binding in the caudal (peripallidal) striatum, a finding that probably explains the small component (10 to 25 percent) of [^{3}H]spiroperidol binding in striatal homogenates that has S_2 characteristics (*10, 11, 18, 19*).

The quantification afforded by digital subtraction autoradiography also allows comparisons to be made between the distribution of pharmacologically defined receptors and the density of their afferent innervation. For example, the striatal complex contains the most dense dopaminergic input of the forebrain as well as the highest concentration of D_2 sites. However, the enrichment of D_2 sites in the lateral caudate-putamen relative to the medial periventricular zone is unexpected. The sharp lateral-to-medial gradient in striatal D_2 density does not appear to be matched by any discernible parallel gradient in dopamine concentration (*20*). Also, the restricted laminar distribution of neocortical S_2 sites contrasts with the more scattered distribution of serotonergic axon terminals throughout the superficial layers (*21*).

The observed pattern of D_2 and S_2 receptor distribution has at least three important implications for the function of dopamine and serotonin in the mammalian forebrain. First, the sharp lateral-to-medial gradient of D_2 binding in the striatal complex suggests a novel neurohistochemical basis for the functional distinction between these subregions (*22*). Second, in view of the high correlation between the potency of antipsychotic drugs in vivo and their affinity for the D_2 binding site (*2*), the paucity of D_2 sites in the neocortex suggests that a subcortical locus for the action of antipsychotic drugs should be reconsidered. Third, the laminar distribution of S_2 sites in the motor cortex may provide a substrate for the well-documented motor actions of serotonergic compounds acting on brain S_2 sites (*23*). In addition to permitting quantitative localization of radioligand-labeled receptor subtypes in tissue sections, digital subtraction autoradiography should help to elucidate regional changes in brain receptors related to ontogeny, senescence, or brain injury (*4, 25*).

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Regenerating Fish Optic Nerves and a Regeneration-Like Response in Injured Optic Nerves of Adult Rabbits

Abstract. *Regeneration of fish optic nerve (representing regenerative central nervous system) was accompanied by increased activity of regeneration-triggering factors produced by nonneuronal cells. A graft of regenerating fish optic nerve, or a "wrap-around" implant containing medium conditioned by it, induced a response associated with regeneration in injured optic nerves of adult rabbits (representing a nonregenerative central nervous system). This response was manifested by an increase of general protein synthesis and of selective polypeptides in the retinas and by the ability of the retina to sprout in culture.*

The central nervous system (CNS) of higher vertebrates, unlike that of lower vertebrates, is not capable of posttraumatic regeneration (*1–4*). Injury in CNS of lower vertebrates alters synthesis and transport of selective molecules (*5–10*) and sprouting activity in culture (*11, 12*). The lack of posttraumatic regenerative response in mammals could stem either from the intrinsic inability of the cell body to undergo the appropriate changes or from a deficient or hostile environment. Indeed, in a nonregenerative CNS, environmental modifications can induce morphological regeneration (*13, 14*). In regenerative systems, axonal in-

jury is accompanied by alterations in the type and the amount of substances originating from the nonneuronal cells (*15–18*).

We have found that alterations in the type and amount of substances originating from nonneuronal cells of a regenerating fish optic nerve (*16*) are associated with the production of factors that can trigger regeneration in a nonregenerative system (rabbit optic nerve). This implies that a nonregenerative system can express characteristics of regeneration if appropriate external factors are provided.

We first established environmental modifications by transplanting segments of regenerating fish optic nerves into an injured optic nerve of adult rabbits (*19*). Because of immunological drawbacks to that approach, we substituted the transplanted nerves with "wrap-around" implants of silicone tubes containing substances derived from nonneuronal cells of regenerating fish optic nerves. We used changes in the retinal protein synthesis and sprouting activity in culture as markers for the regenerative response in this system (*4, 11, 12*). In anesthesized rabbits, we used a conjunctival approach to expose the optic nerves distally from the globe for about 1 cm. The optic nerve was then crushed or cut unilaterally (left side) at a distance of 4 to 5 mm from the optic disk. When a regenerating fish optic nerve (5 to 6 days after injury to the nerve) was transplanted into the cut rabbit optic nerve (the first environmental modification), it was sutured by three absorbable sutures through the respective meninges. When implants were used instead, a sleeve of a silicone tube (6 mm long, 4 mm external, and 2 mm internal diameters) was placed around the injured site of the rabbit optic nerve. Before being implanted, the silicone tube was coated with purified bovine dermal collagen and then soaked in 300 μl of medium conditioned by fish optic nerves (400 μg of protein per milliliter). Conditioned medium was prepared by incubating segments of fish optic nerves in a serum-free medium [two segments per 300 μl of Dulbecco's minimum essential medium (DMEM), Gibco] for 1.5 hour at room temperature.

One week after the injury to the rabbits, retinas on both sides were excised separately and incubated in DMEM free of methionine (Gibco) supplemented with [^{35}S]methionine (40 μCi per retina, 1230 Ci/mmol; Amersham). After an incubation of 1.5 hour the reaction was stopped on ice, the retinas were collected, homogenized and centrifuged (25 pounds per square inch, 10 minutes; Airfuge, Beckman). Specific activity of the supernatant (100,000*g*) was then determined (counts per minute per milligram of protein). This soluble fraction is assumed to contain cytoplasmic proteins including those derived from ganglion cells and destined to be axonally transported. The ratio between specific activities of the labeled proteins in the left (injured) versus the right (control) sides (expressed as *L/R*) was used as an indicator for the total change in protein synthesis, as it has been for the retina of a regenerating nerve from fish (*2, 7*).

Table 1. Increased [^{35}S]methionine incorporation by rabbit retinal proteins induced by substances originating from regenerating fish optic nerve grafted into injured adult rabbit optic nerves. Statistical comparisons are with the control groups. Seventy eight percent of the rabbits in the control group (consisted of rabbits that were injured only), had an *L/R* ratio of less than 1; in the treated groups (implanted and transplanted) 83 percent of the rabbits had a ratio greater than 1; S.E.M., standard error of the mean.

Group	*L/R* ($\bar{X}$ ± S.E.M.)	Animals (No.)
Control*	0.76 ± 0.07	11
Implanted	1.5 ± 0.2†	7
Transplanted	1.8 ± 0.50‡	5

*Injured only. †t = 11.24, $P < 0.001$. ‡t = 6.43, $P < 0.001$.

Injuring the rabbit optic nerve generally decreased methionine incorporation by the corresponding retinal proteins relative to the contralateral side ($L/R < 1$) (Table 1). In contrast, when injured rabbit optic nerves received transplants or implants of substances originating from regenerating fish optic nerves, a significantly increased incorporation of [^{35}S]methionine into the retinal proteins was observed ($L/R > 1$) (Table 1). Implants containing medium conditioned by intact fish optic nerves resulted in a slight increase ($L/R = 0.93 \pm 0.2$; $n = 4$). This may suggest the production of active substances by the intact nerve, albeit at a lower level. A "wrap-around" implant of silicone tubes containing an irrelevant protein such as bovine serum

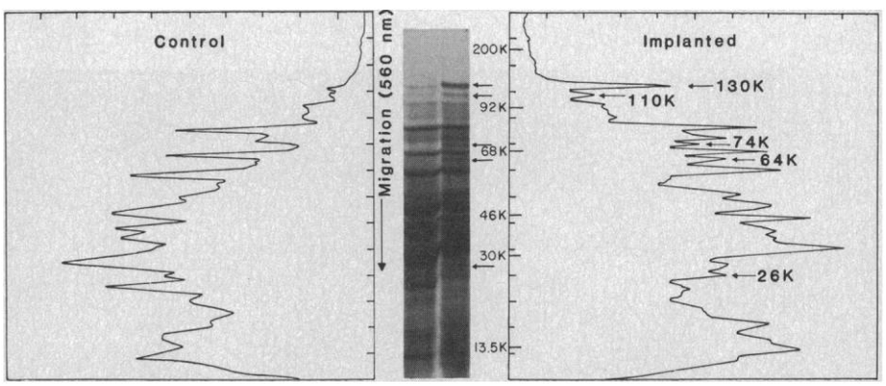

Fig. 1. Gel analysis (SDS-PAGE, 7 to 20 percent) of rabbit retinal protein (100,000*g* supernatant fraction) labeled with [^{35}S]methionine. The retinal preparations were derived from untreated optic nerves (control) and optic nerves that had been injured and implanted with substances originating from regenerating fish optic nerves (implanted). Polypeptides exhibiting major changes (increase or newly appearing) are marked by arrows. This pattern was reproducible in ten tested preparations derived from distinct retinas.

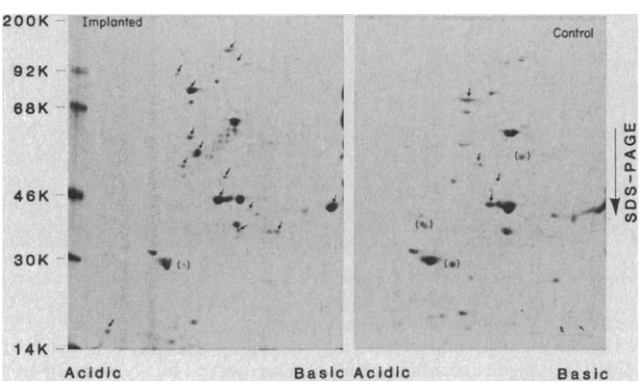

Fig. 2. Two-dimensional gel analysis of the retinal protein prepared as described in Fig. 1. Samples for the first dimension were prepared in denaturing solution containing 9.5*M* urea, 2 percent NP-40 (a nonionic detergent), 2 percent ampholines (1.6 percent *p*H 5 to 7, 0.4 percent *p*H 3 to 10), and 5 percent 2-mercaptoethanol. The second dimension was performed on a slab gel containing 10 percent acrylamide. After electrophoresis in the second dimension, the gels were fixed, stained, and processed for fluorography. Spots demonstrating increased labeling are denoted by arrows. Spots showing reduced labeling in the implanted sample relative to the control are enclosed in parentheses.

albumin (BSA) when introduced to an injured optic nerve had no effect on protein synthesis (L/R = 0.61). Implants of silicone tubes containing media conditioned by injured or intact sciatic nerves of rabbit, also had no effect (L/R ratio of 0.78 and 0.51, respectively).

In addition to the general increase in labeling induced by substances originating from the regenerating fish optic nerve (Table 1), the labeling of several specific polypeptides also increased (Figs. 1 and 2). (The retinal proteins in the contralateral uninjured side were used as a control.) The most prominent changes as analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were in polypeptides of apparent molecular weights 130,000 (130K), 110K, 74K, 64K, and 26K (Fig. 1). Two-dimensional gel analysis revealed the increased labeling of additional polypeptides, especially 50 to 55K; 42 to 46K, and 33 to 37K (Fig. 2). Some of these latter polypeptides are of similar molecular weight to those described as increasing in regeneration (*4–6, 8–10*), including those identified as "growth-associated proteins" by Skene and Willard (*10*).

After being injured, neurons of lower vertebrate CNS sprout prolifically in culture (*11, 12*). To determine if the induced biochemical changes we observed are associated with growth activity, we measured the ability of the retina to sprout in culture. Retinas of injured optic nerves from rabbit into which segments of regenerating fish optic nerves were transplanted did sprout in culture (Fig. 3a). That the fibers are neuritic was confirmed by scanning electron microscopy, which revealed a growth cone at the growing tip (Fig. 3c). No sprouting could be observed in retinas of injured nerves that received the silicone tubes containing BSA (Fig. 3b). Both protein synthesis and sprouting activity were measured in the rabbit retinas. The observed changes are similar to those described in retinas of regenerative systems (*2, 6, 20*). This similarity suggests that our induced regeneration-like response originates from the retinal ganglion cells.

This study demonstrates that the visual system of an adult rabbit (an example of a mammalian CNS) can express regenerative responses if appropriate external factors are provided. The state of the nonneuronal cells providing the factors seems to be crucial. Thus, although medium conditioned by regenerating fish optic nerves was effective, medium conditioned by intact fish optic nerves had only a small effect. This increased activity may stem from production of new molecules or from an increased number of preexisting ones. Alternatively, the higher activity may stem from reduced activity of inhibitors or an increased activity of materials which neutralize them. The ineffectiveness of the medium conditioned by peripheral nerves may suggest that these nerves produce lower amounts of triggering factors or factors with specificity distinct from that provided by the fish optic nerves. Furthermore, the results indicate that the response machinery within the retinas of injured optic nerves of the adult rabbit responsible for receiving the triggering factors is intact and evolutionarily conserved. In mammals the nonneuronal cells and not the neuron seem to be deficient in their ability to provide the active factors. Substantiating this, implantation of media conditioned by injured optic nerves of adult rabbit has no effect on methionine incorporation ($L/R = 0.8 \pm 0.2$; $n = 4$).

Our results suggest that the failure of nonneuronal cells surrounding injured neurons of mammalian CNS to provide the appropriate factors may be a reason for their poor ability to regenerate.

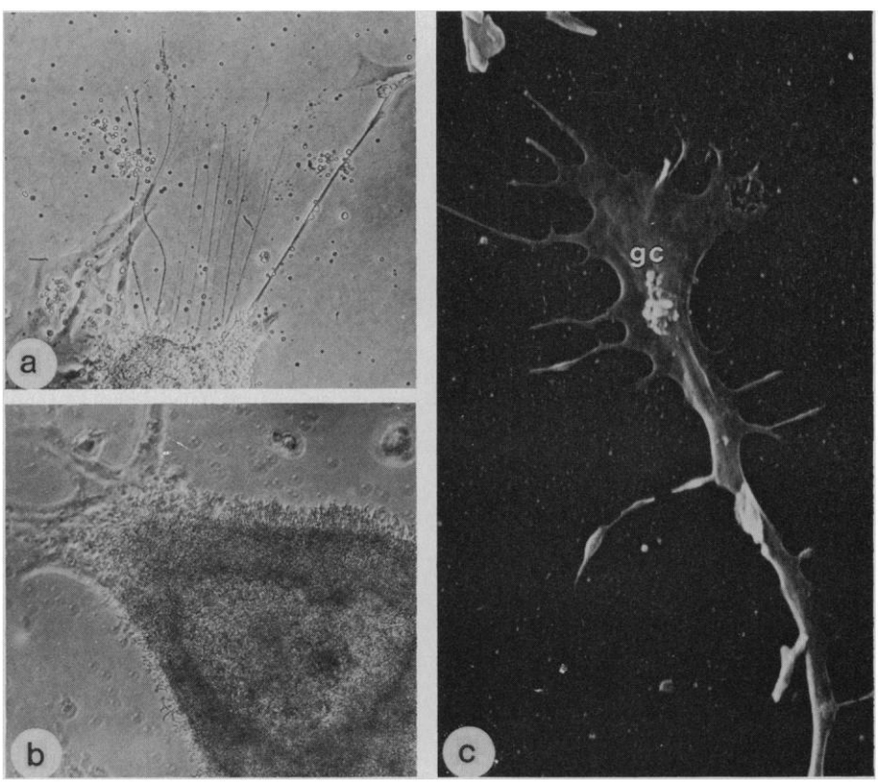

Fig. 3. Neuritic outgrowth from rabbit retinas, in culture. Retinas were cut into 400-μm squares and cultured on dishes coated with poly-L-lysine (0.1 mg/ml, Sigma) in DMEM supplemented with fetal calf serum (5 percent, Biolab) gentamicin sulfate (10 μg/ml, Sigma), Hepes buffer (20 m*M*, Sigma), and glutamine (Biolab). Three retinas in each group were separately examined. (a and c) Photomicrographs of cultured pieces of rabbit retina of optic nerve that was injured and grafted with substances originating from regenerating optic nerve of fish. Abbreviation: gc, growth cone. (a) Approximate magnification, ×60. (c) Scanning electron microscopy of a growing fiber; approximate magnification, ×8050. (b) Micrograph of cultured rabbit retina of an injured optic nerve, implanted with a silicone tube containing BSA; approximate magnification, ×120.

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Habitat Selection in a Clonal Plant

Abstract. *Rhizomatous growth may permit the nonrandom placement of ramets into different environments, but whether clonal plants are able to use this means to exercise adaptive habitat choice is not known. Western ragweed (*Ambrosia psilostachya*) plants are shown to preferentially colonize nonsaline soil over saline soil patches, and clones with the strongest preference for nonsaline soil are those least able to grow when restricted to saline conditions. In clonal plant species, nonrandom associations of genotypes with specific environments may thus reflect habitat selection by plants as well as selective mortality imposed by different habitat patches.*

Active habitat selection is vital to the behavioral ecology of many animals. Although immobility precludes habitat selection by individual growing plants, it has been suggested that phenomena analogous to habitat selection could occur in clonal plant species with rhizomatous growth (*1*). The selective placement of vegetative units into nutrient-rich or otherwise optimal microsites may enable clonal plants actively to choose habitats for future growth and reproduction. However, there is little experimental evidence that adaptive habitat choice indeed occurs in rhizomatous plants (*2*). I report that plants of the perennial herb western ragweed (*Ambrosia psilostachya*: Compositae) (*3*) selectively colonize contrasting habitat patches in a complex environment. Furthermore, genotypes of this species differ in their degree of discrimination between optimal and suboptimal patches, indicating the potential for habitat preference to evolve in response to natural selection.

On natural saline flats in the Great Plains, clones of western ragweed occupy a wide range of soil salinities (*4*). Salt depresses plant growth (*4*), but there is extensive genetic variation in salt tolerance in this species (*5*). Field transplants of clones spanning a range of tolerance levels indicate that ramet survivorship in saline habitats is greater among more salt-tolerant genotypes (*6*). Soil salinities may be sharply heterogeneous on a local scale (<0.5 m) (*4*); thus, a capacity for nonrandom colonization of different soil types would have important consequences for the process of natural selection in ragweed populations.

The following experiment was conducted to evaluate (i) whether western ragweed was able to select between saline and nonsaline microhabitats and (ii) the magnitude of genetic (clonal) variation for habitat preference in this species. Six replicate ramets (stems) of nine distinct clones (*7*) were individually rooted in low-salinity soil in the center of long narrow pots (18 by 46 by 13 cm) in a greenhouse. A gradient in soil salinity was maintained by repeatedly watering one end of the pot (15 cm) with 1 percent NaCl (*8*) and flushing the rest of the pot with tap water to prevent salt accumulation (*4*). Each plant thus had equal opportunity to invade saline and nonsaline patches at the opposite ends of its pot. After 3 months, the plants had produced underground rhizomes in the saline and nonsaline sections of every pot, and numerous shoots began to emerge from the soil surface. Emergence of shoots declined after 4 months; each plant's habitat preference was then calculated as the proportion of all shoots that emerged in nonsaline soil. A value of 0.5 signified equal placement of shoots in both habitats (no preference); values less or greater than 0.5 indicated respective preference for saline or nonsaline soil. Finally, the distribution of shoots that failed to emerge was quantified.

Table 1. Habitat preferences of nine western ragweed clones in the greenhouse experiment. Preference was calculated as the proportion of total shoots emerging in nonsaline soil.

Clone	Rank of salt tolerance*	Proportion of emerged shoots in nonsaline end†
A	1	0.60 (216)‡
B	4.5	0.64 (210)
C	2	0.71 (55)
D	4.5	0.73 (200)
E	6	0.73 (177)
F	3	0.89 (53)
G	8	0.89 (95)
H	9	0.92 (80)
I	7	0.96 (74)
Total		0.74 (1160)

*High tolerance = 1. †χ^2 texts indicate that every clone deviates significantly from equal shoot placement in the two habitats ($P < 0.005$). ‡Numbers in parentheses are total emerged shoots for each clone in saline and nonsaline ends, summed over six pots (five for B).

The initiation and development of new vegetative shoots was strongly biased towards nonsaline soil. Of 2730 total shoots initiated in all 53 pots (average 51.5 shoots per pot), 67 percent occurred in nonsaline soil ($\chi^2 = 319$, $P < 0.0001$). Furthermore, the proportion of shoots that emerged from the soil surface was significantly greater in nonsaline (47 percent) than in saline soil (34 percent) ($\chi^2 = 18.06$, $P < 0.001$). The population of emerging shoots was thus strongly concentrated in nonsaline soil ($\chi^2 = 266$, $P < 0.0001$) (Table 1). Mortality of shoots before emergence from the soil was negligible (<10 percent for all clones in both habitats) and did not account for this bias.

All nine genotypes showed a significant preference for nonsaline habitat, yet the intensity of discrimination varied widely among clones (Table 1), ranging from moderate (60 percent of shoots in nonsaline soil: clone A) to very strict (>90 percent in nonsaline soil: clones H and I). The heritability (broad sense) of habitat preference was 0.39 (95 percent confidence interval: 0.16, 0.73) (*9*), indicating substantial genetic control over variation in this trait. In addition, the nine clones in this experiment had previously been evaluated for salt tolerance (that is, growth rate when confined to saline soil) (*5*). Less salt-tolerant genotypes showed a significantly higher concentration on nonsaline soil (Spearman's $r_s = -0.75$; $n = 9$; $P < 0.05$) (Table 1).

These patterns of differential shoot placement may reflect (i) the direct suppression of plant growth by saline soil conditions or (ii) the facultative reduction by plants of resource allocation for