Effects of topiramate on the ultrastructure of synaptic endings in the hippocampal CA1 and CA3 sectors in the rat experimental model of febrile seizures: the first electron microscopy report

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Abstract
The present study aimed at exploring a potentially neuroprotective effect of topiramate (TPM), one of the most commonly used newer-generation, broad-spectrum, antiepileptic drugs against ultrastructural damage of hippocampal synaptic endings in the experimental model of febrile seizures (FS). The study used male young Wistar rats aged 22-30 days, divided into three experimental groups and the control group. Brain maturity in such animals corresponds to that of 1- or 2-year-old children. Hyperthermic stress was evoked by placing animals in a 45°C water bath for four consecutive days. TPM at a dose of 80 mg/kg b.m. was administered with an intragastric tube before and immediately after FS. Specimens (1 mm3) collected from the hippocampal CA1 and CA3 sectors, fixed via transcardial perfusion with a solution of paraformaldehyde and glutaraldehyde, were routinely processed for transmission-electron microscopic analysis. Advanced ultrastructural changes induced by hyperthermic stress were manifested by distinct swelling of hippocampal pre- and post-synaptic axodendritic and axospinal endings, including their vacuolization and disintegration. The axoplasm of the presynaptic boutons contained a markedly decreased number of synaptic vesicles and their abnormal accumulation in the active synaptic region. The synaptic junctions showed a dilated synaptic cleft and a decreased synaptic active zone. TPM used directly after FS was ineffective in the prevention of hyperthermia-induced injury of synaptic endings in hippocampal CA1 and CA3 sectors. However, “prophylactic” administration of TPM, prior to FS induction, demonstrated a neuroprotective effect against synaptic damage in approximately 25% of the synaptic endings in the hippocampal sectors, more frequently located in perivascular zones. The synaptic junctions showed a dilated synaptic cleft and a decreased synaptic active zone. TPM used directly after FS was ineffective in the prevention of hyperthermia-induced injury of synaptic endings in hippocampal CA1 and CA3 sectors. However, “prophylactic” administration of TPM, prior to FS induction, demonstrated a neuroprotective effect against synaptic damage in approximately 25% of the synaptic endings in the hippocampal sectors, more frequently located in perivascular zones. It was manifested by smaller oedema of both presynaptic and postsynaptic parts, containing well-preserved mitochondria, increased number and regular distribution of synaptic vesicles within the axoplasm, and increased synaptic active zone. Our current and previous findings suggest that TPM administered “prophylactically”, before FS, could exert a favourable effect on some synapses, indirectly, via the vascular factor, i.e. protecting blood-brain barrier components and through better blood supply of the hippocampal CA1 and CA3 sectors, which may have practical implications.

Key words: ultrastructure of synaptic endings, hippocampal CA1 and CA3 sectors, experimental febrile seizures, topiramate, neuroprotection.

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Introduction

Febrile seizures (FS) are the most common form of convulsions in childhood, occurring at body temperature above 38.5°C. These seizure disorders affect 2-5% of children between 6 months and 5 years of age, with a peak incidence between 12 and 18 months of age.

The condition is more common in boys – the male to female ratio is approximately 1.6 to 1.0. It is assumed that 30-40% of paediatric patients with FS will have a recurrence during early childhood [7,19,26]. Simple febrile convulsions are usually benign but children with complex FS are at risk for future epilepsy. It has been reported that complex FS can cause the development of temporal lobe epilepsy later in life and in some patients can be associated with reduced cognition [7,10,16,19,22,26].

Experiments conducted in the last decade, including our own observations using a rat model of hyperthermia-induced febrile seizures, indicate that prolonged febrile seizures early in life have long-lasting effects on the structure of the hippocampus, resulting in its substantial injury [1,8,9,21,27,33-35]. It has been observed that recurrent experimental FS induce a long-lasting change in hippocampal excitability, leading to enhanced seizure susceptibility and structural alterations in hippocampal plasticity. Structural alterations in the hippocampus and altered ion channel expression have both been proposed as mechanisms underlying this decreased seizure threshold [6,14,15,18,29,30]. However, the morphogenesis of the changes in the hippocampus in the course of FS has not been sufficiently elucidated and thus the treatment of febrile seizures still poses a huge challenge for paediatric neurologists [10,16,19,22,26]. Some authors explaining the mechanism of hippocampal injury caused by hyperthermic stress indicate a major contribution of a neurotransmitter, nitric oxide, whose excess is toxic to neurons and may cause neuronal apoptosis. They suggest that nitric oxide mediates neuronal apoptosis in experimental recurrent FS through endoplasmic reticulum stress [1,33]. Others, however, believe that these are changes in GABA(A)R-mediated neurotransmission in the dentate gyrus and CA1 regions of the hippocampus that exert a significant role in the pathomechanism of experimental early-life febrile seizures [18,29,30].

Topiramate (TPM) is one of the most commonly used newer-generation, broad-spectrum, antiepileptic drugs (AEDs) in the therapy of hyperthermic convulsions, especially in the course of preventive treatment of recurrent FS, which produce no adverse and impair cognitive effects [2,10,31]. Despite long-term neurochemical studies on topiramate, literature data still indicate that neuroprotective properties of this AED in febrile seizures remain poorly assessed. Especially electron transmission microscopy-based research in this field is exceptionally scarce.

Therefore, the present study aimed at exploring a potentially neuroprotective effect of TPM against ultrastructural damage to the synaptic endings in the cortex of the hippocampal CA1 and CA3 sectors caused by the experimental model of febrile seizures in young rats.

The current study is a continuation of our previous research into the effect of TPM on the histological picture of the ammonal cortex [21] and electron microscopic assessment of chosen morphological elements of the hippocampal cortex and neocortex, mainly the blood-brain barrier (BBB) components, astrocytes and pyramidal neurons in the hippocampal CA1 and CA3 sectors [8,9,27], and astrocytes of the neocortex of the temporal lobe in an analogous experimental model of hyperthermic seizures [9]. It is also significantly associated with our research into permeability markers of BBB in children with neurological disabilities, especially with epilepsy [23].

Worthy of note is that the rat model of hyperthermia-induced FS used in the current study was first designed in our Centre and is comparable to paediatric FS [21].

Material and methods

Animals

The retrospective electron-microscopic analysis of the effect of TPM on chosen morphological structures of the rat brain, including the neuropil components of the hippocampal cortex, in the experimental model of FS was conducted in the Department of Medical Pathomorphology, Medical University of Białystok.

The experiment used 18 young male Wistar rats aged 22-30 days. Brain maturity in such animals corresponds to that of 1- or 2-year-old children [21]. The rats were divided into four groups – three experimental and one control (five rats in each experimental
group and three in the control group). The animals were pre-selected according to the standard pharmacological screening tests. All procedures were performed in strict accordance with the Helsinki Convention Guidelines for the care and use of laboratory animals. The study was approved by the Ethical Committee of the Medical University of Bialystok.

**Model of febrile seizures**

The FS group contained rats with induced febrile seizures. Hyperthermic stress was evoked by placing the animals in 30 × 30 × 60 cm water bath filled with 45°C warm water. Water temperature was maintained at the same level. The rats were put into water for 4 minutes until convulsions appeared and then moved to a separate container lined with lignin. The animals, except for controls, were placed in the warm water bath for four consecutive days.

In the FS + TPM group, topiramate (Topamax, Janssen-Cilag; 80 mg/kg b.m. dissolved in 2 ml normal saline) was administered with an intragastric tube, immediately after each convulsion episode (every rat received the drug four times).

In the TPM + FS group, topiramate was applied in the same way and at the same dose, prior to the induction of febrile convulsions, i.e. 90 minutes before the animals were put into warm water. Control animals and the FS group received only normal saline. A detailed description of the methodology has been presented in our previous paper [21].

**Preparation for transmission electron microscopy**

Seventy-two hours after the last convulsion episode, the rats were anaesthetized with Nembutal (25 mg/kg b.m., i.p.). Then, they were intravitally, transcardially (through the left heart chamber to the superior aorta and incision of the right atrium) perfused with a fixative solution (approximately 200 ml/animal) containing 2% paraformaldehyde (Sigma) and 2.5% glutaraldehyde (Serva) in 0.1 M cacodylate buffer (Serva), pH 7.4, at 4°C, under pressure of 80-100 mmHg. After removal of the brains, hippocampal samples were taken and fixed in the same solution for 24 h. Postfixation was completed with 1% osmium tetroxide (OsO4) (Serva) in 0.1 M cacodylate buffer, pH 7.4, for 1 h. After dehydration in ethanol and propylene oxide (Serva), small specimens (1 mm³) of the gyrus hippocampal cortex (from the hippocampal CA1 and CA3 areas) were processed routinely for embedding in Epon 812 (Serva) and sectioned on a Reichert ultramicrotome (Reichert UltraCut 5) to obtain semithin sections. The semithin sections were stained with 1% methylene blue (POCH) in 1% sodium borate (POCH) and preliminarily examined under a light microscope to select Epon blocks. Ultrathin sections were double stained with uranyl acetate (Serva) and lead citrate (Serva), and examined with a transmission electron microscope (Opton EM 900, Zeiss, Oberkochen, Germany) and photographed with TRS camera (CCD – Camera for TEM 2K inside). The material obtained from the gyrus hippocampal cortex in the control group was processed using the same techniques as for the experimental groups.

**Results**

**FS group**

All the animals with hyperthermia-induced febrile seizures, as compared to the control (Fig. 1), showed markedly enhanced ultrastructural changes in the neuropil components, especially in the synaptic endings of CA1 and CA3 sectors of the gyrus hippocampal cortex (Fig. 2A-D). Since the synaptic changes observed in the two sectors of the hippocampal cortex were qualitatively similar, they are described jointly.

Seldom, slightly better preserved neuropil components were found next to markedly damaged areas of the neuropil. Apart from damage to the synaptic endings we observed distinct injury to dendritic processes of nerve cells and glial processes lying loosely in the neuropil.

Both the axodendritic and axospinodendritic endings, i.e. formed on the dendritic spines of hippocampal nerve cells (Fig. 2C,D) frequently showed signs of severe damage. In advanced electron-microscopic alterations in the synaptic endings, substantial swelling of the pre- and post-synaptic endings was most pronounced, which is clearly demonstrated in Figure 2A-D. The cytoplasm of enlarged terminal axons, i.e. axonal end-bulbs and the cytoplasm of dendritic endings contained extensive, optically almost empty fields, or filled with very fine residual microfibrillar material, probably remains after degenerated components, dilated smooth endoplasmic reticulum with large vacuolar, electron-lucent,
sometimes pleomorphic multivesicular structures (Fig. 2A-D), and often damaged mitochondria. The appearance of the vacuolar structures within the cytoplasm of the synaptic endings led to their vacuolization (Fig. 2B,D). Moreover, the axoplasm of the presynaptic bulbs contained a markedly reduced number of synaptic vesicles and their abnormal distribution. Frequently, residual quantity of the presynaptic vesicles was found to abnormally accumulate in the form of clumpings, mainly in the vicinity of the synaptic cleft, or were unevenly dispersed throughout the cytoplasm (shown in Fig. 2A-D).

The synaptic junctions presented dilated synaptic cleft, reduced length of the synaptic active zone and decreased curvature of the synaptic interface; the postsynaptic part, on the other hand, displayed reduced postsynaptic density thickness (Fig. 2A,C,D).

Numerous synaptic endings affected by hyperthermic stress showed features of substantial disintegration (Fig. 2B-D).

**FS + TPM group (the antiepileptic administered immediately after the induction of febrile seizures)**

The ultrastructural picture of the synaptic endings of the CA1 and CA3 sectors of the gyrus hippocampal cortex in the rats which after hyperthermic injury received topiramate showed both quantitative and qualitative alterations resembling those found in the FS group (Fig. 3). Thus, in this experimental group the unfavourable effect of TPM was noted after hippocampal synaptic damage induced by hyperthermic stress.

**TPM + FS group (the antiepileptic administered prior to febrile seizures)**

In the majority of observations, no distinct beneficial effect was found on the ultrastructure of the neuropil elements of CA1 and CA2 sectors of the gyrus hippocampal cortex in the rats receiving topiramate at a dose of 80 mg/kg b.m. before the induction of hyperthermic stress, as a presumed neuroprotective agent. In approximately 75%, the synaptic endings did not differ significantly from those observed in the FS group, showing features of distinct swelling. The cytoplasm of axodendritic endings, especially postsynaptic boutons, and the cytoplasm of axospinodendritic endings frequently contained large vacuolar structures and optically empty areas (observed in Fig. 4A). Within the cytoplasm, most of such terminal axons showed a reduced number of synaptic vesicles with a tendency to abnormal clump near the active synaptic region. The reduced length of the synaptic active zone and dilated synaptic cleft was maintained (Fig. 4A).

However, in the experimental group the “prophylactic” administration of TPM showed morphological features indicating a neuroprotective effect of TPM against synaptic damage in the hippocampus in approximately 25% of the synaptic endings. The beneficial effect of topiramate was mainly observed in the endings that were situated directly in the perivascular zones of relatively well-preserved capillaries, or in the vicinity of such zones, which can be seen in Figure 4B. This was manifested by less substantial swelling of the synaptic endings, both in

**Fig. 1.** The ultrastructure of the neuropil elements of the hippocampal cortex in the control group shows well-preserved synaptic endings; the presynaptic (a) as well as postsynaptic (d) parts of synapses are clearly seen, with normal structure. Most axonal-end bulbs contain a large number of synaptic vesicles, evenly distributed within the axoplasm; synaptic active zones are distinct – mainly long with narrow synaptic cleft and well-preserved postsynaptic density thickness; normal mitochondrion (m) within the cytoplasm of axonal end-bulb is seen at the bottom of the figure. Scale bar 100 nm.
the pre- and post-synaptic parts with well-preserved mitochondria, and granular endoplasmic reticulum channels (Fig. 4B-D). Moreover, an increase was observed in the number of synaptic vesicles and their regular distribution within the axoplasm of the presynaptic end-bulbs. Increased length of

Fig. 2. Electronograms demonstrate remarkable swelling and damage to the synaptic endings in the hippocampal cortex in the FS group. A) The neuropil shows substantially enlarged presynaptic (a1) part of the synapse, remarkably reduced content of synaptic vesicles and their abnormal clumping in the active synaptic region, i.e. in the vicinity of the synaptic cleft; reduced synaptic active zone and synaptic interface curvature; d1 – swollen post synaptic part filled with very fine microfibrillar material. In the vicinity, a swollen terminal axon (a2) with vacuolar structures (V) is present. In the margins of the figure, fragments of remarkable swelling of axodendritic junctions can be seen. Scale bar 250 nm. B) Vacuolar, almost electron-lucent large structures (V) can be seen within the cytoplasm of markedly swollen axonal endings (a1, a2), with features of axonal disintegrations and containing very fine microfibrillar material of the axonal (a1, a2) endings; residual quantity of synaptic vesicles. Centrally located and relatively well-preserved single axodendritic junction, with extended but regular granular endoplasmic reticulum channels (> ) visible in the cytoplasm of the postsynaptic part of the synapse; in the axonal end-bulb, quite numerous synaptic vesicles. Scale bar 250 nm. C) The cytoplasm of axonal (a1, a2, a3) endings containing the microfilament elements of the cytoskeleton; the content of synaptic vesicles is significantly reduced, and the cytoplasm of dendritic (d1, d2) endings shows vacuolar structures resulting from the extension of granular endoplasmic reticulum (> ); markedly reduced length of the synaptic active zones; s – dendritic spine. Scale bar 500 nm. D) Morphological details referring to degenerative changes in the axodendritic junction seen in the bottom fragment of Figure 2C in higher magnification. Numerous vacuolar structures (V) present in the axonal (a3) ending; the active synaptic region significantly decreased, with the reduced content of synaptic vesicles in its vicinity. Scale bar 250 nm.
the synaptic active zone was noted (shown also in Fig. 4B-D).

Discussion

Our submicroscopic findings clearly demonstrate serious damage, including vacuolization and disintegration, to the synaptic endings of the CA1 and CA3 sectors of the rat hippocampal cortex induced by repeated FS as well as maintenance of these changes at a similar level in animals given topiramate as a presumed neuroprotective agent at a dose of 80 mg/kg b.m., directly after FS. A distinct protective effect of TPM against damage to the hippocampal synapses was observed in approximately 25% of the synapses in the experimental group receiving TPM as a prophylaxis prior to FS.

In the FS group, the major cytoplasmic alterations in the synaptic endings included large swelling both in the pre- and postsynaptic parts, with features of vacuolization and the presence of optically almost empty fields or with very fine residual microfibrillar material and swollen mitochondria. The axoplasm of the presynaptic bulbs contained a markedly reduced number of synaptic vesicles which accumulated as residue in the form of aggregates in the vicinity of the synaptic cleft, and showed shortened length of the synaptic active zone.

The ultrastructure of the synaptic endings of the hippocampal subfields observed in the current study in rats subjected to repeated FS was similar to that described by Zhou et al. [35] in another experimental model of hyperthermic convulsions. The submicroscopic picture of the synapses with hyperthermia-induced injury was nonspecific and observed in other CNS pathologies. It also resembled experimental valproate encephalopathy induced by chronic application of sodium valproate in rats and presented by Sobaniec-Lotowska [28].

It has been reported, including our earlier elaboration [27], that repeated hyperthermic stress markedly attenuates and impairs the perikarya of pyramidal neurons of the CA1 and CA3 hippocampal sectors, leading to their death and total disintegration. This stress causes substantial metabolic disorders within the hippocampal pyramidal neurons mainly affecting the oxidation-reduction transformations and protein synthesis [27,35].

The current ultrastructural findings are the first in the literature to document the morphological status of the synaptic endings in rats receiving topiramate in the experimental model of FS model. We found that the neuroprotective action of TPM observed in about 25% of the hippocampal synaptic endings, more frequently referred to the synapses located in the vicinity of relatively well-preserved microcirculation. This effect was morphologically demonstrated as smaller swelling of both presynaptic and postsynaptic parts of the synapses, the presence of well-preserved mitochondria, increased number of synaptic vesicles within the cytoplasm of axonal end-bulbs, their regular distribution in terminal axons without clear tendency to abnormal clumping in the active synaptic region and increased length of the synaptic active zone.

Interestingly, in the same experimental model of febrile seizures we observed that the “prophylactic” administration of TPM prior to FS induction, at a dose
Synapses in FS and after topiramate

**Fig. 4.** Electronograms demonstrate morphological details of synaptic endings in the neuropil of the hippocampal cortex in the TPM + FS group (A – the view referring to the majority, i.e. approximately 75%, of synaptic endings which do not exhibit significant signs of TPM neuroprotection; B-D – the views concerning approximately 25% synaptic endings exhibiting features of a neuroprotective effect of TPM against synaptic damage). **A** The picture of preserved degenerative changes in the neuropil of the hippocampal cortex, especially within the postsynaptic (d) parts of synapses, which do not differ significantly from those observed in the FS group. The cytoplasm of dendritic endings (d1, d2) is remarkably swollen and contains fine residual microfibrillar material, pleomorphic vacuolar structure (within d2) and dilated elements of granular endoplasmic reticulum within d1 (>). Although the centrally located single axonal end-bulb (a2) is relatively well-preserved and filled with quite numerous synaptic vesicles, with a clearly seen normal mitochondrion, the synaptic active zone is markedly shortened. Scale bar 250 nm.

**B-D** Electronograms show morphological signs of a favourable effect of TPM on the synaptic endings of the hippocampal cortex. **B** In places, ultrastructural signs are visible of a protective effect of TPM on the synaptic endings located directly in the perivascular zone – in close vicinity of a well-preserved capillary. The picture of the presynaptic (a) and postsynaptic (d) parts of the synapse, with a relatively well-preserved ultrastructure as compared to the FS group which adhere to a fragment of almost unchanged capillary seen at the bottom of the figure; the axonal end-bulb of the presynaptic part (within a) filled with a large quantity of synaptic vesicles; normal mitochondrion (m) clearly visible in the dendroplasm of the postsynaptic part (within d). Other axonal end-bulbs, especially more distant from the vessel (top right corner of the figure), contain less abundant synaptic vesicles; however, the mitochondria present in the cytoplasm of these synaptic endings are unchanged. Notice the long active synaptic zone between (a) and (d) parts of the synapse despite dilatation maintained within the synaptic cleft. L – perfused vascular lumen. Scale bar 250 µm. **C, D** The picture of quite well-preserved, as compared to the FS group, presynaptic (a) and postsynaptic (d) parts of synapses. The cytoplasm of synaptic endings is much less swollen, with no features of vacuolization. Most of the axonal end-bulbs filled with synaptic vesicles, evenly distributed within the cytoplasm. Centrally located axodendritic junction shows an increase in the length of the synaptic active zone, dilatation maintained within the synaptic cleft and relatively well-preserved postsynaptic density; slightly dilated granular endoplasmic reticulum channels within the dendroplasm of the postsynaptic part of this synapse (>). Mitochondria (m) present within the synaptic endings are normal or focally showing swollen matrix. Scale bar 250 nm (fig. 4C) and 100 nm (fig. 4D).
was reported by Zhou of immature rats with repeated febrile convulsions age to the synapses in the hippocampal CA1 area neuroprotective effect against ultrastructure dam-
ag of some of the synapses in the experimental model campal CA1 and CA3 sectors, and support protection of the BBB, improve blood supply of the hippo-
capillary endothelial cells, in rats subjected to hyper-
thermia-induced seizures. They believe that TPN inhibits seizure activity and maintains BBB integrity in the course of febrile convulsions.

Our current observations together with previous findings suggest that TPM administered as a protective agent before FS induction could exert, via the vascular factor, i.e. through an indirect protective action, a beneficial effect on the structural components of the BBB, improve blood supply of the hippocampal CA1 and CA3 sectors, and support protection of some of the synapses in the experimental model of FS in young rats. It should be added that a similar neuroprotective effect against ultrastructure damage to the synapses in the hippocampal CA1 area of immature rats with repeated febrile convulsions was reported by Zhou et al. [35] after administration of another compound, i.e. high-dose fructose-1,6-diphosphate, a metabolic intermediate.

Until now the exact mechanism of topiramate action has still remained unclear. Neurochemical studies suggest that the anticonvulsant and neuroprotective properties of this AED in various experimental models involve its multidirectional effect on the CNS, especially on the hippocampus structure, and are mainly based on the modulation of its GABA-ergic system. This is probably due to the involvement of AMPA/kainate and GABAA receptors, with antagonistic effects of TPM on glutamate receptors of the AMPA/kainate subtype, which play an essential role in excitotoxic neuronal damage [3,5,11-13,17,32]. Worthy of note is that the hippocampal neurons are particularly sensitive to a variety of excitatory amino-acid mediated cerebral damage. Recent observations of Motaghinejad et al. [11-13] concerning neuroprotection of topiramate against methylphenidate-induced neurodegeneration in dentate gyrus and CA1 regions of the rat isolated hippocampus [11,13] and also in isolated rat amygdala [12] suggest that this antiepileptic drug can be used as a neuroprotective agent against apoptosis, oxidative stress and neuroinflammation. This could be partly caused by the activation of GABAA receptor, inhibition of AMPA/kainite receptor and probably via CREB/BDNF pathway [11-13].

In our opinion, the neuroprotective effect of topi-
ramate observed in the current experimental model of FS in only approximately 25% of the synaptic end-
ings is connected with a strong destructive effect of hyperthermic stress on the ultrastructure and func-
tion of synapses, especially both pre-and post-syn-
aptic membranes. However, according to literature data, the neuroprotective or therapeutic effects of various AEDs, and most possibly, topiramate, can be expected only with properly functioning synapses [20,24,25].

Lack of literature data concerning similar mor-
phological research from other centres hinders the interpretation of the ultrastructural alterations observed in the synaptic endings of the hippocampus with regard to potential desirable properties of TPM in experimental hyperthermic convulsions. This issue requires further investigations.

The presented results may serve as a useful comparative material for similar neuropathological observations conducted by other research centres. They may also have practical implications in the pre-
vention of the effects of prolonged and recurrent FS in children.

**Conclusions**

Our ultrastructural findings indicate that TPM at a dose of 80 mg/kg b.m. used “prophylactically”, i.e. prior to experimental FS induction, as a presumed neuroprotective agent, was effective in the prevention of hyperthermia-evoked synaptic damage to the hippocampal CA1 and CA3 sectors in approximately 25% of the synaptic endings in young rats. We assume that the indirect effect of a vascular factor, among other factors, can be of essential importance in the morphogenesis of synaptic neuroprotection, which may have practical implications. However, TMN used directly after FS, was ineffective in the prevention of hyperthermia-induced synaptic damage in the hippocampal sectors studied.

**Acknowledgments**

The paper is dedicated to Professor Wojciech Sobaniec, PhD, MD, former Head of the Department
of Paediatric Neurology and Rehabilitation, Medical University of Białystok, Poland, with deep gratitude for his excellent assistance with our experiment, kindness and inspiration for further research. This work was supported by research grant (Grant Number: 3-68-911P) from the Medical University of Białystok, Poland.

Disclosure

The authors report no conflict of interest.

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