Biocompatibility of silicon-based electrode arrays implanted in feline cortical tissue

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The passive biocompatibility of silicon-based electrode arrays was studied in feline cortical tissue. Three types of arrays were used: uncoated, coated with polyimide, and coated with polyimide over an adhesion promoter. Fifteen arrays were implanted for 24 h to determine early tissue reaction to the implantation procedure, and twelve arrays were implanted for 6 months to determine structural and material biocompatibility. Edema and hemorrhage were present around the short-term implants, but involved less than 6% of the total area of the tissue covered by the array. With chronic implants, leukocytes were rarely present and macrophages were found around roughly one-third of the tracks. Remnants of foreign material from the electrodes could be identified in less than 10% of the tracks. Gliosis was found around all tracks, forming an annulus between 20 and 40 μm thick. A capsule was not always present, and never exceeded a thickness of 9 μm. These results suggest that the implantation procedure produces limited amounts of tissue damage, and that the arrays are biocompatible. However, the arrays insulated with polyimide over a primer had significantly greater involvement of macrophages, gliosis, and capsule formation than uncoated arrays and arrays insulated with polyimide without printer, perhaps indicating a reaction to aluminum oxide in the primer.

INTRODUCTION

Recent work in our laboratories has focused on developing a monolithic, silicon-based array of penetrating electrodes for chronic implantation in the cerebral cortex for neuroprosthetic applications.[1,2] The present study was designed to investigate the biocompatibility of this electrode array and the procedure used to implant the array.

The electrode array is made from phosphorous doped monocrystalline silicon, a material known to be nonreactive in cortical tissue.[3] For use in neuroprosthetic applications, the array needs to be insulated. The insulating material chosen for this study was polyimide, which also has been shown to be nonreactive in neural tissue.[4] One problem with polyimide coatings is adhesion to the substrate.[5] An aluminum chelate primer, which is converted to aluminum oxide after baking, can provide acceptable adhesion for polyimide insulation in a saline environment.[6,7]

We tested our arrays in three configurations—uncoated, coated with polyimide placed directly on the silicon, and coated with polyimide after treating the silicon surface with aluminum chelate primer—with two goals in mind: first, to verify that the implantation procedure was not detrimental to cortical tissue and, second, to study the reaction of cortical tissue to chronically implanted electrode arrays. Because we were interested in basic mechanical and material effects, the arrays were passive (i.e., they were not used to stimulate or record from the cortex during the period of implantation).

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MATERIALS AND METHODS

A total of 27 arrays were implanted in the cerebral cortices of eight cats. Each cat generally had two arrays implanted in one hemisphere of the brain for 6 months and two more arrays implanted in the other hemisphere for 1 day just before sacrifice.

Electrode arrays

Each array consisted of a 10 × 10 matrix of electrodes on a 200 μm thick, 4 × 4 mm square solid base. Each electrode was 1.5 mm long and tapered from 80 μm in diameter at its base to a sharply pointed tip. A drawing of an electrode array is shown in Figure 1. The arrays are described in more detail elsewhere.[1,2]

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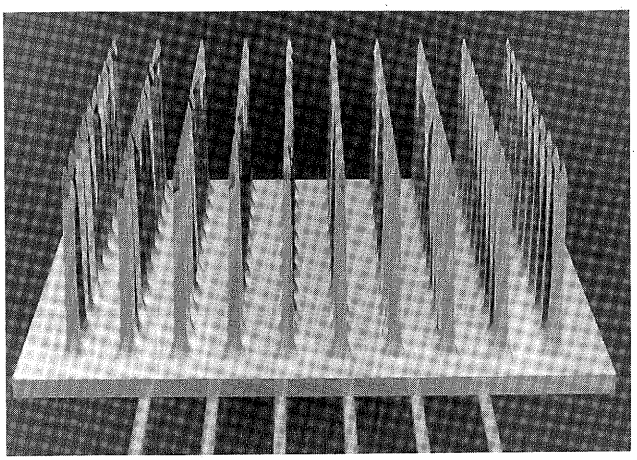

Figure 1. Drawing of a 10 × 10 electrode array. The array is positioned with the electrodes pointing up. The bottom of the picture shows six lead wires coming from the base of the electrode. In the implants used in the present study, no lead wires were present. The spacing between electrodes is 400 μm.

The arrays were fabricated from phosphorous doped monocrystalline silicon (3×10^{14} phosphorous atoms per cubic centimeter), sonically cleaned in acetone for 5 min and isopropyl alcohol for 2 min, and rinsed eight times with deionized water.

One set of implants consisted of uncoated silicon arrays. A second set of implants consisted of arrays coated with polyimide. The liquid polyimide was dripped on the array, the excess was wicked off, and the array was baked, electrode tips down, at 120°C for 30 min. Two more layers were added in the same manner and the array was then baked a fourth time for 1 h at 200°C. A third set of arrays were coated with polyimide in the manner just described, except that a layer of aluminum chelating agent was spun on and baked for 30 min at 350°C prior to application of the first polyimide layer.

All arrays were flash sterilized in an autoclave at 120°C for 5 min with a 10 min drying time just prior to implantation. The numbers and implant durations for the various array types are shown in Table I.

TABLE I
Numbers of Arrays Implanted, as Function of Array Type and Duration of Implant Period

Array Type	1 day	6 months
Uncoated silicon	8	3
Polyimide-coated		
Without primer	0	3
With primer	7	6

Surgical procedures

National Institutes of Health guidelines for the care and use of laboratory animals were observed in conducting these experiments. Cats were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg, i.p.). Sterile surgical techniques were used. Two openings approximately 1 cm across and separated by a few millimeters were made over the cerebral cortex on one side of the skull. The dura was reflected at each site. An array of one type was implanted at one site and an array of a different type was implanted at the other site, using an impact insertion technique as described elsewhere.[8] The dura was closed, each opening in the skull was sealed with silicone rubber, and capped with dental acrylic. The animals were kept for 6 months in a room that allowed freedom of movement and opportunity to feed *ad libitum*.

The surgical procedure was repeated under aseptic conditions 6 months later on the other hemisphere, the dura was closed, the skull openings were sealed with silicone rubber and covered with dental acrylic, and the animal was kept anesthetized for 24 h before sacrifice. At sacrifice the cat was deeply anesthetized, heparinized, and perfused through the heart with a buffered 3.7% formaldehyde solution.

Histological procedures

The tissue was allowed to fix overnight, then the area of the brain containing the implants was exposed and the tissue removed and placed in fixative at room temperature for at least one more day. The array was then removed from the tissue. The tissue was embedded in paraffin, cut in 5 μm sections parallel to the cortical surface (orthogonal to the electrode tracks), deparaffinized, and stained with hematoxylin and eosin. This material was used for quantitative analysis. Other material was embedded in Epon resin and 1 μm sections were stained with methylene blue. This material was used to illustrate details of the tissue reaction to the implants.

Data were gathered from transverse sections of the tissue at three levels: near the tips of the electrodes, near the pial surface, and midway between the tips and bases of the electrodes. The sections were photographed, and several different tissue response parameters were quantified by tracing the area of involvement on a digitizing tablet connected to an IBM PC-compatible computer. Arrays implanted for 6 months were quantitatively examined for macrophages, leukocytes, gliosis, capsule formation, and the presence of extracellular foreign material. Arrays implanted for 24 h were examined for edema and hemorrhage. In analyzing the data, a region of cortex 400 μm on a side was defined as the associated area for each electrode. For each parameter, the number of electrodes involved and the area of involvement were measured at each of the three levels.

The decision as to where the boundary should be drawn to delimit an area of involvement was neces-

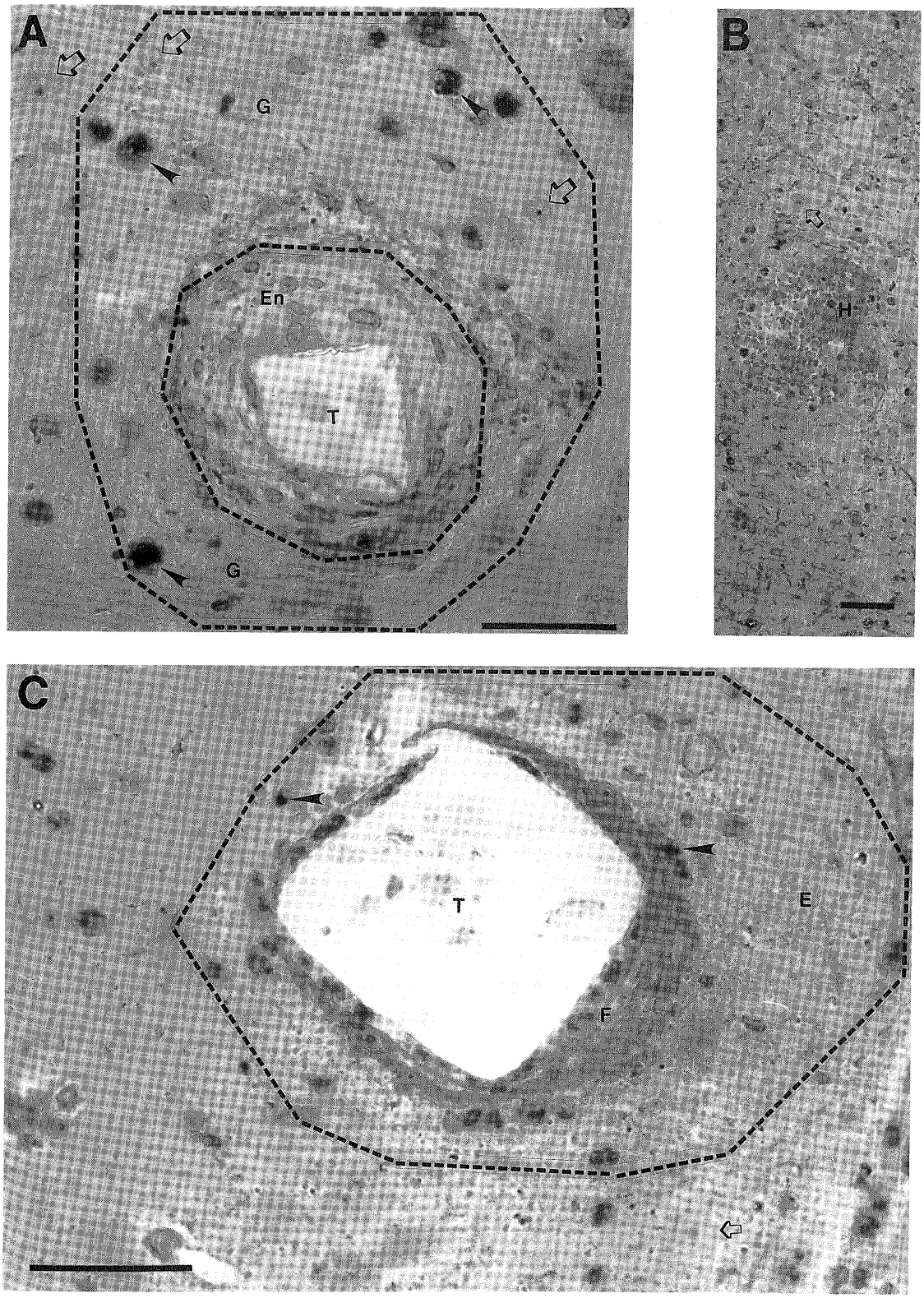

Figure 2. Micrographs of cortical tissue implanted with silicon electrode arrays. **A.** Encapsulation (En) was defined as a compacted barrier of modified fibroblasts around the track (T), shown within the inner dashed line. Gliosis (area G between the two dashed lines) was characterized by astrocyte proliferation appearing as darker stained areas which included swollen astrocytes and modified cells. Macrophages (closed arrows) contained hemosiderin and electrode material. Neuronal bodies, indicated by the open arrows, can be seen near the electrode track. **B.** Hemorrhage (H) was characterized by the presence of extravascular red blood cells. A nearby neuron is indicated with an open arrow. C. Edema (E) appeared as a pronounced lacy area (within the dashed line) resulting from fluid accumulation. The track (T) is surrounded by a fibrin layer (F) in this section from acutely implanted tissue. Leukocytes are indicated by closed arrows. A nearby neuron is indicated with an open arrow. Epon embedded, 1 μm thick sections stained with methylene blue. Scale bars: 30 μm.

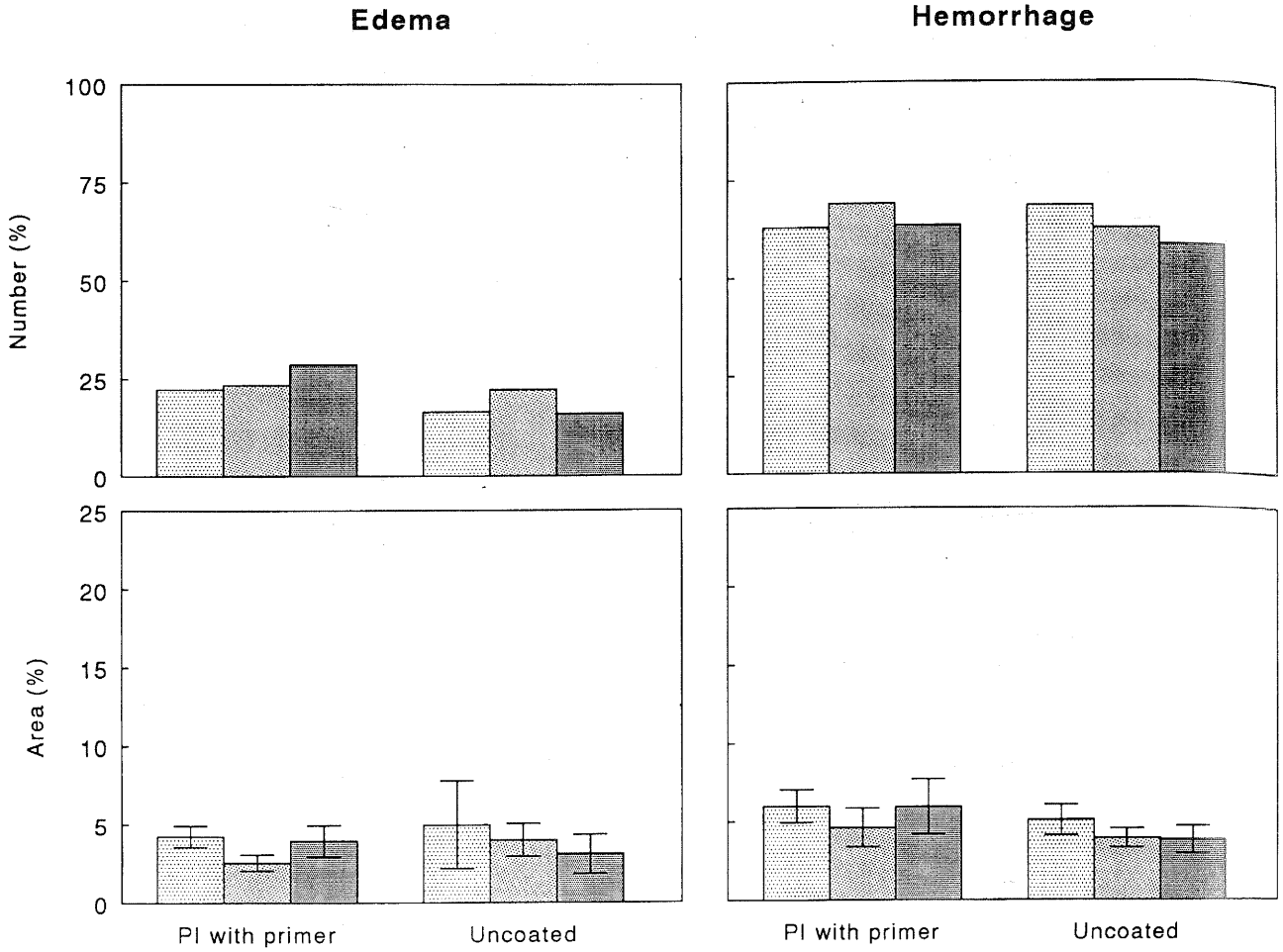

Figure 3. Edema and hemorrhage occurred only in short term implants. The percentages of tracks affected are shown in the upper graphs and the fractions of the total array area affected (with standard deviations) are shown in the lower graphs. Data for two types of arrays are presented: polyimide coated arrays with adhesion promoter and uncoated silicon arrays. Data for three levels along the tracks are shown. The left column in each data set represents data taken from a section near the pial surface. The middle column represents data taken from a section near the middle of the electrodes. The right column represents data taken from a section near the tips of the electrodes.

sarily subjective. We used as a criterion the presence of clearly visible cell or tissue types as illustrated in Figure 2. More sensitive techniques would have expanded the areas somewhat, but we believe the techniques we used are sufficient to show the limit of functionally significant reactions to the implants.

The Mann-Whitney test was used to determine the statistical significance of differences in the extent to which each of these features was present. Comparisons were made between types of arrays within each level and between levels within each type of array.

RESULTS

Acute implants

Edema and hemorrhage were found in acutely implanted tissue. Figure 2(C) shows an example of edema. These areas of tissue had a pronounced lacy appearance as a result of fluid accumulation, but no extravascular red blood cells indicating hemorrhage. Areas of edema often spanned more than one electrode track's area of influence, and so edema was measured as the affected area expressed as percentage of total array area (16 mm^2).

Figure 2(B) shows an area of hemorrhage. This condition is characterized by the presence of extravascular red blood cells. Like edema, areas of hemorrhage generally involved more than one track's area of influence, and so the data for hemorrhage are also expressed as percentage of total array area.

The data for edema and hemorrhage are summarized in Figure 3. The upper pair of plots show the percentages of tracks involved out of the total number of electrode tracks examined at each level, and the lower plots show the averages (with standard deviations) of the percentage of the total array area involved at each level. There were no statistically significant effects of array type or level on either of these parameters for acute implantations.

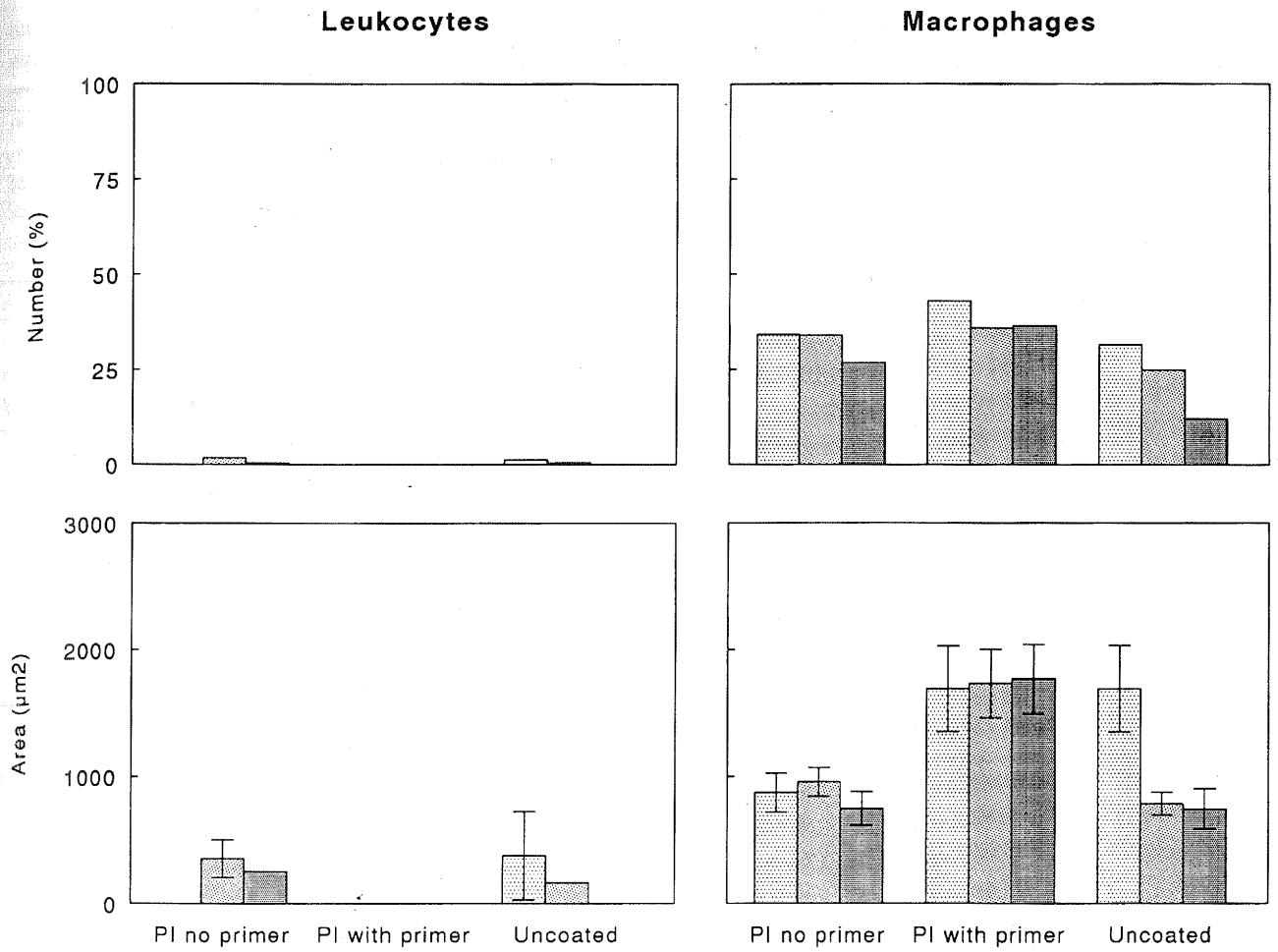

Figure 4. Leukocytes and macrophages were observed in the chronic implants. The percentages of electrodes involved with each characteristic are shown in the upper graphs and the average areas of involvement per affected track are shown in the lower graphs. An area of 3000 μm^2 represents a circle with a radius of approximately 31 μm. Formats are as in Figure 3, with the addition of data from polyimide coated arrays without adhesion promoter.

Chronic implants

Leukocytes and macrophages were found in both acutely and chronically implanted tissue. Figure 2(C) shows examples of leukocytes and Figure 2(A) illustrates macrophages. Leukocytes consisted of lymphocytes and plasma cells, whereas macrophages contained foreign electrode material and hemosiderin. Both leukocytes and macrophages occurred in small clusters around the affected electrodes, and were quantified as the affected area per track.

Figure 4 shows the data for leukocytes and macrophages. The percentages of tracks exhibiting these cell types are shown in the upper graphs and the areas of involvement per affected track are presented in the lower graphs. Note that an area of 3000 μm^2 is equivalent to a circle with a radius of approximately 31 μm. Leukocytes were extremely rare, but were expected to be present at least minimally.[9] Macrophages were commonly associated with all three types of implants, but were found to a statistically significantly greater extent around arrays with primed polyimide coatings than around uncoated or umprimed, polyimide-coated arrays.

Figure 2(A) shows typical examples of gliosis and electrode encapsulation. Gliosis was characterized by astrocyte proliferation appearing as darker stained areas. It included swollen astrocytes and modified fibroblasts. A capsule was defined as a compacted barrier of fibroblastic cells around the track.[10] Both of these features appeared as a ring around the electrode track and are presented as average annular thickness in Figure 5.

Although gliosis occurred around all tracks, primed, polyimide-coated electrodes had a significantly thicker gliotic annulus than unprimed, polyimide-coated electrodes. Similarly, significantly more primed, polyimide-coated electrodes induced capsule formation than did the other two types of arrays. Extracellular foreign material was not seen with the uncoated implants and occurred only infrequently with the polyimide coated arrays.

DISCUSSION

The overall reaction of the tissue to the implants can be regarded as modest. The limited appearance of edema and hemorrhage in the acute implants indicates that the initial trauma of implantation is well tolerated. The restricted amount of tissue reaction in the 6 month implants suggests not only extensive tissue recovery from the initial trauma, but also that the materials used in the arrays do not cause a significant toxic reaction.

Few qualitative differences occurred at different levels for a given type of implant. The most notable effect involved capsule development. In general the thickness of this tissue was reduced from the surface of the cortex toward the tips of the electrodes. This is consistent with the reduction in cross-sectional area of the penetrating implant and the anticipated reduction in tissue trauma along the electrode shaft near the tip.

Even for nonreactive implants, a minimal gliotic reaction may be expected along the tissue-implant border.[3] Differences between types of arrays were most notable for the primed polyimide-coated arrays compared to the unprimed, polyimide-coated arrays. The former were significantly more reactive with regard to macrophages, gliosis, and capsule formation. Primed, polyimide-coated arrays also were generally more reactive than uncoated silicon arrays. Unprimed, polyimide-coated arrays showed no consistent differences from uncoated silicon arrays, except for the occasional presence of extracellular foreign material.

While the primed, polyimide-coated arrays produced less extracellular foreign material than the unprimed, polyimide-coated arrays, the primed arrays induced more tissue reaction than did either type of array without primer. The most likely explanation for this observation is a tissue reaction to the aluminum oxide in the primer.

The present results show a modest reaction, overall, and healthy looking neural tissue was noted within a few micrometers of the electrode shafts. These observations are similar to results obtained with silicon electrode implants in rabbit cortices by Edell and coworkers.[11] Taken together, these findings indicate

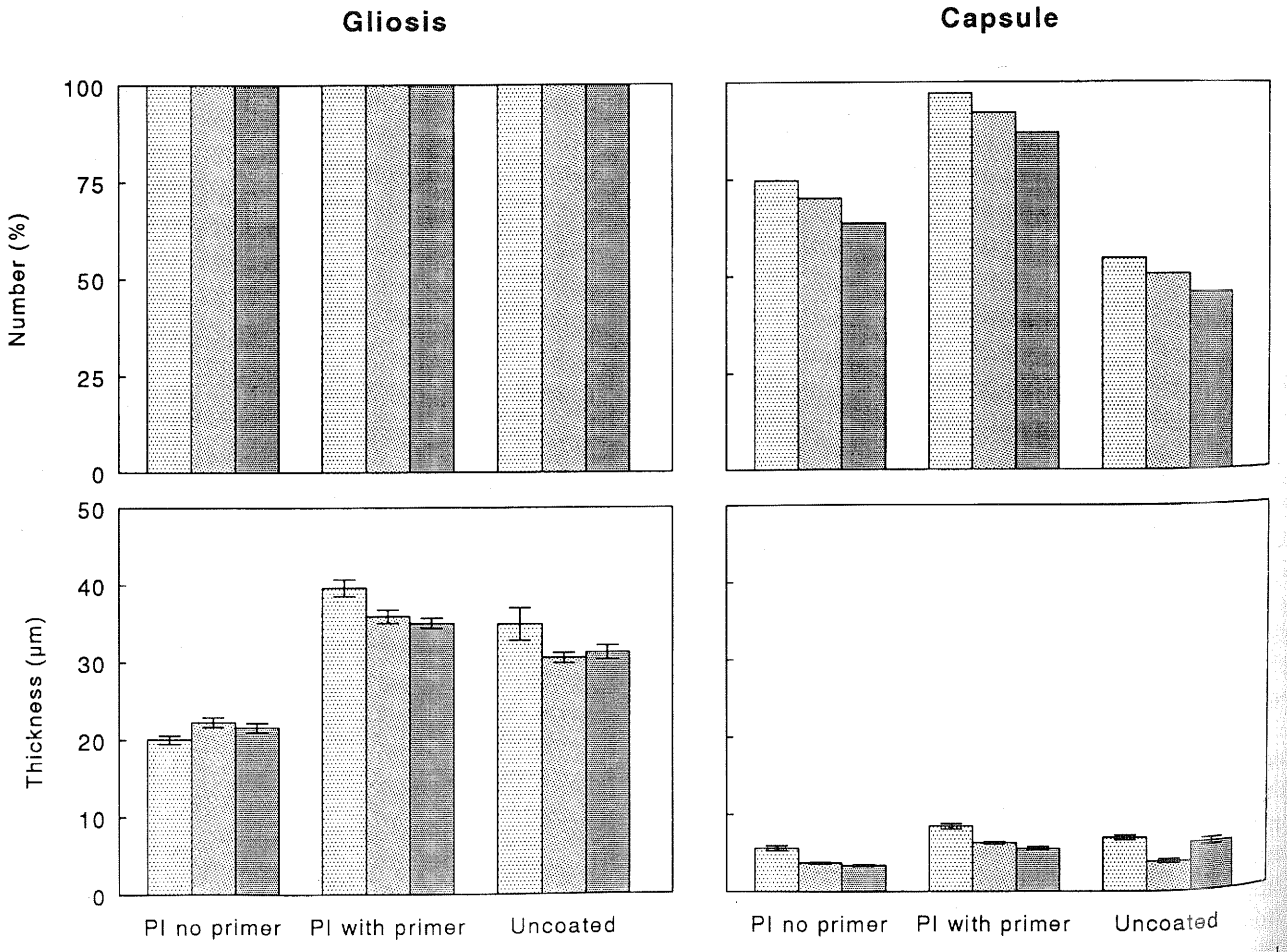

Figure 5. Gliosis and capsule formation occurred only in the long-term implants. The percentages of tracks affected for each measured parameter are shown in the upper graphs. The thickness of the annulus of gliosis and capsule thickness per affected electrode are shown in the lower graphs. Formats are as in Figure 4.

that the materials and structure of our electrode arrays would be potentially acceptable for chronic use as cortical implants.

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