that of mice lacking components of the hepatocyte growth factor/Met signaling pathway (*25*, *26*). In the adult organism, an analogous mechanism could explain the degenerative changes observed in heterozygote old mice. Although in young heterozygote *Dnchc1* mutants, the clinical manifestation of the developmental phenotype is not substantial, the age-related progressive neurodegeneration could be the consequence of a constantly reduced supply of trophic factors (such as NGF) caused by impaired dynein-dependent retrograde axonal transport.

We also identified a specific abnormality in the migration of facial motor neurons. A missense mutation in the p150^{glued} subunit of dynactin has recently been identified in a human kindred displaying a SBMA-like syndrome dominated by facial, bulbar, and distal extremity weakness (*27*). This further supports the hypothesis that subtle alterations of the dynein/dynactin system can lead to human MND; mutations in anterograde axonal transport proteins, including the microtubule motor kinesin (KIF1B), can lead to slow progressive motor neuronopathy (*28*), indicating the potential generality of the link between retrograde and anterograde axonal motor protein deficits and motor neuron degeneration.

The *Loa* and *Cra1* mutations exhibit remarkable similarities to specific features of human pathology, including Lewy body–like inclusions containing SOD1, CDK5, NFs, and ubiquitin. Abnormal accumulation of NFs in the cell body and proximal axons is the most frequently seen early pathological characteristic of ALS (*29*). Retrograde transport of NFs is dynein-dependent, suggesting that the buildup of NFs is secondary to the defect in retrograde transport. SOD1 deposition, which may also be dynein-dependent (*4*), is an unexpected finding pointing to a link between dynein dysfunction and normal SOD1 function.

The mechanism by which SOD1 mutations bring about motor neuron degeneration is unknown. Recent data have demonstrated the slowing of axonal transport as an early event in the motor neuron toxicity of SOD1 mutants (*30*). The disruption of dynein function by interaction with mutant SOD1, creating an aberrant protein complex that interferes with the function of the wild-type complex, may also provide a link between axonal transport, NF accumulation, and cell death.

References and Notes

1. D. W. Cleveland, J. D. Rothstein, *Nature Rev. Neurosci.* **2**, 806 (2001).
2. S. Lefebvre *et al*., *Cell* **80**, 155 (1995).
3. A. R. La Spada *et al.*, *Nature* **352,** 77 (1991).
4. J. P. Julien, *Cell* **104**, 581 (2001).
5. T. Murakami *et al.*, *Neurosci. Lett*. **308**, 149 (2001).
6. F. J. Ahmad *et al.*, *J. Cell Biol.* **140**, 391 (1998).
7. S. Terada, N. Hirokawa, *Curr. Opin. Neurobiol*. **10**, 566 (2000).
8. A. Harada *et al.*, *J. Cell Biol*. **141**, 51 (1998).
9. B. H. LaMonte *et al.*, *Neuron* **34**, 715 (2002).
10. D. C. Rogers *et al.*, *Neurosci. Lett*. **306**, 89 (2001).
11. M. Hrabé de Angelis *et al.*, *Nature Genet*. **25**, 444 (2000).
12. M. Hafezparast *et al*., data not shown.
13. K. Tsuchiya *et al.*, *Acta Neuropathol*. **100,** 603 (2000).
14. A. S. Witherden *et al.*, *Gene* **283**, 71 (2002).
15. D. J. Asai, M. P. Koonce, *Trends Cell Biol*. **11**, 196 (2001).
16. S. M. King, *J. Cell Sci.* **113**, 2521 (2000).
17. S. H. Tynan *et al.*, *J. Biol. Chem*. **275**, 32769 (2000).
18. A. Habura *et al.*, *J. Biol. Chem*. **274**, 15447 (1999).
19. N. R. Morris, *J. Cell Biol*. **148**, 1097 (2000).
20. A. Stieber *et al.*, *J. Neurol. Sci*. **173**, 63 (2000).
21. R. B. Vallee *et al.*, *Trends Cell Biol*. **11**, 155 (2001).
22. J. V. Shah, D. W. Cleveland, *Curr. Opin. Cell Biol*. **14**, 58 (2002).
23. B. A. Eaton *et al.*, *Neuron* **34**, 729 (2002).
24. G. Lalli, G. Schiavo, *J. Cell Biol*. **156**, 233 (2002).
25. A. Ebens *et al.*, *Neuron* **17**, 1157 (1996).
26. F. Maina *et al.*, *Genes Dev*. **11**, 3341 (1997).
27. I. Puls *et al.*, *Nature Genet*. **33**, 455 (2003).
28. C. Zhao *et al.*, *Cell* **105**, 587 (2001).
29. M. E. Gurney *et al.*, *Science* **264**, 1772 (1994).
30. T. L. Williamson, D. W. Cleveland, *Nature Neurosci*. **2**, 50 (1999).

31. Supported by the Motor Neurone Disease Association, Cancer Research UK, the U.K. Medical Research Council, the Violet M. Richards Charity, the Annie Lindsell Trust, the University of Malaya, a German Human Genome Project grant, a CREST Research Project grant, and the Human Frontier Science Program. We thank C. Baker, N. Bermingham, J. Downward, and M. Maden for advice and constructive suggestions; G. Hansen, S. Kohlmann, A. Bareiss, B. Kohnke, and G. Piontek for technical assistance; L. Greensmith and group for samples; and R. Young for graphics.

Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5620/808/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Table S1
References

5 February 2003; accepted 1 April 2003

GABA and Its Agonists Improved Visual Cortical Function in Senescent Monkeys

Audie G. Leventhal,[1]* Yongchang Wang,[1] Mingliang Pu,[1] Yifeng Zhou,[2] Yuanye Ma[3]

Human cerebral cortical function degrades during old age. Much of this change may result from a degradation of intracortical inhibition during senescence. We used multibarreled microelectrodes to study the effects of electrophoretic application of γ-aminobutyric acid (GABA), the GABA type a (GABAa) receptor agonist muscimol, and the GABAa receptor antagonist bicuculline, respectively, on the properties of individual V1 cells in old monkeys. Bicuculline exerted a much weaker effect on neuronal responses in old than in young animals, confirming a degradation of GABA-mediated inhibition. On the other hand, the administration of GABA and muscimol resulted in improved visual function. Many treated cells in area V1 of old animals displayed responses typical of young cells. The present results have important implications for the treatment of the sensory, motor, and cognitive declines that accompany old age.

Aging is known to adversely affect visual function in humans. Senescent humans exhibit decreased visual acuity, binocular summation, contrast sensitivity, motion sensitivity, and wavelength sensitivity. The elderly also respond much more slowly in visual tests and do not perform as well at shape discrimination tests as do the young and middle aged (*1*–*10*). It has been hypothesized that many of the foregoing declines during old age are due to degeneration and/or dysfunction in central visual areas (*10*, *11*).

[1]Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132, USA. [2]School of Life Science, University of Science and Technology of China, Hefei, Anhui 230027, China. [3]Laboratory of Primate Cognitive Neuroscience, Kunming Institute of Zoology, Chinese Academy of Science, Kunming, Yunnan 650223, China.

*To whom correspondence should be addressed. E-mail: audie.leventhal@m.cc.utah.edu

The receptive field properties of cells in the visual cortex (area V1) have been studied for over 40 years. The cells in area V1 are known to respond selectively to both the angular orientation and the direction of motion of lines, bars, and edges (*12*). The orientation- and direction-selective responses of V1 cells are thought to participate in the perception of form and motion.

We reported previously (*11*) that primate visual cortical function declines because V1 cells in old (26 to 30 years old) macaque monkeys exhibit decreased orientation and direction selectivity, accompanied by increased visual responsiveness, increased spontaneous activity, and a decreased ability to signal visual stimuli above background activity (signal-to-noise ratio). We have now studied the effects of electrophoretic application of the inhibitory transmitter γ-aminobutyric acid (GABA), the GABA type a (GABAa) agonist muscimol, and

the GABAa antagonist bicuculline on the receptive field properties of individual V1 cells in old monkeys. We tested the hypothesis that the application of GABA and GABA agonists on individual V1 cells can improve visual function in old animals.

We studied a total of 242 neurons in six young monkeys (7 to 9 years old) and 257 neurons in seven old monkeys (26 to 32 years old) (fig. S1). Both *Macaca mulatta* (75% of cells) and *M. fascicularis* (25% of cells) were studied. The results for the two species did not differ and thus are combined in the figures. Some of the animals included in this study also provided data for a previous one (*11*).

The effects of GABA, muscimol, and bicuculline on the responses of two typical cells in old monkeys and one in a young monkey are illustrated in Fig. 1. Before drug administration, cells in old animals responded equally well to all orientations and directions (Fig. 1, A and D). After GABA and muscimol administration, some of these cells

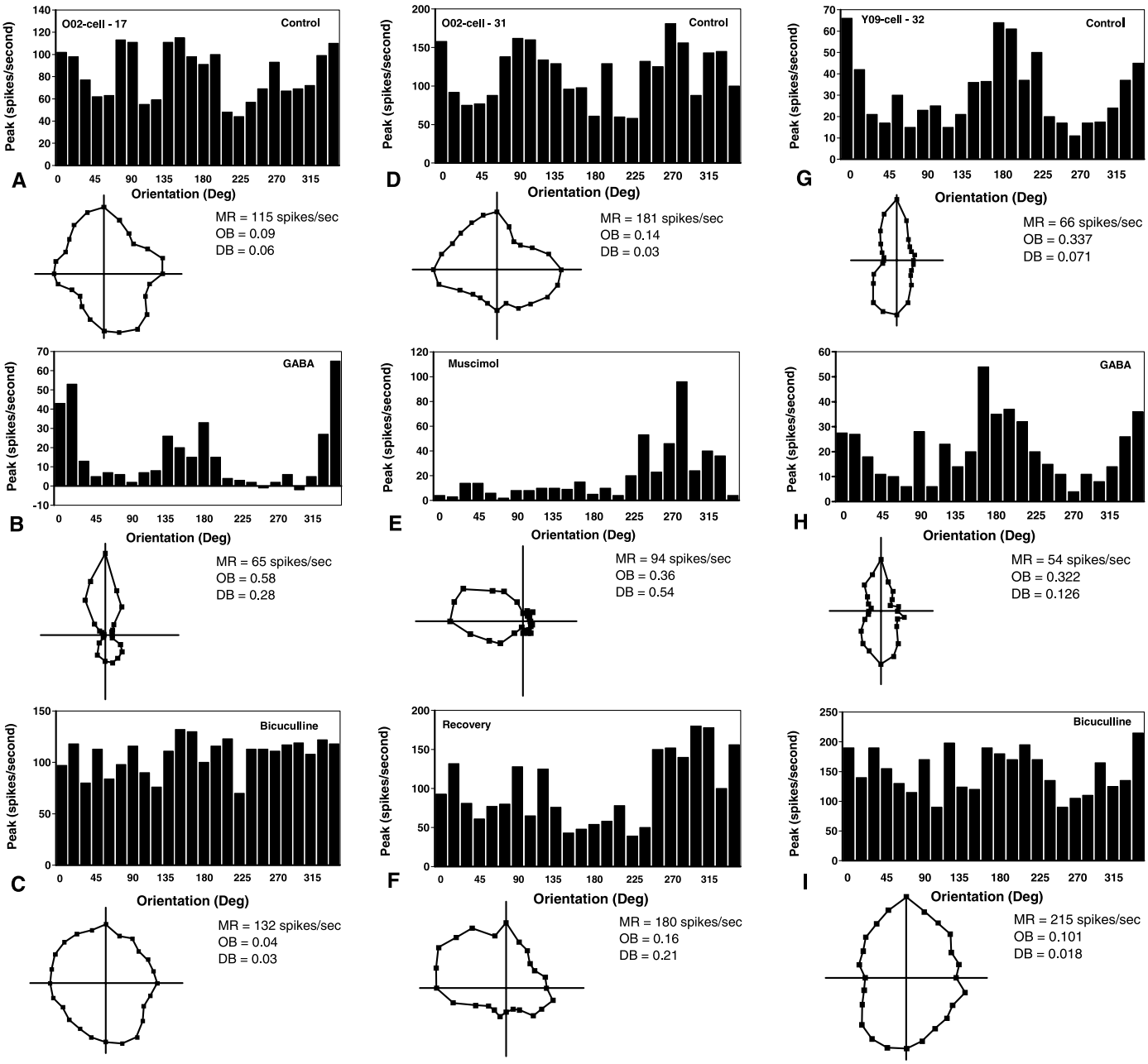

Fig. 1. Tuning curves and corresponding polar plots obtained for two representative cells in old monkeys (A to F) and one typical cell from a young monkey (G to I) that received treatment with GABA, muscimol, and bicuculline. The maximum (peak) responses (MR), orientation biases (OB), and direction biases (DB) are shown for each condition. A typical old cortical cell showing a lack of orientation and direction sensitivity is shown in (**A**). Three minutes after GABA application (**B**), this cell exhibited strong orientation and moderate direction selectivity. The cell's peak response decreased, as did its spontaneous activity. GABA application was then discontinued, and bicuculline application was begun (**C**). Bicuculline reversed the effects of GABA. The responses of a second cell in visual cortex of an old monkey showing a degradation of orientation and direction selectivity are shown in (**D**). Three minutes after muscimol administration (**E**), this cell exhibited moderate orientation selectivity, very strong direction selectivity, a decreased peak response, and decreased spontaneous activity. Five minutes after the discontinuation of muscimol administration, the drug-induced improvement disappeared (**F**). A tuning curve typical of the majority of cells in young monkeys is shown in (**G**). The cell was selective, and its selectivity was not affected by the administration of GABA (**H**). On the other hand, application of bicuculline resulted in a more than 300% increase in peak response and greatly reduced orientation selectivity (**I**).

responded strongly to a narrow range of preferred orientations and directions and exhibited nearly no response to the nonpreferred orientations and directions (Fig. 1, B and E). This differential effect on preferred and nonpreferred orientations and directions suggests that the effect of GABA is not simply to nonselectively decrease the responses of the cell to all visual stimuli. Additionally, as illustrated in Fig. 1H, GABA administration did not affect the selectivity of most of the already strongly selective cells (Fig. 1G) in young animals.

We also investigated the effects of the application of bicuculline on cells in young and old animals. In the old animals, bicuculline did not change stimulus selectivity to any great extent and resulted in a somewhat increased visual response (Fig. 1C). In contrast, as has been reported previously (*13*, *14*), bicuculline greatly diminished selectivity in young animals and greatly increased the magnitude of the visual response (Fig. 1I).

The effects of GABA and muscimol administration on the orientation and direction selectivities of all V1 cells studied in old monkeys are summarized in Table 1. Both GABA and muscimol resulted in increases in the percentage of cells that exhibited significant orientation and direction selectivity. In fact, the percentages of orientation- and direction-selective cells approached those seen in normal animals after the application of GABA and muscimol. On average, however, the degree of selectivity of most cells in old monkeys after drug administration was still lower than that seen in young animals (Table 1, legend). Additionally, unlike in young animals in which bicuculline greatly diminishes selectivity (*13*, *14*), bicuculline did not significantly affect the already low percentage of direction-selective cells in old animals. The number of orientation-selective cells was only reduced slightly (Table 1).

The effects of drug administration on the visually evoked response and spontaneous activity of V1 cells in old monkeys are summarized in Fig. 2, A and B, respectively. GABA and muscimol both decreased peak visual response and spontaneous activity (*t* test, $P < 0.01$ in each case). Muscimol was more potent than GABA, and its application resulted in responses that were within the normal range. Bicuculline had the opposite effect and resulted in increased visual and spontaneous responses (*t* test, $P < 0.001$ in both cases). In each case, 5 to 10 minutes after the cessation of drug administration, the cells reverted to the preapplication state.

Proper brain function requires that stimuli evoke reliable responses that are easily discernable from background activity. We reported previously (*11*) that the ratio of the visually evoked response to background activity was much lower in old than in young monkeys. Histograms showing effects of drug application on the ratio of the peak visually evoked response to the spontaneous discharge rate (referred to here as signal-to-noise ratio) of cortical cells are presented in Fig. 2C. Both GABA and muscimol administration resulted in higher ratios (*t* test, $P < 0.01$ in both cases) and thus an improved ability to signal visual stimuli. On the other hand, bicuculline did not affect signal-to-noise ratios significantly in old animals because ratios were already low.

If GABA-mediated inhibition degrades during old age, then GABA and GABA agonists should be more effective in old than in young animals. Conversely, GABA antagonists should be more effective in young than in old monkeys. The results in Table 2 show that GABA and muscimol both result in larger percentage decreases in visually

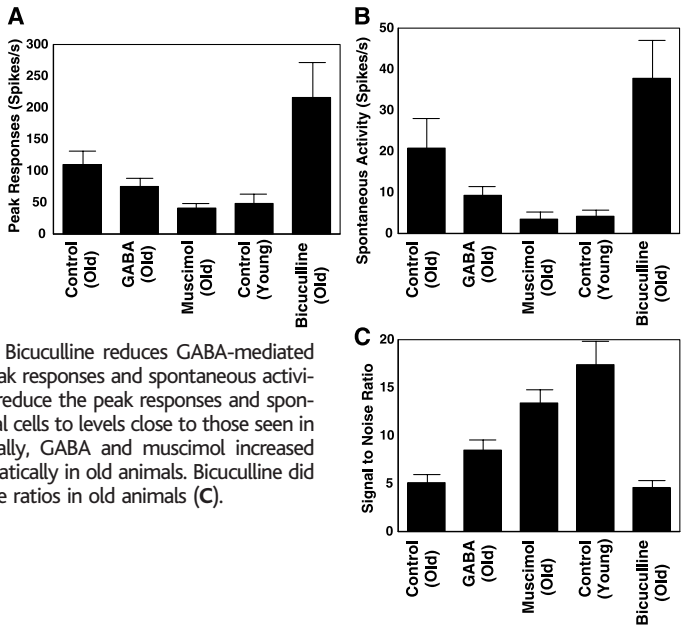

Fig. 2. The maximum visually evoked responses (**A**) and spontaneous activities (**B**) of V1 cortical cells in untreated old monkeys, untreated young monkeys, and old monkeys treated with GABA, muscimol, and bicuculline. Cortical cells in old monkeys exhibit abnormally high peak responses and spontaneous activities as compared to young monkeys. Bicuculline reduces GABA-mediated inhibition and increases peak responses and spontaneous activities. GABA and muscimol reduce the peak responses and spontaneous activities of cortical cells to levels close to those seen in young monkeys. Additionally, GABA and muscimol increased signal-to-noise ratios dramatically in old animals. Bicuculline did not improve signal-to-noise ratios in old animals (**C**).

Table 1. Effects of drug application on the percentages of orientation- and direction-selective cells in area V1 of old monkeys. Old monkeys exhibit a reduction in orientation- and direction-selective cells as compared to young monkeys. Bicuculline results in a small decrease in the number of orientation-selective cells and no change in the number of direction-selective cells. In contrast, GABA and muscimol are capable of increasing the orientation- and direction-selective responses of cortical cells. Although many cells in old monkeys do become selective after drug application, most still do not exhibit the strong selectivities seen in young animals. In old animals, the mean orientation bias increased from 0.098 ± 0.031 to 0.148 ± 0.053 (mean ± SD), whereas the mean direction bias increased from 0.061 ± 0.022 to 0.114 ± 0.035 (mean ± SD) after GABA application. These values are lower than the average biases seen in young animals. Our results for young animals indicate mean biases of 0.37 for orientation and 0.2 for direction (*11*).

	Old monkey				Young monkey control
	Control	GABA	Muscimol	Bicuculline	
Orientation selective	39%	81%	73%	22%	88%
Direction selective	23%	63%	68%	24%	69%

Table 2. Effects of drug application on responses of V1 cells. Changes in peak visually evoked response and spontaneous firing rate induced by GABA, muscimol, and bicuculline in young and old monkeys. GABA and muscimol resulted in larger percentage decreases in neuronal firing rates in old than in young monkeys. Bicuculline resulted in larger percentage increases in neuronal firing rates in young than in old monkeys.

		Control	GABA	Muscimol	Bicuculline
Old monkeys	Peak response	100%	–28.8%	–61.1%	+40.4%
	Spontaneous rate	100%	–57.6%	–83.4%	+88.4%
Young monkeys	Peak response	100%	–20.1%	–28.7%	+214.2%
	Spontaneous rate	100%	–11.2%	–9.1%	+416%

evoked and spontaneous activity in old than in young animals (t test, $P < 0.01$ in both cases). Bicuculline, on the other hand, resulted in larger percentage increases in visually evoked and spontaneous activity in young monkeys than in old ones (t test, $P < 0.01$). Taken together these results strongly suggest a decrease in the amount of GABA-mediated inhibition in cortex.

The results of this study show that the administration of GABA and muscimol results in improved orientation and direction selectivity, accompanied by decreased visual responsiveness, decreased spontaneous activity, and an increased ability to signal visual stimuli. Some cells in V1 of old animals displayed responses typical of cells in young animals after drug application. A restoration of function was evident as soon as 2 minutes after drug delivery. Five to 10 minutes after discontinuation of drug administration, neuronal function reverted to preapplication levels. Application of bicuculline resulted in much smaller changes in the properties of old V1 cells than in the properties of young ones. It should be mentioned that the iontophoretic application of noradrenaline is reported to improve the signal-to-noise ratio of cells in cat visual cortex (*15*) and primate motor cortex (*16*). Whether age affects noradrenaline levels in primate cortex is not known.

The foregoing results are consistent with the hypothesis that reductions in GABA-mediated intracortical inhibition contribute to the degradation of cortical function that accompanies old age. Our finding that GABA agonists exert a weaker effect on cortical cells in young monkeys than in old ones further supports this idea. The finding that bicuculline is more effective in young than old monkeys is also compatible with this suggestion.

A decrease in intracortical inhibition could result from diminished release of transmitter, diminished production of transmitter, a degradation of transmitter receptors, membrane changes, etc. Although our findings cannot pinpoint the changes in old monkeys, the present findings show that simply adding GABA and GABA agonists does facilitate visual function in old animals. Thus, it is tempting to speculate that normal aging results in a decreased ability to produce GABA in cerebral cortex. It is noteworthy that an age-related degradation of GABA-mediated inhibition has also been reported in the inferior colliculus. The effects of age on the inferior colliculus include reductions in the number of GABA-immunoreactive neurons, the concentration of GABA, GABA release, and GABA receptor binding (*17*, *18*). Similar studies in primate cortex need to be carried out.

Some V1 cells in old animals exhibited responses typical of cells in young ones after the application of GABA and GABA agonists. However, most cells exhibited only partial recovery as a result of drug administration. Thus, factors other than a degradation of GABA-mediated inhibition may also be involved. Because GABA-mediated inhibition is prevalent throughout the neocortex (*19*, *20*), it is likely that changes similar to those seen in V1 will exist in many cortical areas in old animals. Thus, the improvement in function of V1 cells after the application of GABA and its agonists has important implications for the treatment of the sensory, motor, and cognitive declines that accompany old age.

References and Notes

1. D. W. Kline, in *The Susceptible Visual Apparatus*, J. Marshall, Ed., vol. 16 of *Vision and Visual Dysfunction*, J. E. Cronly-Dillion, Ed. (Macmillan, London, 1991), pp. 150–161.
2. D. W. Kline, F. Schieber, in *Handbook of the Psychology of Aging*, J. E. Birren, K. W. Schaie, Eds. (Van Nostrand, New York, 1985), pp. 296–331.
3. D. B. Tran, S. E. Silverman, K. Zimmerman, S. E. Feldon, *Graefe's Arch. Clin. Exp. Ophthalmol.* **236**, 269 (1998).
4. V. Porciatti, A. Fiorentini, M. C. Morrone, D. C. Burr, *Vision Res.* **39**, 2157 (1999).
5. D. W. Kline, F. Schieber, L. C. Abusamra, A. C. Coyne, *J. Gerontol.* **38**, 211 (1983).
6. H. C. Weston, *Br. J. Ophthalmol.* **32**, 645 (1948).
7. J. M. Ordy, T. M. Wengenack, W. P. Dunlap, in *The Effects of Aging and Environment on Vision*, D. Armstrong *et al.*, Eds. (Plenum, New York, 1991), pp. 1–12.
8. C. Owsley, R. Sekuler, C. Boldt, *Invest. Ophthalmol. Vis. Sci.* **21**, 362 (1981).
9. C. Owsley, M. E. Sloane, *Br. J. Ophthalmol.* **71**, 791 (1987).
10. P. D. Spear, *Vision Res.* **33**, 2589 (1993).
11. M. T. Schmolesky, Y. C. Wang, M. L. Pu, A. G. Leventhal, *Nature Neurosci.* **3**, 382 (2000).
12. D. H. Hubel, T. N. Wiesel, *J. Physiol.* (*London*) **195**, 215 (1968).
13. A. D. Sillito, *J. Physiol.* **250**, 287 (1975).
14. U. T. Eysel, I. A. Sheveler, N. A. Lazareva, G. A. Sharaev, *Neuroscience* **84** (no. 1), 25 (1998).
15. T. Kasamatsu, P. Heggelund, *Exp. Brain Res.* **45**, 317 (1982).
16. M. Matsumura, T. Sawaguchi, K. Kubota, *Neurosci. Res.* **8**, 138 (1990).
17. D. M. Caspary, J. C. Milbrandt, R. H. Helfert, *Exp. Gerontol.* **30**, 349 (1995).
18. D. M. Caspary *et al.*, *Neuroscience* **93**, 307 (1999).
19. K. Letinic, R. Zoncu, P. Rakic, *Nature* **417**, 645 (2002).
20. M. Galarreta, S. Hestrin, *Science* **292**, 2295 (2001).

21. Supported by National Institute on Aging (NIH) grant AG-17922.

Supporting Online Material
www.sciencemag.org/cgi/content/full/300/5620/812/DC1
Materials and Methods
Fig. S1

28 January 2003; accepted 2 April 2003