these hilar cells, and can vulnerable neurons be rendered resistant by increasing their Ca^{2+} binding capacity experimentally? These questions were examined by means of a model of stimulation-induced cell damage (*4, 5*) modified for the hippocampal slice preparation. We assessed the effects of stimulation on CaBP-negative hilar cells impaled with microelectrodes filled with or without the Ca^{2+} chelator BAPTA [1,2 bis-(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid] (*6*). BAPTA was chosen in preference to other chelators, such as EGTA, since BAPTA is faster acting, is highly specific for Ca^{2+} as opposed to other cations, and has little nonspecific effect on cell function over the short periods of our experiments (*7*).

Hippocampal slices were prepared and maintained as described (*8*). Either granule cells, interneurons, or mossy cells were recorded intracellularly. Granule cells, interneurons, and mossy cells were easily distinguished on the basis of their locations and different physiological properties, properties that had been previously correlated with intracellular staining to define cell morphology (*9*). In addition to intracellular recording, an extracellular recording electrode was used to simultaneously monitor the granule cell population response to stimulation of the perforant path (*10*). The pattern of sustained perforant path stimulation was similar to the "intermittent" pattern of stimulation used in similar studies of cell death performed in vivo (*4, 11*).

Simultaneous extracellular and intracellular recordings from granule cells demonstrated that, after 10 to 90 min of intermittent stimulation, there were no electrophysiological signs of deterioration in granule cells (n = 16; Table 1). Electrophysiological indications of deterioration were defined as (i) over a 20-mV depolarization from prestimulation resting membrane potential (RMP) that persisted after stimulation ceased (until the end of the experiment, from 30 min to 2 hours after stimulation stopped), (ii) a 50% or more loss of input resistance (R_{in}) (*12*), and (iii) a 30-mV or more decrease in action potential (AP) amplitude. In contrast to granule cells, mossy cells were extremely sensitive to stimulation and had a much lower threshold for stimulus-evoked APs than granule cells. When tested with intermittent stimulation, all mossy cells depolarized dramatically (up to 60 mV) within seconds of the first 20-Hz stimulus train; in addition, AP amplitude decreased (Table 1) and AP duration increased. Of eight cells tested, only two cells were able to repolarize and recover healthy AP amplitude after a brief period (less than 5 min) of intermittent stimulation. These two mossy cells were located extremely far (over 1.5 mm) from the stimulating electrode relative to the other, more sensitive mossy cells, and this proximity may have contributed to their lesser sensitivities. However, further intermittent stimulation of these two mossy cells (13 and 19 min, respectively) led to their depolarization without subsequent recovery; AP amplitude and cell R_{in} decreased concomitantly. In these eight experiments, there was no change in the amplitude or waveform of the granule cell population spike at the time when mossy cells showed electrophysiological deterioration. Thus, cells that had been shown, histologically, to degenerate after sustained stimulation in in vivo experiments (that is, mossy cells) also showed electrophysiological signs of deterioration after sustained stimulation in vitro. In contrast, cells that did not suffer histological damage after sustained stimulation in vivo (that is, granule cells) did not show electrophysiological signs of deterioration when recorded during sustained stimulation in vitro.

When mossy cells were impaled with microelectrodes containing BAPTA, they were not as sensitive to intermittent stimulation as when impaled with control pipettes (n = 5; Table 1 and Fig. 1) and were also less sensitive to single stimuli (Fig. 1). During an initial 20 to 30 min of cell impalement with BAPTA-containing electrodes (before intermittent stimulation), mossy cell properties were monitored; there were no changes in RMP, AP amplitude or waveform, or R_{in}. Recording with BAPTA electrodes in granule cells (n = 3) and CA3 pyramidal cells (n = 10), however, showed that processes known to involve intracellular Ca^{2+} increases (for example, spike frequency adaptation, or the afterhyperpolarization af-

Table 1. Effects of stimulation on the RMP, R_{in}, and AP amplitude in neurons of the fascia dentata impaled with or without BAPTA in the microelectrodes, before and 10 to 30 min after continuous intermittent (*11*) stimulation. For all measures with BAPTA-containing electrodes, stimulation was begun 20 to 30 min after cell impalement. Granule cells and insensitive interneurons were stimulated until there was a permanent reduction in paired-pulse inhibition (up to 90 min). Mossy cells and sensitive interneurons were stimulated until there were electrophysiological signs of deterioration (up to 10 min) if BAPTA-containing electrodes were not used and for at least 10 min when BAPTA-containing electrodes were used. Values are the means ± SEM.

Cel type	Before or after stimulation	RMP (mV)	R_{in} (MΩ)	AP amplitude (mV)
Granule cells (no BAPTA) (n = 16)	Before	−76.4 ± 1.2	67.5 ± 4.6	85.1 ± 2.8
	After	−83.8 ± 1.0*	90.6 ± 6.0*	80.3 ± 2.7
Insensitive interneurons (no BAPTA) (n = 10)	Before	−62.7 ± 3.8	138.2 ± 7.1	68.0 ± 4.3
	After	−70.3 ± 3.8	158.0 ± 6.9	68.2 ± 4.1
Mossy cells (no BAPTA) (n = 8)	Before	−64.8 ± 1.9	88.8 ± 6.7	79.0 ± 5.2
	After	−32.5 ± 6.2*	13.3 ± 20.0*	18.5 ± 9.4*
Mossy cells (BAPTA) (n = 5)	Before	−64.3 ± 3.0	94.0 ± 8.3	79.7 ± 0.9
	After	−66.5 ± 6.1	93.3 ± 6.0	81.8 ± 1.2
Sensitive interneurons (no BAPTA) (n = 4)	Before	−66.8 ± 2.6	101.2 ± 7.2	59.8 ± 4.6
	After	−21.4 ± 6.0*	45.2 ± 20.2*	18.2 ± 9.4*
Sensitive interneurons (BAPTA) (n = 2)	Before	−67.2	106.0	71.8
	After	−69.8	100.8	73.0

*Difference between before and after stimulation measures statistically significant, $P < 0.05$, t test.

ter a burst of APs) decreased 20 to 30 min after impalement, suggesting that 20 to 30 min were sufficient to allow for diffusion of BAPTA into the impaled cell. Therefore, mossy cells were activated via intermittent perforant path stimulation 20 to 30 min after impalement. These mossy cells impaled with BAPTA electrodes did not depolarize (or depolarized only slightly) during the initial segment of 20-Hz stimulus trains, and actually hyperpolarized during 2-Hz stimulus trains (Fig. 1B). After 10 min of intermittent stimulation, these cells showed no deterioration by electrophysiological criteria. This response to intermittent stimulation of CaBP-negative cells that were impaled with BAPTA-containing electrodes was in contrast to the response obtained from comparable cells impaled with microelectrodes containing no Ca^{2+} chelator and was similar to the response of CaBP-positive cells such as granule cells.

Two mossy cells that were recorded with BAPTA-containing electrodes were stimulated intermittently soon after impalement (at 10 min, before substantial BAPTA diffusion occurred) and 30 min after impalement (Fig. 1). Their responses to the first period of intermittent stimulation were similar to the responses of mossy cells impaled without BAPTA-containing microelectrodes (Fig. 1A). However, when the same stimuli were delivered 30 min after impalement, the same mossy cells were relatively resistant to stimulus-induced depolarization, decreases in AP amplitude, broadening of APs, and loss of R_{in} (Fig. 1B).

Other neurons of the dentate hilus that were sensitive to sustained intermittent stimulation had electrophysiologic characteristics of interneurons. They were infrequently encountered and difficult to hold in stable penetrations. Like the mossy cells, these sensitive interneurons depolarized, lost R_{in}, and deteriorated during sustained intermittent stimulation ($n = 4$; Table 1). Some interneurons in the hilus responded to intermittent stimulation like the granule cells; these insensitive interneurons did not depolarize, lose R_{in}, or deteriorate during stimulation ($n = 10$; Table 1). No immunocytochemistry was performed in these experiments, so it was not possible to determine whether the sensitive interneurons lacked Ca^{2+} binding proteins, or whether they contained somatostatin, as experiments performed in vivo would suggest (*4, 5*). However, it was possible to differentiate the two populations of interneurons in another way. The interneurons that were sensitive to sustained intermittent stimulation had a very low threshold for AP generation evoked by perforant path stimulation, whereas the insensitive interneurons had a threshold that was similar to granule cells. Two sensitive interneurons with low thresholds were impaled with microelectrodes containing BAPTA, and intermittent stimulation was delivered 30 min after impalement. Intermittent stimulation led to transient, minimal depolarizations (up to 4 mV) and cells completely repolarized after intermittent stimulation; there were no significant changes in R_{in} (Table 1).

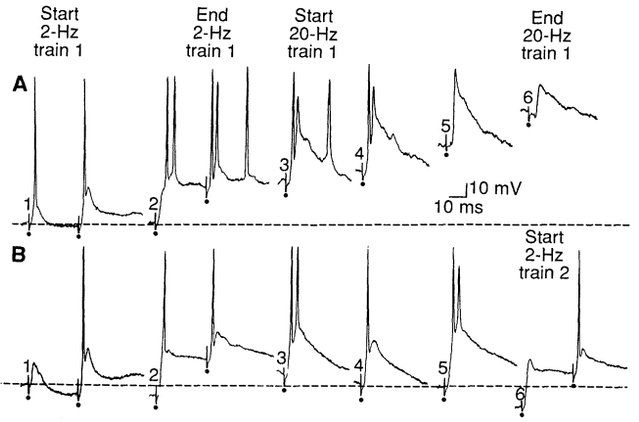

Fig. 1. Effects of intermittent stimulation on mossy cells impaled with intracellular recording electrodes containing BAPTA. (**A**) Responses to intermittent stimulation (*11*) 10 min after impalement. During the 2-Hz period, the cell fired more APs per stimulus than during control stimulation (compare A_1 and A_2). During the 20-Hz period (A_3 through A_6), the cell depolarized dramatically and APs decreased in amplitude and broadened; by the end of the 20-Hz period, the response to stimulation was only a small depolarization (A_6). After the 20-Hz stimulus train stimulation was stopped, the cell eventually recovered its membrane potential, AP amplitude and waveform, and response to stimulation [see trace 1 in (B)]. The RMP before stimulation is indicated by the dotted line. Stimulus artifacts are marked by the closed circles; some APs are truncated by digitization. (**B**) Thirty minutes after impalement, the same mossy cell shown in (A) was stimulated with the same stimulus paradigm. During the 2-Hz train, there was very little increase in the number of APs per stimulus and the cell hyperpolarized (compare RMP at start of traces B_1 and B_2). During the 20-Hz train there was a small initial depolarization (B_3); the cell repolarized quickly and remained at prestimulation RMP for the remainder of the 5-s period (B_4 and B_5). When the 2-Hz stimulation was continued (B_6), the cell hyperpolarized. Alternate 2-Hz and 20-Hz stimulus trains were continued for 15 min, with the same responses to 2-Hz and 20-Hz stimulation as shown. A very small increase in stimulus intensity required to reach threshold for AP generation [evident on comparison of the response to the first stimulus in (A) and (B)] was observed in four of five cells.

The mechanism of the protective effect of BAPTA is most likely the chelation of intracellular free Ca^{2+}, which would limit the rise in Ca^{2+} during stimulation. Calcium levels may rise during stimulation because of the release of free Ca^{2+} from intracellular stores, or as a result of Ca^{2+} influx through voltage-dependent or receptor-linked Ca^{2+} channels. To test whether the deterioration was mediated by *N*-methyl-D-aspartate (NMDA) channels, as suggested by other studies (*2*), we carried out two manipulations. In ten experiments, extracellular Mg^{2+} concentration was raised to 2 m*M*, a level more effective in blocking NMDA-gated channels than our baseline level of 1 m*M* (*13*). In two other experiments, the specific NMDA receptor antagonist DL-amino-phosphonovalerate (APV) (100 μ*M*) (*14*) was added to our standard medium. Both manipulations blocked the damaging effects of intermittent stimulation on electrophysiologically characterized mossy cells, demonstrating that the Ca^{2+}-induced cell deterioration was due to influx via NMDA-gated channels, which were likely opened by the high level of excitatory input produced by perforant path stimulation (*15*).

In summary, we have used the Ca^{2+} chelator BAPTA to reduce the damaging effects of sustained stimulation on cells found in the hilus of the fascia dentata that appear to lack Ca^{2+} binding proteins. Since we have no way of assessing long-term survival of neurons studied in our in vitro experiments, it is not possible to state unequivocally that electrophysiological deterioration is equivalent to cell death. However, the marked and long-lasting electrophysiological changes we encountered have long been associated with dying neurons and correlate well with morphological studies that show death of the same neuron populations (*4, 5*). Thus, we interpret our results to suggest that effective buffering of intracellular free Ca^{2+} during periods of neuronal excitation is crucial to cell survival. Our data indicate that in complex organized central nervous system tissue, high levels of activation of normal synaptic connections can lead to a rise in Ca^{2+} that causes irreversible degenerative changes in neuronal physiology. Supplementing the Ca^{2+} binding capacity of vulnerable cells can prevent cell damage.

REFERENCES AND NOTES

1. W. Y. Cheung, Ed., *Calcium and Cell Function* (Academic Press, London, 1980).
2. F. A. X. Schanne, A. B. Kane, E. E. Young, J. L. Farber, *Science* **206**, 700 (1979); J. L. Farber, *Life Sci.* **29**, 1289 (1981); D. W. Choi, *Trends Neurosci.* **11**, 465 (1988).

3. M. P. Blaustein, *Trends Neurosci.* **11**, 438 (1988); R. M. McBurney and I. R. Neering, *ibid.*, p. 264.
4. R. S. Sloviter, *Brain Res. Bull.* **10**, 675 (1983); J. W. Olney, T. de Gubareff, R. S. Sloviter, *ibid.* **12**, 699 (1983); R. S. Sloviter, *Science* **237**, 73 (1987).
5. R. S. Sloviter, *J. Comp. Neurol.* **280**, 183 (1989).
6. Intracellular electrodes were pulled from borosilicate glass (80 to 120 megohms). Electrodes were filled with either 4*M* potassium acetate or 200 m*M* BAPTA (tetrapotassium salt; Molecular Probes) in 4*M* potassium acetate.
7. R. Y. Tsien, *Biochemistry* **19**, 2396 (1980).
8. Adult female Sprague-Dawley rats were decapitated and a section of brain containing the hippocampus was quickly removed. Longitudinal, 400-μm-thick slices were cut in 4°C buffer with a Vibroslicer (Frederick Haer). Slices were placed immediately after dissection in a warmed (35°C), humidified (95% O_2, 5% CO_2) recording chamber at an interface of air and modified Krebs-Ringer buffer (126 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 1.25 m*M* NaH_2PO_4, 26 m*M* $NaHCO_3$, and 10 m*M* dextrose, *p*H 7.4) [H. E. Scharfman and P. A. Schwartzkroin, *J. Neurosci.* **8**, 3812 (1988)].
9. Granule cells were distinguished from hilar cells by their cell body location in the granule cell layer, as well as by several physiological properties, such as a negative RMP (compared to pyramidal cells, interneurons, and mossy cells), a low level of spontaneous activity, and marked spike frequency adaptation [R. A. Fricke and D. A. Prince, *J. Neurophysiol.* **51**, 195 (1984)]. Mossy cells were impaled only in the hilus proper [D. Amaral, *J. Comp. Neurol.* **182**, 851 (1978)] and were distinguished from other hilar neurons (the interneurons) by their firing properties (similar to area CA3 pyramidal cells), a particularly long time constant and high R_{in}, variability in response to fixed depolarizing current pulses, and high frequency and amplitude of spontaneous depolarizing potentials [H. E. Scharfman and P. A. Schwartzkroin, *J. Neurosci* **8**, 3412 (1988)]. Interneurons were characterized primarily by their lack of spike frequency adaptation and large, brief afterhyperpolarization following each AP, as has been shown for many types of interneurons in other areas of the hippocampus [P. A. Schwartzkroin and L. Mathers, *Brain Res.* **157**, 1 (1978); Y. Kawaguchi and K. Hama, *ibid.* **425**, 351 (1987); J.-C. Lacaille, A. L. Mueller, D. D. Kunkel, P. A. Schwartzkroin, *J. Neurosci.* **7**, 1979 (1987); J.-C. Lacaille, D. D. Kunkel, P. A. Schwartzkroin, *ibid.* **8**, 1632 (1988)].
10. Extracellular recording electrodes (2 to 10 megohms) were filled with 1*M* NaCl and placed 50 to 100 μm deep in the slice. Bipolar electrodes were placed in stratum moleculare to stimulate perforant path fibers. Stimulating electrodes were made of twisted Teflon-coated stainless steel wire; poles were 100 μm apart at the tips. The tips of the stimulating electrode were placed on the surface of the slice, over 500 μm from the recording electrodes.
11. Paired stimuli (interstimulus interval, 30 ms) were delivered at 2 Hz for 25 s, alternated with a 5-s period of single stimuli delivered at 20 Hz. Stimulus intensity (monophasic square pulses, 10 to 100 μA for 50 to 100 μs) was adjusted to evoke a small (1 to 3 mV) population spike.
12. R_{in} was determined from the amplitudes of responses at steady state to hyperpolarizing rectangular current pulses (0.1 to 1.0 nA for 100 ms) and defined as the slope of linear portion of the current-voltage (*I-V*) curve. AP amplitude was measured from RMP to peak.
13. L. Nowak, P. Bregestovski, P. Ascher, A. Herbert, A. Prochiantz, *Nature* **307**, 462 (1984); M. L. Mayer *et al.*, *ibid.* **309**, 261 (1984).
14. APV (Sigma) was added to the perfusate before and during stimulation. APV had no detectable nonspecific effects on cell properties or the population spike.
15. Although receptor binding studies have not indicated that the dentate hilus has a particularly high concentration of NMDA receptors, studies to date have not had sufficient resolution to detect differences among individual hilar cells [D. T. Monaghan and C. W. Cotman, *J. Neurosci.* **5**, 2909 (1985); E. W. Harris *et al.*, *Brain Res.* **382**, 174 (1986); W. F. Maragos, J. B. Penney, A. B. Young, *J. Neurosci.* **8**, 493 (1988)].
16. These studies were supported by National Institute of Neurological and Communicative Disorders and Stroke grants NS-01744, NS-15317, and NS-18895. P.A.S. is an affiliate of the Child Development and Mental Retardation Center, University of Washington.

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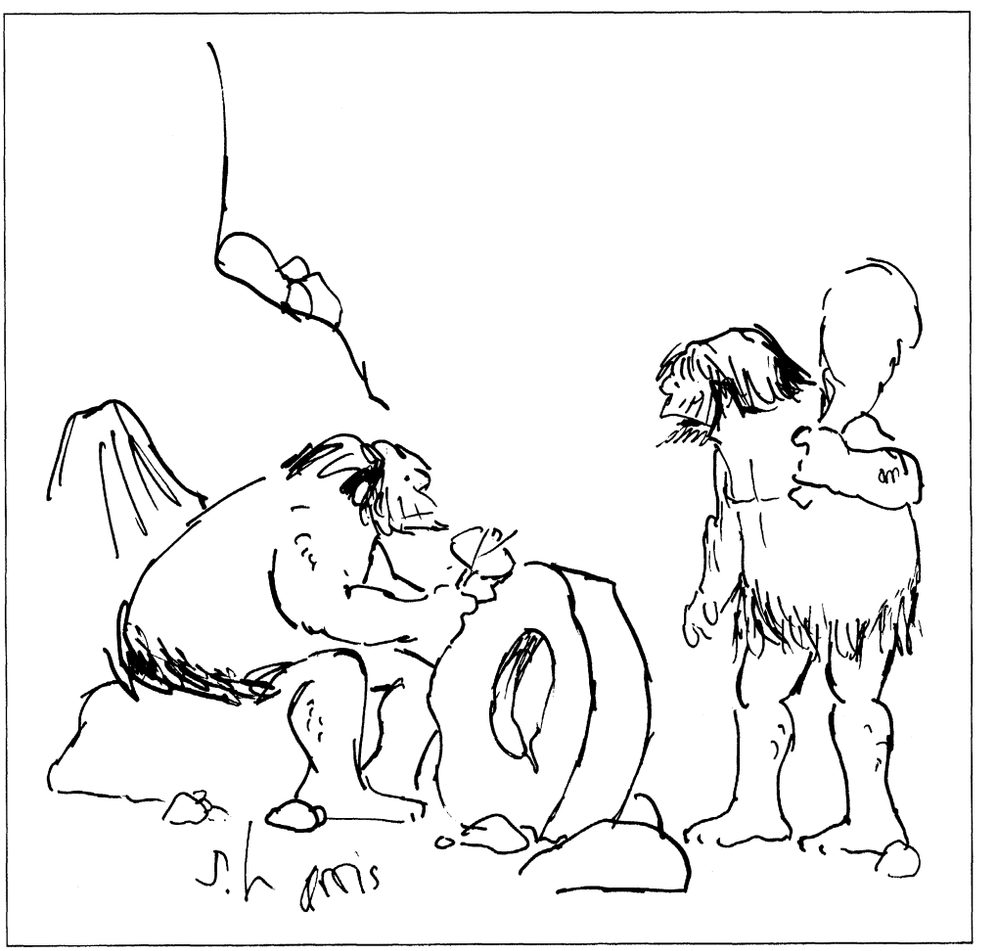

" What you'll need now, I guess, is some sort of brake."