(306 and 613 Hz) and sounds separated by even less than one octave in the HT frequency range (2688 and 4808 Hz) (*14*). The sounds used in the auditory DMS task (243 and 3676 Hz) were separated by almost four octaves.

The three monkeys exhibited differing degrees of impairment. Reconstruction of the lesions showed that although the amount of ablated cortex on the lateral surface of the superior temporal gyrus and the upper bank of the superior temporal sulcus was similar for all three animals, the amount of damage to the lower bank of the lateral fissure was correlated with the degree of auditory deficits. However, it was not clear whether lesion locus or lesion size was the relevant variable.

The results of this study show that when monkeys are trained on a task known to engage auditory processing and retention mechanisms, lesions of the auditory association cortex have dramatic effects on auditory short-term memory. To what extent the deficits reflect impairments in the processing or the storage of auditory information, however, remains to be determined.

The finding of auditory memory impairments that are difficult to explain on the basis of impairments in basic auditory sensory abilities parallels in many ways the visual recognition memory deficits that occur after lesions of the inferior temporal cortex. This result, plus the fact that the two modalities share similar patterns of cortical and subcortical connections (*15*), supports the idea that the mechanisms underlying short-term memory in the two systems are similar. Together with the findings of Heffner and Heffner (*16*) that monkeys may possess a precursor to Wernicke's area located in the superior temporal gyrus, these results may prove relevant to the understanding of the neurological mechanisms underlying auditory memory and language processing in humans.

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7. The successive version of the delayed matching-to-sample task was originally developed by J. Konorski [*Bull. Acad. Pol. Sci. Ser. Sci. Biol.* **7**, 115 (1959)].
8. The front panel of the chamber also accommodated two in-line stimulus projectors situated on the same horizontal plane as the speakers. The projector on the right was used for presenting the visual sample stimulus and that on the left for presenting the visual comparison stimulus. A transparent plastic key, situated directly in front of the projector, served as the response mechanism.
9. All sessions consisted of 39 trials, the first 3 serving as warm-up trials and therefore not included in the data analysis. The remaining 36 trials were equally divided into matching and nonmatching trials. During retention gradient testing, the 36 usable trials were divided into 12 trials dedicated to each of the three delay intervals.
10. Monkeys B and T received lesions of the left auditory association cortex first and monkey M that of the right auditory association cortex first. Surgery was performed in an operating room with sterile procedures. During the operation the monkeys were immobilized with 0.02 mg kg^{-1} $hour^{-1}$ of pancuronium bromide, respirated with 68.5% nitrous oxide and 30% oxygen, and anesthetized with 1.5% halothane.
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14. Only the right speaker and lever were used for the auditory discrimination task. The monkeys were trained on an asymmetrically rewarded "go/no-go" procedure to press the lever in the presence of the positive stimulus and refrain from pressing the lever in the presence of the negative stimulus. All sessions consisted of 48 trials, and the criterion was one session with 43 out of 48 correct responses. Monkeys M and T required 1 and 7 sessions, respectively, to reach the criterion on the 2688- and 4808-Hz pair, and 5 and 15 sessions, respectively, to reach the criterion on the 306- and 613-Hz pair. Although unable to satisfy the criterion, monkey B averaged 73.5% over the last 10 of 27 sessions of 2688- and 4808-Hz testing and 74.2% correct over the last 10 of 20 sessions of 306- and 613-Hz testing.
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Survival of Adult Basal Forebrain Cholinergic Neurons After Loss of Target Neurons

MICHAEL V. SOFRONIEW,* NEIL P. GALLETLY, OLE ISACSON,† CLIVE N. SVENDSEN

Target cells are thought to regulate the survival of afferent neurons during development by supplying limiting amounts of neurotrophic factors, but the degree to which afferent neurons remain dependent on target-derived support in the adult is uncertain. In this study, uninjured basal forebrain cholinergic neurons did not die after excitotoxic ablation of their target neurons in young adult rats, indicating that they are either not dependent on neurotrophic factors for survival or can obtain trophic support from other sources after target neurons are lost. This finding suggests that cholinergic cell death in neurodegenerative conditions such as Alzheimer's disease is not due solely to a loss of target neurons or factors provided by them.

REGRESSIVE EVENTS DURING NEURAL morphogenesis in vertebrates, such as naturally occurring cell death and retraction of collaterals, are regulated by dynamic interactions between afferent neurons and their targets (*1*). Studies on the molecular nature of these interactions in peripheral neurons that innervate nonneuronal targets have demonstrated that a target-derived substance, nerve growth factor (NGF), is required to sustain sympathetic neurons and neural crest-derived sensory neurons (*2*). Evidence that NGF may be active in the central nervous system (CNS)

Department of Anatomy, University of Cambridge, Cambridge, CB2 3DY, England.

*To whom correspondence should be addressed.
†Present address: McLean Hospital/Harvard Medical School, Belmont, MA 02178.

(*3*) and the identification of other putative neurotrophic factors have further strengthened the hypothesis that, during development, trophic factors are elaborated in target tissues in small amounts to regulate the survival of afferent neurons (*2*).

Although neurotrophic factors continue to be produced, secreted, and retrogradely transported in some regions of the adult CNS (*2, 3*), the degree to which afferent neurons remain dependent on target-derived support throughout life is not known. Cholinergic neurons in the medial septal nucleus provide a well-characterized system in which to address this question. They are a subpopulation of the basal forebrain cholinergic system with only one main projection, to the hippocampus (*4*), and they degenerate for unknown reasons in conditions showing loss of hippocampal neurons such as the Alzheimer-type dementias (*5*). These cells bear NGF receptors and retrogradely transport radiolabeled NGF from the hippocampus, where target neurons produce NGF and its mRNA (*3, 6*). Moreover, the majority of these neurons are lost after axotomy of the septohippocampal projection (*7, 8*), and this loss can be prevented pharmacologically by administration of exogenous NGF (*9*). Nevertheless, there is no direct evidence that these neurons are dependent on target-derived support for survival either during development or in the adult (*2*). We have therefore examined the effect on cholinergic neurons in the medial septal nucleus of removing their target neurons.

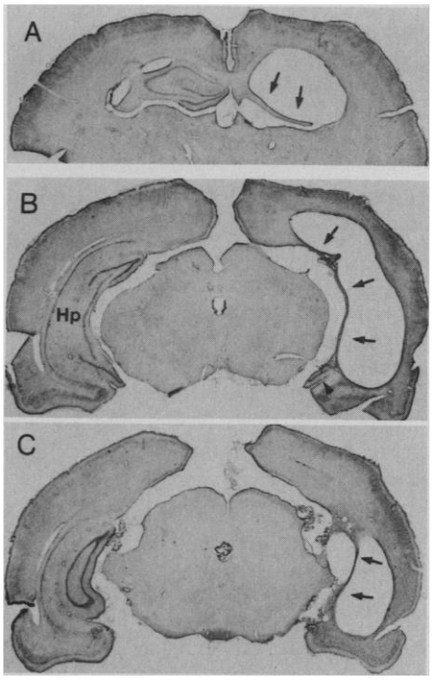

Fig. 1. (**A** to **C**) Photomicrographs of cresyl violet–stained sections through three levels of the hippocampus (Hp) comparing the lesioned and unlesioned sides 90 days after unilateral injections of NMDA into six sites (0.5 μl of 100 nmol/μl in 0.9% NaCl per site) distributed evenly throughout the hippocampal formation (*25*). Arrows indicate the persisting fimbrial remnant on the lesioned side. Arrowhead in (B) indicates a small region of persisting temporal hippocampus. (×3.6).

To ablate target neurons without transecting the afferent fibers of the medial septal cholinergic neurons, the excitotoxic amino acid *N*-methyl-D-aspartic acid (NMDA) was stereotaxically injected unilaterally into multiple sites in the hippocampus in young adult female rats (Fig. 1). Control animals received equivalent injections of vehicle. Injections of a fluorescent tracer into the same sites in other animals resulted in the retrograde labeling of neurons throughout the medial septum (Fig. 2A), demonstrating that the target fields of these neurons had been well infiltrated.

Histological analysis (*10*) showed that injections of vehicle had essentially no effect, whereas injections of NMDA resulted in the death of virtually all neurons throughout the hippocampal formation, including CA1 to CA4 subfields, dentate gyrus, and subiculum. Pronounced gliosis and large numbers of reactive astrocytes positive for glial fibrillary acidic protein (GFAP) were seen after 7 days (n = 2) and 28 days (n = 2), whereas histochemical staining for acetylcholinesterase (AChE) showed no decrease in cholinergic fiber density at these times. Some tissue shrinkage was evident by 28 days and had become massive after 90 to 120 days (Fig. 1). Surface area measurements (*11*) comparing the lesioned with the unlesioned side at 90 to 120 days showed an average of 9.8 ± 2.6% hippocampal tissue remaining. This tissue consisted primarily of a thin band of white matter that persisted as the remnant of the fimbria along its entire rostrocaudal extent in spite of the absence of overlying hippocampal tissue (Fig. 1). A small number of reactive GFAP-positive astrocytes persisted along this remnant, and although many AChE-positive fibers were present within the remnant, their density was somewhat lower than that found in normal hippocampal tissue, indicating that a substantial reduction in the total extent of the original cholinergic axonal network had occurred.

Immunohistochemically stained cholinergic neurons (*10*) in the medial septum of these animals (Fig. 2, B and C) were counted by computerized interactive image analysis (*11*). In the caudal region of the septum, which shows maximal cholinergic cell loss after transection of the fimbria-fornix (*12*),

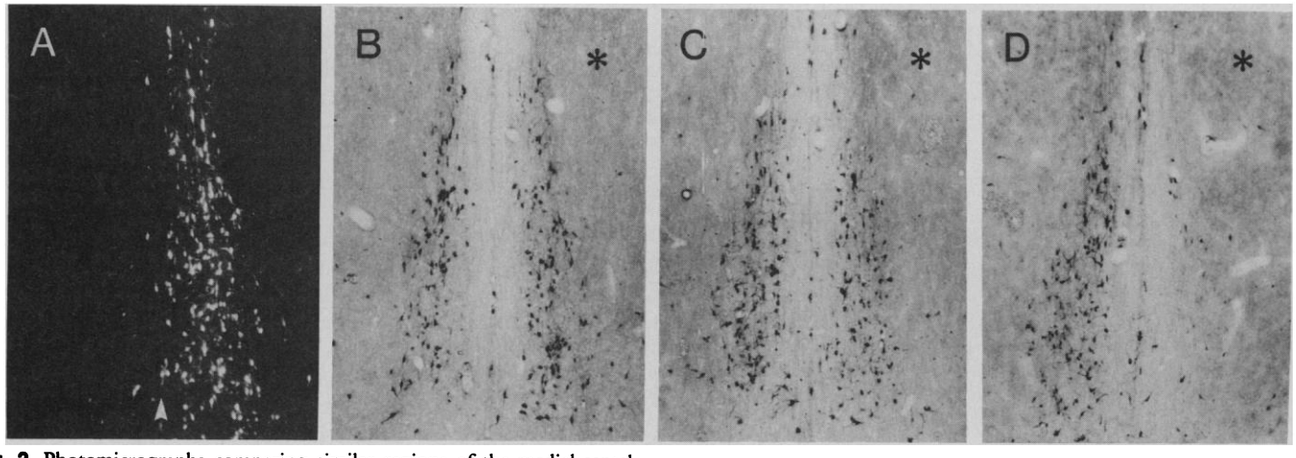

Fig. 2. Photomicrographs comparing similar regions of the medial septal nucleus in four animals. (**A**) Neurons retrogradely transporting the fluorescent tracer True blue (Illing, West Germany) 1 week after injection into six hippocampal sites [0.3 μl of 2% (w/v) solution per site] equivalent to those described in the legend to Fig. 1. Arrowhead denotes the midline. (**B** through **D**) Cholinergic neurons immunohistochemically stained for ChAT (*10*) 120 days after unilateral hippocampal injections (see legend to Fig. 1) of vehicle (B) or NMDA (C and D). Animals in (B) and (C) had no further treatment; animal in (D) received a complete unilateral transection of the fimbrial remnant at a level equivalent to that of Fig. 1A 14 days before being perfused (*7*). Asterisk denotes treated (left) side. (×55).

there was no significant loss of neurons ipsilateral to hippocampal NMDA lesions (Fig. 3A), and no correlation could be demonstrated between cell number and hippocampal surface area (Fig. 3B), up to 120 days after NMDA lesions. Similarly, no significant reduction was found in the number of neurons immunohistochemically stained for NGF receptor (*10*), the majority of which are cholinergic. To look for rostrocaudal shrinkage of the medial septal nucleus, which might influence cell counts, every third section through the entire nucleus was counted in all animals with NMDA lesions. Cholinergic neurons were present in the same number of sections on both sides, with a mean of 97.3 ± 1.6% of the cells on the lesioned sides as compared with the unlesioned sides, indicating that no shrinkage of the nucleus had occurred.

Cell size and optical density of cholinergic neurons were also analyzed (*11*). Cell size has been positively correlated with terminal sprouting in the CNS (*13*), and optical density is an indication of immunohistochemical staining intensity and therefore of intracellular content of choline acetyltransferase (ChAT). There was a small but significant reduction in the mean cell surface area (*14*), but no significant change in the mean optical density (*14*), of cholinergic neurons ipsilateral to NMDA lesions. Histograms plotting cell number versus either cell size or optical density were prepared for individual animals or from data pooled between animals. The histograms showed that the small change in mean cell size was due to a pronounced drop in the number of large neurons (Fig. 3C) and also revealed a similar but smaller decline in the number of darkly stained neurons. Statistical analysis of these trends was conducted on defined subpopulations of cholinergic neurons and showed significant reductions in the number of the largest, and of the most darkly stained, neurons (*14*). These decreases were accompanied by increases in the numbers of smaller cells (Fig. 3C) or less intensely stained cells, respectively, indicating that cell shrinkage and reduced staining rather than cell loss, had occurred. This is reflected in the somewhat atrophied appearance of septal cholinergic neurons ipsilateral to the lesioned hippocampus (Fig. 2C).

The persistence of essentially all septal cholinergic neurons after ablation of their entire field of target neurons is in striking contrast to the death of more than 70% of these neurons reported to occur 14 to 28 days after axotomy of the dorsal septohippocampal projection, the fimbria-fornix (*7, 8*). We therefore examined the response to axotomy of this pathway and found that as extensive a loss of septal cholinergic neurons occurred after transection of the persisting fimbrial remnant 90 to 120 days after hippocampal NMDA lesions as occurred after fimbria-fornix transection in unlesioned animals (Figs. 2D and 3A). This result demonstrates that the majority of, if not all, axons from septal cholinergic neurons are still present in the proximal remnant of the fimbria up to 120 days after the loss of all hippocampal neurons.

To look for collateral projections that might sustain septal cholinergic neurons after loss of hippocampal neurons, we determined the number (*11*) of labeled septal neurons after multiple injections of different fluorescent tracers into the hippocampus and other reported potential target regions (*4*). Fewer than 18% of neurons in the medial septum projected to sites other than the ipsilateral hippocampus, and less than 5% projected both to the hippocampus and another site (Fig. 4), in agreement with other studies (*4*). An increase in AChE staining in the lateral septum after transection of the fimbria-fornix may represent an increase of collateral branching of axotomized cholinergic neurons (*8*), but we found no such increase after hippocampal NMDA lesions. The massive loss of medial septal cholinergic neurons that occurs after axotomy of the fimbria-fornix also indicates that any collateral branches originating proximal to this pathway are unable to support many neurons. Thus, the survival of septal cholinergic neurons after hippocampal NMDA lesions is not due to the presence of axon collaterals to other targets.

These results show that uninjured basal forebrain cholinergic neurons in the medial septum of young adult rats do not die in the absence of target neurons and are either not dependent on neurotrophic factors for survival or can obtain trophic support from other sources for at least 120 days after target neurons are lost. Exogenously applied NGF will prevent the loss of these neurons after axotomy (*9*), suggesting that they may normally require NGF to remain alive. In favor of this is the evidence that these neurons bear NGF receptors and transport NGF from the hippocampus where it is produced (*3*). However, the ability to prevent retrograde cell death is not restricted to NGF; fibroblast growth factor (FGF) will also rescue axotomized septal cholinergic neurons (*15*). In addition, removal of the entire population of target cells that normally produce NGF does not result in retrograde cell death. Thus, uninjured septal cholinergic neurons may not absolutely require NGF for survival in the adult, but substances like NGF and FGF may pharmacologically prevent degenerative changes in neurons not normally dependent exclusively

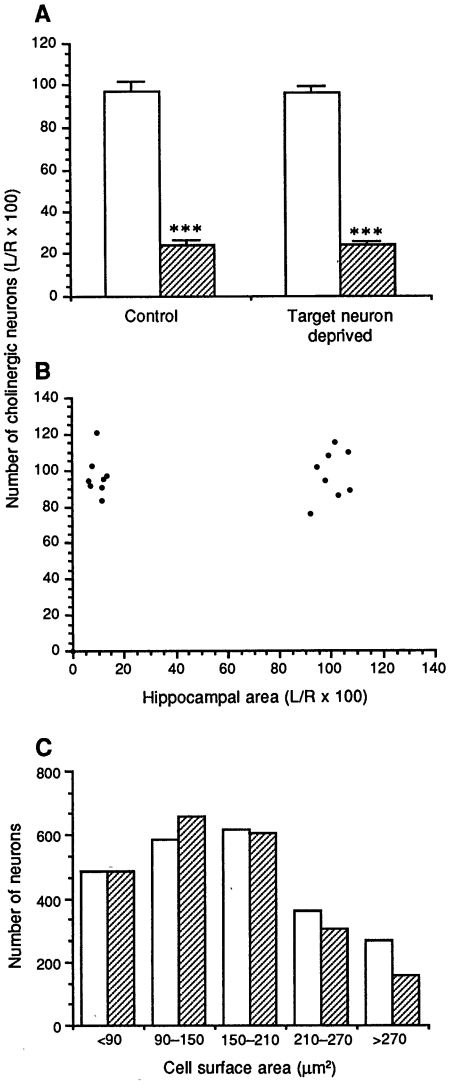

Fig. 3. (**A**) Bar graph comparing the number of cholinergic neurons in the medial septal nucleus in nonaxotomized (open bar) (n = 8) or axotomized (hatched bar) (n = 4) control (vehicle-injected) animals and in nonaxotomized (n = 8) or axotomized (n = 4) animals 90 to 120 days after complete unilateral ablation of target neurons by injection of NMDA into the hippocampus (see legend to Fig. 1). Neuronal counts (*11*) were performed on three sections between 200 and 600 μm rostral to the decussation of the anterior commissure (average of 83 cells per section on unlesioned sides) and Abercrombie corrected (*26*). Values are expressed as the mean percentage of cells counted on the treated, left (L) side versus cells counted on the untreated, right (R) side (that is, L/R × 100) ± SEM. ***P < 0.001 versus nonaxotomized, t test. (**B**) Scattergraph plotting hippocampal area (*11*) as a function of the percentage of cholinergic neurons on the L versus the R sides, in the medial septal nucleus of each nonaxotomized control or NMDA-lesioned animal. (**C**) Pooled histogram plotting cell number as a function of cell surface area for all cholinergic neurons measured (*11*) in the medial septal nucleus of nonaxotomized NMDA-lesioned animals, comparing the numbers of neurons ipsilateral (hatched bar) and contralateral (open bar) to the lesion.

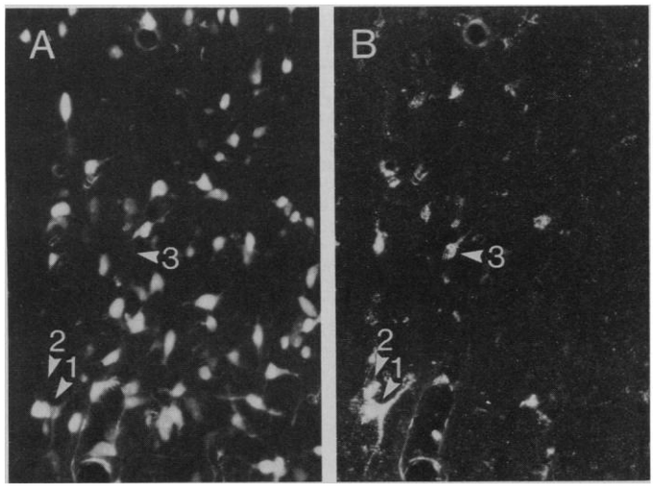

Fig. 4. Photomicrographs of the same field of the medial septal nucleus taken with different filters appropriate to visualize either of the fluorescent tracers: (**A**) True blue or (**B**) rhodamine latex microspheres (LumaFluor, New City, New York). Each animal ($n = 6$) received six injections of True blue into the ipsilateral and six injections of microspheres into the contralateral hippocampus with parameters described in legends to Figs. 1 and 2, plus an additional three to four injections of microspheres into the ipsilateral cingulate cortex, preoptic area, mammillary bodies, and dorsal raphe nucleus (*25*). Arrowheads denote a few labeled neurons. Neuron 1 contains both tracers and projects both to the ipsilateral hippocampus and another site. Neurons 2 and 3 contain only rhodamine latex microspheres and project only to other sites (×180).

on them. This is an important consideration when evaluating the therapeutic potential of growth factors to halt or ameliorate neuronal degeneration (*9, 15, 16*). In the absence of target neurons, basal forebrain cholinergic cells may be able to obtain trophic support from other sources, such as reactive astrocytes, which have been reported to secrete NGF in vitro (*17*), or from other components of injured CNS tissue (*18*). After loss of hippocampal neurons, the majority of cholinergic axons remain in the fimbrial remnant for at least 120 days, where there are a few reactive astrocytes and other cells, and where they are in close proximity to the cerebrospinal fluid. Nevertheless, their ability to obtain NGF, FGF, or other trophic factors from this position is not known.

NGF derived specifically from target neurons does not directly regulate the survival of adult basal forebrain cholinergic neurons. However, it may have other functions in this system. Studies in the adult peripheral nervous system suggest that NGF regulates neuronal morphology. Changes have been demonstrated in axonal branching, dendritic arborization, and cell body size, as well as in amounts of neurotransmitters and their associated enzymes (*2, 19*). Similarly, in vitro studies have shown that NGF will increase both the production of ChAT and the degree of neurite outgrowth in septal cholinergic neurons (*20*). Our findings of cell shrinkage, reduced staining intensity for ChAT, and a reduction of the cholinergic axonal network after loss of target neurons producing NGF are compatible with this notion, and they suggest that NGF may regulate neuronal morphology and transmitter-associated enzyme concentrations in vivo in the adult CNS.

The retrograde reaction to axotomy is often attributed to a loss of target-derived trophic support, but it can vary considerably, from rapid cell death to subtle changes in expression of various molecules in the absence of structural deterioration (*21*). Other cholinergic neurons in the basal forebrain complex that also bear functional NGF receptors do not die after axotomy of their principal projections to the neocortex (*7*). Moreover, axotomy appears more likely to lead to retrograde cell death as the site of axonal injury becomes closer to the nerve cell body, even in the absence of evidence for intervening axon collaterals (*12, 22*). It seems unlikely that the loss of target-derived factors alone could underlie all of these responses. Thus, although some of the differences in the retrograde neuronal reaction to various insults might be mediated by the amount or type of neurotrophic factors lost, other mechanisms such as intracellular disturbances of ion balance due to disruption of axonal membrane integrity might also operate.

Lastly, our results have some bearing on attempts to understand certain neurodegenerative mechanisms. Atrophy and probable loss of both basal forebrain cholinergic neurons and their target neurons in the hippocampus and cerebral cortex are prominent features of Alzheimer-type dementias (*5*). Our findings indicate that loss of target neurons and the trophic substances supplied by them would not alone result in the retrograde death of basal forebrain cholinergic neurons, but might lead to their atrophy. If a loss of neurotrophic support is, as proposed (*23*), a major cause of cholinergic cell death in these conditions, it is unlikely to be due solely to a failure in supply by target neurons (*24*).

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10. Animals were fixed by perfusion with 4% paraformaldehyde and 0.1% glutaraldehyde, and transverse vibratome sections (40 μm) through the septal region and hippocampus were prepared. Free-floating sections were immunohistochemically stained by using antibodies against either ChAT (*7*), NGF receptor [C. E. Chandler, L. M. Parsons, M. Hosang, E. Shooter, *J. Biol. Chem.* **259**, 6882 (1984)], GFAP, or neuron-specific enolase (DACO, United Kingdom) by means of the peroxidase-antiperoxidase procedure (*7*). Mounted sections were either stained with cresyl violet, processed for AChE histochemistry (*8*), or viewed unstained without a cover slip to identify fluorescent tracers.
11. Hippocampal surface area was determined by drawing around the extent of neuronal tissue on the digitized image captured with a ×1 objective and a Seescan (Cambridge, England) image analyzer with frame capture from a black and white video camera attached to a Zeiss microscope. Cell counts, cross-sectional area, and optical density of immunohistochemically stained cholinergic neurons were assessed with the use of a ×10 planapo objective. Objects were accepted for analysis if they had a cross-sectional area greater than 50 μm^2 and less than 500 μm^2, a Feret diameter greater than 8 μm, and a roundness factor (KA/P^2, where $K = 1.257$, A is cross-sectional area, and P is perimeter) greater than 0.35 to eliminate large elongated fibers. Cross-sectional area and optical density were automatically calculated for each counted neuron. Cell counts of septal neurons retrogradely transporting fluorescent tracers were similarly made with a ×16 objective. The same fields were first analyzed with different filters appropriate for each tracer (Fig. 4). Subsequently, the number of neurons containing both tracers was determined by subtracting one image from another.
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14. Mean cell surface area and mean optical density values from the treated, left (L), and untreated, right (R), sides were expressed as the percent L/R (that is, L/R × 100) ± SEM for each animal. Mean L/R values for cell surface area were 101.6 ± 2.3% for control and 93.7 ± 1.8% for NMDA-lesioned animals, a significant reduction of 7.9% ($P < 0.01$, t test). Mean L/R values for optical density were 99.5 ± 1.9% for control and 98.8 ± 0.8% for NMDA-lesioned animals, not significantly different (t test). To test the significance of changes noted in histograms, numbers of cholinergic neurons in de-

fined subpopulations were expressed as the percent L/R for each animal. Mean L/R values for the number of neurons greater than 230 μm^2 (representing the largest 20% of the normal population) were 104.8 ± 4.6% for control and 64.3 ± 5.2% for NMDA-lesioned animals, a significant drop of 40.5% ($P < 0.001$, t test). Mean L/R values for the number of the most darkly stained neurons (upper 25% of the normal population) were 94.8 ± 24.8% for control and 76.8 ± 15.0% for NMDA-lesioned animals, a significant reduction of 18.0% ($P < 0.05$, t test).

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27. We dedicate this paper to the memory of Hans Kuypers, our friend, colleague, and chairman, who died on 26 September 1989. His many scientific contributions included the introduction of fluorescent tracers (as used in this study) to neuroscience. Supported by grants from the British Medical Research Council and Science and Engineering Research Council, the Wellcome Trust, the Research Corporation Trust, the Swedish Medical Research Council, and Merck Sharp & Dohme Research Laboratories. We thank S. Stevens and K. J. Baker for technical assistance, J. Bashford for photography, and P. Campbell for editorial assistance.

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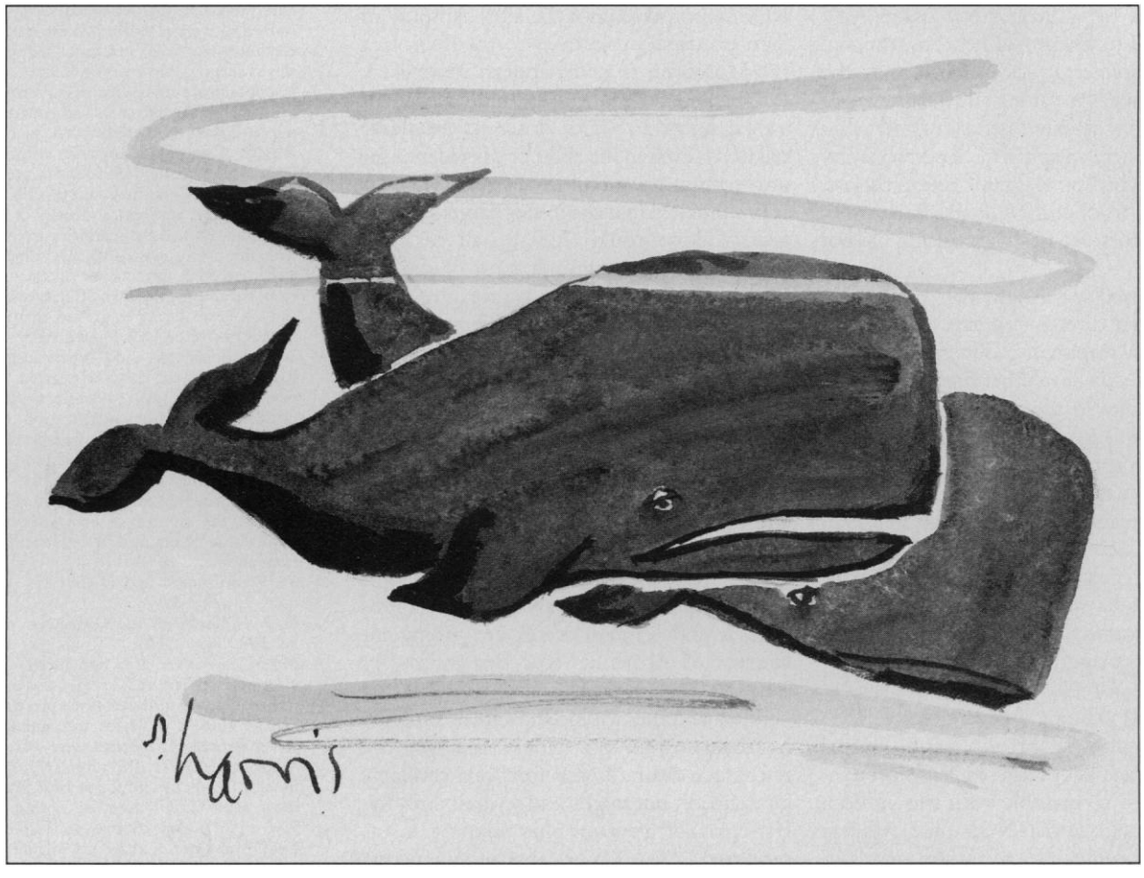

" I'm strictly a squid and seaweed eater. Whenever I have plankton, I'm hungry an hour later."