The Primate Hippocampal Formation: Evidence for a Time-Limited Role in Memory Storage

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20. T. Kiyosue, M. Arita, S. Imanishi, M. Aomine, *Jpn. Heart J.* **23** (suppl.), 51 (1982).
21. *Escherichia coli* possesses at least two Mg^{2+} uptake systems. One of these also transports Co^{2+} and Mn^{2+}, an ion selectivity reminiscent of I_{Mg} [D. L. Nelson and E. P. Kennedy, *J. Biol. Chem.* **246**, 3042 (1971); S. Silver and D. Clark, *ibid.*, p. 569].
22. I_{Mg} tail currents could be elicited with depolarizations of 10 ms or less. Action potential duration in *Paramecium* may exceed 40 ms, depending on stimulus intensity.
23. C. Kung and Y. Saimi, *Annu. Rev. Physiol.* **44**, 519 (1982); Y. Saimi and C. Kung, *Annu. Rev. Genet.* **21**, 47 (1987); H.-D. Görtz, *Paramecium* (Springer-Verlag, New York, 1988); R. D. Hinrichsen and J. E. Schultz, *Trends Neurosci.* **11**, 27 (1988).
24. L. A. P. Miglietta and D. L. Nelson, *J. Biol. Chem.* **263**, 16096 (1988); S. M. Travis and D. L. Nelson, *Biochim. Biophys. Acta* **966**, 84 (1988); P. A. Mason and D. L. Nelson, *ibid.* **1010**, 116 (1989).
25. J. Golowasch, A. Kirkwood, C. Miller, *J. Exp. Biol.* **124**, 5 (1986); G. L. Collingridge and T. V. P. Bliss, *Trends Neurosci.* **10**, 288 (1987); P. R. Stanfield, *ibid.* **11**, 475 (1988); R. E. White and H. C. Hartzell, *Science* **239**, 778 (1988); I. Duchatelle-Gourdon, H. C. Hartzell, A. A. Lagrutta, *J. Physiol. (London)* **415**, 251 (1989); M. Horie and H. Irisawa, *ibid.* **408**, 313 (1989); M. Pusch, F. Conti, W. Stühmer, *Biophys. J.* **55**, 1267 (1989); M. Tarr, J. W. Trank, K. K. Goertz, *Am. J. Physiol.* **257**, H1663 (1989).
26. Action potentials triggered in the presence of physiological external Mg^{2+} concentrations (0.5 mM) lack the strong, K^+-based afterhyperpolarization characteristic of similar responses in Mg^{2+}-free solutions.
27. Although an outward tail that may correspond to loss of internal Mg^{2+} via I_{Mg} was seen occasionally in response to initial membrane potential change, subsequent steps failed to elicit similar currents. It is not certain whether this reflected inhibition of I_{Mg} or depletion of $[Mg^{2+}]_i$. Activation of I_{Mg} in 0.05 mM $[Mg^{2+}]_o$ did not significantly deplete $[Mg^{2+}]_i$, which was 0.35 ± 0.18 mM before and 0.34 ± 0.19 mM after prolonged activation of I_{Mg} with the conditioning protocol (means ± SD from five cells).
28. I thank P. V. Minorsky and B. Martinac for critically reviewing the manuscript, L. Olds for helping prepare the illustrations, and C. Kung and Y. Saimi for their continued encouragement and support of this work and for many helpful discussions. Supported by the Lucille P. Markey Charitable Trust and NIH (GM22714 and GM36386).

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The Primate Hippocampal Formation: Evidence for a Time-Limited Role in Memory Storage

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Clinical and experimental studies have shown that the hippocampal formation and related structures in the medial temporal lobe are important for learning and memory. Retrograde amnesia was studied prospectively in monkeys to understand the contribution of the hippocampal formation to memory function. Monkeys learned to discriminate 100 pairs of objects beginning 16, 12, 8, 4, and 2 weeks before the hippocampal formation was removed (20 different pairs at each time period). Two weeks after surgery, memory was assessed by presenting each of the 100 object pairs again for a single-choice trial. Normal monkeys exhibited forgetting; that is, they remembered recently learned objects better than objects learned many weeks earlier. Monkeys with hippocampal damage were severely impaired at remembering recently learned objects. In addition, they remembered objects learned long ago as well as normal monkeys did and significantly better than they remembered objects learned recently. These results show that the hippocampal formation is required for memory storage for only a limited period of time after learning. As time passes, its role in memory diminishes, and a more permanent memory gradually develops independently of the hippocampal formation, probably in neocortex.

CURRENT UNDERSTANDING OF THE organization and neural foundations of memory has depended importantly on cognitive studies of memory-impaired patients (*1*) and on studies of a primate model of human amnesia (*2*). In humans, neuropathological findings (*3, 4*), together with high-resolution magnetic resonance imaging (*5*), have demonstrated that selective, bilateral damage to the hippocampal formation is sufficient to cause significant memory impairment. Similar findings have been obtained in monkeys (*6–8*) and other mammals (*9*). On the basis of neuropsychological studies of patients with confirmed hippocampal damage, it appears that the hippocampal formation is necessary for establishing a usable record in long-term memory of previously encountered facts and events (*1, 10*).

One useful source of information about the function of the hippocampal formation is the phenomenon of retrograde amnesia, that is, loss of memories acquired before the onset of amnesia. Retrograde amnesia is often temporally graded; patients lose access to the recent past more readily than to the remote past (*11*). Further, as measured by objective tests, memory for the very remote past can be intact in patients with hippocampal damage (*3, 12*), regardless of the difficulty of the test items (*13*). This finding suggests that the hippocampal formation is not a repository of permanent memory. In addition, the phenomenon of temporally graded retrograde amnesia suggests that the role of the hippocampal formation in memory is time-limited. However, more data are needed to confirm and illuminate these ideas. Indeed, the correct interpretation of temporally graded retrograde amnesia depends on the precise shape of the performance curves, which cannot be determined with certainty with the tests available for assessing remote memory retrospectively in humans (*14*).

We have assessed retrograde amnesia prospectively in cynomolgus monkeys (*Macaca fascicularis*) with bilateral lesions of the hippocampal formation (the H^+ lesion) (*15*). Figure 1 shows a cross section from the brain of a monkey in the operated group. Monkeys were trained on five different sets of 20 two-choice object discrimination problems (100 discrimination pairs). Training on each 20-pair set began approximately 16, 12, 8, 4, and 2 weeks before surgery. For training, each object pair was presented for 14 consecutive trials with a 15-s intertrial interval (*16*). Monkeys were trained on two new object pairs each day so that 10 days were required to train monkeys on each of the five sets of 20 object pairs (*17*). The ability to learn simple object discrimination problems like the ones used here is known to depend on the integrity of the hippocampal formation (*7*).

Preoperative performance on the 100 object discrimination problems averaged 54.5% correct (chance, 50%) on the first trial of training and 87.7% correct on trial 14 (average of 18 monkeys and 100 discrimination pairs). The learning curves were numerically very similar for the five training episodes, although some improvement did occur with continuing exposure to discrimination problems (*18*). Tests given at the end of each training episode, which assessed the level of preoperative learning (*17*), showed that virtually the same final level of performance was attained on each of the five sets of discrimination problems. Performance on these tests averaged 78.9, 81.9, 79.4, 79.7, and 78.6% for the first to the last training episode, respectively. A two-way analysis of variance (training episode × group) revealed no significant differences ($F < 2.0$, $P > 0.10$).

Two weeks after surgery, we assessed memory for the preoperatively learned object pairs by presenting a single trial of each of the 100 pairs in a mixed order. This retention test consisted of 50 trials present-

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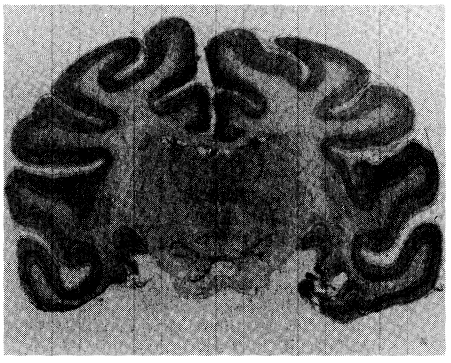

Fig. 1. A thionin-stained coronal brain section midway through the lateral geniculate from one monkey in the operated group. This animal sustained nearly total bilateral ablation of the hippocampal formation, including the dentate gyrus, the subicular complex, and entorhinal cortex. Also, nearly all of the parahippocampal cortex was damaged bilaterally. Overall, the damage was very similar to that described previously in monkeys with the H^+ lesion (7); the amygdaloid complex was completely spared. On the left side of the brain, the lesion extended laterally into the ventral aspect of presumed unimodal visual association cortex (area TE). This damage was moderate, probably the result of an infarction during surgery.

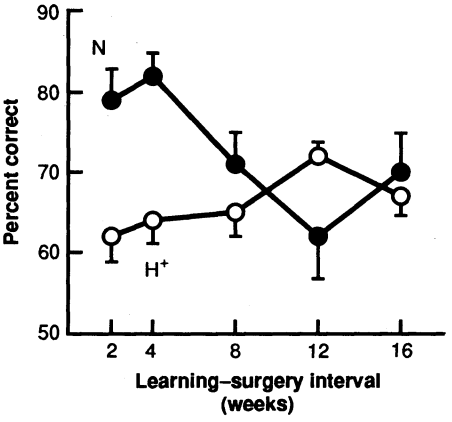

Fig. 2. Retention of 100 object discrimination problems learned approximately 2, 4, 8, 12, and 16 weeks before hippocampal surgery (20 pairs per time period). Retention was assessed 2 weeks after surgery in monkeys with lesions (H^+) (○) ($n = 11$) or after an equivalent interval in unoperated animals (N) (●) ($n = 7$). Brackets show standard error of the mean.

ed on each of two consecutive days. The order of presentation of the objects was the same for each monkey.

Figure 2 shows the mean postoperative retention scores obtained by normal monkeys and monkeys with H^+ lesions as a function of the learning-surgery interval. The operated monkeys performed significantly more poorly overall than the normal group [$F(1,16) = 8.0$, $P < 0.02$], and there was a significant effect of group × time period [$F(4,64) = 5.8$, $P < 0.001$]. Specifically, the operated monkeys performed more poorly than the normal monkeys on object pairs that had been learned either 2 weeks before surgery [$t(16) = 3.2$, $P < 0.01$] or 4 weeks before surgery [$t(16) = 4.1$, $P < 0.01$]. The groups did not differ at any other time periods ($P > 0.10$) (*19*). The normal monkeys exhibited forgetting: recent memories were recollected better than older memories (trend analysis across all five time points, $P = 0.07$; across the interval from 2 weeks to 12 weeks, $P < 0.05$).

Monkeys with H^+ lesions remembered remote information significantly better than recently acquired information. Specifically, the score for object pairs learned 12 weeks before surgery (72.3% correct) was significantly higher than the score for object pairs learned either 2 weeks before surgery (62.3%) or 4 weeks before surgery (64.1%) ($P < 0.05$). Moreover, the scores of the H^+ group for object pairs learned from 2 weeks to 12 weeks before surgery increased monotonically (*20*) and improved significantly across this portion of the performance curve (trend analysis, $P < 0.01$). Only one operated monkey obtained a lower score for object pairs learned 12 weeks before surgery than for object pairs learned 2 weeks before surgery.

There have been two different ways to explain temporally graded retrograde amnesia in patients with hippocampal lesions. Both views suppose that the hippocampal formation has a temporary role in memory. In the first view, the role of the hippocampal formation is temporary because the particular kinds of memory that depend on the hippocampal formation are ordinarily short-lived. No transformation or reorganization occurs in memory; across time there is simply differential attrition of memory by type. As a result, recent memory is always more vulnerable to hippocampal damage than remote memory, and temporally graded retrograde amnesia will occur. However, according to this view, the ability to recall the recent past can never be poorer than the ability to recall the remote past (*21*).

In the second view, the hippocampal formation has a temporary role in memory because information that initially depends on the hippocampal formation can eventually become independent of this structure. As time passes after learning, a process of reorganization and consolidation (*22*) occurs such that temporary storage in the hippocampal formation is eventually replaced by a gradually developing, more permanent memory elsewhere. This view uniquely explains how monkeys with hippocampal lesions can remember the remote past better than the recent past, precisely the result observed in the present study. Accordingly, our findings favor the second of the two explanations; namely, that information in remote memory is unaffected by hippocampal lesions because of a change in the organization of memory storage (from hippocampal-dependent to independent) that occurs gradually with the passage of time after learning (*23*).

It has been proposed that the hippocampus is initially the storage site for a simple memory, a conjunction, or an index (*24*). This storage site is established in the hippocampus at the time of learning through convergent anatomical projections from distributed sites in neocortex, where simultaneous and coordinated neural activity is thought to underlie perception and the capacity for immediate (short-term) memory (*10, 25*). The hippocampus might serve temporarily as a way of binding the distributed neocortical sites that together comprise the record of a whole event so that subsequently a complete memory can be revivified even from a partial cue. The characteristics of retrograde amnesia demonstrated here require in addition a gradual transformation or consolidation process in the organization of memory storage whereby the contribution of the hippocampus gradually diminishes and a more permanent memory gradually develops, probably in neocortex. Although the neural events underlying consolidation remain to be identified, it seems likely that slow changes in synaptic connectivity are involved. The hippocampus is needed at the time of learning and during consolidation.

REFERENCES AND NOTES

1. B. Milner, *Clin. Neurosurg.* **19**, 421 (1972); L. R. Squire, *Science* **232**, 1612 (1986); L. Weiskrantz, *Hum. Neurobiol.* **6**, 93 (1987); D. Schacter, in *Memory Systems of the Brain: Animal and Human Cognitive Processes*, N. Weinberger, G. Lynch, J. McGaugh, Eds. (Guilford, New York, 1985), pp. 351–379; A. R. Mayes, *Human Organic Memory Disorders* (Cambridge Univ. Press, Cambridge, 1988).
2. M. Mishkin, B. J. Spiegler, R. C. Saunders, B. J. Malamut, in *Toward a Treatment of Alzheimer's Disease*, S. Corkin, K. L. Davis, J. H. Growdon, E. J. Usdin, R. J. Wurtman, Eds. (Raven, New York, 1982), pp. 235–247; L. Squire and S. Zola-Morgan, in *The Physiological Basis of Memory*, J. A. Deutsch, Ed. (Academic Press, New York, 1983), pp. 199–268.
3. S. Zola-Morgan, L. R. Squire, D. C. Amaral, *J. Neurosci.* **6**, 2950 (1986).
4. M. Victor, J. Angevine, C. M. Fisher, *Arch. Neurol.* **5**, 244 (1961); W. Penfield and B. Milner, *Arch. Neurol. Psychiatry* **79**, 475 (1958); R. N. DeJong, H. H. Itabashi, J. R. Olson, *Am. Neurol. Assoc.* **93**, 31 (1968); J. L. Cummings, U. Tomiyasu, S. Read, D. F. Benson, *Neurology* **34**, 679 (1984); B. T. Woods, W. Schoene, L. Kneisly, *J. Neurol. Neurosurg. Psychiatry* **45**, 243 (1982); M. Victor and D. Agamanolis, *J. Cognit. Neurosci.* **2**, 246 (1990).
5. G. A. Press, D. G. Amaral, L. R. Squire, *Nature* **341**, 54 (1989); L. R. Squire, D. G. Amaral, G. A. Press, *J. Neurosci.* **10**, 3106 (1990).
6. S. Zola-Morgan and L. R. Squire, *Behav. Neurosci.* **100**, 165 (1986).
7. ———, D. G. Amaral, *J. Neurosci.* **9**, 898 (1989).
8. M. Mishkin, *Nature* **273**, 297 (1978); H. Mahut, S. Zola-Morgan, M. Moss, *J. Neurosci.* **2**, 1214 (1982); E. A. Murray and M. Mishkin, *ibid.* **4**, 2565 (1984).

9. D. S. Olton, J. T. Becker, G. E. Handelmann, *Behav. Brain Sci.* **2**, 313 (1979); C. A. Barnes, *Trends Neurosci.* **11**, 163 (1988); R. J. Sutherland and J. W. Rudy, *Psychobiology* **17**, 129 (1989).
10. L. R. Squire, *Memory and Brain* (Oxford Univ. Press, New York, 1987). Memory for facts and events (declarative memory) can be contrasted with skill-learning, priming, conditioning, and other forms of nondeclarative (implicit) memory, which are independent of the hippocampal formation.
11. T. Ribot, *Les Maladies de la Memoire* (Germer Baillere, Paris, 1881) [English translation: *Diseases of Memory* (Appleton-Century-Crofts, New York, 1882)]. The phenomenon of temporally graded retrograde amnesia has been confirmed repeatedly in rodents with a variety of memory tasks and disruptive treatments [J. L. McGaugh and P. E. Gold, in *Neural Mechanisms of Learning and Memory*, M. Rosenzweig and E. Bennett, Eds. (MIT Press, Cambridge, MA, 1976), pp. 549–560].
12. D. MacKinnon and L. R. Squire, *Psychobiology* **17**, 247 (1989).
13. L. R. Squire, F. Haist, A. P. Shimamura, *J. Neurosci.* **9**, 828 (1989).
14. With most tests available for assessing remote memory in humans, one cannot be sure that past time periods are being sampled equivalently [L. R. Squire, *Neuropsychologia* **12**, 429 (1974)]. Accordingly, for any time period it is difficult to know whether the score on a remote memory test reflects the age of the memories or the difficulty of the questions. Also see E. E. Coons and N. E. Miller, *J. Comp. Physiol. Psychol.* **53**, 524 (1960).
15. The H^+ lesion included the hippocampus proper, the dentate gyrus, the subicular complex, the posterior entorhinal cortex, and most of the parahippocampal cortex (7).
16. Testing was carried out in a Wisconsin General Test Apparatus [H. Harlow and J. A. Bromer, *Psychol. Rec.* **19**, 434 (1938)]. For each object pair, a raisin reward was concealed under the correct object. Assignment of the five sets of training objects to the five preoperative training periods was balanced across monkeys. The 20 object pairs in each set were always presented in the same order to each monkey.
17. The five preoperative training episodes began for all monkeys exactly 109, 81, 53, 25, and 11 days before surgery and were completed 10 days later. To measure how well the object pairs were learned preoperatively, an additional test was administered 1 day after training was completed on each 20-pair set. For these tests, all 20 object pairs were presented once to each monkey in the same random order during a single session.
18. The learning scores for the five training episodes were 70.0, 73.9, 77.1, 75.8, and 75.8% correct (from the first to the last episode, respectively, and averaging across 14 training trials, 20 object pairs, and 18 monkeys). A two-way analysis of variance (learning trials × training episodes) showed marked learning within each episode [$F(6,24) = 177$, $P < 0.001$], a numerically small but significant effect of training episode [$F(4,24) = 17.4$, $P < 0.001$], and no interaction. For this analysis, the 14 learning trials were blocked into seven groups of two trials each. The scores for the monkeys who were later given hippocampal lesions were virtually identical to the scores of the monkeys who served as unoperated controls (within 2.5% for each training episode).
19. At the 12-week time period, the data for normal monkeys were quite variable (standard error = 5.3%), and the comparison between normal and operated monkeys was not significant ($P = 0.12$).
20. The issue of interest is whether the memory scores of operated monkeys increase significantly as one moves from a recent to a more remote time period, not whether such an increase extends to every remote time period in the study. Indeed, in very remote time periods the scores of operated monkeys would be expected to join the forgetting curve of normal monkeys.
21. Assume that some acquired information will be remembered for a long time and that other information will be remembered for a relatively short time. Both kinds of information must be more abundant shortly after learning than at later times. If hippocampal damage affects information that is ordinarily short-lived, then what survives in memory after damage will be relatively long-lasting information, and it will be at least as abundant in recent time periods as in more remote time periods. Accordingly, after hippocampal damage, performance scores should always be at least as good for the most recent time period (that is, for the period just before the time of damage), as for any more remote time period. The limiting case would have performance scores equal across all time periods. The present finding, that retention was significantly poorer for recent time periods than for more remote time periods, rules out this account of temporally graded retrograde amnesia.
22. For earlier versions of the consolidation concept, see G. E. Muller and A. Pilzecker, *Z. Psychol.* **1**, 1 (1900); J. L. McGaugh and M. Herz, *Memory Consolidation* (Albion, San Francisco, 1972); S. E. Glickman, *Psychol. Bull.* **58**, 218 (1961); L. R. Squire, *Annu. Rev. Neurosci.* **5**, 241 (1982).
23. Gradients of retrograde amnesia have been obtained previously, in which the remote past was remembered better than the recent past: with psychiatric patients prescribed electroconvulsive therapy [L. R. Squire, P. C. Slater, P. M. Chace, *Science* **187**, 77 (1975)] and in prospective tests of mice given electroconvulsive shock [L. R. Squire and C. W. Spanis, *Behav. Neurosci.* **98**, 345 (1984)]. However, results with convulsive stimulation cannot be directly related to neuroanatomy or to hippocampal function. Recently, this same kind of temporal gradient of retrograde amnesia was described in rats after hippocampal lesions; in this case, the gradient extended 2 to 5 days [G. Winocur, *Behav. Brain Res.* **38**, 145 (1990); also see R. Sutherland *et al.*, *Soc. Neurosci. Abst.* **13**, 1066 (1987)]. In monkeys with more extensive medial temporal lobe lesions and overtraining of object pairs, temporally graded retrograde amnesia was not observed [D. Salmon, S. Zola-Morgan, L. R. Squire, *Psychobiology* **15**, 37 (1987)].
24. D. Marr, *Philos. Trans. R. Soc. London Ser. B* **176**, 23 (1971); B. McNaughton, in *Neural Connections, Mental Computations*, L. Nadel, L. A. Cooper, P. Culicover, R. M. Harnish, Eds. (MIT Press, Cambridge, MA, 1989), pp. 285–350; E. Rolls, in *An Introduction to Neural and Electronic Networks*, S. F. Zornetzer, J. L. Davis, C. Lau, Eds. (Academic Press, San Diego, CA, 1990), pp. 73–90; T. J. Tyler and P. Discenna, *Behav. Neurosci.* **100**, 147 (1986); P. Milner, *Neuropsychologia* **27**, 23 (1989); E. Halgren, in *Neuropsychology of Memory*, L. R. Squire and N. Butters, Eds. (Guilford, New York, 1984), pp. 165–182; L. R. Squire, N. Cohen, L. Nadel, in *Memory Consolidation*, H. Weingartner, E. Parks, Eds. (Erlbaum, Hillsdale, NJ, 1984), pp. 185–210; L. R. Squire, A. Shimamura, D. G. Amaral, in *Neural Models of Plasticity*, J. Byrne and W. Berry, Eds. (Academic Press, New York, 1989), pp. 208–239.
25. M. Mishkin, *Philos. Trans. R. Soc. London Ser. B* **298**, 85 (1982); A. R. Damasio, *Cognition* **33**, 25 (1989).
26. We thank C. LeClair, R. Clower, and D. Amaral for assistance. Supported by the Medical Research Service of the Department of Veterans Affairs, the Office of Naval Research, NIH grant NS19063, National Institute of Mental Health grant MH24600, the McKnight Foundation, and a bioscience grant for International Joint Research from the New Energy and Industrial Technology Development Organization, Japan.

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Widespread Expression of BDNF But Not NT3 by Target Areas of Basal Forebrain Cholinergic Neurons

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Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) are homologs of the well-known neurotrophic factor nerve growth factor. The three members of this family display distinct patterns of target specificity. To examine the distribution in brain of messenger RNA for these molecules, in situ hybridization was performed. Cells hybridizing intensely to antisense BDNF probe were located throughout the major targets of the rat basal forebrain cholinergic system, that is, the hippocampus, amygdala, and neocortex. Strongly hybridizing cells were also observed in structures associated with the olfactory system. The distribution of NT3 mRNA in forebrain was much more limited. Within the hippocampus, labeled cells were restricted to CA2, the most medial portion of CA1, and the dentate gyrus. In human hippocampus, cells expressing BDNF mRNA are distributed in a fashion similar to that observed in the rat. These findings point to both basal forebrain cholinergic cells and olfactory pathways as potential central targets for BDNF.

THE PROTOTYPIC NEUROTROPHIC fac-tor nerve growth factor (NGF) has recently gained much attention as a potential therapeutic agent for Alzheimer's disease by virtue of its apparent trophic action on cholinergic forebrain neurons (*1*). Although the more recently described neurotrophic factors BDNF and NT3 are present in the central nervous system (*2–4*), little is known about the sources or targets for these molecules in the brain. To localize mRNA for BDNF and NT3 in rat brain, we performed in situ hybridization at high stringency (*5*) with ^{35}S-labeled RNA probes (*6*). An initial survey of the brain revealed a striking pattern of BDNF hybridization in several forebrain regions. Significant labeling for BDNF mRNA was also observed in

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