reflect the nonhomology of simple leaves in eudicots. KNOXI expression of the "complex" pattern was seen in the complex primordia of different eudicot lineages [euasterid II: Apiaceae–*Daucus carota* (Fig. 4, A and B); rosids: Vitaceae–*Cissus* (Fig. 4, C to E)]. Sampling suggests that the molecular hypothesis regarding an association of KNOXI activity with complexity of the leaf primordium is supported across most eudicots [fig. S3 (*12*)]. One exception is a group of legumes, including peas, which have complex leaves but no KNOXI expression in leaf primordia (*29*, *30*). We believe that, in this group, *KNOXI* genes ceased to be part of the genetic cascade leading to the complex leaf form and that a different gene, PEAFLO (*29*, *30*), became part of the cascade (*31*). The unusual KNOXI expression pattern in this group of legumes is striking, given our observation that the correlation of "complex" KNOXI protein expression with primordium complexity was present in ferns and gymnosperms (Fig. 4, F to J), representing stages early in the evolution of vascular plants.

Regardless of final leaf form, KNOXI expression is down-regulated at sites of leaf initiation (P_0) in flowering plants and gymnosperms [Figs. 1, 3, and 4; figs. S1 to S3 (*12*)]. This suggests a mechanism that denotes "determinacy" during initiation of the leaf. Unlike in seed plants, KNOXI is not down-regulated in the P_0 of ferns (Fig. 4G) (*32*). This result is consistent with current understanding that leaves of ferns and seed plants evolved independently (*33*) and may have different developmental characteristics (*34*).

Our results suggest that at least two different modes of development have evolved to generate simple leaves (e.g., *L. africanum* with simple pattern and *Pimpinella anisum* with complex pattern). By contrast, the same, complex, pattern of KNOXI expression characterizes independently evolved complex leaves (e.g., *Cissus congestum* and *Daucus carota*; *N. aquatica*, *L. perfoliatum*, and *L. hyssopifolium*). Complex leaves may thus be partially indeterminate. Several studies on vascular development in leaves suggest that leaf shape and venation patterns parallel each other. However, it is unclear whether one directs the other and, if so, which one. Analysis of venation patterns in developing leaves with secondary morphogenesis may provide some information on this aspect of leaf development. Our results are similar to those for Crustacea, a group of animals, in which Hox expression is correlated with the specialization of limbs into feeding appendages (*35*). As in that case, these results highlight the value of comparative studies in augmenting and/or refuting hypotheses that emerge from experimental studies, and in suggesting new hypotheses that may be tested experimentally.

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Amacrine-Signaled Loss of Intrinsic Axon Growth Ability by Retinal Ganglion Cells

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The central nervous system (CNS) loses the ability to regenerate early during development, but it is not known why. The retina has long served as a simple model system for study of CNS regeneration. Here we show that amacrine cells signal neonatal rat retinal ganglion cells (RGCs) to undergo a profound and apparently irreversible loss of intrinsic axon growth ability. Concurrently, retinal maturation triggers RGCs to greatly increase their dendritic growth ability. These results suggest that adult CNS neurons fail to regenerate not only because of CNS glial inhibition but also because of a loss of intrinsic axon growth ability.

Neurons in the CNS lose the ability to regenerate their axons early in development, but it is not known why. A currently prevailing view is that a strongly inhibitory glial environment causes regenerative failure in the adult CNS (*1*, *2*), as CNS glial cells, both astrocytes and oligodendrocytes, inhibit regenerating axons after injury (*3*–*6*). A crucial question is whether overcoming these inhibitory cues will be sufficient to promote rapid regeneration or whether adult CNS neurons have undergone a developmental loss of intrinsic regenerative ability (*7*–*11*). For example, CNS neurons in slices are less able to

extend their axons into target explants as they age, but as these slices contain glia, this developmental loss of axonal regenerative ability might be accounted for by glial rather than neuronal aging (*10*, *12*).

In the present study, we have taken advantage of our ability to highly purify RGCs away from glia and other cell types (*13*), to directly investigate whether CNS neurons undergo a loss in axonal growth ability with age. We initially compared the axon growth ability of RGCs purified from embryonic day 20 (E20) and postnatal day 8 (P8) rats, ages before and after target innervation, respectively. Purified E20 and P8 RGCs in culture both extend axons of similar caliber that are immunopositive for tau, β-tubulin III, and gap-43 (Fig. 1, A and B). After 3 days in culture, however, E20 RGCs extended their longest axon seven times as far as P8 RGCs [Fig. 1C; (*14*)]. When we measured their axon length daily over this period, we observed an initial lag period of about 1 day for both E20 and P8 RGCs, when axon growth was reinitiated (Fig. 1C). Thereafter, E20 RGCs extended axons about 10 times as fast, at about 500 μm/day, close to the speed they extend during normal development, whereas the axons of P8 RGCs grew at an average rate of only 50 μm/day (Fig. 1C). Despite this slow average growth rate, about 1% of P8 RGCs elongated axons at a rate exceeding 1 mm/day. To determine when RGCs lose rapid axon growth ability, we examined purified RGCs from various ages. The axon growth rate of RGCs decreased sharply within a day of birth (Fig. 1D). Thus, both embryonic and postnatal RGCs are competent to extend their axons in response to peptide trophic signals, but embryonic RGCs intrinsically extend axons far more rapidly than do postnatal RGCs.

To investigate whether this loss of axon growth ability was general, we next explored RGC axon growth in a variety of strongly trophic environments. Much more rapid axon growth by embryonic RGCs compared with postnatal RGCs was observed in response to medium conditioned by either embryonic or postnatal superior collicular (target), retinal, or optic nerve cells (Fig. 1E). Remarkably, more rapid growth of P8 RGCs could not be stimulated even when they were cultured in medium conditioned by (Fig. 1E) or directly in contact with sciatic nerve Schwann cells, strong stimulators of axon growth and regeneration (*15*). We next compared the ability of RGCs to regenerate in vivo after transplantation into developing brain (Fig. 1, F and G). Bcl-2 overexpression was needed to ensure survival of most of the RGCs (Fig. 1G). Both the transplanted E20 and P8 RGCs were able to regenerate their axons, but embryonic RGCs extended their axons significantly farther (Fig. 1G). Taken together, these data indicate that the difference in axon growth ability between embryonic and postnatal RGCs is intrinsic and not dependent on a specific trophic environment.

Why do RGCs lose the ability to rapidly extend axons as they age? We tested three previously reported possibilities: down-regulation of Bcl-2 expression (*9*), loss of laminin responsiveness (*8*), or a decrease in cAMP levels (*16*). First, although overexpressing Bcl-2 by using an adenoviral vector (Ad-bcl-2) (*17*) kept most postnatal RGCs alive in culture in the absence of any trophic support, it was insufficient to induce axon growth on its own (*18*) or to potentiate brain-derived neurotrophic factor (BDNF)–stimulated growth (Fig. 2A). Second, although laminin-1 and merosin enhanced the ability of BDNF to promote survival and axon growth of both E20 and P8 RGCs, E20 RGCs retained a growth rate advantage over P8 RGCs, demonstrating that loss of axon growth ability is not accounted for by loss of laminin responsiveness (Fig. 2, B and C). Third, a postnatal decrease in cAMP levels was also not responsible, as the growth difference was maintained even with high levels of the nondegradable cAMP analog chlorophenylthio-cAMP (Fig. 2D). Furthermore, a loss of cAMP-mediated recruitment of surface TrkB (*19*, *20*) did not account for the postnatal decrease, because with cAMP ele-

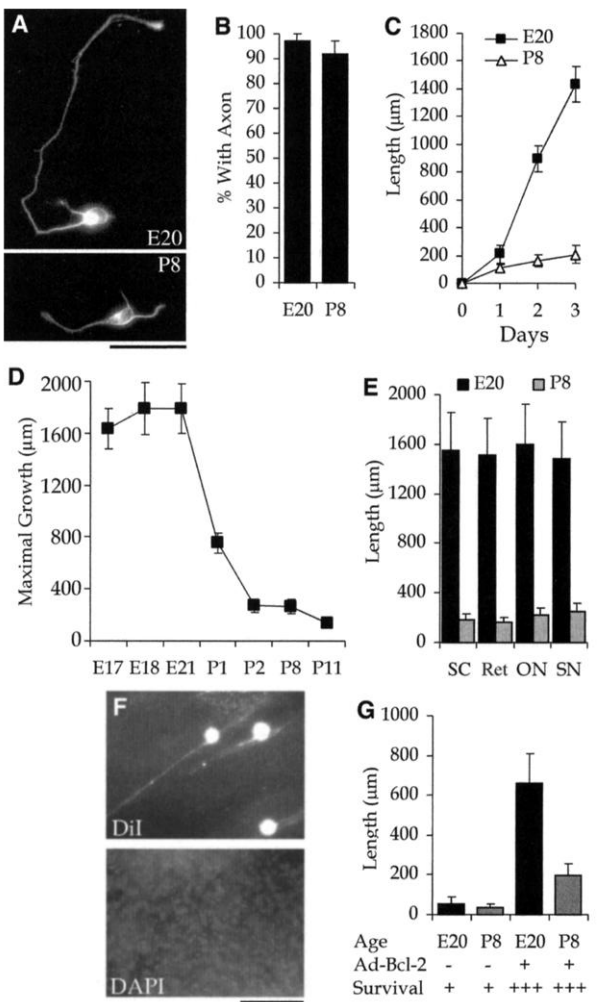

Fig. 1. Difference in axon growth ability of E20 and P8 RGCs. (**A** to **D**) RGCs purified by immunopanning were cultured at clonal density (<5/mm²) on poly-D-lysine (PDL) and laminin in growth medium containing BDNF, ciliary neurotrophic factor (CNTF), insulin, and forskolin (GM). (A) RGC axons immunolabeled for the axonal protein β-tubulin III after 18 hours. (B) Percentage of RGCs at each age elaborating at least one axon greater than 20 μm after 3 days. (C) Average length of each neuron's longest axon at days 1, 2, and 3. E20 and P8 axon lengths did not differ statistically at day 1. P8 axon lengths at days 2 and 3 were longer than on the day before (Dunnett's, $P < 0.05$). (D) Time course of change in intrinsic axon growth ability of RGCs purified simultaneously from rats of different ages and cultured in target-conditioned GM. Means ± SEM. (**E**) RGC axon length after 3 days at clonal density in minimal medium conditioned by superior collicular, SC; retinal, Ret; optic nerve, ON; or sciatic nerve, SN cells. (**F** and **G**) Purified, DiI-labeled RGCs were infected with Ad-bcl-2 or with a control Ad-GFP adenovirus and transplanted into the early postnatal (P2 to P4) corpus collosum, at an age when axons are normally growing in this pathway. After 3 days, transplanted brains were cryosectioned coronally, and sections containing DiI-labeled RGCs (F, top) were counterstained with the nuclear dye 4′,6′-diamdino-2-phenylindole (DAPI) (E, bottom). (G) All singly identifiable axons extending from RGCs visualized as above were quantified from each brain. Shorter axons were too dense to count reliably (not shown), biasing the data toward comparing the longest axons at each age. Survival was estimated on the basis of the presence of pyknotic, DiI-labeled cells. For each condition, RGCs were transplanted into at least six brains, and the average axon growth rates per brain (range $n = 4$ to 26) were averaged to derive a mean and standard errors. Scale bars, 50 μm.

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vation, nearly all P8 RGCs activate mitogen-activated protein (MAP) kinase in response to BDNF (*21*), and overexpressing TrkB in the purified RGCs did not increase axon growth rates [Fig. 2E; (*18*, *22*)]. Thus, the developmental loss of RGC axon growth ability cannot be accounted for by down-regulation of bcl-2 or cAMP levels, or by loss of laminin or neurotrophin responsiveness.

The neonatal decrease in axonal growth ability could be due to an intrinsic aging program or instead could be signaled by neighboring cell types. To find out, we aged purified E20 RGCs in culture for 10 days, long past the equivalent postnatal age at which rapid axon growth ability was lost in vivo, and then we replated them and measured their axon growth rates. Remarkably, the rate of axon growth remained nearly the same as the rate of acutely purified E20 RGCs, at about 500 μm/day (Fig. 3A). Similarly, the rate of growth by purified P8 RGCs cultured for 10 days remained slow at 50 μm/day (Fig. 3A). When the P8 RGCs were aged in medium conditioned by embryonic target, retinal, optic nerve, or postnatal Schwann cells, they failed to revert to the embryonic phenotype (Fig. 3A). These findings indicate that the normal neonatal loss of axon growth ability is not caused by intrinsic aging, is normally signaled in vivo by another cell type (*23*) and, once signaled, is apparently irreversible.

What is the source of this extrinsic signal? During the neonatal period, RGC axons are innervating their superior collicular targets, glia are differentiating in the optic nerve (*24*), bipolar and amacrine cells are generated within the retina (*25*), and hormones are changing systemically. We tested each of these possibilities. Medium conditioned by postnatal mixed retinal cells, optic nerve cells, or superior collicular cells had no effect (Fig. 3A), nor did rat serum, retinoic acid, or growth hormone (*26*). Similarly, when purified E21 RGCs were labeled with the retrograde tracer 1,1′-didodecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate (DiI) and cultured in direct contact with 300-μm sections of superior colliculus for 3 to 6 days, only a small decrease in growth rate was observed [Fig. 3, B and C; (*27*)]. However, when we allowed RGCs to maintain contact with neighboring retinal cells by culturing E20 retinal explants for 3 to 4 days (past the equivalent age of the neonatal switch) before purifying the RGCs and measuring their axonal growth ability, we found that their axon growth rate decreased to one-seventh the rate of acutely purified E20 RGCs (Fig. 3D). This decrease was not abrogated when the retinas were aged in the presence of a cocktail of activity blockers including tetrodotoxin, curare, and kynurenic acid (Fig. 3D). Furthermore, the decrease in growth rate could not be attributed to damage caused by removing the cells from the cultured retinal explant, as RGCs purified from E17 retinas aged 3 days in vitro decreased their axon growth rate only slightly, by 11.2 ± 4.3% (n = 3). Taken together, these results demonstrate that the neonatal loss of ability of RGCs to rapidly grow axons is signaled by retinal maturation, probably involves direct contact with retinal cells, and does not depend on activity.

We next investigated the identity of the retinal cell type that signals RGCs to lose their axonal growth ability. In rodents, RGCs receive synaptic inputs primarily from amacrine cells and at least some bipolar cells. We developed methods to purify amacrines and a subset of bipolars (*28*, *29*). When purified E20, DiI-labeled RGCs were cultured in direct contact with purified amacrine cells or bipolar cells for 3 days and then replated to measure their axon growth, only the amacrine cells induced RGCs to decrease their axon growth ability (Fig. 3E). This decrease was not signaled by retinal suspensions depleted of amacrine cells or by amacrine cell–conditioned medium, but was irreversibly signaled when E20 RGCs were cultured in direct contact with amacrine cell membrane preparations [Fig. 3E; (*30*, *31*)]. Thus a contact-mediated or membrane-associated signal from amacrine cells signals RGCs to decrease their intrinsic axon growth ability.

Because the decrease in RGC intrinsic growth ability occurs at a stage in vivo when they rapidly expand their dendritic trees (*32*), we wondered whether the decrease in axon elongation ability coincided with an increased ability to extend dendrites. We assayed dendritic morphology by immunostaining with monoclonal antibodies against MAP2 (Fig. 4A). Whereas embryonic RGCs generally extended about three dendrites from the cell body, postnatal RGCs extended an average of eight dendrites (Fig. 4B), with an increase in dendritic branching and length (Fig. 4C). After transplantation into adult white matter in vivo, we found that P8 RGCs robustly extended dendrites but not axons, whereas the E20 RGCs extended axons but not dendrites, suggesting a property intrinsic to the cells. Furthermore, when RGCs of either age were cultured for 10 days and then replated and allowed to re-extend axons and dendrites, they maintained their dendritic phenotype, with postnatal RGCs extending significantly more and longer dendrites per neuron than aged embryonic RGCs. The acquisition of this enhanced dendritic growth ability was induced after aging the E20 RGCs in retinal explants, but not after aging RGCs

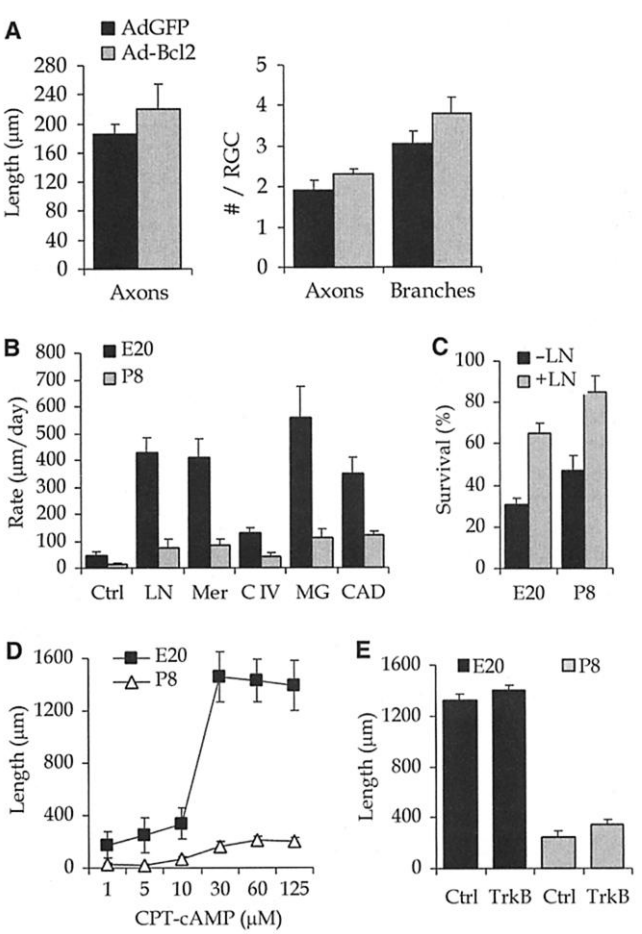

Fig. 2. Effect of bcl-2 expression, matrix substrates, and TrkB expression on axon growth rate of E20 and P8 RGCs. **(A)** Axon length, number of axons extending from the cell body, and number of branches of P8 RGCs infected with Ad-bcl-2 or Ad-GFP as marked and cultured in growth medium were quantified. Means were not different at conventional levels of significance (Student's t test). **(B)** Average axon growth rate of E20 and P8 RGCs cultured on PDL plus laminin, LN; merosin, Mer; collagen IV, C IV; matrigel, MG or N-cadherin, CAD. Differences significant on all substrates; Dunnett's, $P < 0.05$. **(C)** Survival of E20 and P8 RGCs cultured on PDL with or without laminin (LN). **(D)** Average axon lengths of E20 and P8 RGCs in response to BDNF (50 ng/ml) plus increasing concentrations of the nondegradable cAMP analog chlorophenylthio-cAMP (CPT-cAMP). **(E)** Average axon length of E20 or P8 RGCs infected with Ad-TrkB or with a control Ad-GFP adenovirus.

on superior collicular slices (Fig. 4D). Thus, the loss of axon elongation ability as a result of signals by amacrine cells corresponds temporally with an acquisition of a greatly enhanced ability to grow dendrites, which is also triggered by a retinal cue.

Taken together, our results show that retinal maturation triggers neonatal RGCs to irreversibly switch from an axonal to a dendritic growth mode. These findings have important implications. First, they suggest that the ability of neurotrophic factors to stimulate axon and dendrite growth may strongly depend on whether a neuron is in an axonal or dendritic growth mode. Second, they show that a transient signal to a differentiated, postmitotic cell type can induce a permanent phenotypic change. Third, because this switch in growth mode appears to be irreversible and occurs neonatally, concurrent with the loss of regenerative ability observed in vivo, we propose that it is an important contributor to the failure of CNS neurons to regenerate in vivo. An intrinsic loss of rapid axonal elongation ability would help explain why in many previous experiments regeneration proceeds remarkably slowly, even when glial inhibitory cues have been removed. For instance, most RGCs take 2 to 3 months to regenerate through a peripheral nerve graft to the superior colliculus (*33*–*35*), nearly the rate that most P8 RGCs elongate in vitro but far longer than the 10 days that would be predicted if axons grew at their normal developmental growth rate of 1 mm/day. The few RGCs that manage to regenerate long distances rapidly in these and other similar experiments (*36*, *37*) may correspond to the small subset of mature RGCs that we observed retain fast intrinsic growth ability in vitro. In contrast, adult peripheral neurons retain their rapid axon elongation, *18* ability (*38*), a difference undoubtedly critical to their rapid regenerative ability (*39*). Thus, in order to promote robust and rapid CNS regeneration in patients, new strategies to accelerate intrinsic axon growth rate may be crucial (*40*).

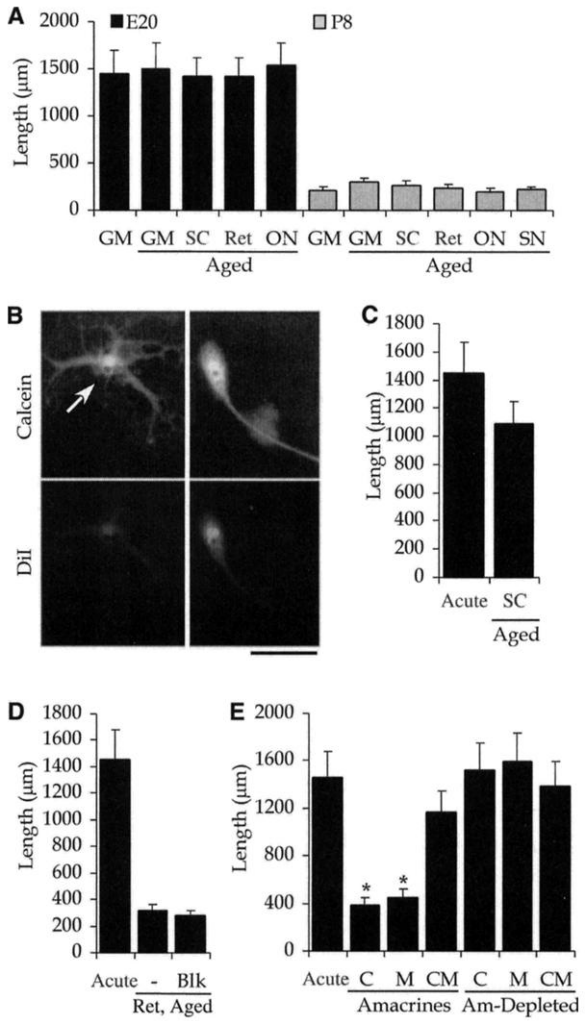

Fig. 3. Effect of aging and of extrinsic signals on intrinsic axon growth ability. (**A**) RGCs were purified and cultured in vitro, and their axon lengths were measured acutely in growth medium or after having aged 10 days in GM or in growth medium conditioned by superior collicular target, SC; retinal, Ret; optic nerve, ON; or sciatic nerve Schwann cells, SN; dissociated and cultured in inserts suspended above the RGCs. (**B** and **C**) E21 RGCs were purified, DiI-labeled, and cultured for 3 days directly on 300-μm slices of superior colliculus. They were then resuspended by gentle pipetting and replated at clonal density. (B) Three days after replating, living cells and axons were visualized with calcein (top), and RGCs were identified among these by the DiI label (bottom). Collicular cells were visualized with calcein but not with DiI (arrow). Scale bar, 30 μm. (C) Average axon length of E20 RGCs examined acutely or after aging on superior collicular target, SC, slices. (**D**) Average axon growth of E20 RGCs either acutely purified or aged 3 to 4 days in retinal explants, Ret, with or without a cocktail of activity blockers, Blk (see text), before purification by immunopanning. (**E**) DiI-labeled E20 RGCs were aged for 3 days in the presence of amacrine cells or retinal cells depleted of amacrines, C, on membranes, M, isolated by sucrose gradient and adsorbed onto the culture dish, or in the presence of conditioned medium, CM, from these two cell populations, as marked. After this aging period, the cultures were dissociated and replated, and the E20 RGC axons were measured after a subsequent 3 days in growth medium, and compared to acutely purified E20 RGC axons. *Dunnett's, $P < 0.05$.

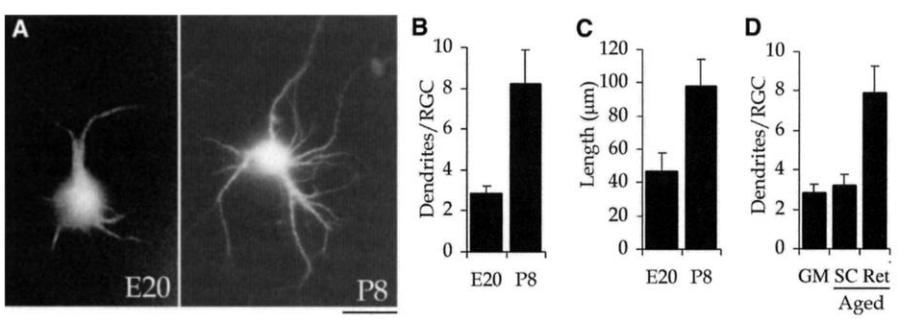

Fig. 4. Differences in dendrite growth between E20 and P8 RGCs. RGCs were cultured in growth medium for 3 days. (**A**) MAP2 immunoreactivity in E20 and P8 RGCs. Scale bar, 20 μm. (**B**) Number of dendrites per RGC extending from the cell soma. (**C**) Total dendritic length per RGC. (**D**) Axon growth of E20 RGCs purified acutely and of E20 RGCs aged for 7 days first in retinal explants before purification by immunopanning, Ret, or on superior collicular target slices and then dissociated and replated, SC. Differences significant by Student's *t* test, $P < 0.05$.

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26. Serum drawn from rats on P0 or P1 was tested at final concentrations ranging from 0.1 to 10%. Retinoic acid and growth hormone were tested at 1 to 100 nM and 1 μM, respectively. Conditioned medium from P8 tissues was also tested and showed no effect.
27. Further confirmation that this developmental switch was not attributable to target interaction was obtained by aspirating the superior colliculus from P0 animals, which did not prevent the decrease in intrinsic axon elongation ability.
28. Rat amacrine cells (9 to 12% of retinal neurons) were enriched to >85% purity by sequential immunopanning (*13*), depleting OX-7$^+$ and RAN-2$^+$ cells and then selecting VC1.1$^+$ cells. Immunostaining with HPC-1, glial fibrillary acidic protein (GFAP), G26 (rhodopsin, courtesy of P. Hargrave), and Per3B6 (peripherin-2, courtesy of R. Molday) showed 85% amacrines, and <1% glia, rod, and cone photoreceptors. Amacrine 3-day survival was 75% on poly-D-lysine (PDL)-coated dishes in RGC growth media. Amacrine-depleted cultures consisted of all retinal cells not removed in the protocol above, largely photoreceptors and bipolar cells. Bipolar cells (4 to 6% of retinal neurons) were enriched from P12 L7-green fluorescent protein (L7-GFP) mouse retina (*29*) by using fluorescence-activated cell sorting. After FACS, 35 to 40% of the sorted cells were strongly GFP$^+$, and another 30% were weakly to moderately GFP$^+$; 38% were protein kinase C (PKC) (MC5)$^+$ bipolar neurons; 22% were B6-30$^+$ rods; 2% were HPC-1$^+$ amacrines; and none were GFAP$^+$ glia. Thus, this procedure enriched bipolar neurons to between 38% (by PKC expression) and 75% (by L7-promoted GFP expression).
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30. Amacrine membranes were adsorbed onto the dish and rinsed before adding RGCs; after coculture, RGCs were trypsinized, pipetted, and centrifuged before replating, making subsequent amacrine membrane carryover unlikely. To examine membrane transfer directly, we labeled amacrine cells before membrane preparation with [^{35}S]methionine for bulk protein and with DiI for membrane lipid. RGCs cultured on these amacrine membranes and then replated per our regular protocol carried over no visible DiI, and ^{35}S was not above background. Thus, an amacrine membrane-associated signal did not simply carry over and inhibit signal axon growth but rather irreversibly signaled the embryonic neurons to decrease their intrinsic axon growth ability.
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40. Detailed step-by-step protocols are available on request. We thank M. Cameron for gifted technical assistance, Regeneron for recombinant BDNF and CNTF, M. Lin and M. Greenberg for the adenoviral TrkB-GFP vector, M. Yuzaki for L7-GFP mice, Y. Xu and N. Davidson for the adenoviral bcl-2 vector, P. Hargrave and R. Molday for valuable antibodies, and S. Brady-Kalley for purified N-cadherin. We also thank L. Luo, J. Dugas, W. Mandemakers, K. Christopherson, and M. Lin for thoughtful comments on the manuscript. This research was possible thanks to the support of the National Eye Institute (RO1 EY11030, B.A.B.), the March of Dimes Foundation (1-FY01-352, B.A.B.), and the NIGMS Medical Scientist Training Program (2T32GM07365; J.L.G.).

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Trans-Synaptic Eph Receptor–Ephrin Signaling in Hippocampal Mossy Fiber LTP

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The site of induction of long-term potentiation (LTP) at mossy fiber–CA3 synapses in the hippocampus is unresolved, with data supporting both pre- and postsynaptic mechanisms. Here we report that mossy fiber LTP was reduced by perfusion of postsynaptic neurons with peptides and antibodies that interfere with binding of EphB receptor tyrosine kinases (EphRs) to the PDZ protein GRIP. Mossy fiber LTP was also reduced by extracellular application of soluble forms of B-ephrins, which are normally membrane-anchored presynaptic ligands for the EphB receptors. The application of soluble ligands for presynaptic ephrins increased basal excitatory transmission and occluded both tetanus and forskolin-induced synaptic potentiation. These findings suggest that PDZ interactions in the postsynaptic neuron and trans-synaptic interactions between postsynaptic EphB receptors and presynaptic B-ephrins are necessary for the induction of mossy fiber LTP.

Although it is widely agreed that expression of LTP at mossy fiber synapses occurs in the presynaptic terminal (*1*, *2*), the site of induction of this *N*-methyl-D-aspartate (NMDA) receptor–independent form of plasticity is still not clear (*3*, *4*). Previous data have both supported (*3*, *5*) and excluded (*4*, *6*, *7*) a role for postsynaptic signaling pathways in the induction process. In contrast, it is generally agreed that the long-lasting alteration in synaptic strength is expressed presynaptically as an increased probability of neurotransmitter release (*1*, *6*). If postsynaptic mechanisms do indeed underlie induction of mossy fiber LTP, retrograde signaling must exist to transduce the signal to the presynaptic nerve terminal.

The goal of our initial experiments was to determine the importance of glutamate receptor–PDZ interactions in stabilizing excitatory transmission at hippocampal mossy fiber synapses. Intracellular perfusion of CA3 pyramidal neurons with a peptide corresponding to the carboxyl-terminal 10 amino acids of GluR2 (R2ct) to disrupt GluR2–PDZ interactions (*8*) had a small effect on basal synaptic transmission in some recordings, as previously described in CA1 pyramidal neurons (*9*, *10*) (Fig. 1B). In contrast, potentiation of the excitatory postsynaptic current (EPSC) measured 25 to 30 min after tetanic stimulation was significantly smaller than in control recordings without peptide [control LTP: 220 ± 30%, $n = 13$ cells; R2ct LTP: 140 ± 11%, $n = 18$ cells, $P < 0.01$ Kolmogorov-Smirnov (K-S) test (Fig. 1)]. In control recordings, the paired-pulse ratio (PPR) of mossy fiber EPSCs, measured at a 40-ms interval between stimuli, was reduced after induction of LTP, consistent with an increase in the probability of glutamate release (*6*) (control PPR: 2.9 ± 0.12; LTP PPR: 2.3 ± 0.16, $n = 12$ cells, $P < 0.01$). Postsynaptic perfusion of the R2ct peptide also reduced this change in PPR (R2ct control PPR: 2.8 ± 0.14; LTP PPR: 2.5 ± 0.12, $n = 16$ cells, $P > 0.05$), in accord with the diminished potentiation after tetanic stimulation (Fig. 1). We also observed that posttetanic potentiation (PTP) immediately after tetanic stimulation was significantly smaller in recordings in

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