well as the developing fetus in utero. It is tempting to speculate that a breakdown of the proposed mechanism may lead to infertility and early abortion.

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Action Potentials in Macrophages Derived from Human Monocytes

Abstract. *The electrical activity of macrophages derived from human blood monocytes was recorded in vitro with intracellular microelectrodes and was analyzed with computer-assisted data acquisition and analysis techniques. In cells impaled 6 to 8 days after the cultures were prepared, the resting potentials reached a maximum value of −72 millivolts. The cells were electrically excitable; spikes exhibited a slow upstroke, a fast downstroke, a discrete threshold, a large overshoot, and a brief undershoot. Repetitive firing was induced by a maintained depolarizing current. A positive relation was observed between transmembrane currents and resting potential. Voltage-current relations were nonrectifying for subthreshold current injections. Since these cells had not been treated with any specific activation factors, the electrical activity recorded is evidence for the presence of voltage-dependent inward and outward currents in the membranes of mature macrophages. The electrical signals generated by these cells may be useful for the assay of sensor and effector functions of macrophages, such as chemotaxis, receptor-ligand interactions, and phagocytosis.*

Macrophages exhibit certain of the characteristics of excitable cells, such as transient hyperpolarizations, switching of the resting potential between stable states, and high-resistance regions in N-shaped current-voltage curves (*1–3*). We now report evidence of overshooting, repetitive, spiking behavior in normal human macrophages, and we consider the significance of these events as possible markers of receptor-effector functions.

Human macrophages were prepared from the peripheral blood of normal donors by the Ficoll-Hypaque centrifugation and adherence method (*4*). Mononuclear cells were washed four times with serum-free medium (RPMI-1640, Gibco) to remove platelets. Tissue culture dishes (60 mm^2, Falcon) were each seeded with 4×10^6 cells in 3 ml of complete medium [RPMI-1640 supplemented with gentamicin (50 μg/ml) plus 10 percent autologous serum that was not inactivated with heat]. The cells were incubated at 37°C in 5 percent CO_2 and 95 percent room air for 2 hours. The nonadherent cells were then removed by gentle washing with RPMI-1640, and the adherent cells were fed with complete medium and incubated as above for at least 5 days. The resultant cultures contained more than 95 percent macrophages, as determined by morphology and nonspecific esterase staining (*5*). For studies of electrical activity, cultures were fed with Hepes-buffered (15 m*M*) complete medium, and the culture dish was mounted on a heat-controlled stage of an inverted phase-contrast microscope (Zeiss). Intracellular recordings were carried out with glass microelectrodes filled with 3*M* KCl (40 to 45 megohms) coupled to an electrometer (model KS 900, W-P Instruments) with a Ag-AgCl probe. A similar probe connected the bath to ground. Pulses of constant current were injected into a cell through the recording electrode. Recordings of transmembrane potentials and injected currents were transmitted directly to a microcomputer (New England Digital, Able-40) for storage and analysis.

As the monocyte macrophage became attached to the surface of a plastic culture dish, the cytoplasm spread radially to form an oblate spheroid with a central nucleus. The diameter, or degree of spreading, increased with time. The mean ± standard deviation (S.D.) of the resting potentials recorded from a sample of 28 cells obtained from six donors was −42 ± 14 mV. In this sample, 15 cells exhibited spiking behavior. The voltage-current dependence (*E*/*I*) of the monocyte macrophage membrane was examined by injecting a series of hyperpolarizing and subthreshold depolarizing currents. The slope of the line, representing *E*/*I*, by definition, is the input resistance (R_0) averaged 32 ± 4 megohms. In 85 percent of cells tested ($N = 30$), the resistance to inward and outward test pulses was the same, indicating no rectification. In the rest of the cells, there was slightly less resistance to depolarizing than to hyperpolarizing currents, indicating outward rectification. Estimates of the surface area of the cells averaged 3.93×10^{-5} cm^2 which, when combined with measurements of R_0 (32 ± 4 megohms), gave a calculated value for specific membrane resistance (R_m) of 1258 ohm/cm^2. The membrane time constants were short, seldom ex-

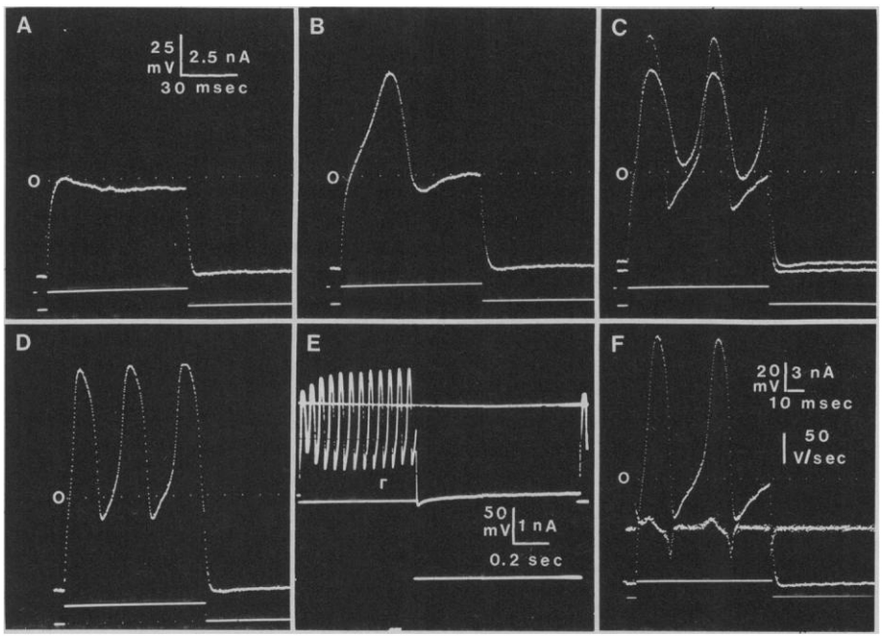

Fig. 1. Electrical responses of single macrophages to injected pulses of depolarizing current. Recordings (A to C) are from the same cell; calibrations in (B) to (D) are as in (A). (A) Subthreshold stimulus; (B) threshold for single spike; (C) variable spike generation in response to repeated stimulation. In the smaller spikes, the membrane potential has decreased by 7 mV. (D) Cell with slower firing rate; (E) repetitive firing in response to a long pulse (680 msec); (F) computer reconstruction of record illustrating *dV/dt* trace (sampling rate, 200 μsec).

ceeding 5 msec. Small depolarizing current injections elicited graded responses (Fig. 1A) similar to those recorded in peritoneal macrophages (*2*). At threshold (Fig. 1B), the membrane depolarized electrotonically for the first few milliseconds and then depolarized abruptly to produce an action potential. Action potential, in this context, refers to an abrupt, phasic change in membrane potential, and those reported here showed an overshoot, a rapid repolarization, and a postspike hyperpolarization. The latency of the action potential varied from 3 to 15 msec and depended on the time constant and excitability of the cell. The duration of the action potentials from stimulus onset to repolarization ranged from 55 to 90 msec.

Repetitive activity was induced by sustained current injection (Fig. 1, C to F). The transition from a single spike to a burst of repetitive action potentials occurred when stimulus current was increased or when threshold fell. After the postspike hyperpolarization, the membrane depolarized in a slow linear ramp until it reached threshold or until it repolarized and oscillated about a plateau potential. When the stimulus current was maintained for 720 msec, a spike train was generated (Fig. 1E); the frequency of firing ranged from 20 to 50 spikes per second. Both the amplitude of the action potentials and the level of membrane polarization during the interval between spikes increased with repetitive firing.

The first derivative of transmembrane voltage with respect to time (*dV/dt*), an index of transmembrane ionic current, displayed an S-shaped relation to the resting potential. Resting potentials measured over the range −49 to −72 mV had corresponding *dV/dt* values of 12.7 and 30.5 V/sec, respectively. At membrane voltages between these two values, the data were significantly linear, with *R* values for upstroke and downstroke of +0.778 and −0.905, respectively. At membrane potentials between −75 and −49 mV, the curve leveled off in a manner indicative of a typical inactivation process. On-and-off steps of the stimulus current produced brief capacitative membrane currents (Fig. 1F). During the action potential, the peak inward current during the upstroke was smaller (24 V/sec) than the subsequent peak outward current during the downstroke (−40 V/sec). This activity suggests that either a slow inward current (Ca^{2+}?) and/or a partially inactivated fast current (Na^{+}?) is flowing during the rising phase of the action potential. The activation of a rapid outward K^{+} current follows.

Human alveolar macrophages obtained by lung lavage were also tested for rectification and excitability. The membrane of the alveolar macrophage was nonrectifying and showed regenerative, repetitive firing, similar to the membrane of the monocyte-derived macrophage. There were no observable qualitative differences in the basic electrical responses to injected currents between macrophages from these two sources.

Macrophage membranes showed short-term and long-term changes in potential, both of which may be associated with accompanying functional states. Short-term changes in potential include spiking and hyperpolarization. Spiking could allow for an influx of calcium ions which, in turn, could regulate exocytosis of enzymes and other packaged secretory products. Hyperpolarizations have been induced by serum activated with endotoxin (*1*) and by lymphokines from stimulated spleen cells or from lymphocytes (*6*). Normal, untreated macrophages undergo spontaneous hyperpolarization during impalement which could lead to increased intracellular calcium. This, in turn, could trigger a Ca^{2+}-activated K^{+} current, with resulting hyperpolarization (*7, 8*).

Long-term processes, such as development of the resting potential and certain macrophage effector functions, appear to be closely linked to the number of days in culture. Indices of macrophage maturation include phagocytosis of erythrocytes coated with the third component of complement (*9*) and Fc receptor activity (*10*), which can be modulated by lymphokines (*11*). These processes attain peak functions by 7 to 8 days in culture. Preliminary evidence from this study indicates that this time span coincides with that at which spike amplitude and resting potentials attain their maximum voltages. Since monocytes have a limited half-time in the bone marrow and subsequently in the circulation (*12*), the electrophysiological state of blood monocyte membranes during the first several days in vitro, may be an important marker of the functional state of maturation.

Fluorescent dyes sensitive to changes in membrane potential now make possible an assay with flow cytometry of the relative resting potentials of large numbers of macrophages (*6*), and intracellular recordings can now be made in parallel with flow cytometry to quantify these potentials under a variety of conditions.

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Two-Component Hearing Sensations Produced by Two-Electrode Stimulation in the Cochlea of a Deaf Patient

Abstract. *Dissimilarities in perception elicited by stimulation with two electrodes were estimated. A two-dimensional spatial configuration was found to be suitable to represent the dissimilarity data, and the two dimensions could be interpreted as corresponding to the position of the apical and basal electrode of the two-electrode combination. A speech-processing strategy that converts acoustic, first and second formants to two-electrode stimulation is proposed.*

Previous speech studies in a number of research centers have shown that useful speech information could be presented to deaf patients by electrical stimulation of the residual auditory nerve fibers with the use of single- or multiple-electrode prostheses implanted in the cochlea (*1–4*). In our laboratory, electrical stimulation was produced by electrodes implanted in the scala tympani of the human cochlea, and a speech-processing strategy was formulated on the basis of psychophysical information obtained from our patients (*5, 6*). In the speech-processing strategy currently used by our patients with multiple-electrode cochlear implants, fundamental frequency of voicing is converted to the repetition rate of electric pulses delivered to the electrodes, and the second formant frequency of speech is converted to electrode position. Only one electrode is activated during a stimulus period (inverse of repetition rate).

There are four major psychophysical observations (*5, 6*) that support this speech-processing strategy: (i) individual electrodes produced pitch sensations in accordance with the tonotopic organization of the cochlea, (ii) pitch increased with repetition rate, (iii) the pitch sensations produced by repetition rate and the electrode position are perceived separately in the same way as Plomp (*7*) described for acoustic fundamental rate and single formant frequency, and (iv) the auditory system was able to discriminate short duration electrode transitions but not short transitions of electric repetition rate. Electrode transitions are therefore suitable for encoding the rapidly changing segmental speech information contained in the second formant frequency, and transitions of electric repetition rate are more suitable for encoding the slowly time-varying suprasegmental information contained in the fundamental frequency.

We investigated the possibility that, in addition to the fundamental and second formant frequencies, activation of an additional electrode during a stimulus period would present further speech information such as the first formant frequency (*8, 9*). The subject (MC1), a 50-year-old male who suffered from total hearing loss after a car accident, was our first cochlear implant patient. Pure-tone and speech audiometry, both under headphones and in a monitored sound field, had elicited no hearing responses in either of his ears for acoustic stimuli at the maximum output levels of the audiometers, and evaluation showed that this patient received no benefit from a conventional hearing aid (*4*).

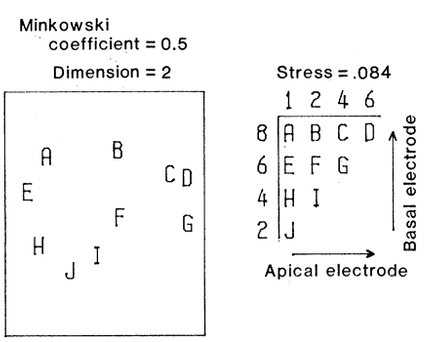

Fig. 1. Two-dimensional configuration representing the dissimilarities among the ten two-electrode stimuli: spatial configuration obtained by multidimensional scaling on the left half, and the stimulus matrix showing correspondence between the alphabetical symbols and the electrode pairs on the right.

In the implant operation (*4*), an array of ten electrodes was inserted through an opening in the round window membrane for a distance of 15 mm around the scala tympani. The electrodes were numbered from 0 to 9 in the apical to basal direction. These electrodes, spaced 1.5 mm apart, were driven by an implanted receiver stimulator. Residual auditory nerve fibers were activated by biphasic current pulses with each phase fixed at 180 μsec.

For this study, pulse trains 300 msec in duration with 50 msec rise-decay times were used. The current levels for a two-electrode stimulus were chosen so that the loudness produced by each electrode in isolation was approximately the same. The pulse trains delivered to the electrode pair were at the same repetition rate, with the pulses on the more apical electrode leading by 0.5 msec. There was, therefore, no temporal overlap between pulses on the two electrodes. This is necessary because it is difficult to balance the loudness contributions of the two electrodes from overlapping pulses due to current summation in the cochlea.

The perceptual dissimilarities among ten two-electrode stimuli were estimated by triadic comparisons (*7*). The ten electrode pairs were: 1-2, 1-4, 1-6, 1-8, 2-4, 2-6, 2-8, 4-6, 4-8, and 6-8. The repetition rate was 166 repetitions per second, and loudness was balanced across the ten stimuli. Three stimuli chosen in random order were presented to the patient, and he was asked to judge which two stimuli from the triad were the most similar and which two were the least similar. A dissimilarity value of 2 was assigned to the least similar, 0 to the most similar, and 1 to the remaining combination of two stimuli. A matrix of dissimilarity values was constructed for the ten stimuli after the presentation of all possible triads. Three matrices obtained in separate sessions were combined, and the resulting matrix was analyzed by a nonmetric multidimensional scaling (MDS) procedure (*10*) for distance functions specified by Minkowski coefficients