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SHORT COMMUNICATION

Ion-selective Microelectrode Technique for Simultaneous Measurements of Small and Rapid Concentration Changes and Biopotentials with Computer Evaluation

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Summary
A microelectrode technique is described for simultaneous measurements of biopotentials and small and rapid ionic changes using double-barrel ion-selective coaxial microelectrodes (ISCM) evaluated by computer analysis.

Key words
Ion-selective microelectrodes – Double-barrel coaxial microelectrodes – Differential DC amplifier – Registration computer analysis

A microelectrode technique enabling simultaneous measurements of biopotentials and concentration changes in the extracellular space with rapid graphic registration of records employing a computer depends closely on the availability of a suitable sensor, i.e. a double-barrel ion-selective coaxial microelectrode (ISCM). The method of fabrication of these electrodes was described in previous reports (Ujec *et al.* 1979, 1980, 1981) together with technical parameters and specific conditions of electrical (τ_e) and concentration time constants (τ_c).

Technical description of recording apparatus

The new type of differential amplifier is a modification of the previous version (Ujec and Beránek 1967, Ujec 1988) and concerns mainly the connection of the input probe (the part encircled by a broken line in Fig. 1A). Both input hybrid integrated circuits A_1 and A_2 (HiO type WSH 223) amplify the signals from each electrode channel independently. Identical voltage amplification (in this case 10 fold) can be adjusted by selecting appropriate resistors (R_a, R_b and $R_{a'}$, $R_{b'}$). By careful selection of resistors, a rejection factor of up to 10^4 can be attained with this type of connection.

The differential amplifier A_3 itself (10 type MAA 741A) with unit amplification is placed in the main body of the amplifier, together with the control elements, e.g. for presenting the DC level (P_l, $P_{l'}$,), for expanding the frequency range (P_f, C_f, $P_{f'}$, $C_{f'}$), for measuring the resistance of individual microelectrode channels (C_r, $C_{r'}$,) and for presenting the output DC level etc. By using precise values of resistors R_c, R_d and $R_{c'}$, $F_{d'}$ it is possible to adjust the required amplification.

Three identical asymmetrical amplifiers A_4-A_6 (see Fig. 1B) are a part of the described differential amplifier circuits (HiO type WSH 526). These amplifiers have a gain of up to 100x of the signal voltage (R_f) from the differential amplifier (Fig. 1A, $OUT_{1,2,3}$). However, it is possible to attenuate high amplitude signals by a factor of 10 by selecting appropriate R_e values and thus to adjust the most suitable amplitude of signals for the computer recording. It is then possible, through the input (-IN), to adjust the output DC level (P_2). The frequency range of all the above amplifiers is from 0 to 50 kHz/3 dB. The temperature shift of the DC level on the input is lower than 5 μV/1 °C.

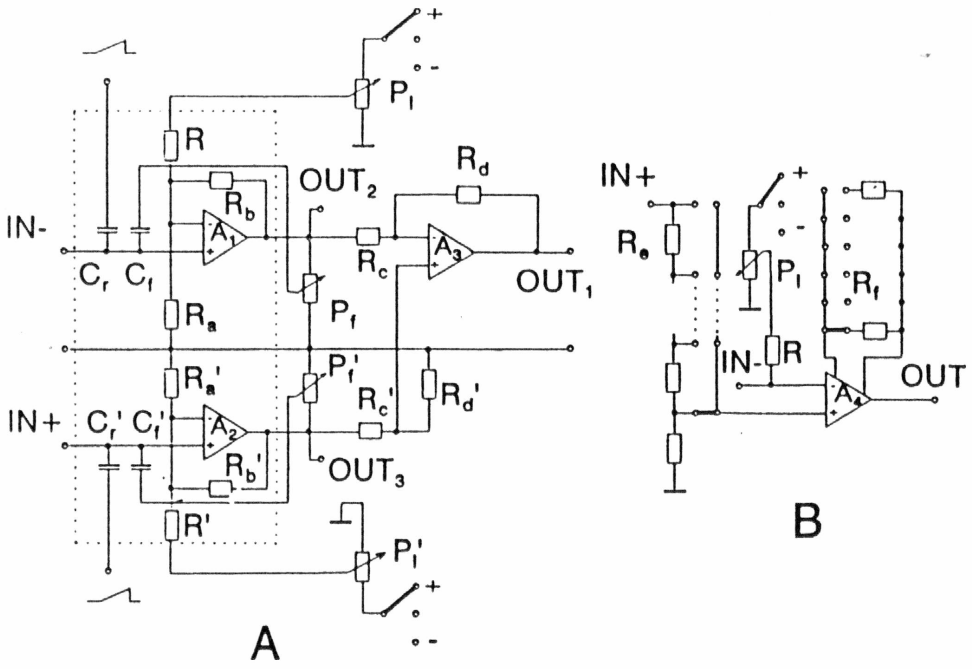

Fig. 1
Block schema of electronic equipment for simultaneous recording of biopotentials and rapid concentration changes. A: DC differential amplifier for measurements with double-barrel ion-selective (coaxial) microelectrodes with compensation of the frequency range. B: Outputs OUT_{1-3} are connected to three amplifiers, enabling the setting of optimal amplitude and a suitable DC level for computer evaluation. (For further details see text).

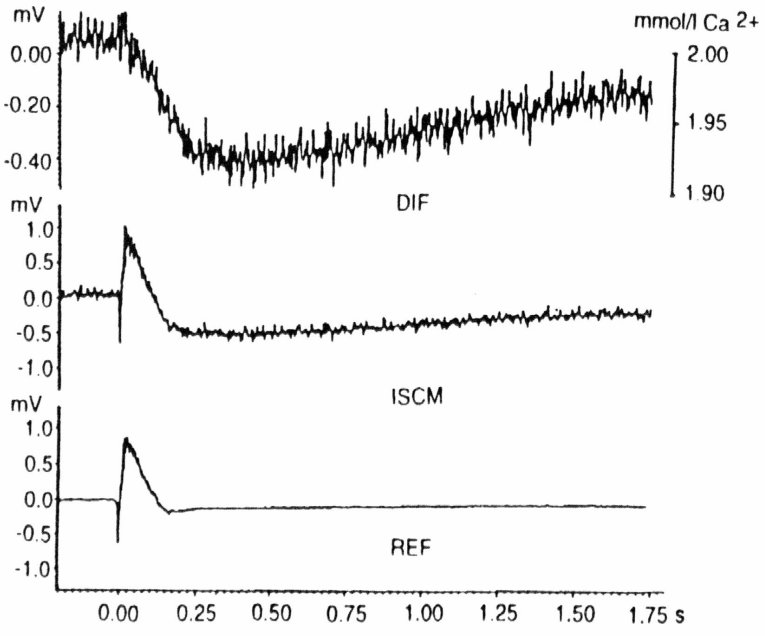

Fig. 2
Changes in $[Ca^{2+}]_e$ during a single interictal epileptiform discharge. Bicuculline (20 μmol/l) was bath-applied 15 min before this recording. Stimulation of the Schaffer collaterals induced epileptiform discharges (lower record, REF). Middle record (ICSM): potential recorded by the ion-selective channel. Upper record (DIF): change in $[Ca^{2+}]_e$ induced by epileptiform activity, greatly exceeding the electric signal in duration (maximum change −80 μmol/l).

Special filters have been designed for measuring very small concentration and potential changes, which improve the signal to noise ratio. This includes three second order filters connected in series (i.e. DFP of the sixth order with a slope of 36 dB per octave). The filter is intended especially for differential records (OUT_1), i.e. for reducing the noise level in small concentration changes.

Registration of signals employing the computer technique

Output signals $OUT_{1,2,3}$ in Fig. 1A are modified by amplifiers as shown in Fig. 1B to a voltage level suitable for I/O card PCL 718 with an A-D converter HDAC 674, which is connected with a PC AT computer. The range of measured potentials was preset at ± 5 V on the card (resolution 2.44 mV). Both signals obtained from points OUT_2 and OUT_3 were sampled using a multiplexor. Furthermore, the output OUT_3 was sampled for both analog comparison (output from the differential amplifier) and digital evaluation of the signal. The fourth sampled voltage was the stimulus artifact. A positive pulse in this channel triggered the recording (external synchronization). Quick Basic was the program utilized for storing and evaluating concentration changes. This program provides the possibility of selecting the sampling speed up to 40 kHz. The length of one data file was restricted to 64 kB. Immediately after sampling, the three above mentioned records were displayed on the monitor (one above each other) and, furthermore, the time course of concentration changes was obtained by calculating the values at points $OUT_{2,3}$. The data file is at first compressed onto one screen, for the sake of comparison. It is, however, possible to use the cursor for indicating places which should be displayed on a more suitable time scale and thus to obtain access to crucial segments in more detail. The calculated time course of concentration changes can be filtered digitally (with a I. order recursive filter). The recorded data are stored on a disk for subsequent off-line evaluation of crucial segments. The immediate graphical display makes it possible to modify the actual experimental set-up of the experiment. A comparison of the analog and digital evaluation of concentration changes has shown good agreement.

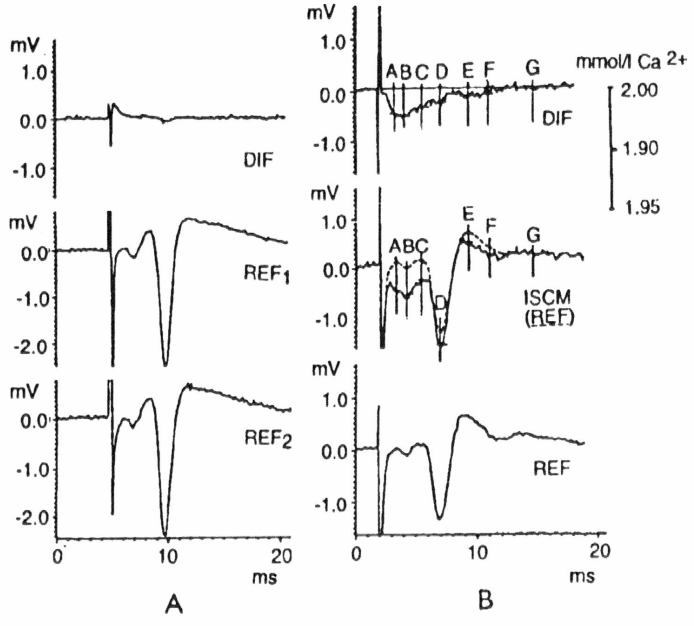

Fig. 3

A: Model measurement using double-barrel electrode with different longitudinal resistances. The barrels were filled with different ionic solutions resulting in a 5fold difference in longitudinal resistance (REF 1: 60 MΩ, REF 2: 1988: 300 MΩ) to simulate the difference in transfer properties between ion-selective and reference electrode. Upper record (DIF): differential signal with small residual transfer artifact (REF 1 and REF 2): voltage recording of population through both electrodes.

B: Demonstration of differential recording of ion concentration changes (−20 μmol/l), performed by a simple graphic method with evaluation of the amplitudes from several loci of recording (A−G), obtained by the overlapping of signals from the ISCM and REF channel (see middle record). The actual time course of the ion concentration is shown in the upper record. The amplitude correspondence of the top and middle records is documented in A−G.

Verification of the method and measuring procedures

A number of measurements have been carried out to check the technique described above which was intended for measuring minute and rapid concentration changes and biopotentials. The measuring system makes it possible to record three records simultaneously. These are registered and analysed by computer according to the equation C = A − B, where C is the time course of concentration changes, B is the biopotential change and A is the summated record of the concentration and biopotential changes.

In Fig. 2, actual records of Ca^{2+} in the extracellular space of the guinea-pig hippocampus are shown, which belong to moderately rapid biopotential responses, where the most rapid components correspond approximately to 100 Hz. These experiments were intended to reveal Ca^{2+} concentration changes during individual epileptic discharges. The discharges were evoked by electrical stimulation 15 min after application of bicucullin (20 μM) (Behrends *et al.* 1991). It may be seen from the differential (upper) trace that both the rapid ascending and the slower descending part of the biopotential are recorded. The ISCM electrode recorded these potential changes from the region of stratum pyramidale (CA_1).

In order to measure more rapid changes (Ujec and Behrends 1991, Behrends *et al.* 1991) we first performed a number of model measurements, when both barrels were filled with an aqueous solution only. The higher impedance of the ion-exchanger barrel channel was simulated by filling it with an aqueous solution of lower concentration than the reference barrel. When recording biopotentials from the tip of the double-barrel electrode, we were careful to make the frequency compensation of the signals from the two barrels (OUT_2, OUT_3) to be as similar as possible. After algebraic summation of the two signals, we attained practically a zero level on the differential output (OUT_1). This is documented in Fig. 3A, where the middle record of the biopotential was obtained *via* the reference channel – REF_1 (60 MΩ); the lower record is the result of the high value of longitudinal resistance of the second barrel (300 MΩ) substituting for the ion-selective channel, denoted as REF_2. The uppermost record then shows the "zero" level after subtracting the two signals. The stimulation artifact undergoes a five to tenfold reduction, depending on the suitable selection of the isolated stimulation unit.

After these model measurements, we began to record concentration changes using coaxial ion-selective double-barrel microelectrodes (Ujec and Behrends 1991, Behrends *et al.* 1991). A representative record from the above mentioned biological preparation is shown in Fig. 3B. The same site was stimulated with Adrian's metal coaxial needle. The measuring resistance of the Ca^{2+} ion-exchanger is considerably higher than, for example, that of the potassium ion-exchanger. The longitudinal resistance of the channel filled with the ion-exchanger up to 100 – 200 μm from the tip, 2 – 3 μm in diameter, were in the range of 1 – 5 GΩ. By using the coaxial type of electrode, there was a 5 – 10fold decrease of its resistance. This made it possible to measure small and rapid biopotentials as well as ionic concentration changes. Such records which occur in a frequency range higher than 1 kHz are shown in Fig. 3B. This has been supplemented in the middle record (summation of the biopotential and concentration) with a broken line indicating the time course of the biopotential. It is possible to demonstrate, by drawing seven vertical lines A – G through the records, that the individual segments in the two records fit the upper differential recording. This recording was selected intentionally because its noise level was close to the extreme differentiating possibility of the amplifier. In this case, the time course can be determined precisely, even though its maximum change is – 20 μmol/l.

It may be concluded that the registration of Ca^{2+} concentration changes in the extracellular space of hippocampal slices demonstrated the possibility of simultaneous measurements of potential changes which are several times greater than the concentration changes. The records used in this report were obtained in the Institute of Physiology, University of Munich, GFR while developing this technique (Ujec and Behrends 1991, Behrends *et al.* 1991).

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