Low-Noise, Interference-Resistant Amplifier Suitable for Biological Signals

Abstract. *Minor changes in conventional low-noise amplifier circuits decrease circuit noise and attenuate the unwanted effects of varying impedances and potentials which exist between commonly employed electrodes and the tissues of biological subjects. The resulting reduction of intrinsic amplifier noise and reduced susceptibility to external interference is helpful in the study of low-frequency signals of microvolt level.*

The study of low-voltage biological signals is complicated by the impedances and potentials which exist between the electrodes and the biological subject. Extracellular potentials of electrically active tissues are frequently recorded at microvolt level from metallic electrodes inserted within tissue masses or applied to convenient surfaces. Between each electrode and the subject, there commonly exists an impedance to electrical current in the range of 500 to 10,000 ohms, and a chemically induced direct-current offset potential in the range of 0.1 to 100 mv.

The impedance associated with the grounding electrode permits interference sources to impress relatively large artifactual voltages upon the subject. Differential amplifiers are thus required to suppress this unwanted voltage common to both signal electrodes. The magnitude of the common mode rejection ratio expresses the ability of the amplifier to favor the desired voltage difference between the signal electrodes, and to reject the common interfering signal.

The impedances associated with the signal electrodes of the differential amplifier are less important for their magnitude than their inequality, since amplifier input impedances will produce unequal voltage drops across these differing electrode impedances. Interfering signals common to all parts of the subject thereby lose their equality at the amplifier input so that differential rejection is incomplete. The magnitude of the common mode input impedance expresses the overall immunity of the amplifier system to interference signals obtained through differing electrode impedances.

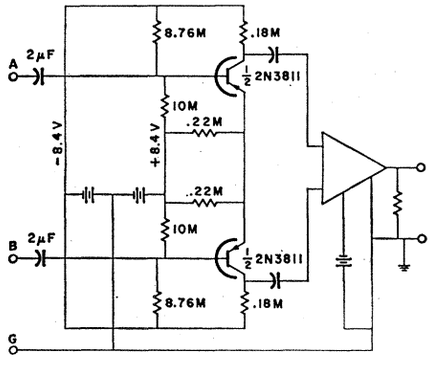
The direct-current potentials associated with metallic electrodes have become more important with the use of bipolar transistor differential amplifiers which, in providing the lowest noise amplification for signals at impedances under 5000 ohms, have found favor in the study of many low-level biological signals. Conventional bipolar transistor amplifier circuits (Fig. 1) often use matched transistors such as the Motorola 2N3811; they employ capacitors in each input lead, both to isolate the amplifier from direct-current electrode potentials and to prevent electrode polarization by amplifier circuit currents.

As the low-noise operation of this circuit demands a low-impedance path to the signal source, large value capacitors are required at low frequencies. Considerable noise power and voltage leakages are associated with large capacitors, and as conventional circuits employ one in each signal input lead, the feature of low-noise amplification is in double jeopardy.

A modification in circuitry (Fig. 2) of the conventional amplifier (Fig. 1) combines the well-known features of the floating transistor preamplifier and capacitive emitter coupling in a unique manner which should significantly aid in the study of low-level signals.

The effects of impedances between electrodes and subject are largely overcome with the use of an interference reference electrode (R) which is applied on the subject near the signal electrodes A and B, to acquire the interference signal which is common to electrodes A and B. This interference signal, in replacing the zero-potential ground reference of conventional first stage amplifiers, obviates a first stage discrimination against the relatively large interference voltage existing between the signal electrodes and ground. Following first-stage amplification of a desired microvolt level signal, discrimination with a millivolt level interference signal is easily accomplished at the second stage of amplification. This modification results in a much improved common mode rejection ratio, and greatly reduces the effect of interference resulting from impedance at the grounding electrode. As the interference signal from the reference electrode is common to all components of the first-stage amplifier, there occurs an almost infinite common mode input impedance, and a much reduced susceptibility to impedance inequality at the signal electrodes.

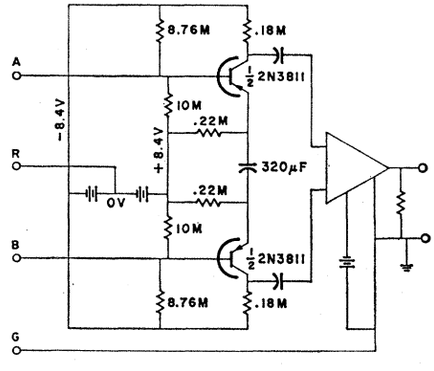
The effects of signal electrode potentials are overcome with the use of a single interemitter capacitor which in replacing the two conventional input capacitors not only reduces by half the sources of capacitor noise, but specifically reduces low-frequency amplifier noise. At low frequencies, the increasing reactance of the conventional input capacitors increases amplifier input impedances so that amplifier noise increases. The low-frequency increase in reactance of the single interemitter capacitor effectively reduces amplifier gain so that noise generation decreases.



Voltage gain 18,000; Frequency response, 3db 0.2-60,000 hz; Differential input impedance 2.5 × 10^6 ohm; Common mode input impedance 4 × 10^6 ohm; Common mode rejection ratio 7 × 10^4 : 1; Inter-electrode current, A–G 5 × 10^{-11} amp; Inter-electrode current, B–G 5 × 10^{-11} amp

RMS noise referred to shorted input	0.2— 10 hz	1.3 μv
	2.0— 100 hz	0.3 μv
	20 —1000 hz	1.2 μv

Fig. 1. Conventional amplifier circuit.



Voltage gain 18,000; Frequency response, 3db 0.2-60,000 hz; Differential input impedance 2.5 × 10^6 ohm; Common mode input impedance 10^{12} ohm; Common mode rejection ratio 5 × 10^6 : 1; Inter-electrode current A–R 6 × 10^{-9} amp; Inter-electrode current B–R 5 × 10^{-9} amp

RMS noise referred to shorted input	0.2— 10 hz	.05 μv
	2.0— 100 hz	0.1 μv
	20 —1000 hz	1.1 μv

Fig. 2. Modified amplifier circuit.

Combined use of the reference electrode and single interemitter capacitor enables stable, independent operation of the input transistors so that base-emitter voltages need not be matched. Transistors with matched gains or balance controls are not mandatory, as there is no first-stage discrimination against the interference signal. Circuit stability allows the signal inputs to be maintained close to a zero-potential so that very little current flows through the electrodes.

The tabulated performance features of Figs. 1 and 2 afford a meaningful comparison except in common mode capabilities which, due to the limitations of routine measurement techniques, are better compared under actual operating conditions. In examining a human scalp for brain potentials with a 1000-ohm impedance at all electrode junctions (save for one signal electrode of 5000 ohms) and with the subject placed in a uniform electrostatic field (50 mv of 60 hz interference impressed upon his body), the standard amplifier produced an interference output of 560 mv, while the modified amplifier produced 30 mv. In this practical test, an 18-fold reduction in susceptibility to interference and the effects of unequal electrode impedance is obtained by the modification.

With the modified amplifier circuit, low-noise electroencephalograms, electromyograms, electronystagmograms, and abdominal lead fetal electrocardiograms have been recorded. However, with signal impedances less than 10,000 ohms, any extracellular potential recording in the frequency range of 0.01 to 10,000 hz will be aided by the modified circuit.

GRAHAM SCHULER, GORDON PARK
Radio and Electrical Engineering Division, National Research Council, Ottawa, Canada

JOHN P. ERTL
Faculty of Psychology, University of Ottawa, Canada

8 August 1966

Noncollagenous Nature of the Proteins of Shark Enamel

Abstract. *The proteins, soluble and insoluble at neutral* p*H, of relatively mature enamel of two different species of sharks were distinctly different from both collagen and embryonic enamel proteins and similar to the proteins isolated from mature human and bovine enamel.*

The amino acid compositions of the proteins of the organic matrix of embryonic and developing enamel from a number of different species have been characterized by their relatively high contents of proline, leucine, histidine, and glutamic acid (*1–3*). During the maturation and development of enamel, more than 95 percent of the proteins, especially components rich in proline and histidine, are lost from the enamel (*4, 5*), so that the overall amino acid composition of mature enamel proteins is distinctly different from that of the embryonic proteins (*5, 6*). Although both the embryonic and mature enamel proteins contain small amounts of both hydroxyproline and hydroxylysine (*2–4, 7*), their amino acid composition, molecular structure, and physical chemical properties (*8, 9*) distinguish them from collagens. In contrast with these findings, however, it has been reported that the organic matrix of enamel from the mature shark consists principally of an ectodermal collagen (*10*). Because of the significance of this latter finding for the comparative biology of enamel (*10*), and because of the difficulties (*4, 5*) encountered in obtaining "pure," mature enamel free of dentinal or cemental collagen, we felt that these results should be independently confirmed, preferably on freshly obtained teeth, rather than on teeth obtained from commercial sources, which are usually treated with chlorine-containing bleaches and other strong reagents in order to improve their appearance. Consequently arrangements were made to obtain the teeth of several sharks immediately after their death.

The jaws of hammerhead sharks (2.4 to 3.0 m) were dissected free immediately after decapitation of live animals caught in the waters off the Florida Keys. The jaws were frozen with dry ice and kept frozen until used. After the jaws were thawed, the first three rows of unerupted teeth, which were adjacent to the already erupted teeth, were dissected from the mandible and maxilla. All soft-tissue attachments were carefully scraped and dissected free under the dissecting microscope, and the coronal surfaces were wiped clean with gauze. The teeth were placed in 0.3*M* EDTA (ethylenediaminetetraacetate), *p*H 7.7, at 2°C for 24 hours, and very small fragments of tissue were removed from the surfaces of the crowns. The surfaces of the crowns were brushed with gauze in EDTA and placed in fresh EDTA for an additional 12 hours. The surfaces were again cleaned, and the crowns were rinsed with water and immersed immediately in 0.5*M* EDTA, *p*H 8.3, at 25°C, with toluene or thymol crystals added for bacteriostasis. After 24 to 36 hours, small, partially decalcified, thin fragments of the enamel could be dissected free, with a small amount of enamel still present on the tooth. These small, partially demineralized fragments of enamel were placed in dialysis bags in 0.5*M* EDTA, *p*H 8.3 and dialyzed against EDTA at 2°C for 3 days, and then against 0.05*M* NH_4HCO_3, *p*H 7.7, at 2°C for 3 days. The clear supernatant representing the neutral-soluble, nondiffusible proteins was lyophilized. The fraction insoluble at neutral *p*H was washed with water and dried.

In addition to the hammerhead sharks, six near-term embryonic blacktip Bermuda sharks were obtained live, frozen immediately with dry ice, and kept frozen until used. The first and second rows of teeth, which were partially erupted, were dissected free and carefully cleaned of soft tissues. Because the teeth were so small, it was not possible to dissect the separate tissue layers. Therefore the teeth were placed in 0.3*M* EDTA, *p*H 7.7 at 25°C for 24 hours, the surfaces and pulp chambers were cleaned, and the teeth were washed with water. The teeth were then placed in fresh 0.3*M* EDTA, *p*H 7.7 at 2°C for 1 week and then fragmented in the EDTA solution with mortar and pestle. The entire contents including the EDTA solution were transferred to a dialysis bag and dialyzed for 3 days against 0.3*M* EDTA, *p*H 7.7 at 2°C. The nondiffusible proteins soluble at neutral *p*H were obtained as described. Portions were hydrolyzed in constant boiling 6*N* HCl, at 105°C for 24 hours and analyzed on an automatic amino acid analyzer.

Small fragments of the organic matrix, insoluble at neutral *p*H, of the enamel of the hammerhead shark teeth were washed with 0.15*M* NaCl, *p*H 7.4, rinsed quickly in water, and dried on siliconized glass slides. Thin sheets of the dried material were removed with a razor blade, and x-ray diffraction