pension culture were prelabeled with [2-^{3}H]-glycerol (6μCi/ml, Amersham, Ontario). The cells were treated with IFN and assayed for diacylglycerol levels as described by Habenicht *et al.* (*14*). Similarly, IFN-treated fibroblasts prelabeled with myo-[2-^{3}H]inositol (10 μCi/ml, Amersham, Ontario) were assayed for inositol phosphate levels according to the method of M. J. Berridge, J. P. Heslop, R. F. Irvine, K. D. Brown, *Biochem. J.* **222**, 195 (1984). IFN's α, β, and γ used in this study were recombinant human IFN's obtained from Triton Biosciences and Dr. W. Berthold. The rabbit polyclonal antibodies to human IFN β were produced in this laboratory. IFN activity was standardized to NIH-WHO-GO2-901-527 (IFN α), NIH-WHO-GO2-902-527 (IFN β), and Gg-901-530 (IFN γ).

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Central Projections of Identified, Unmyelinated (C) Afferent Fibers Innervating Mammalian Skin

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Unmyelinated (C) fibers are the most numerous sensory elements of mammalian peripheral nerve and comprise many of those responsible for initiating pain and temperature reactions; however, direct evidence has been lacking as to where and how these fibers terminate in the central nervous system. A plant lectin (*Phaseolus vulgaris* leukoagglutinin) was applied intracellularly by iontophoresis as an immunocytochemical marker. This permitted visualization of the central terminations of cutaneous C sensory fibers that had been identified by the nature of stimuli that excited them. The central branch of C-fiber units arborized and terminated mainly in the superficial layers of the spinal dorsal horn in defined patterns that related to their functional attributes. Thus, the superficial dorsal horn seems to act as a processing station for signals from fine sensory fibers.

NEURONAL FUNCTION IS DETERmined not only by the characteristics of the individual nerve cell but also by the connections made by the neuron. Therefore, the absence of direct information as to how and where the unmyelinated (C) fibers (the most numerous primary sensory fibers of vertebrates) terminate in the central nervous system has been a hindrance to understanding neural arrangements for the sensory systems of mammals.

The central terminations of myelinated fibers of particular functions have been established by marking individual fibers intracellularly with the tracer substance horseradish peroxidase (HRP) after determining the nature of the afferent messages of the fiber during electrophysiological recordings (*1*). Unmyelinated fibers are extremely fine (less than 1 μm in diameter), and stable intracellular recordings of and intracellular injections into such small fibers have not been possible. An alternative, injecting the larger cell bodies of these neurons in the dorsal root ganglia (DRG), has been problematic because the distance from the spinal cord appears too great for orthograde HRP transport in the very fine fibers (*2*). To circumvent the transport problems we have used a plant lectin, *Phaseolus vulgaris* leukoagglutinin (PHA-L), which readily fills distant processes by orthograde transport (*3*) and can be iontophoresed into cell bodies from micropipette electrodes.

A small rodent (guinea pigs, 150 to 300 g) was used for these experiments to minimize the transport distance between the DRG and the spinal cord. Under surgically clean conditions and deep pentobarbital anesthesia, a laminectomy and dissection exposed either the cervical 2 (C2) or the lumbar 6 (L6) ganglion and a major nerve supplying it—the great occipital nerve for C2 and the sciatic nerve for L6. The nerve was stimulated electrically (once every 2 to 3 seconds) with brief pulses at an intensity sufficient to excite C fibers to establish the afferent fiber's conduction velocity. Fine micropipettes (less than 0.2 μm in diameter at the tip) containing a 2.5% solution of PHA-L dissolved in 0.1*M* phosphate buffer (*p*H 7.4) were used to record intracellularly from the DRG cell bodies. When a recording was obtained from the C-fiber neuron (conduction velocity, 0.3 to 1 m per second), the receptive field of the nerve was explored with a systematic progression of "natural" stimuli—static and moving contact of the skin, skin cooling, radiant heat, mechanical pressure, and noxious mechanical stimulation (*4*). We were only able to classify neurons with cutaneous or immediately subcutaneous receptive fields. Previously established criteria were used to identify each type of C-fiber sensory unit (*5*).

After determining the nature of the stimuli that effectively excited the unit, PHA-L was deposited intracellularly by positive iontophoretic current (10×10^{-9} A for 2 minutes or more). One cell was labeled in each animal as verified by determining that PHA-L appeared in only one DRG cell body. The wounds were closed with standard surgical techniques. An antibiotic was administered and the animal's general condition was monitored while it recovered from anesthesia and for 2 to 7 days afterwards. (Surviving

Table 1. Terminal domains of each type of C fiber. The cells stained in the C2 ganglion were immunohistologically processed after 2 days of survival, and those in the L6 ganglion after 5 to 6 days of survival. The extension of each terminal area (the dense areas of enlargements and terminals in Figs. 1 and 2) in the histological sections was measured with an ocular micrometer or calculated by a computer three-dimensional graphics program. The results with these seven fibers were consistent with observations on less complete arborizations in other similar units stained with PHA-L or HRP. The extent and distribution of the central branches of a fiber were greater than these terminal domains. Laminae of the terminal area were classified according to Rexed (*12*) and Light and Perl (*1*).

Sensory modality of receptive field	Segmental level (ganglion)	Conduction velocity (m/sec)	Terminal area			
			Rostrocaudal extension (μm)	Dorsoventral extension (μm)	Mediolateral extension (μm)	Laminae
High-threshold mechanoreceptor	C2	0.5	280	100	150	II_o, part of II_i
Polymodal nociceptor	C2	0.5	300	50	200	II_i
	L6	0.5	600	150	200	I, II_o, III, IV
Mechanical cold nociceptor	C2	0.5	400	300	150	I, part of II_o
	L6	0.5	450	100	150	I, part of II_o
Low-threshold mechanoreceptor	C2	0.5	380	50	120	II_o, part of II_i
	C2	0.6	300	50	100	II_i, II_o

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animals moved freely and drank or ate spontaneously within 12 hours after the end of the electrophysiological session.) Under deep sodium pentobarbital anesthesia, a transcardiac perfusion with 4% paraformaldehyde and 10% picric acid fixative terminated the experiment.

Histological sections (50 μm thick) of the ganglion, its dorsal root, and adjacent spinal cord were tested for the presence of the lectin by the binding of a specific antibody directed against PHA-L. The antibody was visualized by the avidin-biotin–HRP method (*6*). Composite camera lucida drawings were made of the arborization of the stained fiber in the spinal cord, from all of the histological sections, and three-dimensional reconstructions were produced by tracing the arborizations with a digital interactive graphics system (*7*).

The majority (>80%) of the approximately 100 C-fiber units that were characterized functionally were nociceptors; that is, their activation thresholds were high for all forms of stimulation compared to other sensory units of the tissue, and maximal discharge was evoked only by tissue-damaging stimuli. These included mechanical nociceptors (excited only by strong mechanical stimuli), polymodal nociceptors (responsive to noxious heat, mechanical stimuli, and irritant chemicals), and cold nociceptors [excited by marked skin cooling (<15°C) and by noxious mechanical stimuli]. In addition, we observed C mechanoreceptors (a low threshold mechanoreceptor responsive to gentle mechanical stimulation of the skin and not exhibiting a greater response to noxious stimuli) and, least often, cooling receptors (excited most effectively by innocuous cooling, for example, 25°C). Central termination patterns were established for four types of units—cold nociceptor, polymodal nociceptor, high threshold mechanoreceptor, and C low-threshold mechanoreceptor—from seven PHA-L–labeled C fibers for which apparently complete arborization labeling was obtained. The conclusions are supported by observations on another 15 markings of identified C fibers with PHA-L or HRP for which the labeling of the central terminals was incomplete.

Two major differences were noted in labeling central processes with the PHA-L molecule as compared to earlier experience with HRP. PHA-L was transported much more slowly along the axon than HRP; HRP transport rates are reported as 0.5 mm per hour or more (*1*). Although we did not analyze details of PHA-L transport, it was carried centrally along C-fiber axons at apparent rates of 2 to 3 mm in 24 hours. A second difference was that the lectin tended to cross the dorsal root–spinal cord junction without the apparent block or fading commonly noted with HRP labeling of C-fiber DRG neurons. As in the case of HRP marking of myelinated fibers, immunocytochemical visualization of PHA-L demonstrated an extensive arborization of the fiber's central processes as seen with classical Golgi staining. En passant and end terminal synaptic enlargements (Figs. 1D and 2B) were seen for each well-stained fiber.

The central branch and collaterals of the C-fiber neurons were very fine, often at the limit of light microscopic resolution (0.2 μm). Nonetheless, the arborization of a well-marked fiber generally could be traced unambiguously, without fading, to a terminal enlargement. Therefore, we were confident that a complete pattern was observed for many branches. On the other hand, it was more difficult to be certain about the full rostrocaudal distribution of fibers with the more extensive longitudinal projections exhibited by one lumbar polymodal nociceptor; this is because the concentration of label diminished gradually with distance along a fiber, and after several millimeters of trajectory inside the spinal cord a branching of a faintly stained fiber could be missed. In zones of extensive arborization, numerous

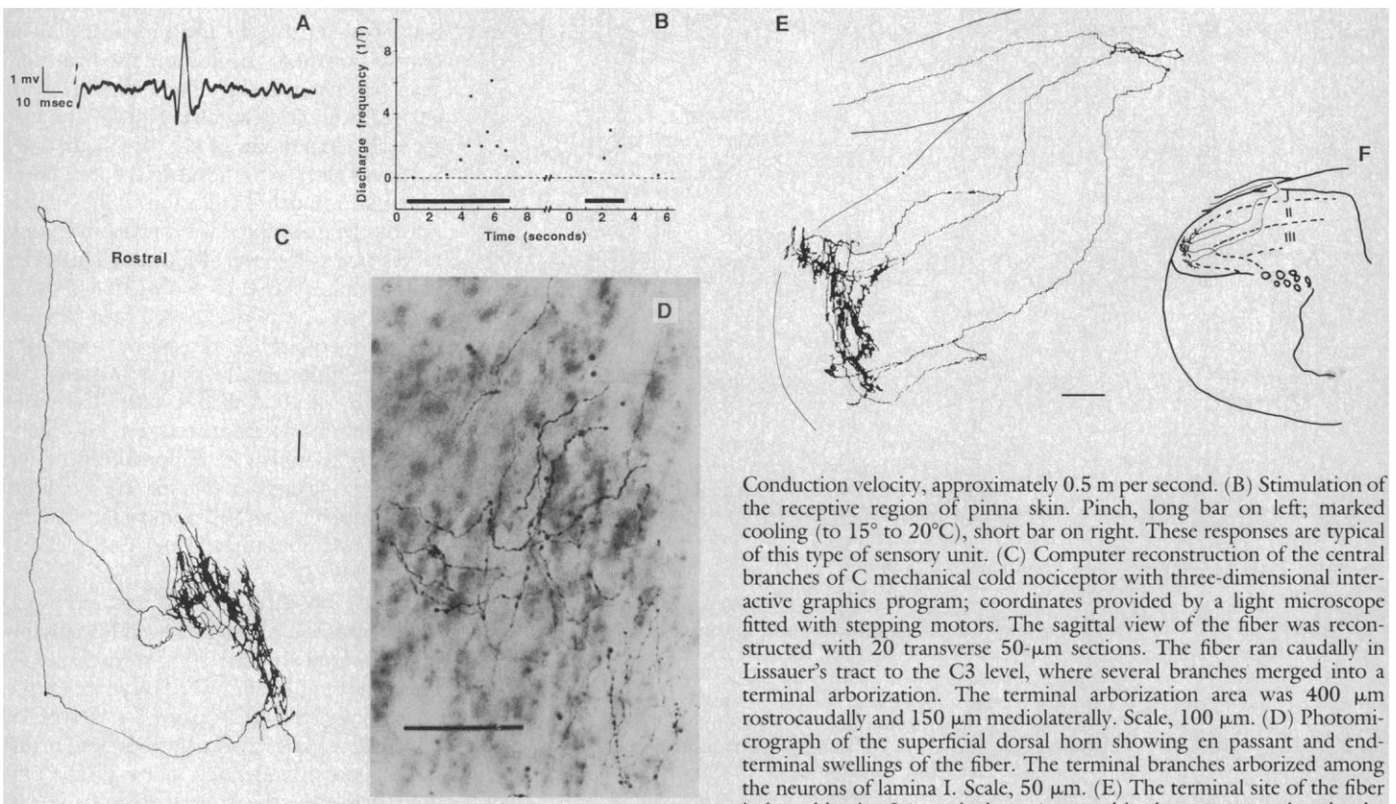

Fig. 1. (A) Responses recorded from the cell body of a C mechanical and/or cold nociceptor in the C2 ganglion. Response to a single electrical pulse to the great occipital nerve recorded at conduction distance of approximately 20 mm. Conduction velocity, approximately 0.5 m per second. (B) Stimulation of the receptive region of pinna skin. Pinch, long bar on left; marked cooling (to 15° to 20°C), short bar on right. These responses are typical of this type of sensory unit. (C) Computer reconstruction of the central branches of C mechanical cold nociceptor with three-dimensional interactive graphics program; coordinates provided by a light microscope fitted with stepping motors. The sagittal view of the fiber was reconstructed with 20 transverse 50-μm sections. The fiber ran caudally in Lissauer's tract to the C3 level, where several branches merged into a terminal arborization. The terminal arborization area was 400 μm rostrocaudally and 150 μm mediolaterally. Scale, 100 μm. (D) Photomicrograph of the superficial dorsal horn showing en passant and end-terminal swellings of the fiber. The terminal branches arborized among the neurons of lamina I. Scale, 50 μm. (E) The terminal site of the fiber in lateral lamina I (marginal zone) rotated in the transverse plane by the graphics system. Scale, 100 μm. (F) Diagram showing arborization of the cold nociceptor terminals relative to boundaries of the dorsal horn laminae.

en passant enlargements of 1 to 7 μm in diameter were seen; a branch typically terminated in a larger swelling at the upper end of this range. The larger swellings were of the size of the central profile found in glomerular arrangements common in the superficial dorsal horn (*8*). In studies of myelinated fiber projections, synaptic contacts have been found only at such swellings (*9*); whether this also applies to C-fiber projections will require studies by electron microscopy. All of the fibers showed both the small en passant enlargements along the course of branches in the region of arborization and larger swellings at the end of most of the branches. Typically the en passant swellings were more numerous in the region of terminal arborization and all but a few enlargements of one unit (a polymodal nociceptor from the lumbar region) appeared in the outer two laminae (I and II) of the dorsal horn.

All but one of the C fibers passed from the dorsal root and ran for some distance in either Lissauer's tract of the dorsolateral spinal cord or rostrocaudally in the superficial dorsal horn itself. Branches left the parent fiber at several loci. This general arrangement, in which a rostrocaudally directed parent fiber periodically branches into the cellular zones of the spinal cord, mimics that established for myelinated primary afferent fibers. In contrast to the arrangement of the C fibers, the longitudinal main branch for most myelinated fibers except those associated with nociceptors is located in the dorsal column (*1*).

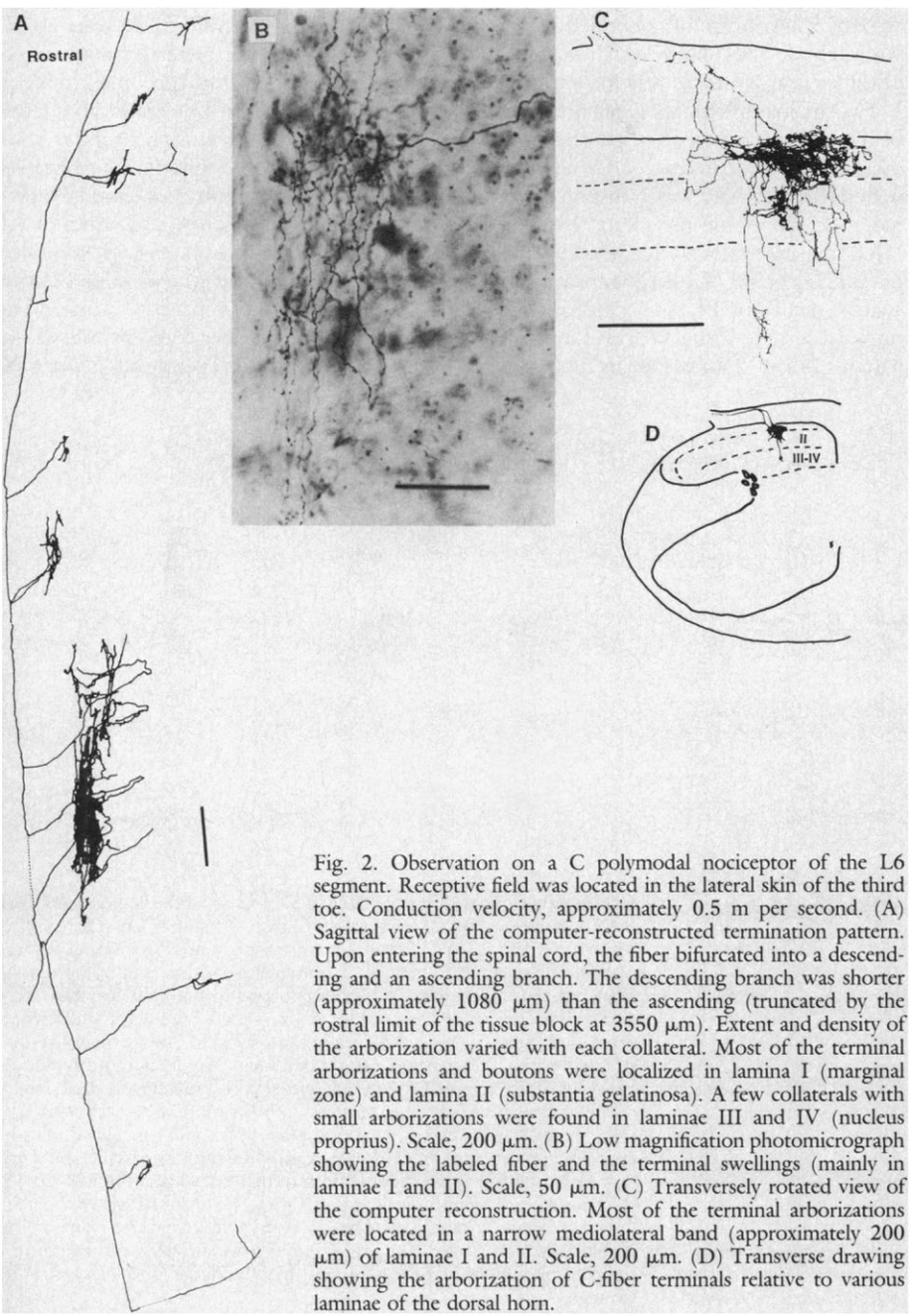

Fig. 2. Observation on a C polymodal nociceptor of the L6 segment. Receptive field was located in the lateral skin of the third toe. Conduction velocity, approximately 0.5 m per second. (A) Sagittal view of the computer-reconstructed termination pattern. Upon entering the spinal cord, the fiber bifurcated into a descending and an ascending branch. The descending branch was shorter (approximately 1080 μm) than the ascending (truncated by the rostral limit of the tissue block at 3550 μm). Extent and density of the arborization varied with each collateral. Most of the terminal arborizations and boutons were localized in lamina I (marginal zone) and lamina II (substantia gelatinosa). A few collaterals with small arborizations were found in laminae III and IV (nucleus proprius). Scale, 200 μm. (B) Low magnification photomicrograph showing the labeled fiber and the terminal swellings (mainly in laminae I and II). Scale, 50 μm. (C) Transversely rotated view of the computer reconstruction. Most of the terminal arborizations were located in a narrow mediolateral band (approximately 200 μm) of laminae I and II. Scale, 200 μm. (D) Transverse drawing showing the arborization of C-fiber terminals relative to various laminae of the dorsal horn.

Terminal projections of the C-fiber units were remarkably circumscribed and distinctive (Table 1). Our sample does not include observations on sensory units with receptive endings in subcutaneous tissues.

The mechanical cold nociceptors of the cervical and of the lumbar region had virtually equivalent termination patterns, located mostly in lamina I with a few enlargements in the outer part of lamina II (II_o). The termination region for these cells was a limited zone measuring some 400 μm rostrocaudally and 150 μm transversely.

Terminations of a mechanical nociceptor of the cervical region were concentrated in lamina II_o, again in a circumscribed distribution. There were essentially no terminals visible superficially in lamina I or more deeply in inner lamina II (II_i). A similar unit was not successfully marked in the lumbar region.

In the cervical region, terminals from the polymodal nociceptor were mostly concentrated in a region of lamina II_i. At the lumbar level, the same type of unit had a much more extensive and complex projection largely to more superficial layers (principally lamina I but with a substantial number of enlargements in lamina II_o). The lumbar polymodal nociceptor also had a few branches passing through lamina II with enlargements in lamina III and IV and was the only C-fiber unit of our sample that had terminals deep to the superficial dorsal horn. The rostrocaudal distribution of the lumbar polymodal nociceptor was remarkably long; it was traced for over 4600 μm. However, the computer-based reconstruction showed that the arborization extended only 200 μm in the mediolateral direction, consonant with the thin sheets of terminations described by classical studies (*10*). The more extensive distribution seen for this unit, including terminals in regions deep to the substantia gelatinosa (lamina II), confirms observations made by fragmentary staining of certain C-fiber units in our earlier efforts with HRP (*2*).

C-fiber low-threshold mechanoreceptors, units responsive to the most gentle mechanical disturbance of the skin, were found in the guinea pig ear but were very rare on the lower limb; the frequency of this type of sense organ varies markedly with both mammalian species and body location. The two units stained in the cervical region had nearly identical central projections concentrated in a circumscribed field centered in lamina II, extending into both II_o and II_i.

From these observations we conclude the following. First, unmyelinated afferent fibers of the somatic nerves follow general rules for central termination that also apply to myelinated fibers. (i) The termination of the functionally defined kinds of sensory unit appears in a specific region of the spinal gray matter; (ii) the termination pattern of each functionally defined kind of C-fiber sensory unit appears to be characteristic, although regional differences in spinal cord organization, such as those distinguishing cervical from lumbar levels, can influence the pattern. Second, the superficial layers of the spinal dorsal horn—particularly lamina II, the substantia gelatinosa—appear to be the main projection zone for unmyelinated primary afferent fibers from the skin. (Whether this area is also a termination zone for C-afferent units from visceral and other subcutaneous tissues is unknown.) This result is consistent with deductions based on indirect evidence from classical morphological analyses and correlations between functional properties and dendritic arborization of spinal neurons (*8, 11*). Third, a given unmyelinated primary afferent fiber in a central termination zone can have significantly different size enlargements appearing along its course. The size variation may be in the en passant enlargements themselves or between the terminal bouton and the en passant varicosities, a point noted in the past for one type of myelinated sensory axon (*9*). Some en passant enlargements along a collateral were noted to be 0.5 μm in diameter while others were over 2 μm in diameter. Not all branches of an arborization showed such variation in enlargement size. The latter observation suggests that different kinds of synaptic contacts may be made by a given fiber.

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The Little Ice Age as Recorded in the Stratigraphy of the Tropical Quelccaya Ice Cap

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The analyses of two ice cores from a southern tropical ice cap provide a record of climatic conditions over 1000 years for a region where other proxy records are nearly absent. Annual variations in visible dust layers, oxygen isotopes, microparticle concentrations, conductivity, and identification of the historical (A.D. 1600) Huaynaputina ash permit accurate dating and time-scale verification. The fact that the Little Ice Age (about A.D. 1500 to 1900) stands out as a significant climatic event in the oxygen isotope and electrical conductivity records confirms the worldwide character of this event.

IN 1983 TWO ICE CORES, ONE 154.8-M summit core containing a record of 1350 years and another 163.6-m core (core 1) containing a record of 1500 years, were recovered from the Quelccaya ice cap (13°56′S, 70°50′W) with a solar-powered drilling system. About 6000 samples, cut from pits, shallow cores, and the two deep cores were melted in closed containers by passive solar heating in a laboratory tent, placed in polyethylene bottles, sealed with wax, and shipped to Ohio State University. Samples were divided so that microparticle concentrations, size distributions, conductivity, and oxygen isotope ($\delta^{18}O$) measurements could be made on identical samples. Total β radioactivity and pollen and chemical analyses were conducted for 1500 samples. The microparticle and conductivity measurements were made under class 100 clean-room conditions at the Ohio State University, and $\delta^{18}O$ analyses on the summit core were conducted at the University of Copenhagen and on core 1 at the University of Washington. We discuss the 1000-year record, since A.D. 1000, of microparticle concentrations, conductivities, and oxygen isotopes (*1–3*).

Dating of the ice cap cores was accomplished by using several stratigraphic features that exhibit seasonal variability. The initial age for the bottom of the ice cap was estimated from flow-model calculations (*4*) that depend heavily on initial assumptions and boundary conditions. Alternative stratigraphic dating of the core was made possible in the field by examination of visible annual dust layers (Fig. 1) (*1*). The thickness of the annual layers ranged from 1.24 m (ice equivalent) at the surface to 0.01 m at the base. In both cores, the visible stratigraphy was complemented by the preservation of annual variations in microparticle concentrations, conductivity, and oxygen isotope ratios (Fig. 1). The combination of ice core parameters exhibiting seasonal cycles allows clarification of ambiguous features and results in the most reliable time scale. Further time scale refinement results when the two core records are compared.

In general, visible dust layers, characteristic of the dry season (*3*), are associated with high microparticle concentrations, less negative $\delta^{18}O$ ratios, and high conductivities. Increased dry-season particle concentrations are attributed to (i) receipt of intense radiation accompanied by little accumulation (minor sublimation, which leaves the insoluble particles concentrated at the surface); (ii) dominant wind direction from the west and northwest, which transports material from the high, dry altiplano; and (iii) higher dry-season wind speeds, which facilitate entrainment and transportation.

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