winter birds consumed less than 20 kcal/day (*10*). Thus, activity level and energy output also cannot be the proximate cause for increased torpor in the winter.

The duration of a bout of torpor may be determined by the energy state of the bird in an interaction between "biological clock" and "biological fuel gauge" (*2*, *4*). This is probably true for animals in certain emergency situations. However, the fact that in my study the *Eucalyptus* population of birds used torpor much more extensively than did summer birds regardless of unusually mild ambient temperatures and abundant food suggests that energy depletion is not used as a cue for torpor in the winter. Instead, an innate circannian pattern of torpor is suggested for this species. In the study areas there is about a 1.5-hour difference in daylight between the two seasons, which is adequate to serve as a photoperiodic cue for a circannian rhythm (*11*). The selection pressure for the evolution of a circannian torpor rhythm has been suggested above: the low ambient temperatures that usually occur in the winter. A circannian cycle of torpor is of special value to an organism living in a region where nocturnal temperatures are seasonally low but where the daytime climate gives no indication of the low nocturnal temperatures to come. Such a climate exists in the Peruvian Andes, where winter days are sunny and mild. In this study, when an individual *O. estella* entered its roost on a winter evening, the temperature was usually no lower than that in a typical summer roost. Yet in most winters the roost temperature probably falls to near freezing by midnight. If the bird enters torpor immediately upon roosting, it will have saved energy that can be spent later that night if ambient temperatures fall low enough to force T_b regulation. Thus, the peculiar climate of the tropical Andes has apparently selected for both a relatively low temperature limit to nonregulated torpor and an innate annual cycle of torpor in *O. estella*. The genetic responses of this species to this environmental selection pressure have enabled it to live year-round on the energetically demanding slopes and plains of some of the highest mountains in the world despite the fact that it is the smallest member of the avifauna of that region.

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Regeneration Electrode Units: Implants for Recording from Single Peripheral Nerve Fibers in Freely Moving Animals

Abstract. *Implantable electrode assemblies that become penetrated by regenerating axons were used to record signals from single sensory and motor nerve fibers associated with leg movement in unrestrained amphibians* (Xenopus laevis). *Such neuroimplants may provide a means for establishing the roles of various muscle afferents and efferents in posture and locomotion, and have potential clinical applications.*

Little direct information is available on the roles in locomotion of the individual sensory and motor nerve fibers supplying mammalian muscles. This is partly due to the difficulty of recording simultaneously from several single peripheral axons during free movement —a procedure requiring implantable arrays of electrodes that stay in place despite movement, while picking up signals from identified sensory and motor neurons. Regeneration electrode units were designed to satisfy these requirements.

We thought that it might be possible to separate small bundles of axons, for either recording or stimulation, by encouraging severed nerve fibers to regenerate through an implant perforated by cylindrical channels (*1*) having built-in electrodes (*2*). Ideally, such channels would be narrow enough to allow selective recording from single units while not greatly impeding regeneration, and would be sufficiently long to permit action potential currents to develop a recordable voltage between a central electrode and the tissue fluid; the peak-to-peak amplitude of the triphasic spike expected from one of the fibers in a channel of length L and diameter is D is $k(L/D)^2$, for small L and D (*3*).

Channeled implants were fabricated by etching silver strands 25 μm in diameter out of epoxy wafers with ferric nitrate, by boring holes through photoengraved electrode patterns, or by drilling through wires embedded in epoxy slabs with 100-μm bits (Sphinx microdrills; Swiss Instruments, Toronto).

The latter technique was simplest and gave satisfactory results. One end of a ribbon formed from ten Teflon-coated silver wires with core diameters of 77 μm (Medwire Corp., Mount Vernon, New York) was dipped in Epon 812 (Ladd Research Industries, Burlington, Vermont). The bulb of hardened epoxy was milled down to two flat faces, parallel to the ribbon of wires and 0.7 mm apart. A channel perpendicular to the flat faces was drilled through each wire (Fig. 1a), then cleaned ultrasonically. Thus, each channel was left with its own central wire electrode. The impedances of these electrodes were determined, and their noise level was predicted (*4*). Units with several leads having impedances less than 20 kilohms or more than 300 kilohms were discarded.

Figure 1a shows how regeneration electrode units were implanted in the thighs of *Xenopus laevis*, anesthetized by immersion in tricaine methanesulfonate (0.2 g/liter) (Fraser, Vancouver, British Columbia). A period of 12 to 25 weeks was allowed for regeneration.

The first evidence that bundles of axons had penetrated the implants was provided by light microscopy. This was corroborated by electron micrographs of channel cross sections (Fig. 1b), which confirmed that axons grow into channels as narrow as 25 μm. Of 17

channels in implants dissected out of 3 animals, 9 contained from 1 to 29 clear axon profiles. A total of 85 penetrating fibers in the cross sections were measured at their greatest dimension; values ranged from 1 to 14 μm, but 80 percent fell between 1 and 5 μm. Although Schwann cells had apparently migrated into the channels, no clear, laminated myelin figures were evident. Patches of electron-opaque material (Fig. 1c) and mesaxons, similar to those believed to characterize early remyelination (5), were sometimes observed. In any case, remyelination of implant-penetrating axons appeared to lag significantly behind regeneration. High magnification (Fig. 1c) showed typical axoplasm with mitochondria surrounded by microtubules in a speckled field of neurofilaments. It seemed likely that the axon profiles were representative of penetrating sensory and motor fibers, but this hypothesis was tested in electrophysiological studies.

Figure 2 summarizes the results of these studies, conducted at 20°C in six reanesthetized animals in which the implant leads had been retrieved from the dorsal lymph space and connected for differential recording. Figure 2a shows the response of an implant-penetrating afferent fiber to elongation of the gas-

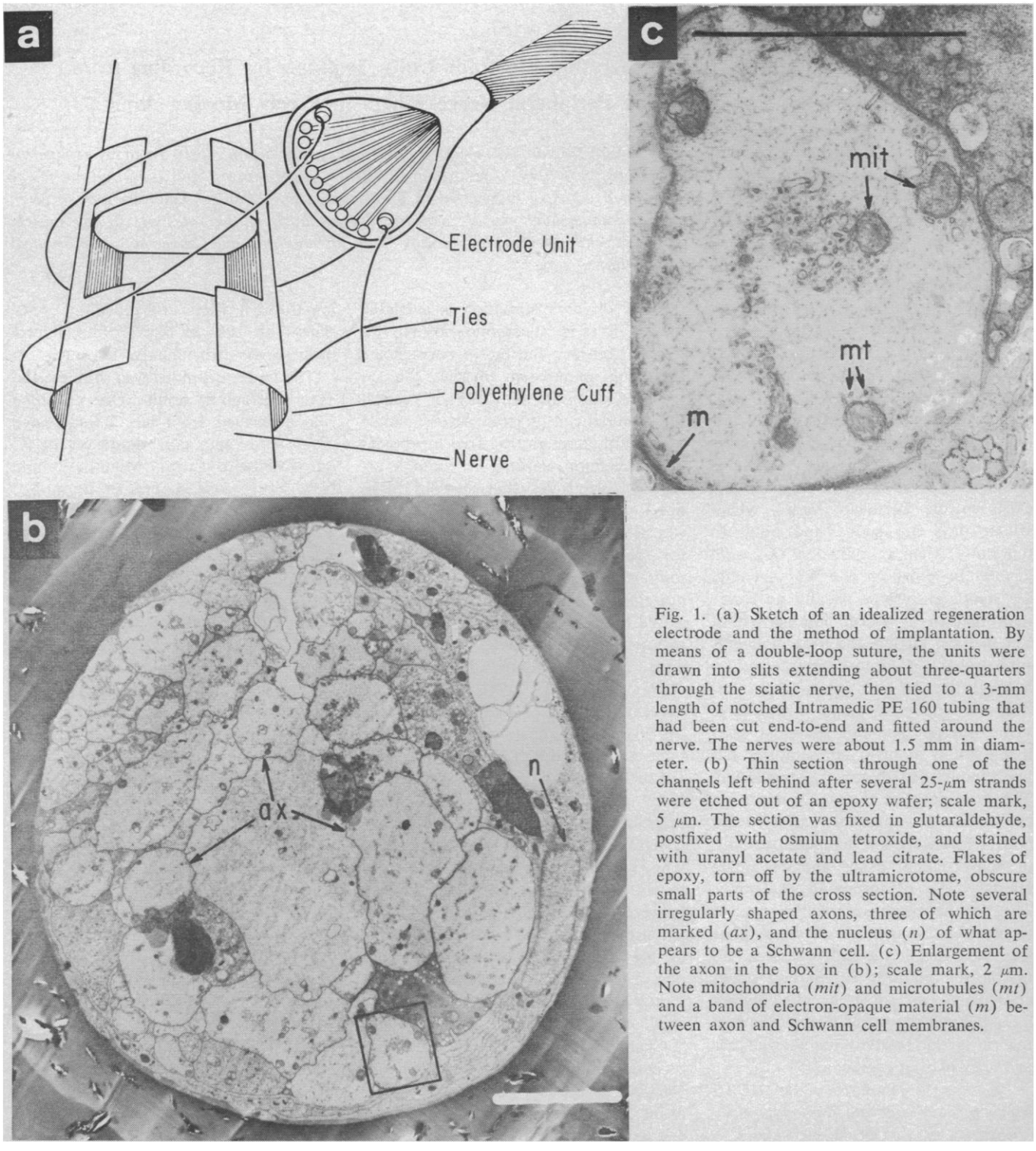

Fig. 1. (a) Sketch of an idealized regeneration electrode and the method of implantation. By means of a double-loop suture, the units were drawn into slits extending about three-quarters through the sciatic nerve, then tied to a 3-mm length of notched Intramedic PE 160 tubing that had been cut end-to-end and fitted around the nerve. The nerves were about 1.5 mm in diameter. (b) Thin section through one of the channels left behind after several 25-μm strands were etched out of an epoxy wafer; scale mark, 5 μm. The section was fixed in glutaraldehyde, postfixed with osmium tetroxide, and stained with uranyl acetate and lead citrate. Flakes of epoxy, torn off by the ultramicrotome, obscure small parts of the cross section. Note several irregularly shaped axons, three of which are marked (*ax*), and the nucleus (*n*) of what appears to be a Schwann cell. (c) Enlargement of the axon in the box in (b); scale mark, 2 μm. Note mitochondria (*mit*) and microtubules (*mt*) and a band of electron-opaque material (*m*) between axon and Schwann cell membranes.

trocnemius muscle, produced by rotating the ankle with a solenoid. The latencies (from onset of stretch to response) of 29 afferent spikes, recorded in two animals, ranged from 10 to 80 msec (mean, 38 msec) (*6*). Single spike responses were recorded from six channels, bursts of two or three spikes from several channels, and a burst of eight spikes from one channel. The activities of the several contributing penetrating axons were separable because of differences in threshold and in the latency and amplitude of spikes, but unequivocal separation may present a problem in some channels in freely moving animals. It is not clear why only a third of the implants contained functioning afferents; inadequate time for receptor reinnervation, shifting of the implant with damage to penetrating fibers, or lead breakage are possible explanations. The afferent action potentials (Fig. 2b) were triphasic and their amplitudes were as great as ten times the noise level (*3, 4*). The duration of the first two phases, between where the linearly extrapolated edges of the wave form crossed the middle of the noise, measured 1.2 to 3.0 msec (mean, 2.1 msec) in nine cases. The third phase was sometimes small and difficult to measure. The single-unit properties of the spikes were all-or-none disappearance as stimulus strength was smoothly reduced; intermittent but complete failure during repetitive stimulation; and a jitter in latency at near-threshold stimulation. A particular spike recorded from one pair of leads was not recordable from the leads supplying adjacent channels; this implies that the action potentials originated in implant-penetrating fibers, not in those that circumnavigated the electrode unit.

In four channels in three animals that were allowed to recover from anesthesia, we recorded bursts of electrical activity synchronous with swimming or kicking movements (Fig. 2c). Each burst contained 2 to 20 spikes, apparently from single units, with wave forms resembling those recorded from afferents. The bursts had temporal patterns similar to motor unit action potentials recorded with needle electrodes in unoperated *Xenopus*. This activity was distinguishable from slower waves of variable amplitude seen on six occasions, which presumably represented the mass activities of muscles near the implant site. Further evidence that the bursts did not originate in muscles or bundles of nonpenetrating axons lies in the fact that the properties of any given burst train were recordable from only one of the channels. The bursts presumably originated in penetrating motor (rather than sensory) fibers, because stimuli delivered through the leads from which they were recorded evoked twitches (Fig. 2d), with latencies between 20 and 25 msec. These twitches, recorded by a transducer connected to the dorsum of the foot, did not arise from excitation of nonpenetrating fibers, for attempts to stimulate through neighboring channels failed to elicit twitches despite a tenfold increase in stimulus strength.

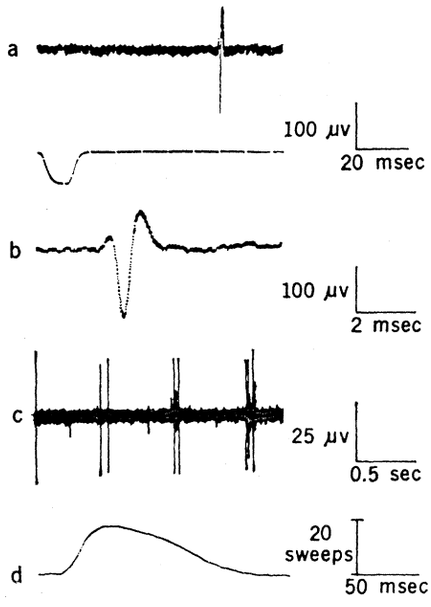

Fig. 2. (a) Response (upper trace) of a single implant-penetrating sensory nerve fiber to a brief muscle stretch (lower trace). (b) Expansion of action potential in (a). (c) Burst discharge recorded from a single implant-penetrating motor nerve fiber in a swimming animal. (d) Computer average showing the time course of a twitch evoked from an unknown number of gastrocnemius motor units, with the neuroimplant as a stimulating device.

Regeneration electrode units may also be useful for recording from nerve fibers in mammals, but we have yet to determine whether mammalian peripheral axons can regenerate through the narrow channels in these devices, let alone mature and be identified by electrophysiological means. If this technique is successful in mammals, the devices offer the intriguing possibility of permanently interfacing electronic components to sensory and motor axons in man. For instance, in amputees, a regeneration electrode unit might isolate enough channels of motor information to separately activate several servomotors in a highly sophisticated prosthetic arm, even one with independent, powered digits. There are other difficulties bearing on the avoidance of tissue reactions and the transmission of neural information through the skin. However, we are encouraged at this early stage that muscle nerve fibers, at least in amphibia, will regenerate back to their end organs through channels as narrow as 25 μm and that action potentials from single sensory and motor axons can be recorded through neuroimplants.

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4. The resistance from the center of a saline-filled channel of dimensions L and D is $\rho L/(\pi D^2) = 22$ kilohms; where $L = 700$ μm, $D = 100$ μm, the saline resistivity $\rho = 100$ ohm-cm. The resistance of the bare tip of a Teflon-coated silver wire in saline is 50 to 100 kilohms at 1000 hertz. Assuming a bandwidth of 10 to 20 kilohertz for an electrode of 100 kilohm total impedance at 20°C gives a minimum Johnson noise level of 5 μv.
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