tion site for the shorter mRNA may be heterogeneous, possibly the result of polyadenylation signals at more than one position in apoB mRNA. The same signals are present in the hepatic apoB-100 mRNA but evidently are not utilized since 100% of hepatic apoB mRNAs are full-length 14-kb structures (Fig. 1). The 14-kb intestinal apoB mRNAs (~15% of the total) must also extend for an additional 7 kb before a polyadenylate tail is added (*15*) even though they have the same in-frame stop codon as the more abundant, shorter mRNAs.

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8. The chylous fluid was centrifuged at a density of 1.006 g/ml at 55,000 rpm (20 hours, 10°C, Ti60 rotor) in a Beckman L5-65 ultracentrifuge. The chylomicrons were collected and further purified by centrifugation at a density of 1.006 g/ml. They were delipidated by extraction with acetone and ethanol (1:1, by volume) at −20°C for 18 hours. The precipitate was extracted with the same solvent, washed with diethyl ether, and dried under N_2 and subsequently at reduced pressure at 4°C.
9. Immunoblot analysis of the purified apoB-48 was performed against the following monoclonal antibodies: 2D8 (R. Theolis *et al.*, *Arteriosclerosis* **4**, 498 [1984]), MB47 (S. G. Young *et al.*, *ibid.* **6**, 178 [1986]), and M-34, which binds to apoB-100 and apoB-48 at a site other than the 2D8 binding site (S. R. Silberman, unpublished). Chylous and plasma chylomicron apoB-48 both reacted with 2D8 and M-34 but not with MB47, which is consistent with the finding that chylous apoB-48 is immunologically similar to plasma chylomicron apoB-48.
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Behavioral Recovery Induced by Applied Electric Fields After Spinal Cord Hemisection in Guinea Pig

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Applied electric fields were used to promote axonal regeneration in spinal cords of adult guinea pigs. A propriospinal intersegmental reflex (the cutaneous trunci muscle reflex) was used to test lateral tract function after hemisection of the thoracic spinal cord. An electrical field (200 microvolts per millimeter, cathode rostral) applied across the lesion led to functional recovery of the cutaneous trunci muscle reflex in 25 percent of experimental animals, whereas the functional deficit remained in control animals, which were implanted with inactive stimulators.

MANY ATTEMPTS HAVE BEEN MADE to induce axonal regeneration in injured mammalian spinal cord, with the ultimate goal of restoring behavioral function (*1*). Yet, even in the few cases where clear anatomical evidence of axonal regeneration has been obtained, unequivocal demonstration of functional recovery has not been possible (*2–4*). It is well established that applied electric fields affect development and regeneration of neurites in vitro (*5–7*) and in vivo (*8, 9*) and that they induce regeneration of dorsal column axons in adult mammalian spinal cord (*3*). The present study addresses the effect of electric fields on functional return in mammalian cord by analyzing a simple, quantifiable behavioral response that requires the integrity of a thoracic sensory tract.

The cutaneous trunci muscle (CTM) reflex is a contraction of the back skin in response to cutaneous stimulation (*10*). The CTM originates around the base of the forelimb on either side and spreads backward in a thin sheet, closely applied to the dermis of almost the entire back skin. The motoneurons that innervate it are contained in the cervical spinal cord and project through the brachial plexus in the lateral thoracic nerve (*11*). The reflex is driven by sensory fibers in segmental dorsal cutaneous nerves (DCN). In the rat, the reflex requires a pinch stimulation and is mediated by A-delta and C fibers (*10*). In the guinea pig, the reflex can be elicited by light touch and produces a stereotyped twitching of the skin centered 2 to 3 cm rostral to the point of stimulation. The afferent pathway projects

Fig. 1. Photomicrograph of a horizontal section at the level of the central canal (CC) showing a right lateral hemisection of a guinea pig spinal cord. The hole (m) in the tissue was left by a marker, a short length of Prolene monofilament inserted into the lesion immediately after section. To make the hemisection, an insect pin (000) was pushed dorsoventrally completely through the spinal cord at the midline, then the right side of the cord was cut and the locating pin removed laterally to confirm the section. The dashed line indicates the plane of section, cutting completely through the white matter of the right side. This animal was electrically treated and showed functional recovery by day 56. Rostral, top of photograph. Scale bar, 700 μm.

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ipsilaterally in the ventrolateral cord (*12*). Lateral hemisection of the thoracic spinal cord produces permanent loss of the reflex below the level of the lesion without affecting the response on the opposite side. The receptive field of the CTM reflex is therefore a sensitive indicator of the functioning of lateral tract axons.

Small dc constant-current stimulators were implanted within the peritoneal cavity of anesthetized guinea pigs, and wick electrodes were routed beneath the skin of the left side to the back (*3*). These stimulators delivered 50 μA for no more than 4 weeks (*13*). The field associated with this current flow was estimated to be about 200 μV/mm at the cord surface [from previous measurements (*3*)]. The spinal cord was hemisected at the mid- to lower thoracic level (Fig. 1), and electrodes were sutured to muscle so that the ends rested in small laminectomy sites about 1 cm rostral and caudal of the hemisection, without touching the cord.

After they recovered from surgery, all animals were monitored at 10- to 14-day intervals for the CTM response, both visually and electromyographically by an observer unaware of their experimental status (*14*). Recovery of the CTM response below the lesion within 2 weeks of surgery indicated incomplete lesion, and three such animals were eliminated from the study. Two groups were studied: one in which the stimulator delivered no current (29 animals) and one in which the active cathode was situated rostral to the lesion (28 animals). All known responses of growing nerves to applied fields are directed toward the cathode (*5–9, 15*), and we tried to influence ascending sensory axons.

Table 1. Number of animals recovering CTM reflex in response to applied electric fields after thoracic spinal cord lateral hemisection. Animals were killed at different times after injury and were therefore divided into three groups. Since recovery was first apparent at 56 to 100 days in animals with applied fields, the recovery rate may be underestimated at earlier times.

Response	Number of animals surviving after injury			Total animals
	50–59 days	60–69 days	70–140 days	
Experimental group				
CTM reflex	2	2	3	7
No CTM reflex	5	7	9	21
Total	7	9	12	28
Control group				
CTM reflex	0	0	0	0
No CTM reflex	12	5	12	29
Total	12	5	12	29

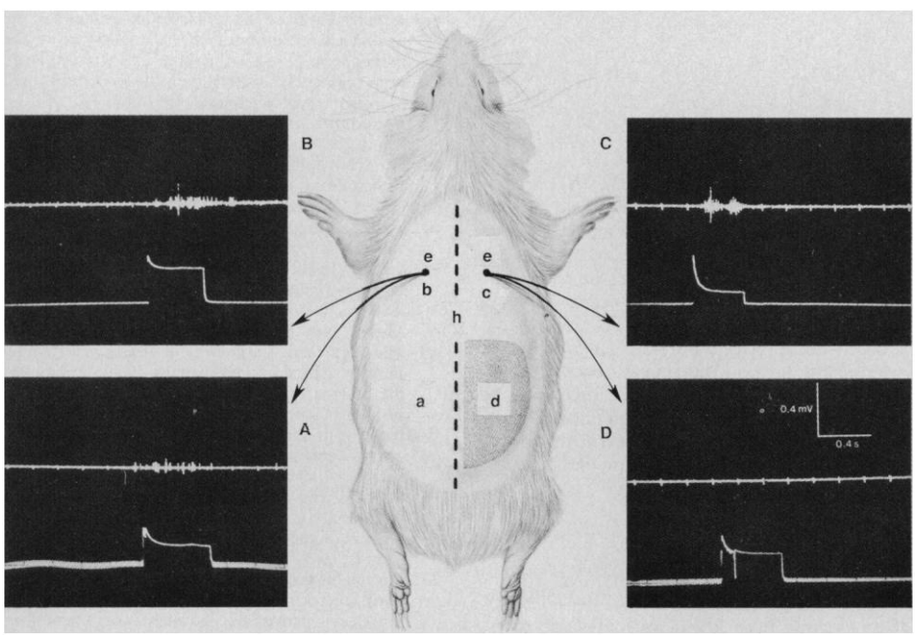

Fig. 2. Electromyograph (EMG) recordings of CTM responses in a control animal at 61 days after hemisection; EMGs were recorded from subdermal wire electrodes, amplified with a Grass P15D preamplifier, and displayed on a Tektronix 5113 oscilloscope. Electrodes (e) were placed in the brachial region on either side of the midline (dashed line). Stimulation was performed by lightly touching the shaved skin with a pair of watchmaker's forceps (without pinching). Timing of stimulus contact (upward deflection in each of the four lower traces) was obtained by connecting the forceps in a circuit, which included a battery, and was completed through the animal to a moist ground electrode beneath the foot. Current was limited to <1 μA. The surface of the skin was dampened at the stimulus site for electrical contact. Traces A, B, C, and D show examples of EMG with stimuli at sites a, b, c, and d. Trace D shows lack of response to stimulation below the hemisection (h). The unresponsive area (stippled area) was a persistent deficit after hemisection. The small repetitive signal on all traces represents the electrocardiogram.

The CTM reflex could still be elicited from the side contralateral to a hemisection of the thoracic cord and from the skin rostral to the lesion on the ipsilateral side. Stimulation of the skin more than 1 cm below the lesion on the ipsilateral side did not normally elicit a response except a withdrawal of the whole body at relatively high threshold (*16*). The CTM responsiveness in a guinea pig 61 days after hemisection is shown in Fig. 2. There was no spontaneous return of sensitivity to light touch below the lesion for the duration of the experiment in controls (Table 1). In preliminary studies, we found no return of the reflex in five animals that were hemisected (but without implants) and survived 6 to 10 months after injury.

In the experimental group, 25% of the animals showed a return of CTM sensitivity below the level of the lesion. This return of sensitivity involved a gradual decrease in the area of insensitivity, beginning between 56 and 100 days after injury (Table 1) and (Fig. 3). Two animals showing functional recovery were killed shortly afterward. In five animals recovery was observed to persist from its initial observation until they were killed [10 days in one animal, 35 days in two more, 50 and 77 days in the remaining two (*17*)].

We tested the possibility that the applied fields affected the peripheral innervation of more rostral or contralateral DCNs rather than central processes of those that had been transected. In one recovered animal, we exposed the large DCNs under pentobarbital anesthesia. A 4-cm skin incision was made, 1 cm to the right of the midline. We then redefined the area of recovery, where tactile stimulation below the level of the lesion elicited contraction of the skin (Fig. 3). We recorded this and the CTM response on the intact side, then severed three DCNs below and ipsilateral to the hemisection and recorded complete loss of responsiveness in the skin normally innervated by these nerves. This left an island of sensitivity caudal to the cut DCNs. Severing the two nerves innervating this area resulted in complete loss of the evoked CTM response on the right side below the hemisection. After perfusion fixation the most rostral cut DCN was found to enter the spinal cord through the T11 dorsal root, 7 mm below the original hemisection. We confirmed these observations on a second animal. Thus, functional recovery initiated by the applied field was mediated by changes induced in central processes of the reflex loop and was not mediated by changes in the sensory innervation.

Applied electrical fields can (i) increase the rate of regeneration (*8, 18*), (ii) decrease the degree of retrograde degeneration (*9*), (iii) increase nerve branching (*8*), (iv)

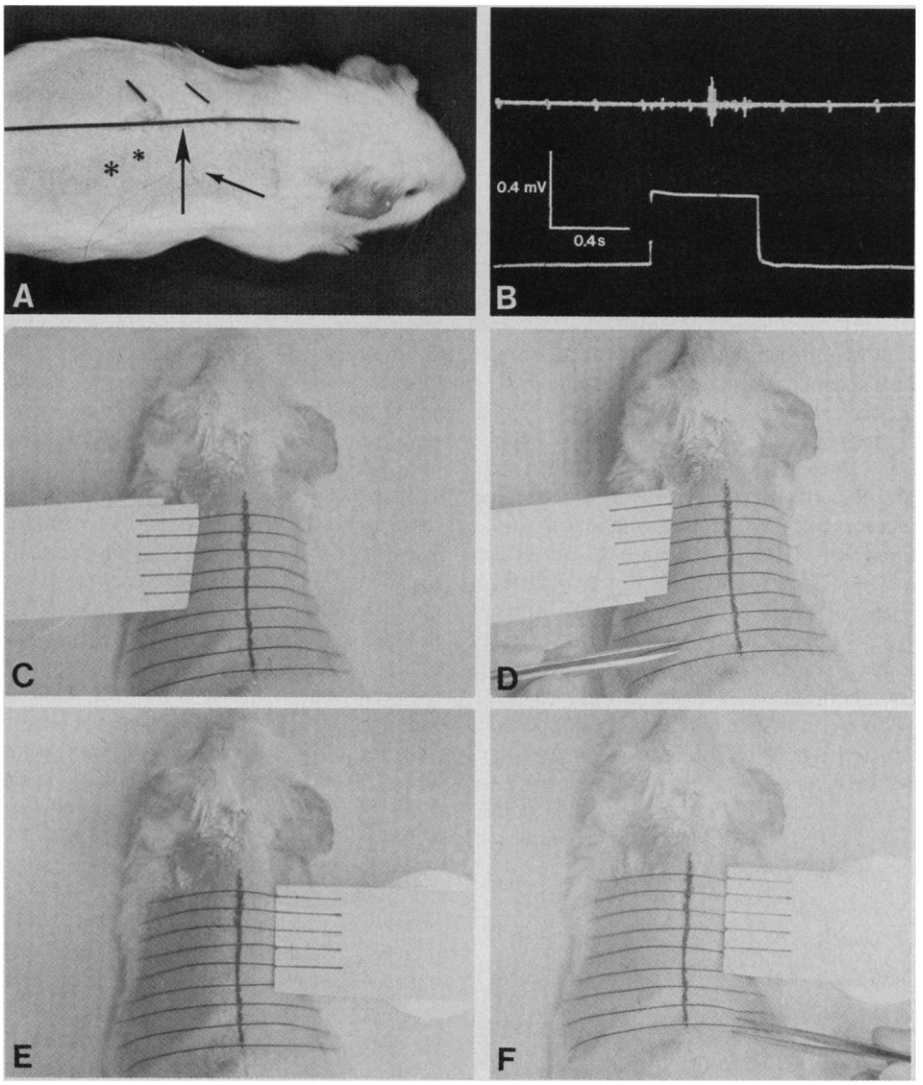

Fig. 3. CTM responses in an animal showing functional recovery with applied electrical field, 65 days after hemisection. (**A**) Placement of a right lateral EMG electrode (small arrow), the hemisection (large arrow), and representative sites of stimulation below the level of the lesion that elicited clear CTM responses (asterisks). Small pieces of tape were used to mark the animal's midline and the ends of the subdermal wick electrodes. (**B**) EMG from the upper right quadrant of the CTM. Lower trace shows stimulus contact at the site of the more lateral of the two asterisks depicted in (A). (**C**) The upper half of the animal was shaved and depilated, and drafting tape lines were applied to the skin. The midline was marked with a pen. Lines drawn on card were aligned with the grid on the animal's back (*10*). (**D**) Tactile stimulation at the level of the lesion on the intact side. Contraction of the CTM caused grid lines on the back to draw away from the index lines. This response could be elicited by stimulation at any level on the left flank. (The wick electrodes underneath the skin did not interfere with the normal reflex.) (**E** and **F**) Tactile stimulation below the hemisection elicited clear CTM response in this animal, drawing grid lines on the skin away from the reference lines.

strongly orient neurites toward the cathode (*6, 7, 19*), (v) increase degeneration in fibers facing the anode (*9, 20*), (vi) increase filopodial activity in developing neurites (*19*), and (vii) increase the proportion of neuroblasts that continue development in culture (*6*). Therefore, it was not unexpected that applied electric fields to some extent sustain axonal regeneration in the mammalian spinal cord (*3*). We provide evidence that such regeneration can be associated with functional recovery, although precise mechanisms are unknown. This recovery of function in the CTM reflex is probably the result of axonal regeneration of the kind observed after electric field application to the dorsal column lesion, where axons regenerated around the lesion through relatively undamaged parenchyma (*3*).

In summary, applied electric fields appeared to affect axons that were severed by lateral hemisection, and this facilitated regeneration was sufficient to yield functional recovery of the intersegmental reflex in a significant proportion of animals. This finding implies that the regenerated axons are able to make relevant synaptic connections, although they may be anatomically inappropriate. Recovery of function based on apparently inappropriate synaptogenesis occurs after spinal cord lesions in goldfish (*21*) and lamprey (*22*). Facilitated regeneration in the adult mammal may yield functional return similar to endogenous recovery in nonmammalian vertebrates.

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12. The reflex is unaffected by cutting thoracic dorsal and dorsolateral tracts but is abolished by lateral sections that spare the ventromedial quadrant of the cord. Hence, the sensory fibers responsible for the reflex appear to run in the ventrolateral tracts. There is no evidence of bilaterality in the sensory pathway to ipsilateral motoneurons.
13. According to the battery capacity (35 mA/hour), the stimulator could deliver 50 μA for 29 days.
14. For CTM reflex testing, animals were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally). The reflex is very resistant to this anesthesia, and the responsiveness of the contralateral side allowed any oversedation that might occur to be detected readily.
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Phylogenetic Relations of Humans and African Apes from DNA Sequences in the ψη-Globin Region

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Sequences from the upstream and downstream flanking DNA regions of the ψη-globin locus in *Pan troglodytes* (common chimpanzee), *Gorilla gorilla* (gorilla), and *Pongo pygmaeus* (orangutan, the closest living relative to *Homo*, *Pan*, and *Gorilla*) provided further data for evaluating the phylogenetic relations of humans and African apes. These newly sequenced orthologs [an additional 4.9 kilobase pairs (kbp) for each species] were combined with published ψη-gene sequences and then compared to the same orthologous stretch (a continuous 7.1-kbp region) available for humans. Phylogenetic analysis of these nucleotide sequences by the parsimony method indicated (i) that human and chimpanzee are more closely related to each other than either is to gorilla and (ii) that the slowdown in the rate of sequence evolution evident in higher primates is especially pronounced in humans. These results indicate that features (for example, knuckle-walking) unique to African apes (but not to humans) are primitive and that even local molecular clocks should be applied with caution.

THE THREE CONTEMPORARY SPECIES of African apes [common chimpanzee (*Pan troglodytes*), pygmy chimpanzee (*Pan paniscus*), and gorilla (*Gorilla gorilla*)] are the closest living relatives of human (*Homo sapiens*). These species collectively represent a natural monophyletic group, which in turn is closely related to the orangutan (*Pongo pygmaeus*) of southeastern Asia. This genealogical arrangement is no longer in serious dispute, but the phylogenetic relations among humans and African apes are still open to debate (*1, 2*). Questions concerning human origins (man's closest living relative or relatives) remain despite earlier efforts to more fully resolve their relations from both molecular and anatomical evidence (*3*).

The β-globin gene family in primates has been well characterized in terms of its structure and evolution (*4*). In humans and great apes, the ψη-locus is one of six β-related globin genes linked 5′-ε-(embryonic)-^{G}γ-^{A}γ(fetal)-ψη (inactive)-δ-β (adult)-3′ (*5, 6*). By means of clones previously described (*7*), the 5′ and 3′ noncoding flanking regions of the ψη-globin gene were sequenced from common chimpanzee, gorilla, and orangutan [the closest living relative to *Homo*, *Pan*, and *Gorilla* (*1, 2*)], in an attempt to resolve the phylogenetic relations of humans and African apes. These newly sequenced orthologs (an additional 4.9 kbp for each species) were combined with published ψη-gene sequences (*5, 8*) and then compared to the same orthologous region available for humans (*9*). The four aligned sequences spanning nearly 7.1 kbp of noncoding DNA constituted the longest continuous stretch of orthologous DNA currently available for humans and great apes (Fig. 1). Furthermore, the nucleotide sequence from a second human allele of the ψη-globin locus (2.2 kbp) was included in the study (*5, 8*). This sequence allowed us to consider the significance of intraspecific polymorphism in reconstructing phylogenies (*10, 11*).

Pairwise comparisons among the ψη-locus and ψη-flanking sequences of human, chimpanzee, and gorilla reveal that these orthologs have diverged very little from one another (Table 1). The aligned DNA sequences of these three varied by only 1.6 to 2.1%, with human and chimpanzee being most alike. On average, the nucleotide sequence of orangutan differed from those of the other three by 3.6%. These values conform closely to the divergence estimates reported from DNA-DNA hybridization of single-copy DNA (*12*) and from nucleotide sequencing of other noncoding genomic regions (*8, 13–15*).

Only three dichotomous branching patterns are possible for human, chimpanzee, and gorilla (Fig. 2). Phylogenetic analysis of these possibilities by the parsimony method established that the tree with the human and chimpanzee clade is more parsimonious than its two alternatives (*Gorilla* grouping with *Pan* or *Homo* first) by five and six extra mutations, respectively (*16, 17*). Human and chimpanzee are united in the most parsimonious phylogeny by eight putative synapomorphies [shared derived features (*18*)] that represent two transitions (positions 1338 and 4473), three transversions (positions 560, 5480, and 6971), and three gap events (two deletions at positions 1287 and 3054 to 3057 and one insertion in a homonucleotide repeat at position 3272). The two less parsimonious solutions (gorilla grouping with chimpanzee or human first) are supported by only three base substitutions [one transversion (position 5153) and two transitions (positions 5156 and 6808)] and two transitions (positions 34 and 6368), respectively.

Thus, the 7.1-kbp sequences from the ψη-globin region demonstrate that human and chimpanzee are more closely related to each other than either is to gorilla. This arrangement is most heavily supported from independent sources by the DNA-DNA hybridization data of Sibley and Ahlquist [(*12*), and discussion (*19*)]. Other data sets in agreement with this phylogeny include α- and γ-globin protein sequences (*20*), and, to a somewhat lesser extent, the mitochondrial DNA sequences of Brown *et al.* (*21*) [as analyzed by Andrews (*2*) and Hasegawa and Yano (*22*)].

The time of divergence for the initial separation of human, chimpanzee, and gorilla is usually placed somewhere between 5 million and 10 million years ago (*23, 24*). Rates of ψη-globin evolution, as calculated with these dates and the branch lengths of the most parsimonious phylogeny (Fig. 2),

Table 1. Pairwise comparisons of the 7.1-kbp ψη-globin sequences for *Homo sapiens* (HSA), *Pan troglodytes* (PTR), *Gorilla gorilla* (GGO), and *Pongo pygmaeus* (PPY). The following abbreviations regarding base substitutions are used: BP, base positions under comparison; TS, transitions; TV, transversions; and TS/TV, ratio of transitions and transversions. Gaps refer to both insertion and deletion events (*17*). Pairwise percentages of divergences are calculated by the equation: [(TS + TV + gaps)/(BP + gaps)] × 100%.

Species comparison	Substitutions				Gaps	% Divergence
	BP	TS	TV	TS/TV		
HSA/PTR	6974	72	29	2.5	12	1.6
HSA/GGO	6984	77	23	3.3	17	1.7
HSA/PPY	6913	139	61	2.3	33	3.4
PTR/GGO	7020	90	36	2.5	19	2.1
PTR/PPY	6929	151	77	2.0	38	3.8
GGO/PPY	6945	159	66	2.4	33	3.7

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