includes the second half of exon 2, intron 2, and exon 3 of the HLA class C genes. The presence of *HLA-Cw6*, and the related allele *-c1.10*, was identified by SSOP hybridization with CI-326 (CTCCAGTGGATGTATGGCT) that detects a codon for Met at residue 97 specific for these two alleles. An additional set of 14 SSOPs spanning other polymorphic codons (70 to 75, 76 to 81, 91 to 97, 95 to 101, 111 to 117, and 151 to 157) was used to distinguish *Cw6* from *c1.10* and to genotype the other *HLA-C* locus alleles. Amplified genomic DNA from 8 homozygous B cell lines carrying *HLA-Cw6* and from 10 B cell lines carrying other C-locus alleles was used to monitor the specificity of the SSOP.

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7 January 1994; accepted 1 April 1994

Long-Distance Neuronal Migration in the Adult Mammalian Brain

Carlos Lois and Arturo Alvarez-Buylla

During the development of the mammalian brain, neuronal precursors migrate to their final destination from their site of birth in the ventricular and subventricular zones (VZ and SVZ, respectively). SVZ cells in the walls of the lateral ventricle continue to proliferate in the brain of adult mice and can generate neurons in vitro, but their fate in vivo is unknown. Here SVZ cells from adult mice that carry a neuronal-specific transgene were grafted into the brain of adult recipients. In addition, the fate of endogenous SVZ cells was examined by microinjection of tritiated thymidine or a vital dye that labeled a discrete population of SVZ cells. Grafted and endogenous SVZ cells in the lateral ventricle of adult mice migrate long distances and differentiate into neurons in the olfactory bulb.

During brain development, most neurons are born in the VZ and SVZ. From these proliferative regions, cells migrate to reach their appropriate targets where they differentiate into neurons. The generation, migration, and differentiation of neurons are generally thought to end soon after birth (*1*, *2*). However, in adult birds in which neurogenesis persists (*3*), precursor cells that divide in the walls of the lateral ventricles migrate to distant targets within the forebrain before they differentiate into neurons (*4*). Neurogenesis also continues in the dentate gyrus of the hippocampus and in the olfactory bulb of adult rodents (*5*). In contrast to adult birds, newly generated neurons in adult mammals are thought to be derived from precursor cells that proliferate close to their site of differentiation instead of in the ventricle walls (*5*).

In mammals, proliferating cells persist through adulthood in the SVZ of the lateral ventricles (*1*, *6*), and these proliferating SVZ cells from the brain of adult mice can generate neurons in vitro (*7*). These cells are probably the epidermal growth factor–responsive neuronal precursors recently isolated from the brain of adult mice (*8*). The fate of these neuronal precursors in vivo remains unknown. Whereas earlier work suggested that SVZ cells in adult mammals may differentiate into glial cells or neurons (*1*, *6*, *9*), a recent study indicates that SVZ cells in adult mammals die soon after mitosis (*10*).

To investigate whether SVZ cells from adult mice could differentiate into neurons in vivo, we grafted SVZ cells from adult transgenic mice that carry the reporter gene β-galactosidase attached to the promoter of the neuron-specific enolase (NSE) gene (*11*). This transgene is only expressed in differentiated neurons (*11*). SVZ explants from transgenic animals were stereotaxically grafted into the lateral wall of the lateral ventricle of adult immunocompatible nontransgenic mice (Fig. 1) (*12*). Animals were killed 30 days after grafting, and transplanted cells that differentiated into neurons were detected by X-gal histochemistry (*12*). Cells that were X-gal–positive (X-gal$^+$) were detected only in the graft site and the ipsilateral olfactory bulb (*13*). We found no evidence of X-gal$^+$ cells in the

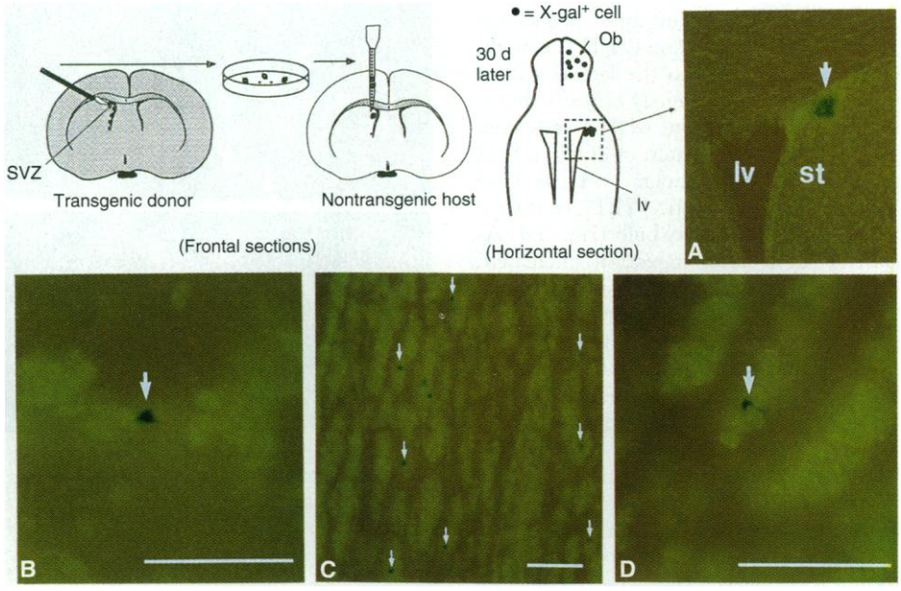

Fig. 1. Transplantation of transgenic SVZ cells close to the lateral ventricle of nontransgenic mice (*11*). X-gal histochemistry produced a blue precipitate in the perinuclear cytoplasm of the NSEp transgenic cells. Cell nuclei stained with Hoechst 33258 appear green. (**A**) Transgenic cells at the site of transplantation (arrow). (B, C, and D) show X-gal$^+$ neurons in the olfactory bulb 30 days (d) after transplantation. (**B**) X-gal$^+$ periglomerular neuron (arrow). (**C** and **D**) X-gal$^+$ neurons (arrows) in the granule cell layer. Ob, olfactory bulb; lv, lateral ventricle; st, striatum. Scale bars, 50 μm.

The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

corpus callosum, cortex, striatum, septum, or hippocampus. In the olfactory bulb, X-gal$^+$ neurons were found in the granule cell layer and around glomeruli (Fig. 1) (*14*). From the position of the X-gal$^+$ cells in the olfactory bulb, we infer that SVZ cells grafted close to the lateral ventricle differentiated into granule and periglomerular neurons. The migration and differentiation of SVZ cells after grafting are robust phenomena (Fig. 1C). We counted the total number of X-gal$^+$ cells in the olfactory bulbs of two of the grafted animals and found 1971 X-gal$^+$ cells; the ratio of X-gal$^+$ granule to periglomerular neurons was 30:1. Thus, SVZ cells from the brain of adult mice can migrate 3 to 5 mm through the brain of adult hosts and generate new neurons.

When SVZ explants from transgenic animals were grafted into the caudate nucleus lateral to the SVZ of nontransgenic hosts, no X-gal$^+$ cells were seen outside of the transplantation site (*12, 13*). Similarly, when parietal cortex explants from transgenic animals were grafted into the SVZ of nontransgenic hosts, X-gal$^+$ cells were found only at the site of transplantation (*12, 13*). Under our conditions, the migration from the wall of the lateral ventricle to the olfactory bulb occurred only when cells from the SVZ were grafted on the SVZ. Similar grafts in neighboring regions did not result in the migration of transplanted cells.

The migration of cells from the SVZ that we observed after transplantation may parallel the normal migration of their counterparts in the host. To test this hypothesis, we examined the fate of endogenous proliferating SVZ cells in adult mice. We injected 10 nl of [^{3}H]thymidine ([^{3}H]T), a marker of cell division, into the lateral wall of the lateral ventricle (Fig. 2) (*15*). Six hours after the microinjection, [^{3}H]T$^+$ cells were restricted to a small patch of dividing SVZ cells (Fig. 2A). Six hours or 1 day after [^{3}H]T microinjection, no [^{3}H]T$^+$ cells were detected in the olfactory bulb (Fig. 2D). As survival time after injection increased, [^{3}H]T$^+$ cells were detected at progressively more rostral locations in a stream of cells extending from the lateral ventricle to the olfactory bulb (Fig. 2, C, D, and E and Fig. 3). Two days after microinjection, some [^{3}H]T$^+$ cells were found in the core of the olfactory bulb (Fig. 2D). The [^{3}H]T$^+$ cells moved from the lateral ventricle to the olfactory bulb at an average rate of 30 μm per hour (*16*), a rate similar to that reported for tangentially migrating young neurons during development (*17*). The number of autoradiography grains overlying the nuclei of [^{3}H]T$^+$ cells decreased as cells migrated rostrally (Fig. 3), suggesting that cells continued to divide between the injection site and the olfactory bulb. This inference is further supported by the labeling of dividing cells in the migratory pathway and the core of the olfactory bulb when [^{3}H]T or bromodeoxyuridine (BUdR, an analog of thymidine) are administered systemically (*18*). The number of labeled cells in the SVZ of the lateral ventricle 6 hours after injection (17,258 ± 1,757) was higher than the number of labeled cells in the olfactory bulb 15 days later (12,884 ± 2,637) (Fig. 3). The reduction in the number of [^{3}H]T$^+$ cells that reached the olfactory bulb was more substantial taking into account that these cells continue to divide during migration. We do not know whether this reduction in the number of [^{3}H]T$^+$ cells was due to cell death, or to the inability to detect [^{3}H]T in cells that diluted the label after continuing division, or to both (*19*).

Fifteen days after [^{3}H]T microinjection most labeled cells were found in the granule cell layer and around glomeruli in the olfactory bulb (Fig. 3). The [^{3}H]T-labeled cells in the granule and periglomerular layers of the olfactory bulb stained for NSE (*7*), but [^{3}H]T$^+$ cells in the walls of the lateral ventricle, the migratory pathway between the lateral ventricle and the olfactory bulb, and in the core of the olfactory bulb were not stained by NSE antibodies.

Two observations indicate that diffusion of [^{3}H]T does not account for the apparent migration of SVZ cells from the lateral ventricle into the olfactory bulb: (i) Microinjected [^{3}H]T was no longer available to label dividing SVZ cells 12 hours after microinjection. This was shown by the combination of [^{3}H]T microinjection with a systemic injection of BUdR (*20*). All of the [^{3}H]T-labeled cells were also labeled by BUdR after the simultaneous injections, but only 3.6% of the [^{3}H]T-labeled cells were labeled by BUdR when the injections were 12 hours apart (*20*). (ii) When [^{3}H]T was systemically injected, endothelial and meningeal cells were labeled throughout the brain, including the olfactory bulb. In contrast, at no time after [^{3}H]T microinjection did we find labeled endothelial or meningeal cells in the olfactory bulb.

To reveal the morphology of the migrating cells and the newly generated neurons in the olfactory bulb, we microinjected the lipophilic dye DiI into the SVZ of the lateral ventricle of adult mice and examined their brains at various times between 6 hours and 30 days after injection (*21*). The injection labeled a volume of tissue with a radius of 150 μm (Fig. 4A). Two, 4, and 6 days after injection spindle-shaped cells with a thick leading process and a thin trailing process were detected in the migratory pathway (Fig. 4B). The morphology of these cells is similar to that described for migrating young neurons (*17, 22*). Processes were oriented in the direction of migration. Three days after injection, some DiI-labeled cells were found in the core of the olfactory bulb, and their processes were

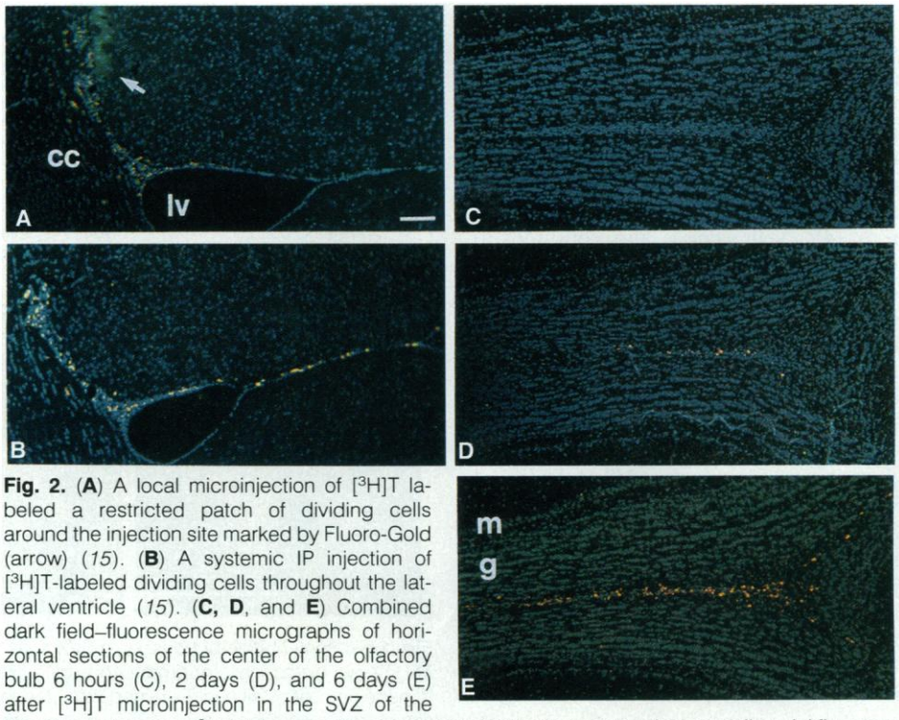

Fig. 2. (**A**) A local microinjection of [^{3}H]T labeled a restricted patch of dividing cells around the injection site marked by Fluoro-Gold (arrow) (*15*). (**B**) A systemic IP injection of [^{3}H]T-labeled dividing cells throughout the lateral ventricle (*15*). (**C, D**, and **E**) Combined dark field–fluorescence micrographs of horizontal sections of the center of the olfactory bulb 6 hours (C), 2 days (D), and 6 days (E) after [^{3}H]T microinjection in the SVZ of the lateral ventricle. The [^{3}H]T-labeled cells appear as bright yellow spots, whereas cell nuclei fluoresce in blue (Hoechst 33258 staining). Anterior is left. cc, corpus callosum; lv, lateral ventricle; g, granule cell layer; m, mitral cell layer. Scale bar, 100 μm.

oriented parallel to the long axis of the bulb. The morphology and orientation of some of the migrating cells in the olfactory bulb began to diverge 6 days after DiI microinjection. DiI-labeled cells changed their orientation, left the core of the olfactory bulb, and migrated radially to more superficial layers. Fifteen and 30 days after DiI microinjection many cells with clear neuronal morphology were found in the granule cell layer and around glomeruli (Fig. 4, C, D, and E). The DiI-labeled cells in the granule cell layer had radially oriented processes that branched in the external plexiform layer and synaptic spines (gemmules) characteristic of granule neurons in the olfactory bulb (*23*). The DiI-labeled cells around glomeruli had processes oriented parallel to the surface of the bulb that branched in neighboring glomeruli. The position and morphology of these DiI-labeled cells is characteristic of periglomerular neurons (*23*). The identity of DiI-labeled neurons confirmed the results presented above and previous work that showed that only granule cells and periglomerular cells are generated in the olfactory bulb postnatally (*5*, *23*). No DiI-labeled cells were detected in the ipsilateral hippocampus or in the contralateral olfactory bulb.

Granule and periglomerular cells are interneurons that do not project outside of the olfactory bulb (*23*). Projection neurons (mitral or tufted cells) in the olfactory bulb were not labeled by DiI at any survival time. Furthermore, DiI-labeled neurons in the olfactory bulb appeared only after labeled cells with migratory morphology reached this region (Fig. 4B). DiI-labeled neurons that appeared in the olfactory bulb 15 or 30 days after DiI injection were derived from DiI-labeled migrating cells that differentiated into neurons, and not from diffusion or transport along axons.

Thus, in adult mice precursor cells that divide close to the lateral ventricle migrate considerable distances (up to 5 mm) before they differentiate into neurons in the olfactory bulb. This process is similar to the one described for neonatal development (*24*). The migration of SVZ cells into the olfactory bulb is parallel to the surface of the ventricle. This is in contrast to the migration of young neurons in adult song birds, where the dispersal seems to be largely radial to the ventricles (*4*). Thus, both radial and nonradial migration occur through the brain of adult vertebrates. The migration of SVZ cells to the olfactory bulb in rodents could serve as a model system to study the factors that control nonradial neuronal dispersion (*17*, *25*). In addition, because the factors that guide SVZ cell migration in the adult brain can also steer grafted cells from adult donors, our data may suggest approaches for adult mammalian brain repair.

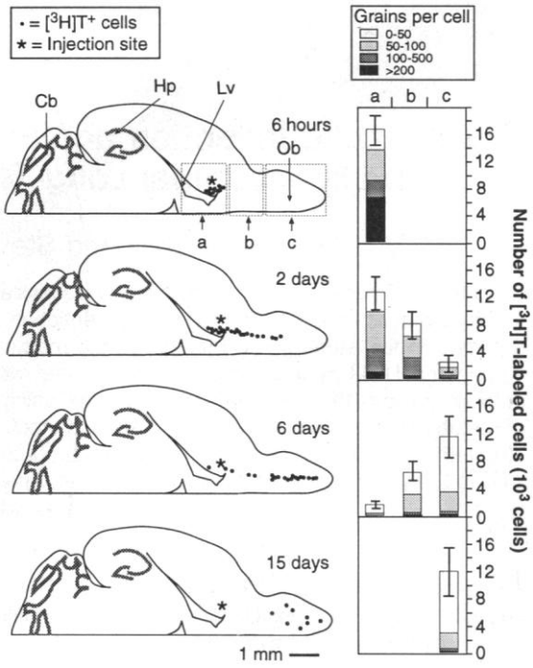

Fig. 3. Distribution and number of [^{3}H]T-labeled cells 6 hours, 2, 6, and 15 days after local injection in the SVZ of the lateral ventricle (*15*). Maps indicate the position of heavily labeled cells (>50 grains) from two superimposed horizontal hemisections 2.3 and 3 mm deep from the pial surface. Histograms indicate the number of cells and the number of grains per cell in the three regions (a, b, and c) boxed in the 6-hours map. Error bars represent the range between two animals analyzed for each survival time (*19*). Ob, olfactory bulb; Lv, lateral ventricle; Hp, hippocampus; Cb, cerebellum.

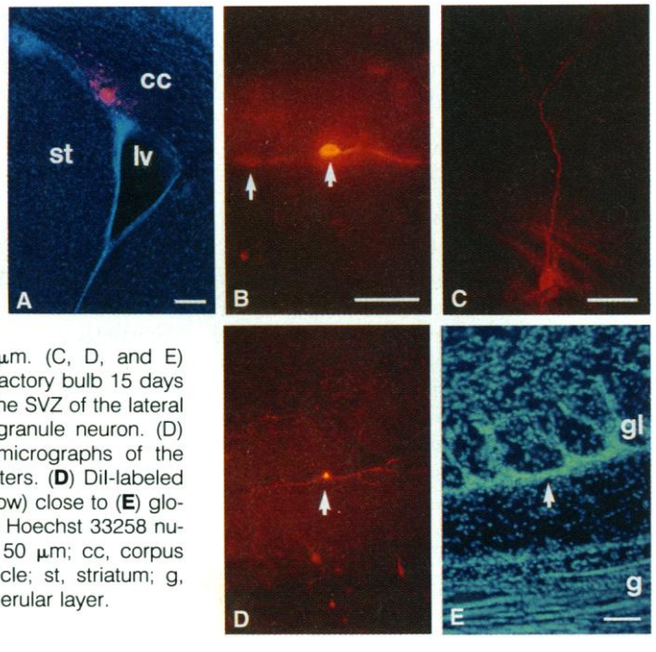

Fig. 4. (**A**) Double exposure microphotograph of the DiI injection site in the SVZ of the lateral ventricle (*21*). DiI fluoresces in red, cell nuclei stained by Hoechst 33258 fluoresce in blue. Scale bar, 100 μm. (**B**) Two DiI-labeled migrating cells (arrows) in the core of the olfactory bulb 6 days after microinjection. Scale bar, 20 μm. (C, D, and E) Labeled neurons in the olfactory bulb 15 days after DiI microinjection in the SVZ of the lateral ventricle. (**C**) DiI-labeled granule neuron. (D) and (E) are fluorescent micrographs of the same field with different filters. (**D**) DiI-labeled periglomerular neuron (arrow) close to (**E**) glomeruli (arrow) revealed by Hoechst 33258 nuclear staining. Scale bar, 50 μm; cc, corpus callosum; lv, lateral ventricle; st, striatum; g, granule cell layer; gl, glomerular layer.

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12. SVZ and cortex were dissected from transgenic animals (*10*) and minced into ~100-μm-diameter explants (*7*). Explants were suspended in Leibovitz L-15 medium plus glucose (6.5 mg/ml), and loaded into 100-μm beveled glass pipettes. We grafted 200 nl of the explant suspension into the SVZ (n = 4) (1 mm anterior to bregma, 0.9 mm lateral, and 2.3 mm deep from the pial surface) or caudate nucleus (n = 2) (0.6 mm anterior to bregma, 1.5 mm lateral, 2.3 mm deep). After 30 days the brains were fixed, cut into 50-μm-thick sections, and processed for X-gal histochemistry [M. G. Kaplitt *et al.*, *Molecular Cell. Neurosci.* **2**, 320 (1991)]. Both donors and hosts were males between 3 and 8 months old. Hosts were CB6/F_1 nontransgenic mice. When SVZ explants from CB6/F_1 nontransgenic animals were grafted into the SVZ of hosts (n = 2), X-gal$^+$ cells were not detected in any region of the brain. Surgery was done under Nembutal (0.6 mg per gram of body weight) anesthesia.
13. The X-gal$^+$ cells in the graft site may have derived from two sources: (i) neurons from the striatum that surrounds the SVZ that survived after transplantation and (ii) neurons that differentiated from grafted SVZ cells.
14. No X-gal$^+$ cells were found between the olfactory bulb and the graft site. Migrating neural cells do not express NSE [P. J. Marangos and D. E. Schmechel, *Annu. Rev. Neurosci.* **10**, 269 (1987)].
15. We microinjected 10 nl of [^{3}H]T (NEN) (6.7 Ci/mmol, 1 mCi/ml) into the SVZ (1 mm anterior to bregma, 1 mm lateral, and 2.3 mm deep from the pial surface) of adult male CD-1 mice under anesthesia (2 to 8 months old). Animals were killed by a lethal dose of anesthetic 6 hours, 12 hours, 1 day, 2, 4, 6, and 15 days after [^{3}H]T microinjection (n = 2 per group), perfused, and their brains cut in horizontal sections 6 μm thick. Sections were processed for autoradiography (*5*) and counterstained with Hoechst 33258 (2.5 μg/ml). The [^{3}H]T-labeled cells were quantified with a computer-based mapping microscope [A. Alvarez-Buylla and D. S. Vicario, *J. Neurosci. Methods* **25**, 165 (1988)]. Fluoro-Gold was included (0.2%) with the [^{3}H]T to mark the position of microinjections; at this concentration Fluoro-Gold did not label SVZ cells. For systemic injections (Fig. 3B), mice (n = 3) received a single intraperitoneal (IP) injection of 50 μl of [^{3}H]T. Animals were killed 6 hours later and processed for autoradiography.
16. To calculate the rate of cell migration, we measured the distance between 10 leading [^{3}H]T$^+$ cells and the injection site with a computer-based microscope (*15*) at different survival times after [^{3}H]T microinjection.
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19. The number of [^{3}H]T-labeled cells and the number of autoradiographic grains per labeled cell were counted in three zones (Fig. 3): SVZ, olfactory bulb, and migratory pathway between the SVZ and the olfactory bulb. For each zone and survival time (two animals for each survival time), labeled cells were counted in 10 sections taken at different levels throughout each zone. The number of labeled cells counted in one animal ranged from 324 (15-days survival) to 1439 (6 hours survival). The total number of labeled cells was estimated from the average number of labeled cells per 6-μm section multiplied by the number of sections that encompassed each region where labeled cells were found. Given the size range of labeled cell nuclei found in our material, it is not necessary to correct for cell splitting between 6-μm sections [S. J. Clarck, J. Cynx, A. Alvarez-Buylla, B. O'Loughlin, F. Nottebohm, *J. Comp. Neurol.* **301**, 114 (1989)].
20. Adult mice (under anesthesia) received stereotaxic microinjections of [^{3}H]T (*15*) and a systemic IP injection of 100 μl of BUdR (100 mg/ml, Sigma). Animals that received simultaneous injections (n = 3) of [^{3}H]T and BUdR were killed 8 hours later. Animals that received injections 12 hours apart (n = 3) were killed 1 hour after BUdR injection. Polyethyleneglycol (PEG) sections were processed for BUdR immunocytochemistry [R. S. Nowakowski, S. B. Lewin, M. W. Miller, *J. Neurocytol.* **18**, 311 (1989)]. BUdR remains available to label dividing cells for less than 1 hour after systemic injection [D. S. Packard, R. A. Menzies, R. G. Skalko, *Differentiation* **1**, 397 (1973)].
21. A 5% solution of DiI (10 nl) [DiIC$_{18}$(3), Molecular Probes] dissolved in vegetable oil (Wesson) was stereotaxically injected into the SVZ of anesthetized adult mice (*15*). Six hours, 3 days, 6, 10, 15, and 30 days after DiI injection, animals (n = 3 per group) were killed, perfused with 3% paraformaldehyde in 0.1 M phosphate buffer, and their brains cut into 50-μm-thick sections with a Vibratome. Sections were counterstained with Hoechst 33258 and wet-mounted with 80% glycerol plus 0.06% gelatin.
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26. All treatments on experimental animals were in accordance with institutional guidelines. We thank S. Forss-Petter and P. Danielson for the NSEp transgenic mice and F. Nottebohm, M. E. Hatten, and G. Fishell for comments on the manuscript. Supported by NIH grant NS 24478 and a Sinsheimer award to A.A.-B. C.L. is a recipient of a La Caixa Foundation graduate program fellowship.

3 December 1993; accepted 31 March 1994

Postsynaptic Induction and Presynaptic Expression of Hippocampal Long-Term Depression

Vadim Y. Bolshakov and Steven A. Siegelbaum

Long-term depression (LTD) is an activity-dependent decrease in synaptic efficacy that together with its counterpart, long-term potentiation, is thought to be an important cellular mechanism for learning and memory in the mammalian brain. The induction of LTD in hippocampal CA1 pyramidal neurons in neonatal rats is shown to depend on postsynaptic calcium ion entry through L-type voltage-gated calcium channels paired with the activation of metabotropic glutamate receptors. Although induced postsynaptically, LTD is due to a long-term decrease in transmitter release from presynaptic terminals. This suggests that LTD is likely to require the production of a retrograde messenger.

Long-term potentiation (LTP) in the hippocampus and neocortex (*1*) and LTD in the cerebellum (*2*) are two important examples of activity-dependent synaptic plasticity in the mammalian brain that is long-lasting. Both forms of synaptic plasticity are induced by an increase in the concentration of Ca^{2+} in the postsynaptic cell. However, the sources of Ca^{2+} are different. LTP in the CA1 region of the hippocampus requires Ca^{2+} influx through the *N*-methyl-D-aspartate (NMDA)–type glutamate receptors (*1*), whereas LTD in the cerebellum requires Ca^{2+} influx (*3*) through voltage-gated Ca^{2+} channels (*4*). The two forms of plasticity also differ in their sites of expression. LTD in the cerebellum results from a decrease in the postsynaptic response of Purkinje neurons to glutamate (*2*). LTP is thought to result from both enhanced presynaptic release of glutamate (*1, 5, 6*) in response to a retrograde messenger produced in the postsynaptic cell (*7*) and from an increased postsynaptic response (*1, 6, 8*).

Relatively little is known about hippocampal LTD in comparison to LTP (*9*). However, the recent finding that prolonged, low-frequency (1 to 5 Hz) stimulation induces LTD (*10–12*) makes it possible to investigate the mechanism of induction and site of expression of hippocampal LTD. Although the induction of LTD in the hippocampus (*10*) and cerebral cortex (*13*) requires postsynaptic Ca^{2+} [see (*14*) for an opposing view], its source is less certain and may depend on influx through NMDA receptors (*10, 11, 15*), voltage-gated Ca^{2+} channels (*16*), or internal Ca^{2+} release after activation of metabotropic glutamate receptors (mGluRs) (*13, 17*). Moreover, it is not known whether hippocampal LTD is due to a decrease in transmitter release or to a decrease in the postsynaptic response to glutamate. Our study shows that hippocampal LTD resembles cerebellar LTD (*4*) because it requires the paired activation of mGluRs and postsynaptic Ca^{2+} entry through L-type voltage-gated Ca^{2+} channels. However, in contrast to the cerebellum, LTD in the hippocampus is expressed presynaptically and results from a long-lasting decrease in transmitter release.

LTD was studied without contamination from LTP in 3- to 7-day-old rats, a stage before the development of LTP (*12*), at the

Center for Neurobiology and Behavior, Department of Pharmacology, Howard Hughes Medical Institute, Columbia University, New York, NY 10032, USA.