ELECTRO- AND MAGNETOBIOLOGY, 20(1), 1–13 (2001)

ELECTRIC ACTIVITY OF NON-EXCITABLE BIOLOGICAL CELLS AT RADIO FREQUENCIES

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ABSTRACT

The importance of cellular electrical phenomena is well known. Most investigations have been limited to static and quasi-static effects. Very few studies deal with the occurrence of RF fields, which can be expected for theoretical reasons. Indirect evidence arises from microdielectrophoretic work that delivers information about the spatial distribution and temporal development of AC electric fields around biological cells. A more detailed investigation is possible by an electronic detection of these fields. A survey of both microdielectrophoretic and electronic work is given, together with some principles that are crucial for the design and interpretation of experiments. We present a multichannel frequency analysis system that for the first time allows the continuous monitoring of cellular RF activities over a wide frequency range. For the fission yeast *Schizosaccharomyces pombe* rhythmic signal changes with period lengths of about 400 s have been found that could be reproduced in a number of experiments.

INTRODUCTION

Electrical phenomena play a key role in the functioning of any organism. Well known are the concepts of voltage gated channels and membrane potentials in all kinds of cells, as well as action potentials and electrical signal transduction in excitable cells. DC (direct current) electric currents can be found in the vicinity of and inside growing cells, often exhibiting spatial structure. While much is known about electrical activities at DC and low frequencies, i.e., below some kHz,

very few investigations have been performed at higher frequencies, leaving out optical frequencies. Still, there are good theoretical reasons for the existence of oscillations well above the kHz range, which could be of great importance to cellular and multicellular function as well as to the interaction between environmental electromagnetic fields and biological cells. Starting from solid state physics, Fröhlich has put forth an exhaustive theory of coherent excitation in biological systems (1–3) that has been supported by numerous experiments (4), most of which give indirect evidence.

The purpose of this article is to give a survey of experimental fundamentals and results concerning the direct detection and characterization of endogeneous cellular AC (alternating current) fields. A measuring system is described that has been developed in our laboratory for the continuous monitoring of cellular RF (radio frequency) activity. Experimental results on the fission yeast *Schizosaccharomyces pombe* are presented.

MICRODIELECTROPHORESIS

The action of nonuniform AC electric fields on neutral, polarizable objects is called dielectrophoresis (DEP) (5). This effect is exploited, for example, for the separation of different cell types according to their complex, frequency-dependent polarizability (6). An extension of this technique has been established by Pohl and coworkers (7–11). Living cells are suspended together with highly polarizable particles, which typically are about a tenth or less of the cell size. These particles are attracted by the cells, which is interpreted as a dielectrophoretic force exerted on the particles by a strongly inhomogeneous cellular AC field, hence the term microdielectrophoresis, or μDEP. By this process not only can the existence of a field be shown, but also its spatial distribution relative to the cell, and its temporal development. Usually the particles are about 2 μm in size, so that they are small enough to probe the field geometry. Smaller objects are not suitable because they are increasingly influenced by Brownian motion (12,13). Additionally, DEP forces are proportional to the particle volume (14); therefore, they diminish quickly with decreasing size. Powders of inorganic salts such as barium and strontium titanate are employed, which exhibit dielectric constants of ca. 1000, while minerals with a permittivity below that of water (about 78) such as quartz or barium sulfate are useful as controls, being repelled by the cells. Since the specific gravity of these materials is far higher than that of water, they sediment very fast in a suspension and—located then on a solid surface—will not be moved by weak μDEP forces. Therefore, a drop of the suspension of cells and particles is positioned at the bottom surface of a cover slide, which is then placed on a microscope slide with a concave surface (''hanging drop technique''). The suspension's electrical conductivity should be rather low to achieve an optimal DEP effect. Therefore, combined with the very small volumes involved, great care has to be taken to avoid any impurities. Further, precise control of the medium's conductivity allows added conclusions about field frequency, strength, and temporal occurrence with respect to the cell cycle (10,15).

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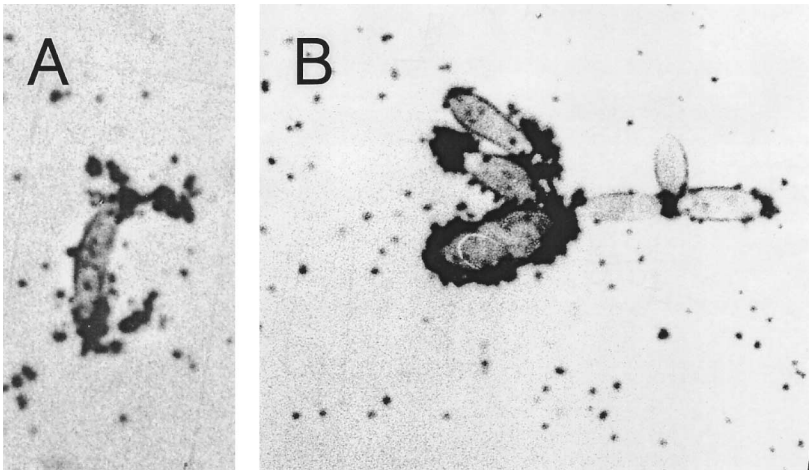

Figure 1. Microdielectrophoretic collection of barium titanate particles (black dots, diameter about 2 μm) by the fresh water alga *Monoraphidium setiforme*. (A) Particles are arranged in a manner indicating field lines. (B) Particles are collected at the cell ends.

μDEP has been demonstrated for various cell types like bacteria, yeasts, algae, and mammalian cells (16,17). It can be shown that the particles' collection is not due to DC effects (7,8), because static fields would be screened by mobile charges in the medium over distances in the order of the Debye relaxation length, which is well below 1 μm. From variations in suspension conductivity, Pohl and Pollock (15) have calculated a lower frequency limit of 5 kHz. By this, μDEP not only evidences the existence of cellular AC fields; it also supports the electronic characterization of these fields by rendering information about optimal electrode size and orientation as well as frequency ranges to be expected.

We have successfully used barium titanate for the investigation of yeasts and algae (12,13). Quite suitable for these studies are fresh water algae, because they allow the application of low-ion-concentration media that are necessary for μDEP experiments. Figure 1 shows the attraction of barium titanate particles by cells of the fresh water alga *Monoraphidium setiforme*. Clearly the particles are collected preferentially at the ends of the cells. Although one might expect this intuitively, this is not self-evident, since indications about the location and orientation of the oscillating units have been very scarce. While Figure 1B is a quite typical μDEP result, the indication of field lines as in Figure 1A can be observed rather seldom.

ELECTRONIC DETECTION

The most obvious approach for the investigation of cellular electric activity is to probe it with electrodes, to amplify the signals, and to display them on an

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oscilloscope. However, there are a number of obstacles to be worked around: From Fröhlich's work (1–3), which is mainly based on solid state physics, vibrations at extremely high frequencies in the Terahertz range follow (18,19), which, however, might result in much lower frequencies by nonlinear interactions (20). Should microtubules be involved in the vibrations, frequencies below 1 GHz can be expected (19,21). Fröhlich's concept of a Bose–Einstein condensation leads to longitudinal electric modes producing fields that decrease very quickly with distance (18), making these fields quite difficult to access. Further, the average metabolic rate of, e.g., a single yeast cell amounts to only about 10^{-11} W under optimal growth conditions, a tiny proportion of which can be expected to be channelled into electric RF activity.

This might explain why only a few reports about such investigations can be found in the literature. In the following we will give a survey of the literature as well as a discussion of experiments and their optimal design, and will present the latest data obtained in our laboratory.

Biological Conditions

Fröhlich's theory predicts the excitation of coherent vibrations if energy is supplied above a critical value (1,2). This energy can only result from the cell's metabolism. Therefore, mainly organisms with a high metabolic rate like fast-growing yeasts (13,22–25) and algae (13,26) have been studied yet. Bacteria are too difficult to access by electrodes due to their small size and due to the correspondingly strong Brownian motion. We have started calculations to determine whether it might be sensible to study bacteria with a defect in septum formation, which form cells of up to 100 μm length and can be transformed to spheroplasts (27).

Yeasts and algae are surrounded by a cell wall, a rigid network of macromolecules, that supports the underlying cell membrane. This makes it possible to perform measurements in media of low ionic strength, which attenuate the electrical signals less than typical growth media. Still, the wall is about 0.2 to 0.5 μm thick, hindering direct access to the cell membrane. This additional distance between electrodes and cell membrane presumably leads to further signal attenuation (18). The use of sharp needle electrodes (25) or partial enzymatical removal of the wall avoids this problem.

In most studies of this type, cells are suspended in media of very low ionic strength, sometimes with sugars added, in order to minimize field attenuation due to currents flowing between the electrodes and around the cells (22–24). However, this leads to a reduced metabolism, worsening the prospect of reaching the critical energy supply mentioned above. From model calculations carried out for yeast cells (25), it follows that from an electronic view such media are advantageous only in the lower MHz range and below, although this also depends on the actual electrode shape and on the cell position and orientation. Thus, we prefer conventional growth media for the majority of measurements.

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The baker's yeast *Saccharomyces cerevisiae* is the best-studied organism in this field so far. Its culture is uncomplicated, there are strains available whose growth can be synchronized, and cells are relatively robust with respect to environmental conditions. The latter facilitates taking into account electronic needs more thoroughly when designing the experimental setup. However, the cells' ellipsoidal and often nearly spherical shape makes it difficult to align them with the electrodes. For this reason we are also studying the fission yeast *Schizosaccharomyces pombe*, which is of elongated cylindrical shape and about twice the size of *S. cerevisiae* (ca. 5 μm × 13 μm as compared to 5 μm × 7 μm).

Electrodes have to be shielded against interference from external sources. Therefore, observing the cells in the course of a measurement is extremely difficult, and has not been performed yet. Nevertheless, gaining information about the cells' state is still possible by using a synchronized culture. This has been done by the groups of Smith (22,23) and Pokomy (24). The latter employ a cold, sensitive mutant of *S. cerevisiae*. Therefore, their electrode arrangement is kept at elevated temperatures around 26°C.

Electrodes

Based on Fröhlich's concept of coherent excitation (2) and its extension by Pokorny and Fiala (18), the fields are expected to decrease very sharply within a few tenths of a micrometer from the oscillating units. This seems to contradict the microdielectrophoretic observations, especially for cells surrounded by a cell wall. Therefore, the question about the need for close contact between electrodes and cell membrane is still open. Sharp needle electrodes, as used by several authors (22,25,28), allow quite close contacts; but positioning the cells with respect to the electrodes is difficult. Although temporary application of RF voltages to the electrodes helps to collect cells at the electrode surface (22,28,29), this introduces the risk of affecting or even killing those cells that are within the actual electrode gap (30), which is only about 5 μm wide. This is a consequence of the field strength being much larger within the gap than at a distance of only a few μm. Alternatively, a cell can be positioned with some skill between needle electrodes that are supported by a tapered glass microcapillary (25).

Planar electrodes are much easier to handle. However, their manufacture is not trivial (13,29) and is best done by photolithography, as in (24). By using a very long and still narrow gap it is possible to study many cells simultaneously. Depending on medium conductivity, frequency, and proportion of electrically active cells, this can lead to an increased signal level as the electric power of all cells is summed (25). On the other hand, the corresponding increase in electrode capacitance weakens the signal, and at higher medium conductivities, attenuation is possible, especially if signal frequencies differ between individual cells. Therefore, both narrow and wide electrodes are used (24,25). In order to minimize the current flow through the medium between the electrodes, their surfaces can be isolated with, e.g., a silicon dioxide layer of some micrometers' thickness (24)

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except at the very tips. We are using platinum-plated glass slides with a 5 μm electrode gap produced by diamond tools. Instead of silicon dioxide, we have applied a paraffin layer of about 50 μm thickness, resulting in better reduction of capacitive currents. This thick layer also helps in positioning the cells in the electrode gap just by sedimentation along the layer's slope.

Alternative approaches for the detection of cellular RF activity may be, for example, photolithographically produced microcoils. An interesting means might be available in the near future as the maximum usable frequency of SQUID (superconducting quantum interference device) magnetometers reaches tens of Megahertz (31).

Amplifiers

As it is well known from electrophysiology, the source impedance of biological cells amounts to around 10^5 Ω and above. A detailed calculation of the resistances and capacitances of the biological cell, the electrodes, and the first stage of the electronic part reveals that the sensitivity of the experiment is determined mainly by the input impedance of the input stage of the electronic equipment (25). Input resistances of 10^6 Ω and more do not pose any technical problem. However, due to the high frequencies involved, very low input capacitances well below 1 pF are optimal, which is quite difficult to achieve.

Interestingly, in the first publications on cellular AC fields spectrum analyzers with a nominal input impedance of only 50 Ω were applied successfully (22,26). In the following, purpose built preamplifiers have been employed with silicon FETs as the active component (13,24,25,28,29) exhibiting 1 to 10 M Ω in parallel with 1.5 to 4 pF. Obviously, low-noise semiconductors as well as low-noise passive components must be used. A further decrease of input capacitance can be achieved by positive feedback in the input stage. However, the noise of this stage would be fed back equally, so that no real increase in the signal-to-noise ratio would be attained. Alternatively, GaAs FETs can be employed, which typically exhibit capacitances an order of magnitude lower than silicon FETs (13,25,29). However, their noise performance is optimized for low impedance function at GHz frequencies, showing quite a high noise level below 1 MHz (32). As with all high-impedance high-frequency circuits, special care must be taken to exclude any oscillation that could be misread to be of cellular origin. Although additional shielding reduces such a risk, it also leads to an increase in input capacitance.

Our latest amplifier design consists of three consecutive silicon FET stages, the first and second working in a common drain configuration for lowest input capacitance. A bipolar stage drives the output with a standard 50 Ω source impedance. Parasitic effects are minimized by the application of components of very small geometrical size (SMDs) and by mounting most of the circuit about 5 mm above the ground plane. The amplifier is powered by rechargeable batteries com-

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bined with voltage regulators. Cells, electrodes, amplifier, and power supply are contained in a closed aluminium case of 2 mm wall thickness, which sufficiently shields any external noise. In order to use the dynamic range of the available signal-analysis instruments efficiently, two wide-bandwidth amplifiers and an equalizer stage follow. The latter is necessary to level out the frequency dependence of the input stage's noise. Without it, the downstream equipment could be driven into saturation merely by noise, due to the wide bandwidth of the system.

In the future, improvements both in sensitivity and frequency range are possible by the application of GaAs FETs, which presumably have to be selected individually for low noise at low frequencies. Low-noise microwave semiconductors based on a combination of silicon and germanium (SiGe transistors) have become available recently, and might be an alternative (33). Still several orders of magnitude more sensitive is the so-called single electron transistor, which has been shown to work up to at least 100 MHz (34), but is still in development.

Data Acquisition and Analysis

Although at first sight, low noise electronics seems to be the crucial factor in the direct detection of cellular AC fields, much information can be lost in the course of data analysis. Displaying the amplified signals with an oscilloscope, i.e., presenting the data in the time domain, makes them nearly impossible to discern in the presence of noise. The strong point of the oscilloscope, i.e., its ability to show phase relations, is not of interest thus far. A spectral presentation, i.e., in the frequency domain, reduces the noise level significantly. This is because the noise voltage is proportional to the square root of the resolution bandwidth, which can be much narrower than the complete spectrum itself. Moreover, numerous signals at different frequencies can be separated easily.

A typical laboratory device for spectral analysis is the spectrum analyzer which works like a superheterodyne receiver, i.e., rather similar to a bandpass filter with continuously variable frequency. In all the work published so far on cellular AC fields, spectrum analyzers have been employed (13,22–24,26,28,29). However, noise reduction by bandwidth limiting is only achievable at the expense of reduced temporal resolution, because the filters involved require a finite time for excitation which is proportional to the inverse of the filter bandwidth. This makes the time needed for one frequency sweep increase with the inverse square of the resolution bandwidth. From this it follows that reducing the noise voltage by bandwidth narrowing, e.g., by 50%, requires a 16-fold increase in sweep time. Typical sweep times of about two minutes are used (13,24). This means that any given frequency is monitored for only about 30 ms within that period, taking the data given in (13,24). Thus, events shorter than the sweep time can be simply lost. This might be one reason for the difficulties so far in detecting cellular AC activity in a reproducible manner.

A possible way out of this dilemma is a Fourier transformation of the signal.

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Here the complete information content is transformed into the frequency domain. However, there are a number of technical obstacles; because of the huge amount of data, intermediate storage and subsequent evaluation do not seem practicable. Therefore, the signal should be transformed in real time and averaged over a period of time that can be chosen arbitrarily, thereby reducing the amount of data to be stored. This calls for very fast analogue-to-digital converters (ADCs) and digital signal processors (DSPs), which, fortunately, are becoming available for the frequency range of interest. Basically, the information loss is mainly limited by the DSP's processing time. Having the data in a digital format opens the possibility of even further improvements. Under special assumptions, for example, presuming only a limited number of frequency bands present in the signal, the signal-to-noise ratio can be improved significantly by model-based frequency analyses (35). Also special digital filters such as the Kalman type could be employed for noise reduction (36).

A simpler approach has been realized in our laboratory employing 14 fixed bandpass filters in parallel (Fig. 2). The filters' center frequencies and widths have been adjusted so that they cover the complete frequency range from 3 kHz to 60 MHz evenly on a logarithmic scale. This is based on the assumption that the probability of a signal being present is the same between 1 and 2 MHz as it is between 10 and 20 MHz. For the same reason the relative resolution, i.e., the ratio between resolution bandwidth and center frequency, has been kept constant, in contrast to a spectrum analyzer, in which the relative resolution varies during a sweep, being finest at the high-frequency end. The filter outputs each are demodulated and are integrated with a time constant, which presently has been chosen as 0.5 s. The demodulated signals are then recorded by a personal computer equipped with a 16-channel data acquisition card at 12-bit resolution. The small number of channels only allows rather approximate frequency information, with an error of about a factor of two. However, at the present stage the experiments

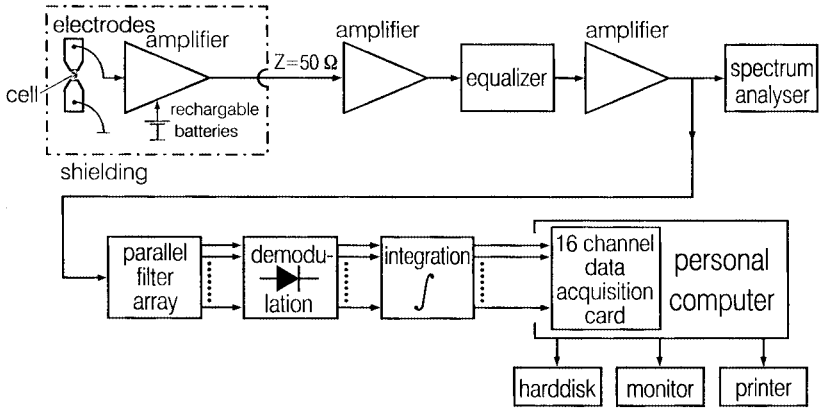

Figure 2. Principal structure of the detection system for cellular radio frequency oscilations.

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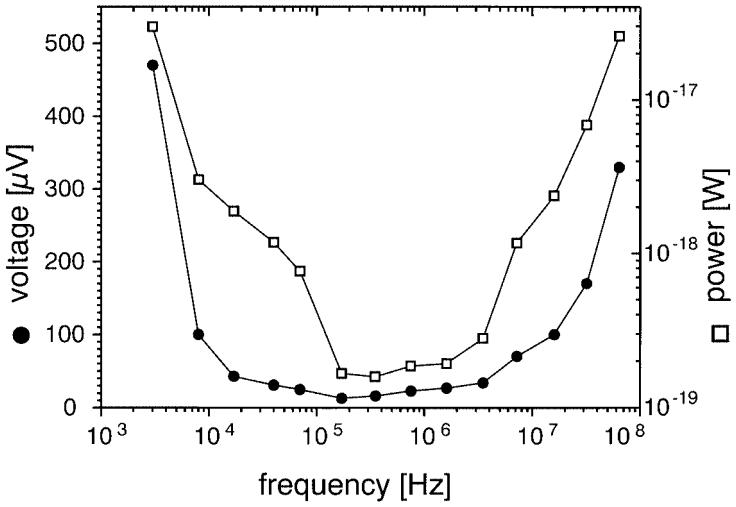

Figure 3. Minimum detectable signal level of the measurement system of Figure 2 at an integration time of 0.5 s. The biological cell is modelled by a signal source in series with a 0.2 pF capacitor and two 1 M Ω resistors.

are still aimed at proving the existence of cellular AC fields and reproducing previous results. Therefore, this restriction is of minor importance yet.

The system's sensitivity has been measured and optimized with a laboratory signal source connected to the input amplifier via a shielded 0.2 pF capacitor and two surface mount 1 M Ω resistors in series, in order to simulate a biological cell. The lowest signal levels that are clearly discernible are centered around 0.5 MHz (Fig. 3) at below 10^{-18} W between about 50 kHz and 5 MHz. This represents an improvement of one to two orders of magnitude in power level as compared to the combined use of preamplifier and spectrum analyzer published before (24), and is mainly a consequence of the integration time being a factor of 150 longer. Still, the temporal resolution is 200 times better, leaving room for even further sensitivity improvement.

EXPERIMENTAL RESULTS

So far, most detection experiments have been done with *Saccharomyces cerevisiae*. This is because handling of this organism is uncomplicated, it withstands hypo-osmolar media, and because the first experiments of this kind (22) were done with this yeast and have been undertaken to be reproduced. Although there seem to be differences in the frequencies found, all authors report RF activity in the region between 6 and 9 MHz (Table 1). Jelinek *et al.* (24) confirm the finding of Jafary-Asl and Smith (22) that maximal activity is recorded during the M phase

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Table 1. Electrical RF Oscillations of Micro-organisms

Author	Frequency [MHz]	Organism
Smith *et al.* (22,23)	1, 7 50 (60)–80	*Saccharomyces cerevisiae*
Pohl (26)	<1	*Netrium digitus*
Hölzel and Lamprecht (13,29)	1.5, 2.6, 5.7, 18, 52	*Saccharomyces cerevisiae*
	3.1, 4.8	*Schizosaccharomyces pombe*
	2.8, 8.5, 16, 35	*Monoraphidium griffithii*
Jelinek *et al.* (24)	8–9, 8.2	*S. cerevisiae*

of the cell cycle, which is also in agreement with the microdielectrophoretic studies of Pohl *et al.* (10). The remaining differences may be just a consequence of different strains used and of different environmental conditions like medium composition, cell concentration, and temperature.

The results attained with our latest experimental system cannot be instantly compared with the literature data due to the different way of representation. Figure 4 shows a 35-minute part of the temporal course of the electric activity of the fission yeast *Schizosaccharomyces pombe*. Cells were grown to the late phase of exponential growth and then diluted with fresh growth medium, of which about 50 µl were pipetted into a gasket around the electrodes. The volume was then sealed with a glass cover. The saw-tooth-like development of the signal level for the frequency bands between 170 kHz and 7 MHz is clearly visible. Such behavior could be observed in several experiments on *Schizosaccharomyces pombe*. In that special case 15 consecutive periods appeared within 98 minutes, corresponding to a duration of 392 s each, with a standard deviation of 8%. The amplitudes are on the order of 10^{-18} to 10^{-17} W. Such a regular oscillation over more than a frequency decade need not be real electrical cell activity, but might be explained by impedance changes of the system formed by the cells, the medium, and the

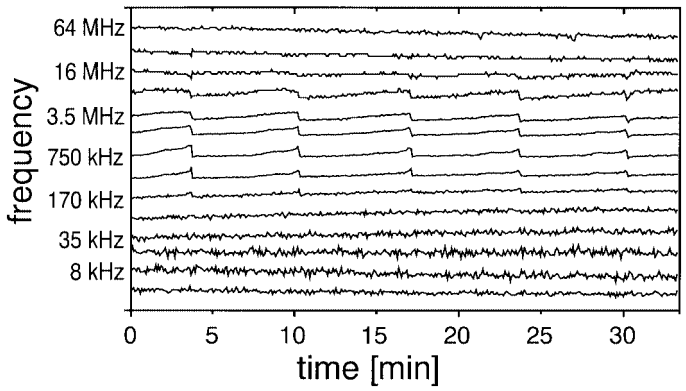

Figure 4. Temporal course of signals detected for the fission yeast *Schizosaccharomyces cerevisiae* in growth medium for 14 frequency bands centered between 3 kHz and 64 MHz.

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electrodes. For example, a spatial cycling of the cells could modulate the noise level at the input stage. This effect would be similar to the method of detecting minute changes of cell contracts by electric impedance monitoring (37). However, the cause for such a rhythmic behavior is not clear, although glycolytic oscillations might be involved. Still, also coherent excitations combined with nonlinear interactions could cause wideband oscillations (20) that would appear as a temporarily increased noise level.

CONCLUSIONS

The further development of concepts about coherent excitations and nonlinear behavior of biological matter, combined with the progress in microelectrode manufacturing and circuit design, have made a direct measurement of these phenomena increasingly feasible. Extending the accessible frequency range into the GHz range remains a challenging task. Pioneering experiments have been reproduced in principle now, and with quite sensitive systems ready, more effort can be put into proper control of experimental and biological conditions to achieve a better understanding of the already-observed phenomena.

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