Biophys. Struct. Mechanism 1, 273—283 (1975)

Dielectric Properties of Synaptosomes Isolated from Rat Brain Cortex

Akihiko Irimajiri, Tetsuya Hanai*, and Akira Inouye

Department of Physiology, Kyoto University School of Medicine, Kyoto 606, Japan

and

* Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan

Received February 18, 1975/Accepted March 27, 1975

Abstract. Dielectric measurements were performed on the suspensions of synaptosomes isolated from rat brain cortex. The synaptosomes in buffered salt media showed typical dielectric dispersions caused by the presence of a thin limiting membrane of sufficiently low conductivity. An analysis of the dielectric data revealed that the electric conductivity of the synaptosome interior was about 37% of the external medium conductivity under isotonic conditions and that the dielectric constant for the interior phase was about 35. The membrane capacitance (0.7 $\mu F\ cm^{-2}$) remained constant irrespective of nature and concentration of the univalent salts examined. Significant reduction in both the conductivity and the dielectric constant of the internal phase can be explained theoretically provided that some intrasynaptosomal structure (synaptic vesicles and/or small mitochondria) of non-conducting nature occupies about 50% of the particulate volume, the remainder being in equilibrium with the external salt medium.

Key words: Dielectric Constant — Conductivity — Synaptosomes.

Introduction

Synaptosomes, first introduced as an *in vitro* neural system by Gray and Whittaker [3] and de Robertis *et al.* [2], are isolated particles of about 1 μm in diameter, covered by the cytoplasmic membrane which encloses a mass of synaptic vesicles and often small mitochondria, and obtained in good purity by the density gradient fractionation technique. Because of their characteristic appearance revealed with electron microscopy, the isolated synaptosomes are supposed to have derived predominantly from presynaptic nerve endings, and hence have been employed by a number of investigators to study both synaptic and neural membrane functions *in vitro* (for review, see *e.g.* Refs. [13] and [14]).

With the studies so far made, however, much interest has centred on the neurochemical aspects of synaptosomes or on the utilization of these particles as a simplified model system for nerve cells of mammalian origin. In contrast, relatively little attention has been paid to the physical properties of synaptosomes except for their microscopic morphology. It appears desirable, therefore, to obtain information about the electrical properties of the limiting membrane by some non-destructive means of investigation.

The present study on the dielectric behaviour of rat brain synaptosomes in suspension is intended to provide one of these approaches in parallel with the light scattering studies that have already been reported [8].

Materials and Methods

Preparation

The synaptosomal fraction was prepared by the method of Gray and Whittaker [3] with slight modification. Male Wistar strain rats weighing 200 to 250 g were decapitated and the whole brains were removed. Cerebral cortex was carefully separated and homogenized in 10 vol of ice-cold 0.32 M sucrose by 7 strokes (up and down) of a loose-fitting Teflon/glass homogenizer. The homogenate was spun down at $1000 \times g$ for 10 min. The supernatant was centrifuged at $10000 \times g$ for 20 min to sediment the crude mitochondrial fraction, which was resuspended in the sucrose solution, placed over layers of 0.8 M and 1.2 M sucrose in tubes for a Hitachi SW 25 rotor, and centrifuged at $64000 \times g$ for 60 min. The band at the interface between 0.8 M and 1.2 M sucrose was collected, diluted with 0.32 M sucrose containing 10 mM Tris buffer (pH 7.3), and then pelleted by centrifugation at $12000 \times g$ for 30 min.

The synaptosomal pellets, after resuspension in a small volume of the buffered sucrose, were divided into aliquots (0.5 ml for each), diluted with 20 vol of the respective test media, and spun down as before. Following such washing procedure repeated twice, the last wash was performed after an interval of equilibration for at least 3 hrs[1]. The final pellets thus obtained were recombined with aliquots of the corresponding supernatant fluids and homogenized by use of tuberculin syringes.

All the procedure described was performed below 4° C.

Estimation of Average Diameter

A portion of the washed pellet of synaptosomes was examined by electron microscopy. The micrographs of thin sections showed that the synaptosomal particles were more or less ellipsoidal rather than spherical in their appearance; so the equivalent diameter was calculated for each particle by assuming a sphere of the equivalent volume. By normalizing to unity the sum of volume thus estimated over a population of 317 particles, a histogram for relative volume fraction was obtained as shown in Fig. 1. From the histogram the volume-averaged inner diameter of the synaptosomal specimen was calculated to be 0.678 μm, this figure being used throughout the analyses presented below.

Dielectric Measurement

A Boonton RX meter type 250 A was used over a frequency range 0.5 to 230 MHz. The measuring cell was composed of a pair of Pt disc electrodes clamped between separable lucite blocks (Fig. 2). The sample volume required was less than 0.3 ml. The electrode leads, cut as short as possible, were connected to the bridge terminals *via* mercury contact, as recommended by Schwan [12]. The meter

[1] As far as the dielectric parameters were concerned, the "3-hr" equilibration period secured no detectable difference between the washings and the original fresh media employed.

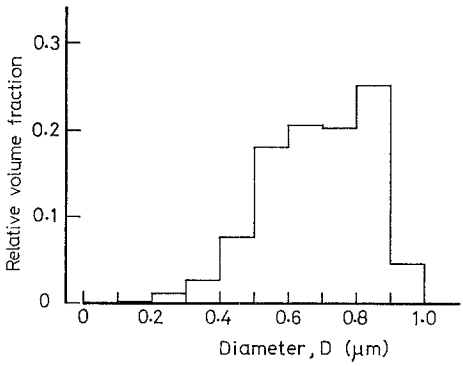

Fig. 1. Distribution of the apparent diameter of synaptosomes as estimated from electron microscopy (*see* text)

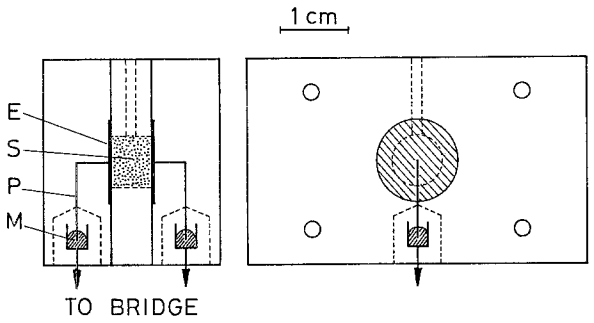

Fig. 2. Schematic of the cell assembly used. *E*, bright Pt electrode; *S*, sample cavity; *P*, Pt wire, and *M*, mercury contact. Polished lucite blocks

readings were corrected for a series inductance L according to Eqs. (29) and (30) in Ref. [12]. An L-value of 4.18×10^{-8}H was determined with the cell *plus* bridge system by using various concentrations of KCl [12]. The correction was an essential procedure[2] to get reasonable values of ε and $\varkappa$, and was quite effective up to about 200 MHz as exemplified in Fig. 3 (*see* broken lines for the medium alone). All the measurements were made at room temperature regulated at $25 \pm 1°$ C.

In the case of relatively conducting samples, measurements were affected by inaccuracies due either to electrode polarization at lower frequencies or to unreliable operation of the meter at higher frequencies. To overcome these difficulties, the limiting dielectric constants ε_l and ε_m (or ε_h) were read from extrapolation in the Cole-Cole plots [1], an example being depicted in Fig. 4.

Analysis of Data

The phase parameters of the suspended particles were determined by applying our curve-fitting method [4] which was originally based on the general

[2] *Uncorrected* readings from the RX meter showed always low values of ε at all frequencies as well as apparent dispersions of ε and $\varkappa$, especially in the high-frequency region, even for a simple salt solution like KCl.

equation of Pauly and Schwan [10]. Calculations were carried out with the aid of a Yokogawa-Hewlett-Packard personal computer Model 10.

List of Symbols

ε	dielectric constant	ω	angular frequency, $2\pi f$
$\varkappa$	electric conductivity	j	$\sqrt{-1}$
ε^*	complex dielectric constant	D	inner diameter of particle
ϵ_0	dielectric constant of free space	d	shell thickness
f	frequency of applied a.c. field	C_M	specific membrane capacity
f_P	characteristic frequency of the *P*-dispersion (*cf.* Ref. [4])	Φ	volume fraction of a whole suspension
		ϕ	volume fraction of a partial suspension

Subscripts refer to:

a	external medium phase	m	plateau value at intermediate frequency
i	internal phase of particle	h	limiting value at high frequency side
s	shell phase	k	different species with respect to D or $\varkappa_i$
l	limiting value at low frequency side	v	intrasynaptosomal structure (*e.g.* vesicles)

Results

Assignment of Dispersion Curves

Fig. 3 illustrates the frequency dependence of dielectric constant and conductivity for a suspension of synaptosomes. The dielectric dispersion observed in the present frequency range was assigned exclusively to the *P*-dispersion [4] or the β-dispersion according to a nomenclature by Schwan [12], as discussed in detail previously [4].

On the other hand, theoretical consideration [5, 12] indicates that the *P*-dispersion in general can be ascribed to an interfacial polarization effect occurring at the shell phase whose dielectric properties differ from those of the external or the internal phase of the suspended particles, and that this effect becomes manifest in the case that the shell phase is composed of poorly conducting material like biological membrane. In fact, such a statement was confirmed experimentally by observing that the addition of surfactant (saponin, 20 mg/ml) to the synaptosomal suspension led to a substantial reduction in the magnitude of dispersion established so far.

Hence it is concluded (i) that synaptosomal membrane resealing was almost complete in spite of the drastic preparatory procedure including the pinch-off of nerve endings by homogenization, (ii) that, to a first approximation, the isolated synaptosomes are represented by a model of shell spheres with a shell phase which is far less conductive compared to the aqueous phases separated by it, and (iii) that the data of our measurements may be analyzed in terms of the dielectric theory of Pauly and Schwan [10].

To obtain information on the limiting membrane and the internal phase of the synaptosomes, the following two series of experiments were conducted.

Variation of Volume Fraction

A well equilibrated pellet of synaptosomes was transferred to the cell and impedance measurements were made at various volume fractions Φ, from 0.54 to 0.10, by sequentially diluting the specimen with an isotonic medium consisting

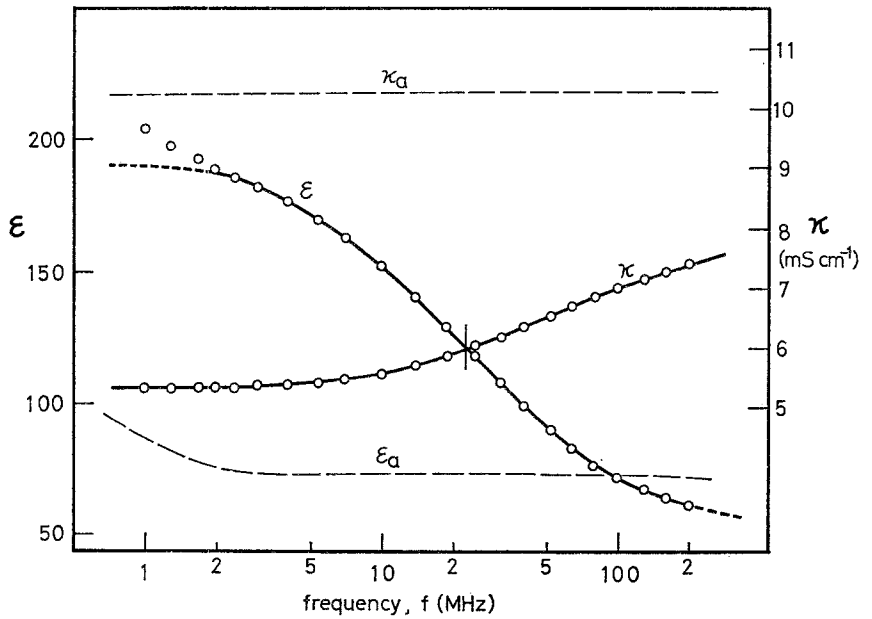

Fig. 3. Plots of dielectric constant ε and conductivity $\varkappa$, as a function of frequency f, for a suspension of synaptosomes with volume fraction $\Phi = 0.38$. The suspending medium contained (in mM): 100 NaCl, 92 sucrose, and 10 Tris-HCl (pH 7.3); its electric data are shown by broken lines (ε_a and $\varkappa_a$)

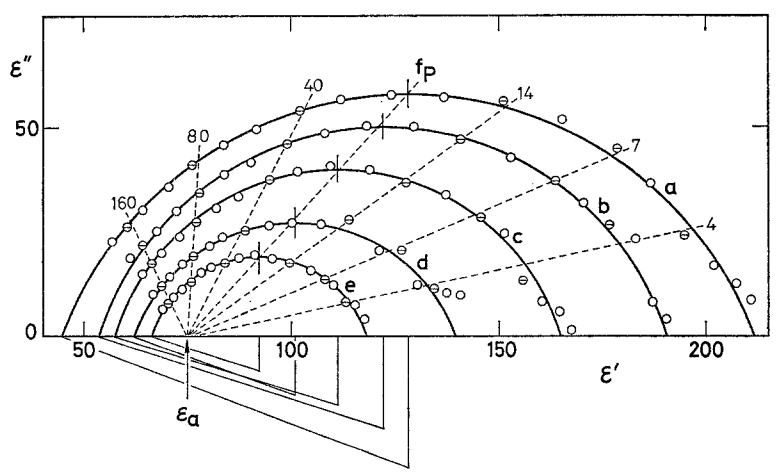

Fig. 4. Cole-Cole plots for the synaptosomal suspensions of volume fractions varied as: (a) $\Phi = 0.54$, (b) 0.38, (c) 0.27, (d) 0.17, and (e) 0.10. *Abscissae*, $\varepsilon' = \varepsilon$; *Ordinates*, $\varepsilon'' = (\varkappa - \varkappa_l)/\omega\epsilon_0$. Parameters are frequencies in MHz; vertical bars denote the characteristic frequencies f_P. The composition of the medium used is as in Fig. 3

Table 1. Electric parameters of synaptosomes in dilution series[a]

Specimen	Φ[b]	$\varkappa_l$ mS cm^{-1}	ε_l	ε_m	f_P MHz	ε_i	$\varkappa_i$ mS cm^{-1}	ε_s[c]	C_M μF cm^{-2}
a	0.54	3.72	211	45	22.8	32	3.6	3.7	0.65
b	0.38	5.33	191	54	22.6	35	3.9	3.7	0.66
c	0.27	6.57	165	57	23.7	38	4.0	3.7	0.65
d	0.17	7.83	140	62	24.6	27	4.3	3.8	0.68
e	0.10	8.86	118	66	23.7	28	4.5	4.0	0.72
Mean					23.4	32	4.1	3.8	0.67

[a] The suspending medium contained (in mM): 100 NaCl, 92 sucrose, 10 Tris-HCl (pH 7.3). Osmolality, 0.30 Osm kg^{-1}. Electric parameters: $\varepsilon_a = 74$, $\varkappa_a = 10.3$ mS cm^{-1}.

[b] Calculated from ratios $\varkappa_l/\varkappa_a$ by using Eq. (3) of Paper I.

[c] Calculated by assuming d = 50 Å.

of 100 mM NaCl and buffered sucrose. The result is shown in Fig. 4 as adapted to complex dielectric plane plots. It can be seen that the data as a whole fitted satisfactorily a circular arc pattern of Cole-Cole type with depressed centres. The phase angle ($\sim$72°) as well as the characteristic frequency f_P ($\sim$23 MHz) were both found to remain fairly constant irrespective of Φ, while the dispersion magnitude, $\Delta\varepsilon_P = \varepsilon_l - \varepsilon_m$, decreased as Φ was decreased.

The depressed centres indicate contribution from multiple relaxation mechanisms operating in the specimen. This point will be considered in some detail in the Discussion. The relative constancy in phase angle and f_P, on the other hand, appears to reflect a fact that the relaxation mechanisms and hence the dielectric properties of the suspended particles were hardly ever affected by the simple dilution technique employed here. If this is the case and if the data are properly analyzed, then the resultant parameters, ε_i, $\varkappa_i$, ε_s, and $\varkappa_s$, all should be independent of Φ. The result of analysis together with the raw data is summarized in Table 1. It is apparent that the electric phase parameters of synaptosomes equilibrated under the specified medium condition were relatively constant. Thus we obtain the mean values: $\varepsilon_i = 32$, $\varkappa_i = 4.1$ mS cm^{-1}, and $\varepsilon_s = 3.8$ or alternatively $C_M = 0.67$ μF cm^{-2}. A finite value of $\varkappa_s$ was not accessible by our method because we started the analysis assuming $\varkappa_s = 0$ or several orders of magnitude smaller than $\varkappa_a$. Indeed, a choice between $\varkappa_s = 0$ and $\varkappa_s = \varkappa_a \times 10^{-6}$ made no discernible difference in the determination of the other parameters.

Variation of Salt Concentration

Aliquots from a synaptosomal preparation were equilibrated separately with four different concentrations of NaCl each supplemented with buffered sucrose to maintain the external osmolality at the isotonic level ($\sim$0.30 Osm kg^{-1}). As shown in Fig. 5, lowering of salt concentration caused the dispersion regions simply to shift toward the lower frequency side while the overall profiles of the dielectric dispersion curves were kept unaltered. This behaviour is quite similar to that reported by Pauly and Packer [11] for isolated heart mitochondria. Analysis

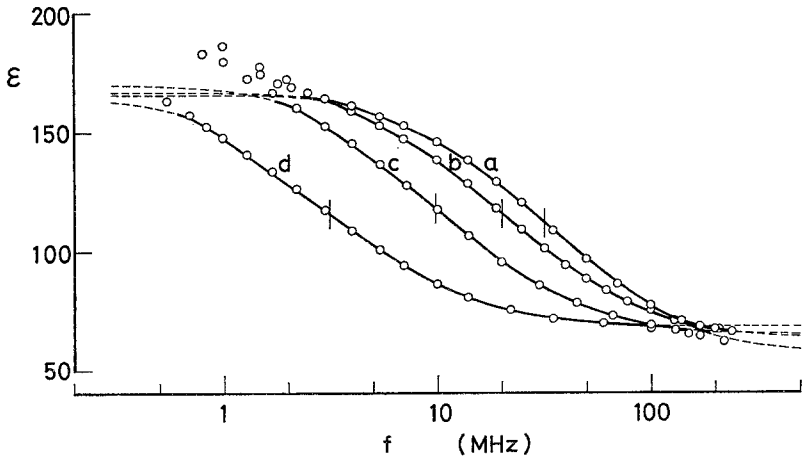

Fig. 5. Dielectric dispersion profiles of the synaptosome suspensions in which the salt (NaCl) concentration of the suspending media was changed as: (a) 150 mM, (b) 100 mM, (c) 50 mM, and (d) 10 mM. Volume fractions Φ ranged over 0.29 (a) — 0.17 (d). Vertical bars indicate the characteristic frequencies f_P estimated from Cole-Cole plots covering the frequency ranges denoted by the solid curves

Table 2. Effect of varying ionic concentrations with fixed medium osmolality[a]

Specimen	Medium NaCl mM	ε_a	$\varkappa_a$ mS cm^{-1}	ε_l	ε_m	f_P MHz	$\varkappa_l$ mS cm^{-1}	Φ[b]	ε_i	$\varkappa_i$ mS cm^{-1}	ε_s[c]	C_M μF cm^{-2}
a	150	75	15.2	165	58	32	9.5	0.29	29	5.3	3.5	0.62
b	100	75	9.7	167	63	20	6.9	0.22	32	4.1	4.2	0.74
c	50	76	5.2	170	65	9.8	3.6	0.22	38	2.0	4.2	0.74
d	10	77	1.5	163	68	3.2	1.2	0.17	37	0.74	4.7	0.84
Mean		75							34		4.2	0.73

[a] Osmolality was adjusted, with varying amounts of sucrose added, to 0.30 Osm kg^{-1}.
[b] Calculated from ratios $\varkappa_l/\varkappa_a$ by using Eq. (3) of Paper I.
[c] Calculated by assuming d = 50 Å.

of the raw data yielded the parameters which are listed in Table 2. Among the phase parameters tabulated, only the internal conductivity $\varkappa_i$ changed systematically with the salt concentration or with the external parameter $\varkappa_a$.

An essentially comparable result was obtained when NaCl, the principal electrolyte in the external media, was replaced by KCl.

Discussion

The results of dielectric measurements clearly demonstrate that the dielectric dispersions of the synaptosomal suspensions can be regarded as a *P*-dispersion which is closely associated with the presence of a virtually non-conducting shell

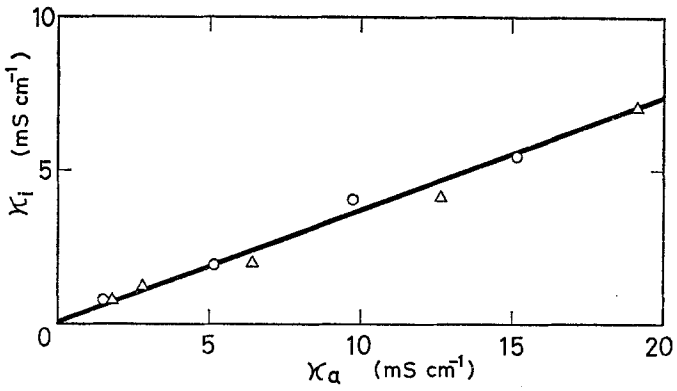

Fig. 6. A relationship between $\varkappa_i$ and $\varkappa_a$ for the synaptosomes equilibrated with the suspension medium containing NaCl (○) or KCl (△)

phase (*i.e.* the synaptosomal limiting membrane) with electric capacity of about 0.7 μF cm^{-2}, and that both the dielectric constant and the conductivity of the synaptosome interior were significantly smaller than those of the external aqueous phase, *i.e.*, $\varepsilon_i/\varepsilon_a \simeq 0.45$ (Table 2) and $\varkappa_i/\varkappa_a \simeq 0.37$ (Fig. 6). No further inference could, however, not be drawn from these results without paying due consideration to the method of analysis employed.

Broadening of Dispersion Curves

In analyzing the raw data obtained, we chose the Pauly-Schwan theory as the most plausible among the theories currently available and so used the general expression of the form of Eq. (1) given in the preceding paper (henceforth referred to as Paper I). Since the expression has been derived for a system of dielectrically homogeneous shell-spheres, it might be impracticable, strictly speaking, to apply to a nonhomogeneous system like the synaptosomes which gave rise to broadened dispersion curves that could not be explained without taking into account more than one relaxation times (Fig. 4).

Indeed, our method was only successful in fitting at the three points (ε_l, f_P, and ε_m) of the theoretical curves. In this regard the curve-fitting method employed was somewhat crude. As a consequence the results of analysis should by no means be taken to be rigorous enough, but it is still conceivable that they represent the most probable values of parameters around which some distribution may well take place. In an attempt to bridge over the discrepancy between the theoretical and the experimental curves (*see* Fig. 3 of Paper I), we first examined possible contribution from the distribution of diameter D.

According to the principle of superposition, the general expression [Eq. (1) of Paper I] is extended to allow for a system of particles having n different diameters (D_k's), so that an extended form of the equation can be written as

$$\frac{\varepsilon_a^* - \varepsilon^*}{2\varepsilon_a^* + \varepsilon^*} = \sum_{k=1}^{n} \frac{(\varepsilon_a^* - \varepsilon_s^*)(2\varepsilon_s^* + \varepsilon_i^*) + (\varepsilon_a^* + 2\varepsilon_s^*)(\varepsilon_s^* - \varepsilon_i^*)(1 + 2d/D_k)^{-3}}{(2\varepsilon_a^* + \varepsilon_s^*)(2\varepsilon_s^* + \varepsilon_i^*) + 2(\varepsilon_a^* - \varepsilon_s^*)(\varepsilon_s^* - \varepsilon_i^*)(1 + 2d/D_k)^{-3}} \phi_k, \qquad (1)$$

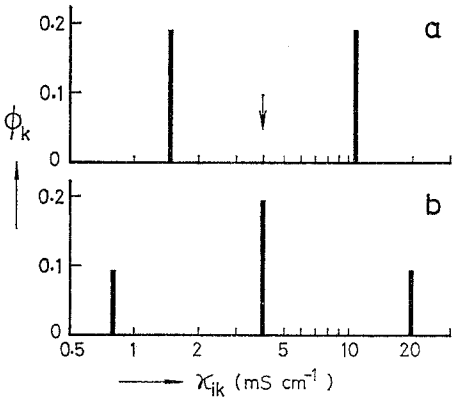

Fig. 7. Assumed distributions of intrasynaptosomal conductivity κ_i

where $\sum_{k=1}^{n} \phi_k = \Phi$ and the notations used are the same as those defined in Paper I. Now we can plot the frequency dependence of ε for any system of size distribution provided that an actual relationship between ϕ_k and D_k is available. As an example, computation was carried out for the case of Specimen b in Table 1 assuming the size distribution depicted in Fig. 1. The result was that the effect of such a size distribution was unexpectedly too small to account for the observed broadening of the dielectric dispersion. The effect of size heterogeneity can thus be ruled out.

The next trial made was to introduce a distribution of the internal conductivity κ_i into the general equation. The reason for this trial was two-fold: (i) because the electron micrographs showed that the synaptosomal specimen contained not only the typical synaptosomes which obviously enclosed synaptic vesicles and/or mitochondria (suggesting low κ_i) but also the ghosts in which these subsynaptosomal structures were entirely lacking (suggesting high κ_i), and (ii) because the parameter κ_i, among others, was the most effective in changing the relaxation frequency f_p without affecting appreciably the other parameters such as ε_l and ε_h (or ε_m) (*cf.* Table 2 of Paper I). To this end the following form of expression is assumed on the basis of the superposition principle here again:

$$\frac{\varepsilon_a^* - \varepsilon^*}{2\varepsilon_a^* + \varepsilon^*} = \sum_{k=1}^{n} \frac{(\varepsilon_a^* - \varepsilon_s^*)(2\varepsilon_s^* + \varepsilon_{ik}^*) + (\varepsilon_a^* + 2\varepsilon_s^*)(\varepsilon_s^* - \varepsilon_{ik}^*)(1 + 2d/D)^{-3}}{(2\varepsilon_a^* + \varepsilon_s^*)(2\varepsilon_s^* + \varepsilon_{ik}^*) + 2(\varepsilon_a^* - \varepsilon_s^*)(\varepsilon_s^* - \varepsilon_{ik}^*)(1 + 2d/D)^{-3}} \phi_k, \qquad (2)$$

where $\varepsilon_{ik}^* = \varepsilon_i + \kappa_{ik}/j\omega\epsilon_0$ and $\sum_{k=1}^{n} \phi_k = \Phi$, the other notations being the same as in Paper I.

Now that the exact distribution of κ_i for synaptosomes is not known, however, it is to a certain extent arbitrary to assume the relation of ϕ_k *vs.* κ_{ik}. Accordingly, we sought to incorporate the simplest ones such as shown in Fig. 7. Included there are only two κ_i's in (a) and three κ_i's in (b); either was proved successful in giving rise to the broadened dispersion curves for Specimen b of Table 1. It is to be noted that another distribution will explain equally well in so far as a symmetrical distribution of log κ_{ik} holds around the most probable value of κ_i (3.9 mS cm^{-1} for the case of Specimen b).

As regards the other phase parameters, it is reasonably expected that some diversity in $\varkappa_s$, ε_s and/or ε_i may be responsible for the observed distribution of relaxation times of the system. At any rate, since the aim of the present study is to find a clue to the knowledge of the gross electrical characteristics of the synaptosomes as obtained, the approach based on the method of analysis proposed in Paper I might not be extremely unsuitable.

Electrical Estimate for Intrasynaptosomal Structure

Judging from morphological evidence by electron microscopy, the interior of a typical synaptosomal particle can be regarded, in turn, as a concentrated suspension of organelles, notably, the synaptic vesicles in this case. It is for such a suspension that we have so far referred to ε_i and $\varkappa_i$. In other words, these parameters should be taken to represent the equivalent static properties of this particular suspension.

On the other hand, Hanai [6], extending Maxwell-Wagner's dielectric theory on a dilute two-phase system to a more concentrated one, has already derived convenient expressions for the dielectric constant and conductivity of an oil-in-water type emulsion in which the conductivity of the dispersed oil droplets is vanishingly small compared to that of the continuous water phase.

If we assume here that regarding the synaptosome interior the static electric conductivity of the dispersed organelles (which are all known to be actually membrane-bounded) be negligibly small relative to the sap phase conductivity, and that the sap phase parameters can be approximated by those of the external solution[3] (*i.e.*, ε_a and $\varkappa_a$), then Hanai's theory [6] could be applied to our system in the following expressions[4]:

$$\varkappa_i/\varkappa_a = (1-\Phi_v)^{3/2} \tag{3}$$

and

$$(2\varepsilon_i - 3\varepsilon_v)/(2\varepsilon_a - 3\varepsilon_v) = (1-\Phi_v)^{3/2}, \tag{4}$$

where Φ_v stands for the volume fraction of dispersed organelles having an equivalent homogeneous dielectric constant ε_v. The value of Φ_v for the synaptosome specimen is calculated, by putting $\varkappa_i/\varkappa_a = 0.37$ (Fig. 6) in Eq. (3), as $\Phi_v = 0.49$. Insertion of this figure together with $\varepsilon_i = 34$ and $\varepsilon_a = 75$ (Table 2) into Eq. (4) yields $\varepsilon_v = 6.6$.

In conclusion, it is suggested from these results that the intrasynaptosomal solid material, such as synaptic vesicles and small mitochondria, occupies just about one half of the internal space of an isolated typical synaptosome and that the equivalent dielectric constant of the solid material is approximately 7. The former finding is in good agreement with the fact that the osmotic dead space of

[3] This assumption, though not directly checked by any ion analysis on the specimen used, seems quite reasonable since equilibration of the synaptosomal particles with the test (suspending) media was usually performed for more than 7 hrs at 0° C.

[4] Eqs. (3) and (4) correspond respectively to Eqs. (62) and (63) (in Ref. [6]) which were derived for O/W emulsions under a low-frequency condition. The choice of the "low-frequency" condition in interpreting ε_i and $\varkappa_i$ mentioned here may be justified, since numerical estimation by using the resulting values of parameters, Φ_v and ε_v, indicated that the possible frequency dependence of ε_i or $\varkappa_i$ was almost negligible, as already pointed out by Hanai [6].

isolated synaptosomes was about 46% of their initial volume at an external osmolality of 0.3 Osm kg^{-1} [7]. Whether or not the latter prediction ($\varepsilon_v \simeq 7$) is decisive should, of course, await further experimental verification. Because of low yield of purified synaptic vesicles, no direct dielectric measurements have as yet been made in our laboratory. Nevertheless, this might be a figure not so unrealistic in view of the reported chemical composition of the isolated synaptic vesicles that the lipid/protein ratio amounted to 0.82 with the highly purified vesicles [9].

Acknowledgments. The authors are grateful to Professor N. Koizumi of Kyoto University for useful suggestions on the dielectric measurements. Our thanks are also due to Drs. K. Kamino and H. Ozaki of our laboratory for electron microscopy and helpful discussion. This research was supported in part by a grant from the Ministry of Education, Japan.

References

1. Cole, K. S., Cole, R. H.: Dispersion and absorption in dielectrics. I. Alternating current characteristics. J. Chem. Phys. **9**, 341–351 (1941)
2. De Robertis, E., De Iraldi, A. P., De L. Arnaiz, G. R., Salganicoff, L.: Cholinergic and non-cholinergic nerve endings in rat brain. I. Isolation and subcellular distribution of acetylcholine and acetylcholinesterase. J. Neurochem. **9**, 23–35 (1962)
3. Gray, E. G., Whittaker, V. P.: The isolation of nerve endings from brain: An electron microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. (Lond). **96**, 79–88 (1962)
4. Hanai, T., Koizumi, N., Irimajiri, A.: A method for determining the dielectric constant and the conductivity of membrane-bounded particles of biological relevance. Biophys. Struct. Mechanism. **1**, 285–294 (1975)
5. Hanai, T.: Electrical properties of emulsions. In: Emulsion science, chapt. 5 (ed. P. Sherman). London-New York: Academic Press 1968
6. Hanai, T.: Theory of the dielectric dispersion due to the interfacial polarization and its application to emulsions. Kolloid-Z. **171**, 23–31 (1960)
7. Irimajiri, A., Hanai, T., Inouye, A.: Evaluation of a conductometric method to determine the volume fraction of the suspensions of biomembrane-bounded particles. (Submitted to Experientia.)
8. Kamino, K., Inouye, K., Inouye, A.: Potassium ion-induced swelling of nerve-ending particles by light-scattering measurement. Biochim. biophys. Acta (Amst.) **330**, 39–52 (1973)
9. Lapetina, E. G., Soto, E. F., De Robertis, E.: Lipids and proteolipids in isolated subcellular membranes of rat brain cortex. J. Neurochem. **15**, 437–445 (1968)
10. Pauly, H., Schwan, H. P.: Über die Impedanz einer Suspension von kugelförmigen Teilchen mit einer Schale. Z. Naturforsch. **14b**, 125–131 (1959)
11. Pauly, H., Packer, L.: The relationship of internal conductance and membrane capacity to mitochondrial volume. J. biophys. biochem. Cytol. **7**, 603–612 (1960)
12. Schwan, H. P.: Determination of biological impedance. In: Physical techniques in biological research, vol. 6, part B (ed. W. L. Nastuk), pp. 323–407. New York: Academic Press 1963.
13. Whittaker, V. P.: The synaptosomes. In: Handbook of neurochemistry, vol. 2 (ed. A. Lajtha), pp. 327–364. New York: Plenum Press 1969
14. Whittaker, V. P.: The use of synaptosomes in the study of synaptic and neural membrane function. In: Structure and function of synapses (eds. G. D. Pappas, D. F. Purpura), pp. 87–100. New York: Raven Press 1972

Dr. A. Irimajiri
Department of Physiology
Kyoto University School of Medicine
Kyoto 606, Japan