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Electrically induced gel-to-gel phase-transition in neurons

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Abstract

At the end of transcardial perfusions with ice-cold physiological saline for 30 min or with isoosmotic potassium chloride for 5 min, but immediately before perfusion fixation, condenser-discharge electric shocks were administered to rats through surface electrodes pressed onto the temporal muscles of the scalped skull. As a result, striking ultrastructural compaction came about in numerous neurons thinly scattered in certain brain areas. Its features displayed a high degree of similarity to those previously observed following the in vivo administration of the same kind of electric shocks. This surprising independence from the actual state of metabolism questions whether the ultrastructural compaction, induced either in vivo or post mortem, is the result of any cascade of enzyme-mediated processes. On the other hand, a physical mechanism, phase transition propagated by non-covalent free energy stored in a cytoplasmic gel structure, which was proposed recently to explain a mechanically induced similar ultrastructural compaction, appears to apply also to the present case.

Keywords: Post-mortem experiments; ‘‘Dark’’ neurons; Ultrastructural compaction; Non-enzymatic mechanism; Stored non-covalent free energy

1. Introduction

In connection with the physical state of the material filling all cytoplasmic spaces in eukaryotic cells that are not occupied by the ultrastructural components ‘‘visible’’ in the conventional transmission electron microscope (‘‘aqueous cytoplasm’’; Clegg, 1984) many conceptions between the extremes outlined below coexist in the relevant literature. The ‘‘dissolved-state’’ conception regards the aqueous cytoplasm as a concentrated solution in which the overwhelming majority of proteins, metabolites, inorganic ions and water molecules can diffuse freely. According to the ‘‘organized-state’’ conception, the overwhelming majority of the molecules of all these substances are bound by non-covalent interactions both to each other and also to the ‘‘visible’’ ultrastructural elements, forming a continuous gel-like structure throughout the cell. Although the organized-state conception has not been favored during recent decades, it has been supported by an abundance of observations, partly from non-biological fields of research (referenced in Pollack, 2001). Among others, polymer chemistry has invented synthetic gels that can assume two or more metastable phases, each having a distinct free-energy minimum, a distinct set of macromolecular conformations and a distinct degree of water content (Annaka and Tanaka, 1992), and therefore a distinct volume. Initiated at a single point, transition from one gel phase to another can spread throughout the gel, propelled by the difference in free energy (Tanaka et al., 1992). The initiation can be performed by a subtle change around

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a critical value in the chemical composition, pH or temperature of the surrounding medium, and also by illumination, an electric field or a mechanical stress (Hoffman, 1991). Such gel-to-gel phase transitions have already been suggested to play essential roles in certain activities of the living cell, such as muscle contraction (Pollack, 1996), action potential (Tasaki, 1999, 2002), mucin secretion (Verdugo et al., 1992) and the formation of ''dark'' neurons (Gallyas et al., 2004).

As regards the latter process, various paradigms modeling human neurological diseases, such as ischemia, hypoglycemia and status epilepticus (referenced in Gallyas et al., 1990), can cause a common type of morphological damage to a number of neurons thinly scattered among neurons without this damage in the brains of experimental animals. The common morphological features acquired by the soma-dendrite domain of each affected neuron are (a) massive shrinkage, hyperbasophilia, type-III argyrophilia and an increased electron density, (b) marked compaction of the ultrastructural elements, and (c) aggregation of the nuclear chromatin with a pattern other than that characteristic of apoptotic cells (Attilo et al., 1983; Petito and Pulsinelli, 1984; Auer et al., 1985; Dietrich et al., 1992; Csordás et al., 2003; Ishida et al., 2004). Such neurons are traditionally designated as ''dark''. Many other cell types of mammalian tissues can also be brought into the ''dark'' morphological state (referenced in Harmon, 1987).

Mechanical injuries are known to momentarily produce ''dark''-cell formation (ultrastructural compaction) in scattered neurons of the rat brain. This ensues even when the injury is inflicted under post-mortem circumstances that are extremely unfavorable for enzyme-mediated biochemical processes: after a 90-min immersion in icy water, which was preceded by a 30-min perfusion fixation (Gallyas and Zoltay, 1992; Gallyas et al., 2004). To explain this surprising fact, an enzyme-independent process, gel-to-gel phase transition has been suggested to propel the ultrastructural compaction (Gallyas et al., 2004). Despite their century-long history (e.g. Nissl, 1886), no other mechanism for the formation of ''dark'' neurons has been proposed so far.

Condenser-discharge electric shocks with various parameters, administered in vivo through surface electrodes pressed onto the temporal muscles of the scalped rat skull, are also known to momentarily produce ''dark'' neurons (Gallyas et al., 1993; Csordás et al., 2003). The affected neurons are scattered in a non-impaired environment among normal neurons of the same phenotype. Similarly to mechanical injuries, such electric injuries might also be able to initiate the formation of ''dark'' neurons under highly non-physiological circumstances, since they are also physical in nature. Should this supposition prove to be right, the gel-to-gel phase-transition theory of the whole-cell ultrastructural compaction, and also the gel-like nature of the ''aqueous cytoplasm'', would be supported.

In pilot experiments, electric shocks failed to produce ''dark'' neurons under the circumstances applied in the above mechanical-injury study. For this reason, we have now tested other non-physiological circumstances: transcardial perfusion with chilled physiological saline or with isoosmotic potassium chloride at room temperature. These will be referred to as post-mortem circumstances, though individual cells may be able to recover.

2. Materials and methods

2.1. Animal experiments

A total of 16 Wistar rats weighing between 190 and 210 g were anesthetized by the intraperitoneal administration of 2 ml/kg of a 1:1 mixture of 25 mg/ml Thiopental (Biochemie GmbH, Austria) and 5 mg/ml Seduxen (Richter Gedeon Rt, Hungary). Four groups of rats were studied, with five rats each in groups 1 and 2, and three rats each in groups 3 and 4. In the middle of the caudate putamen (0.5 mm rostral to the bregma, 2.5 mm lateral to the midline and 5.0 mm ventral to the cortical surface) of each group-3 rat, the bead probe of a digital thermometer was implanted. Subsequently, in a room kept just below the freezing point, the group-3 and group-1 rats were transcardially perfused for 30 min with around 1000 ml of physiological saline previously cooled down to just above the freezing point. The rats in groups 2 and 4 were transcardially perfused first with physiological saline for 1 min, then with 0.15 mol/l (''isoosmotic'') potassium chloride for 5 min at room temperature.

Following these perfusions, the rats in groups 1 and 2 were exposed to a single condenser-discharge electric shock (350 V, 500 μF), administered by a laboratory-built apparatus (Gallyas et al., 1993) through surface electrodes (4×4 mm^2) pressed bilaterally onto the temporal muscles of the exposed calvaria (Csordás et al., 2003). Finally, all the rats were transcardially perfused for 30 min with 500 ml of an electron-microscopic fixative. This was prepared by mixing 250 ml of 0.2 mol/l sodium cacodylate, 50 ml of 20% paraformaldehyde, 50 ml of 25% glutaraldehyde, 25 ml of 0.1 mol/l calcium chloride and 125 ml of 10% polyvinylpyrrolidone K25, followed by adjustment of the mixture to pH 7.5 with a few drops of 0.1 mol/l hydrochloric acid. For the rats in groups 1 and 3, the fixative had been cooled down to just above the freezing point. After fixation, the rats were left untouched at room temperature for 24 h before removal of the brain from the skull (Cammermeyer, 1961).

2.2. Tissue processing and staining

The cerebra and cerebella were vibratome-sectioned coronally at 150 μm. Every tenth vibratome section was stained by a special silver method (Gallyas et al., 1990) that gives reproducible results (Newman and Jasani, 1998). Briefly, following dehydration with graded 1-propanol, vibratome sections were incubated for 16 h at 56 °C in 1-propanol containing 0.8% sulfuric acid and 2% water, then rehydrated with graded 1-propanol, treated with 3% acetic acid for 10 min and finally immersed in a special physical developer until the background had become light-brown. The sections were then washed with three changes of 1% acetic acid, dehydrated with graded 1-propanol, cleared with clove oil and covered with Canada balsam. By visualizing type III argyrophilia, i.e. a capability of tissue elements to catalyze the formation of metallic silver grains in physical developers (Gallyas, 1982), this method selectively demonstrates the neuronal somata, dendrites and axons that have undergone ultrastructural compaction (Gallyas et al., 2002; Csordás et al., 2003).

From other vibratome sections, 2×2-mm^2 parts of the brain areas that contained numerous affected neurons or axons in the adjacent silver-stained sections were dissected. These specimens were postfixed with a 1:1 mixture of 2% osmium tetroxide and 3% potassium ferrocyanide for 1 h at room temperature, and then flat-embedded in Durcupan ACM. Semithin sections were cut at 1.0 μm and air-dried onto microscopic slides previously coated with Vectabond adhesive. They were stained in a solution containing 0.05% toluidine blue, 0.05% sodium tetraborate and 0.1% saccharose (pH ~ 9.5) for 1 min at 90 °C. This method visualizes basophilia, i.e. a capability of tissue elements to bind positively charged dye molecules, in osmicated and then Durcupan-embedded 1-μm sections (Csordás et al., 2003). Thin sections were cut at 40 nm, and stained with uranyl acetate and lead citrate in the usual manner.

2.3. Methodological comments

Intracranial mechanical manipulation such as the implantation of a thermometer bead probe could lead to ultrastructural compaction in neuronal somata, dendrites and axons (Gallyas et al., 2002; Csordás et al., 2003). For this reason, the brain temperatures were not measured in the rats perfused with chilled physiological saline before the electric shock. However, it can be taken as highly probable, but without direct proof, that their brain temperatures at the moment of the electric shock were approximately as low as in the chilled but not electric-shocked control rats.

Probably because of the contraction of the blood vessels, the rate of transcardial perfusion with isoosmotic potassium chloride rapidly decreased with time. After a 30-min perfusion, the brain could no longer be fixed transcardially any more. This was the reason why this kind of perfusion was terminated after 5 min.

As demonstrated in a previous study dealing with the morphological changes caused by in vivo electric shocks (Gallyas et al., 1993), the distribution pattern of the electrically produced "dark" neurons markedly changed as a function of the condenser voltage. In contrast with most brain areas, where "dark" neurons were more numerous at higher voltages, the number of "dark" granule neurons displayed a maximum at 250 V in the caudolateral part of the hippocampal dentate gyri. On the other hand, in the rats perfused with chilled physiological saline, the maximum incidence of the "dark" granule neurons in the same area was found at 350 V (unpublished data). This was the reason why this condenser voltage was chosen in the present study. Furthermore, independently of the condenser voltage in the interval 125–750 V, "dark" granule neurons were inconsistently present in this area in the rats perfused with isoosmotic potassium chloride.

The latter rats were involved in the present study in order to answer the question whether different post-mortem circumstances of electric shocks produce the same intracellular morphological changes.

3. Results

3.1. Control rats

At the end of the 30-min transcardial perfusion with chilled physiological saline, the brain temperature was below 2 °C in each rat tested. Except for the immediate vicinity of the implanted thermometer bead probe in these rats, the morphological characteristics that were consistently observed in the neuronal somata, dendrites and axons in the electric-shocked rats were not detected in any rat in either control group.

3.2. Light-microscopic observations

In the brain of each rat transcardially perfused with chilled physiological saline before the electric shock, but not in any of the control rats, the silver technique stained black a proportion of the neuronal somata and dendrites that were thinly scattered in an apparently undamaged environment. The silver-stained somata were markedly shrunken. If the soma of a neuron was silver-stained, its dendrites were also silver-stained, and vice versa (referred to as "all-or-nothing nature"). The axonal appendage of the silver-stained somata remained unstained. Numerous brain areas in both the cerebrum and the cerebellum contained affected neurons, but their distribution pattern was not exactly the same in each rat. However, a large proportion of the granule cells in the hippocampal dentate gyri were consistently affected. Interestingly, the neurons with more dendrites pointing toward the positive electrode than toward the opposite direction were affected with a much higher probability to the electric shock (referred to as "direction-dependent vulnerability"). This phenomenon was most clearly visible in the caudolateral part of the hippocampal dentate gyri (Fig. 1a), where the main direction of most dendrites of the granule cells residing in the outer blade is opposite to that of those residing in the inner blade. In Durcupan-embedded semithin sections, the affected neuronal somata and dendrites were hyperbasophilic (intensely stained with toluidine blue) and were scattered among apparently normal ones. The hyperbasophilic somata were shrunken and exhibited irregular outlines (Fig. 1b).

In the rats that were transcardially perfused with isoosmotic potassium chloride before the electric shock, only an insignificant number of dentate granule neurons were affected, which obscured the establishment of any "direction-dependent vulnerability". However, a large number of neuronal soma-dendrite domains in the caudate putamen and the neocortex and many Purkinje cells were consistently silver-stained in a scattered distribution among non-impaired neurons of the same type. In addition, several axons were also silver-stained (Fig. 1c) and hyperbasophilic (Fig. 1d). In most cases, only long segments, but not the whole lengths of axons were affected, and their parent somata remained unstained. Such axonal segments were consistently present in the neocortex, the mesencephalon and the cerebellum.

3.3. Electron-microscopic observations

In all examined areas of the rats transcardially perfused with chilled physiological saline before the electric shock, but not in any of the control rats, the neuronal somata and dendrites exhibited an increased electron density and a pronounced compaction of seemingly normal ultrastructural elements (Fig. 2). Markedly reduced distances were observed between the outer surfaces of the endoplasmic reticulum cisternae, the Golgi cisternae, the mitochondria, the lysosomes, the vesicles and multivesicular bodies, the nuclear and plasma membranes, the free ribosomes and ribosome rosettes, and also the components of the filamentous cytoskeleton. Moreover, the interior of the endoplasmic reticulum cisternae became contracted whereas that of the Golgi cisternae was dilated. No change was apparent in the volumes of the mitochondria, lysosomes, multivesicular bodies and vesicles. In the non-compacted neuronal soma-dendrite domains, all the ultrastructural elements able to take part in the compaction appeared to be interconnected with fine side arms. In parallel with the compaction, these side arms became shorter and more electron dense (Fig. 2d). The degree of compaction appeared to be the same throughout the affected electron-microscopic profiles. The nuclei of the neurons with compacted somata were also compacted. The nuclear chromatin aggregated to form numerous small clumps with irregular outlines and a myriad of minute granules. No tear or hole through which the excess intracellular fluid could have run out of the compacted neurons was observed in the plasma membrane. Interestingly, the intracellular space around the compacted neuronal elements was hardly dilated, but markedly swollen

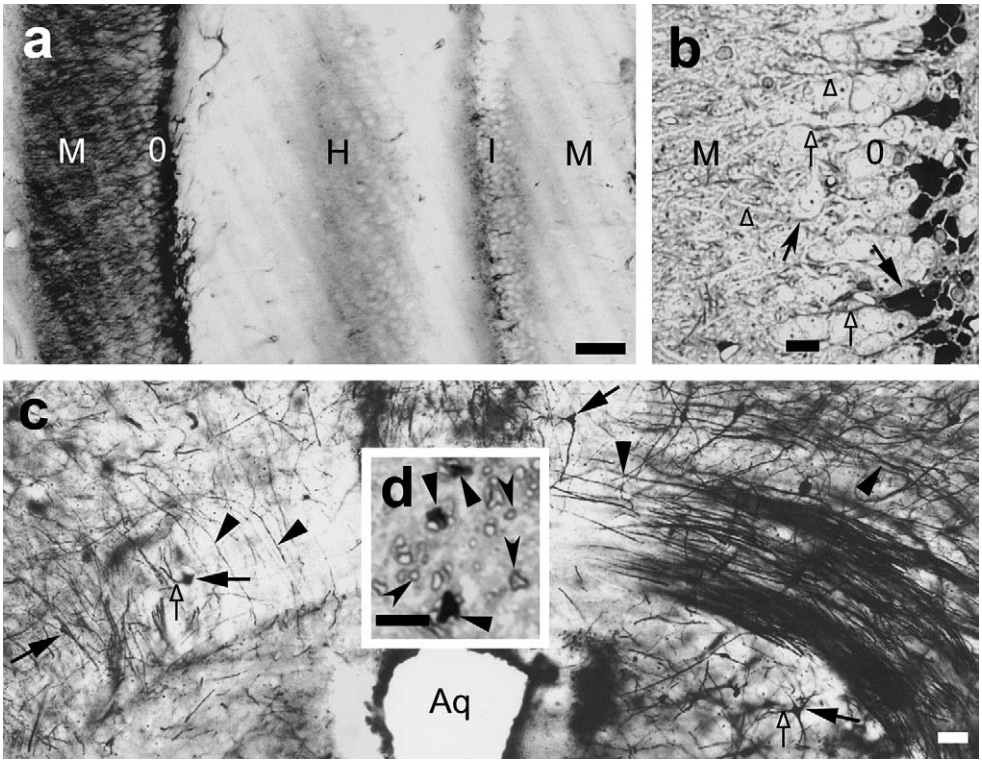

Fig. 1. (a,b) ‘‘Dark’’ granule neurons produced in the hippocampal dentate gyrus of a rat by an electric shock administered at the end of a 30-min transcardial perfusion with chilled physiological saline. (c,d) ‘‘Dark’’ neurons and axons produced in the mesencephalon of a rat by an electric shock administered at the end of a 5-min transcardial perfusion with isoosmotic potassium chloride. (a) and (c): 150-μm vibratome sections stained by the silver method. (b) and (d): 1-μm osmicated and Durcupan-embedded sections stained with toluidine blue. M denotes the molecular layers, O the outer granule cell layer, I the inner granule cell layer, H the hilus of the dentate gyrus, and Aq the cerebral aqueduct. Arrows with closed heads point to ‘‘dark’’ somata, those with concave heads to normal somata, and those with open heads to ‘‘dark’’ dendrites. Closed arrowheads point to ‘‘dark’’ axons, concave arrowheads to normal axons, and open arrowheads to normal dendrites. Scale bars: a,c = 100 μm; b = 10 μm; d = 5 μm.

astrocytic processes were always found next to the compacted neuronal elements (Fig. 2b). These occasionally contained loosely furled multimembranous formations. Infrequently, the non-compacted neurons contained swollen cytoplasmic areas in the close vicinity of the compacted neurons (Fig. 2c). In other respects, the surrounding tissue appeared intact.

In addition to the above electron-microscopic features, in the rats perfused with isoosmotic potassium chloride before the electric shock (Fig. 3a,b), a low proportion of myelinated axons (Fig. 3c,d) and axon terminals (Fig. 3e,f) also displayed a compacted ultrastructure. Compacted axons were never seen to be connected with compacted axon terminals or compacted neuronal somata. The degree of compaction appeared to be the same throughout the affected profiles.

4. Discussion

4.1. Comparison of the present results with those of in vivo electric shocks

The present findings bear marked similarities to those induced by electric shocks administered in vivo (Gallyas et al., 1993; Csordás et al., 2003): (i) The dendrites, but not the axons, of the affected somata were also affected, and vice versa (all-or-nothing nature). The affected soma-dendrite domains momentarily acquired (ii) type-III argyrophilia, (iii) hyperbasophilia and (iv) an increased electron density. (v) The distances between any two neighboring parts of the ultrastructural elements visible in the conventional transmission electron microscope were strikingly reduced. Although the volumes of most membrane-bound ultrastructural elements, such as mitochondria, multivesicular bodies, vesicles and lysosomes, remained unchanged, the interior of the endoplasmic reticulum cisternae was contracted, while that of the Golgi cisternae was dilated. (vi) The degree of compaction appeared to be similar throughout each affected electron-microscopic profile. (vii) Despite the fact that no tear or hole was apparent in either the nuclear or the plasma membrane, a relatively large volume of fluid must have passed through these membranes. (viii) The excess fluid was taken up mainly by nearby astrocytic processes, but to an insignificant extent also by the extracellular space or other cell types. (ix) The nuclear chromatin was aggregated with a non-apoptotic pattern. These points will be referred to as ‘‘intracellular morphological features’’.

On the other hand, there were considerable differences in the distribution of the affected cells. (a) While most dendrites of the affected neurons pointed toward the negative electrode in the in vivo case, in the chilled rats they pointed toward the positive electrode. (b) In the hippocampal dentate gyrus, mainly the granule neurons that bordered the hilus became affected in the chilled rats, whereas the affected neurons were evenly distributed in the in vivo case. (c) The maximum

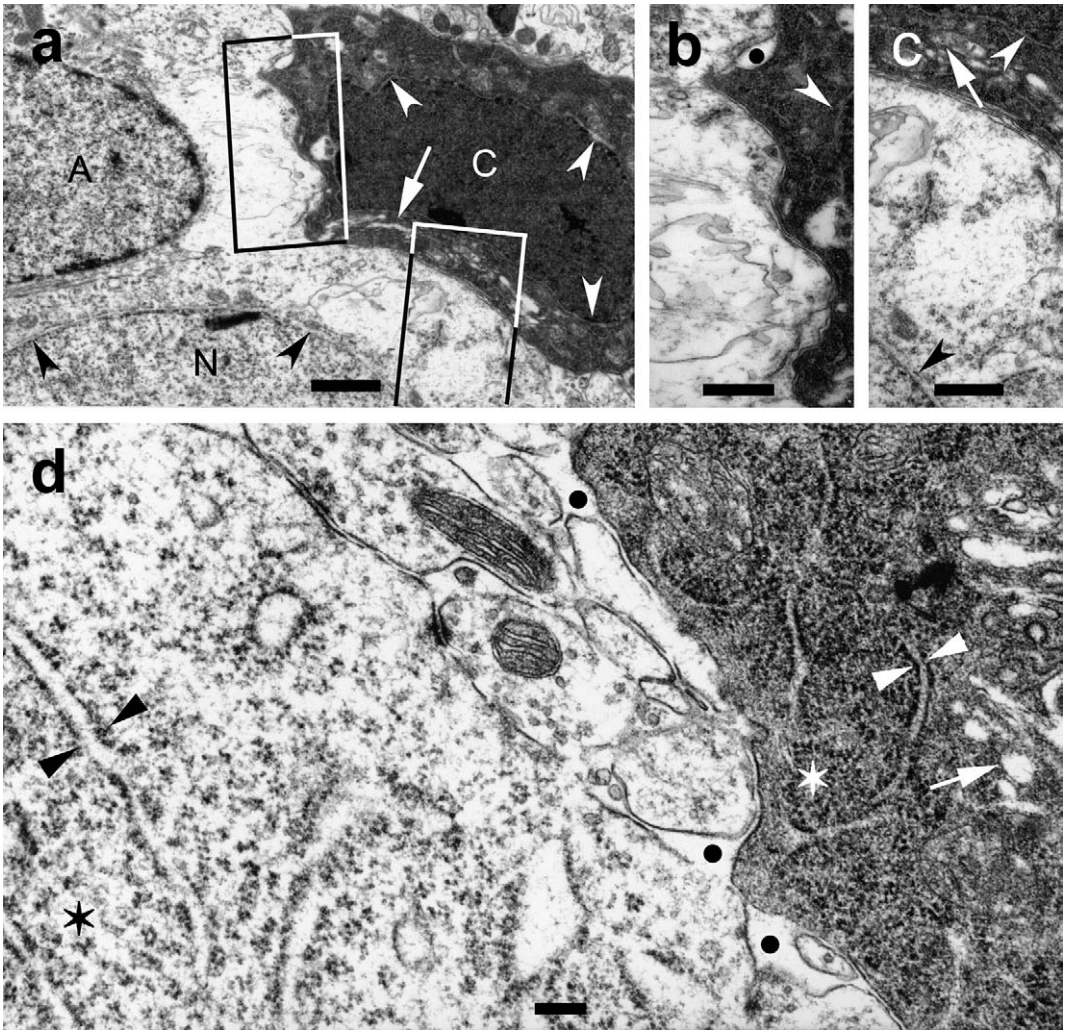

Fig. 2. (a) Compacted (C) and normal (N) neurons and an astrocyte (A) in the hippocampal dentate gyrus of a rat subjected to electric shock at the end of a 30-min transcardial perfusion with chilled physiological saline. (b,c) Magnifications of the areas framed in (a). (d) Cytoplasmic areas of a normal (left) and a compacted (right) neuron in juxtaposition. Closed circles denote dilated extracellular spaces. White or black asterisks are surrounded by ribosome rosettes. Arrows point to dilated Golgi cisternae, white or black concave arrowheads to nuclear envelopes, and white or black closed arrowheads to endoplasmic reticulum cisternae. Scale bars: a = 1 μm; b,c = 500 nm; d = 200 nm.

incidence of affected granule neurons in the caudolateral dentate gyri was found at 250 V in the in vivo case, whereas at 350 V in the chilled rats. (d) Myelinated axons and axon terminals displaying a compacted ultrastructure were present only in the rats perfused with isoosmotic potassium chloride before the electric shock. (e) In the same rats, direction-dependent vulnerability could not be established. These points will be referred to as ''distribution differences''.

4.2. Brief description of the gel-to-gel phase-transition theory of whole-cell ultrastructural compaction (''dark''-cell formation)

The gel-to-gel phase transition theory (Gallyas et al., 2004) postulates that numerous non-covalent binding sites of proteins in the ''aqueous cytoplasm'' are engaged not in maintaining their in vitro observed conformation, but in mutually chaperoning each others' in vivo conformation, binding potassium ions and adsorbing water molecules in multiple ordered layers. The resulting gel structure, which is continuous in the whole cell and is anchored to the ''visible'' ultrastructural elements, stores free energy in the form of non-covalent bonds. If released at any intracellular point by some exogenous activation energy, this stored free energy serves as activation energy at the neighboring points (domino principle).

If we assume the existence of the above cytoplasmic gel structure as true, the whole-cell ultrastructural compaction in neurons consists of two stages, as follows (Gallyas et al., 2004). A physical force or a pathometabolic process transmits activation energy to only a single point in each neuronal soma-dendrite domain (initiation stage) the ultrastructure of which will become compacted in the next stage. Subsequently, the conformational change of protein molecules at this point, which is accompanied by the liberation of water molecules and potassium ions, spreads throughout the whole gel structure, propagated by the release of stored non-covalent free energy (spreading stage). With other words, a cooperative conformational change in the matrix proteins of the assumed

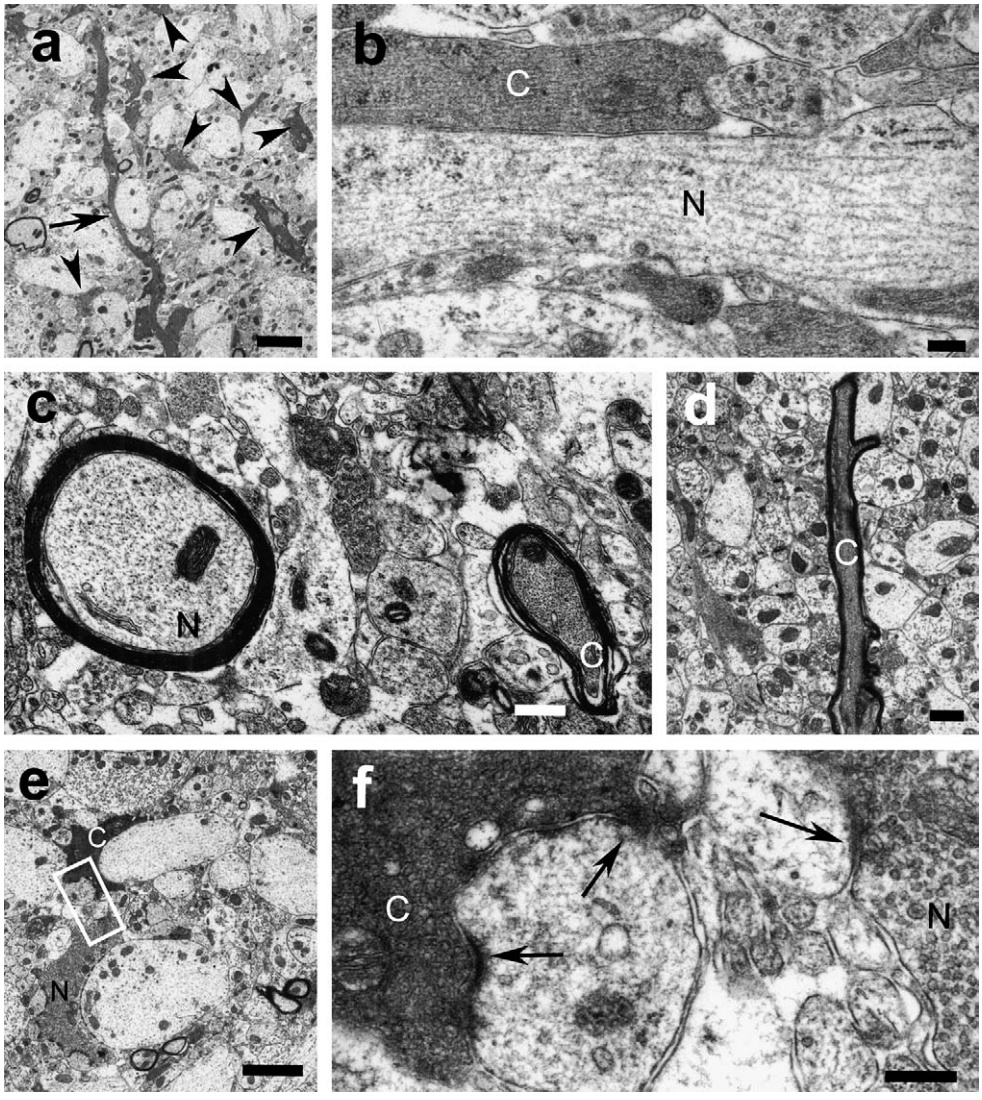

Fig. 3. Compacted (C) and normal (N) neuronal dendrites (a,b), axons (c,d) and axon terminals (e,f) in the neocortex, mesencephalon and hippocampus, respectively, of a rat subjected to electric shock at the end of a 5-min transcardial perfusion with isoosmotic potassium chloride. In (a), an arrow points to a long ramified dendrite, and arrowheads to dendritic profiles of various measures. In (f), which is a magnification of the area framed in (e), the arrows point to synapses. Scale bars: a,d,e = 2 μm; c = 500 nm; b and f = 200 nm.

cytoplasmic gel spreads throughout the cytoplasmic gel. As a final result, the gel becomes shrunken, causing the anchored ''visible'' ultrastructural elements to come closer to each other (compaction) and squeezing the liberated water molecules and potassium ions out of the cell through numerous non-visible pores in the plasma membrane. Bearing all these in mind, it is the spreading process that determines the initiation-independent intracellular morphological features. On the other hand, by determining which neurons will become compacted, it is the initiating process that plays an important role in shaping the distribution pattern of the affected neurons.

4.3. Consistency of the present results with the spreading process of gel-to-gel phase-transition

The high degree of similarity of the ''intracellular morphological features'' observed in the in vivo and the post-mortem electric-shock cases strongly suggests that ultrastructural compaction in neurons cannot be caused by any cascade of enzyme-mediated processes. Otherwise, different intracellular morphological features would have been found, since the in vivo harmony of action among the individual enzyme-mediated processes involved would have been seriously disturbed by changing in different directions and/or to different degrees under the post-mortem conditions applied. Furthermore, the staining properties and the ultrastructural characteristics would have been different in different parts of the soma-dendrite domain, since it is improbable that any complex enzyme-mediated process, started from one single point, should spread over long cytoplasmic distances without fading. Under the circumstances unfavorable for enzyme-mediated processes, large-scale formation in protein molecules of negatively charged side groups and of catalytic sites, which are responsible for hyperbasophilia (Zsombok and Tóth, 2005) and type-III argyrophilia (Gallyas, 1982), respectively, is also improbable.

On the other hand, the ultrastructural compaction, its all-or-nothing nature and its independence from the initiation process and also from the actual state of metabolism are consistent with the gel-to-gel phase-transition theory. Furthermore, the acquirement of hyperbasophilia and type III argyrophilia by the affected neurons can be explained in the same manner as was done in the case of the mechanically initiated formation of ''dark'' neurons (Gallyas et al., 2004). Briefly, as a result of cooperative conformational changes in the matrix proteins of the assumed cytoplasmic gel, pre-existing structure-bound anionic groups can become detached from the counter-ions (K^+?), escape from a hydrophobic environment or become more electronegative. The same conformational changes may favorably rearrange pre-existing chemical side groups of matrix proteins, which produce catalytic sites for the formation of metallic silver particles in a physical developer. It should be mentioned that all the intracellular morphological features found in this study were also displayed in the ''dark'' neurons produced by both in vivo and post-mortem mechanical injuries (Gallyas et al., 2004), and also by mild hypoglycemia (Gallyas et al., 2005). On the other hand, the distribution patterns of the affected neurons in these cases were quite different.

4.4. Electroporation cannot explain the spreading process of the ultrastructural compaction

Electroporation, i.e. rendering the plasma-membrane porous by high-voltage electric pulses, is routinely used in cell biology for transferring foreign materials not only into freely moving cells but also into cells in tissues (Weaver, 1993). Electroporation should be considered as the cause of electrically induced ultrastructural compaction, since this is an enzyme-independent phenomenon, and destabilization of the plasma membrane by making it porous can lead to the collapse of the cytoplasmic architecture (Schliwa et al., 1987). However, the all-or-nothing nature of the electrically induced ultrastructural compaction contradicts this possibility. Specifically, it is impossible that the electric field, which must be quasi-homogenous in the area of a few, neighboring neuronal soma-dendrite domains, should make the plasma membrane porous everywhere in one of them, but nowhere in the others. As already mentioned, the all-or-nothing affection of the neuronal soma-dendrite domain in ultrastructural compaction needs the involvement of a spreading process in addition to a momentary initiating process, which might be electroporation (Csordás et al., 2003). Furthermore, (i) small holes in the plasma membrane do not result in the collapse of the cytoplasm (Kellermayer et al., 1986), (ii) electron microscopy did not reveal holes in the plasma membrane of the electrically compacted neurons either in vivo or post mortem, and (iii) besides electric shocks, ultrastructural compaction can be initiated by numerous other noxae of either pathometabolic (Gallyas et al., 1990) or physical (Gallyas et al., 2004) nature, which noxae do not render the plasma membrane porous.

4.5. Electrical initiation of gel-to-gel phase transition

Since all the morphological changes that are different between the in vivo and the post-mortem results are ''distribution differences'', they must be caused by the initiation process of gel-to-gel phase transition. Consequently, they are not inconsistent with the gel-to-gel phase-transition theory of whole-cell ultrastructural compaction.

If this theory holds true, the initiation occurs at a single point in each affected neuron. Consequently, it appears hopeless to search for either morphological or biochemical signs of the initiation.

The dependence of the vulnerability of neurons on the direction of their main dendrites with respect to the polarity of electrodes puts forward the idea that it must be a direction-dependent electrical property of the neuronal dendrites that plays a decisive role in the initiation. The surprising reversal of this kind of vulnerability in the rats perfused with chilled physiological saline suggests that this electrical property of dendrites also reverses. However, such a property is not yet known.

An established electrical phenomenon, the membrane potential, is known to greatly decrease under the circumstances in our experiment with chilled physiological saline (Mihalovic, 1972). However, its possible role is not supported by the experiment with isoosmotic potassium chloride, which is also known to depolarize excitable cells (Hodgkin and Horowitz, 1959).

The replacement of blood with physiological saline or isoosmotic potassium chloride results in differences in the intracerebral distribution of electric field intensity. This may play some role in the development of the distribution differences observed between our in vivo and post-mortem cases.

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