

Outsourcing in the brain: do neurons depend on cholesterol delivery by astrocytes?

Frank W. Pfrieger

Summary

Brain function depends on the cooperation between highly specialized cells. Neurons generate electrical signals and glial cells provide structural and metabolic support. Here, I propose a new kind of job-sharing between neurons and astrocytes. Recent studies on primary cultures of highly purified neurons from the rodent central nervous system (CNS) suggest that, during development, neurons reduce or even abandon cholesterol synthesis to save energy and import cholesterol from astrocytes via lipoproteins. The cholesterol shuttle may be restricted to compartments distant from the soma including synapses and may be regulated by electrical activity. Testing these hypotheses will help to improve our still insufficient understanding of brain cholesterol metabolism and its role in neurodegeneration.

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Introduction

Specialization and cooperation of cells are fundamental principles of operation in tissues and organs of multicellular organisms. The nervous system provides an outstanding example for cellular differentiation and share-of-labor: neurons specialize in the generation and exchange of electrical signals, whereas different types of glial cells provide structural and metabolic support for the neuronal network.⁽¹⁾ Notably,

neurons and glia also cooperate during brain development by controlling mutually their proliferation, survival and differentiation.⁽²⁾

Here, I propose a new type of neuron–glia interaction in the CNS. My hypothesis states that, after differentiation of astrocytes, neurons reduce their cholesterol synthesis and rely constitutively on cholesterol delivery by astrocytes via lipoproteins. In the following paragraphs, I will present experimental evidence for this hypothesis and suggest possible ways to test it. Furthermore, I will highlight still unanswered questions concerning glial and neuronal metabolism and describe implications for neurodegenerative diseases. The idea of an obligatory cholesterol shuttle from astrocytes to neurons goes beyond previous proposals that non-neuronal cells including astrocytes participate in the recycling of cholesterol after injury in the peripheral⁽³⁾ or central nervous system.⁽⁴⁾ Summaries on the metabolism of cholesterol,^(5,6) lipoproteins^(4,7) and neurosteroids^(8,9) in the brain can be found in recent reviews.

Experimental evidence for neuronal dependence on glia-derived cholesterol

The hypothesis outlined above has been provoked by a series of studies that aimed originally to define the role of glial cells in synapse development. This glial function is indicated by the fact that throughout the CNS, most synapses develop after the differentiation of astrocytes.⁽¹⁰⁾ To study a possible influence of glia on synapses, Pfrieger and Barres⁽¹¹⁾ separated neurons from glial cells and studied their development in isolation. This reductionist approach became possible with the establishment of methods to highly purify retinal ganglion cells (RGCs) from postnatal rats and to culture them for several weeks under defined conditions.⁽¹²⁾ Pfrieger & Barres⁽¹¹⁾ observed that neurons form glutamatergic synapses under these conditions, but that a soluble factor secreted by macroglial cells strongly increases the level of spontaneous synaptic activity without affecting neuronal growth or survival. Subsequent studies showed that this effect was due to a strong increase in the number and the efficacy of synapses.^(13,14) Finally, Mauch et al.⁽¹⁵⁾ identified this synapse-promoting factor as cholesterol secreted by glial cells in apolipoprotein E- (ApoE-) containing lipoproteins. Their discovery suggests

Max-Planck/CNRS Group, UPR2356, Centre de Neurochimie, Strasbourg, France. E-mail: fw-pfrieger@gmx.de
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Abbreviations: ABCA1, ATP binding cassette A1; ApoA1, apolipoprotein A1; ApoD, apolipoprotein D; ApoE, apolipoprotein E; ATP, adenosine triphosphate; CNS, central nervous system; CSF, cerebrospinal fluid; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl coenzyme a; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NPC1, Niemann Pick C Protein 1; RGCs, retinal ganglion cells; SREBP, sterol regulatory element binding protein.

that RGCs depend on an external supply of cholesterol to form numerous and efficient synapses.^(16–18)

Given the large number of studies on primary neuronal cultures, one may ask why this dependency has gone unnoticed. A possible answer is that the medium of conventional cultures contains cholesterol-rich lipoproteins due to the addition of serum or the presence of glial cells. Furthermore, most cultures are prepared from embryonic brain, whose neurons may not need external cholesterol. Further support that cholesterol is a limiting factor for neurons comes from studies on conventional cultures prepared from fetal or newborn mammals. They showed that addition of cholesterol promotes⁽¹⁹⁾ and inhibition of cholesterol synthesis impairs neuronal survival and growth.^(20–22)

Astrocytes as cholesterol-producing factories

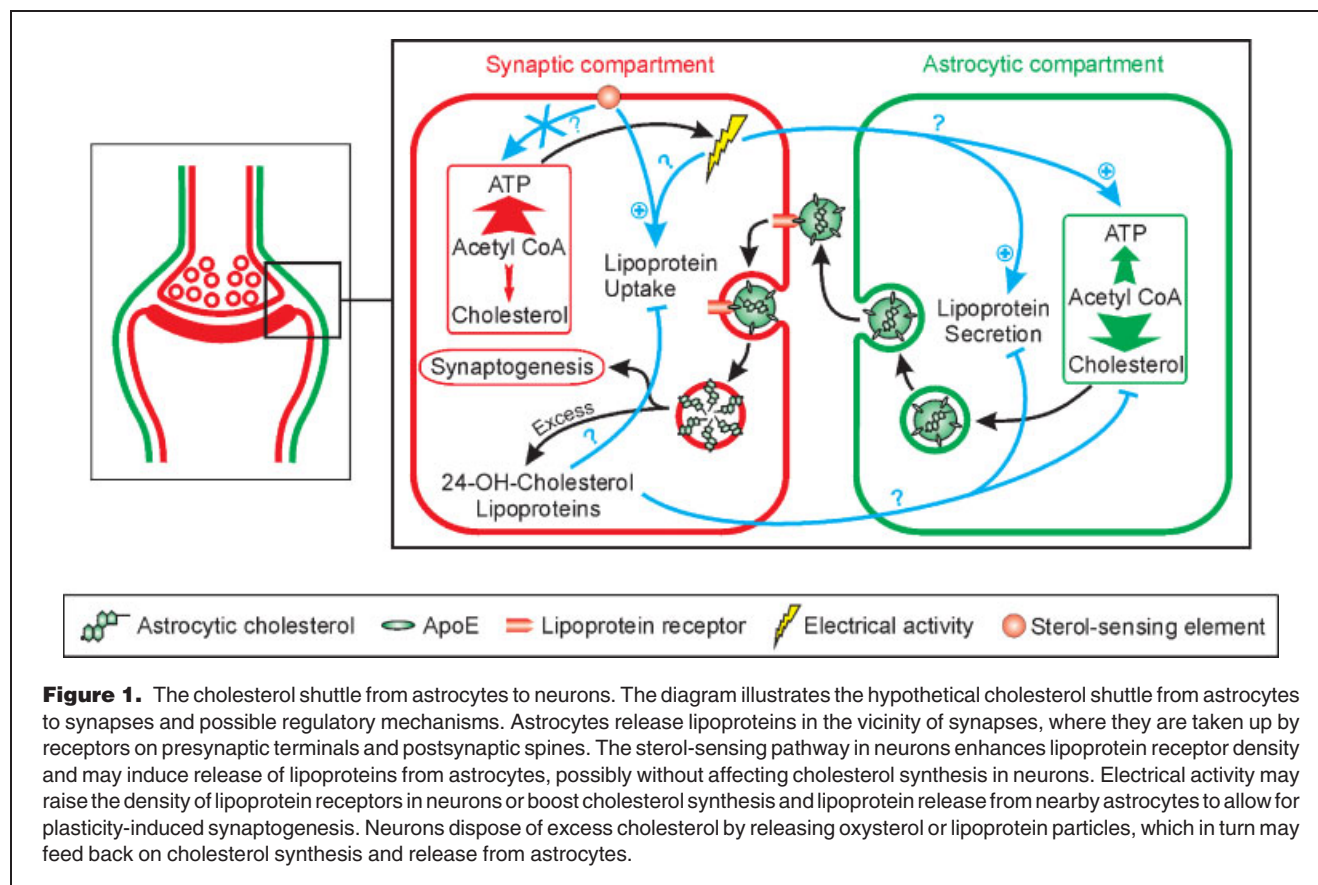
It is well established that glial cells produce and secrete surplus cholesterol (Fig. 1). In vitro studies revealed that astrocytes synthesize two- to three-fold more cholesterol than neurons or fibroblasts⁽²³⁾ and secrete lipoprotein particles, which serve as cholesterol carriers, into the culture medium.^(24,25) Notably, DeMattos and colleagues showed that astrocytes secrete lipoproteins in vivo. They detected human

ApoE in the cerebrospinal fluid (CSF) of transgenic mice that express human ApoE in a subclass of astrocytes.⁽²⁶⁾ So far, however, it is less clear, whether and how the release of lipoproteins from astrocytes is regulated. Removal of lipoproteins from culture medium causes upregulation of cholesterol synthesis in different glial culture preparations,^(27,28) but it is not known whether this also affects lipoprotein release. Interestingly, secretion of ApoE and ApoD is differentially regulated in cultured astrocytes⁽²⁹⁾ suggesting that astrocytes secrete different types of lipoproteins depending on the extracellular lipid milieu.

So far, it has remained enigmatic why astrocytes express specific apolipoproteins and why they secrete cholesterol-rich lipoprotein particles.⁽⁴⁾ The idea that neurons depend on glia-derived cholesterol may provide an explanation.

Cholesterol metabolism in neurons

The finding that cultured CNS neurons depend on an external cholesterol supply raises several questions concerning the neuronal cholesterol metabolism, which I will discuss in the following paragraphs. It will become apparent that, despite the wealth of information about cholesterol, surprisingly little is known about how neurons handle this essential membrane component.



Do neurons synthesize cholesterol?

Although this question appears trivial, it is hard to answer. All cholesterol contained in the brain is probably synthesized locally rather than imported from the blood.^(5,30,31) So far, however, it is not known whether neurons contribute to the brain pool of cholesterol.

My hypothesis postulates that neurons switch from production during embryonic stage to outsourcing after birth. Previous studies demonstrated that CNS neurons from embryonic brain^(23,32) and sympathetic neurons from newborn rats^(20,33) synthesize cholesterol *in vitro*. Little is known about cholesterol synthesis in older neurons. Radioactively labeled cholesterol appeared in axons of RGCs after intraocular injection of tritiated acetate in adult rats,⁽³⁴⁾ but it is not clear whether the sterol was synthesized by glia or neurons. Several studies detected mRNA encoding cholesterol-producing enzymes in the adult brain^(35,36) and revealed developmental changes in their expression levels,^(37,38) but they did not examine whether the enzymes are expressed by neurons. Swanson et al.⁽³⁹⁾ showed expression of 3-hydroxy-3-methylglutaryl coenzyme a (HMG-CoA) synthase in hippocampal and sensory neurons of young rabbits, but did not study its expression in adult animals. Notably, the presence of this enzyme is not sufficient to establish cholesterol synthesis, since it is also required for isoprenoid production. So far, it is not clear, whether neurons express enzymes that are specific for cholesterol synthesis. Future studies need to address this issue and the question whether the neuronal cholesterol metabolism changes during development.

Why should neurons import rather than synthesize cholesterol?

A possible reason could be the enormous logistical costs of sterol synthesis. Formation of the sterol molecule requires a whole battery of enzymes that are distributed in different subcellular organelles and consumes large amounts of energy substrates. Therefore, neurons, which specialize in the generation of electrical activity, may reduce or even abandon cholesterol synthesis. This may apply particularly to neuronal compartments like axon terminals and dendritic spines, which are distant from the soma (Fig. 1). They may import cholesterol rather than relying on local synthesis or intracellular transport from the soma. In agreement with this, Vance and coworkers showed that distal axons of sympathetic neurons cannot produce cholesterol.^(20,33) The idea that neurons outsource cholesterol synthesis to glia is supported by other examples of metabolic cooperation between these cells.^(40–42) Future studies will show whether cholesterol synthesis and energy metabolism are coupled. To accomplish this, it may be timely, to dissect these interactions by a system analysis approach using quantitative computational models that integrate different metabolic pathways in neuronal and glial compartments.⁽⁴³⁾ This may help to formulate testable hypotheses

about metabolic job-sharing and to predict changes in metabolite levels due to pathological conditions or pharmacological interference.

How and where do neurons take up cholesterol?

In general, cellular uptake of cholesterol requires lipoprotein binding to receptors in the plasma membrane, endocytosis of ligand-receptor complexes and their processing in the endosomal–lysosomal pathway^(6,44,45) (Fig. 1). Numerous studies have shown that neurons express different members of the lipoprotein receptor family.^(46,47) As stated above, lipoprotein uptake may occur preferentially in presynaptic terminals and dendritic spines, which are unable to synthesize cholesterol (Fig. 1). Lipoprotein receptors are present on dendrites^(48,49) and axons and dendrites contain endosomal-lysosomal organelles⁽⁵⁰⁾ indicating that these compartments could process lipoproteins. Direct evidence that neuronal processes can handle lipoproteins comes from studies on neuron-like pheochromocytoma cells⁽⁵¹⁾ and on sympathetic neurons.⁽²⁰⁾ I should mention that cells can also acquire cholesterol by an alternative, endocytosis-independent pathway that involves specific lipoprotein receptors and direct transfer of cholesterylester from high-density lipoproteins to the plasma membrane.⁽⁶⁾ Future studies will show which types of lipoprotein receptors mediate the cholesterol import in neurons and where they are localized.

Do all neurons depend on cholesterol import?

The structural and functional specialization of brain regions suggest that their cholesterol metabolism is not uniform.⁽³⁰⁾ Thus, some neurons may depend entirely on an external cholesterol supply, whereas others may be autonomous. Support for this idea comes from studies revealing regional and cell-specific differences in the cholesterol content⁽⁵²⁾ and in the expression profile of cholesterol synthesizing enzymes,^(35,36) sterol-sensing components,⁽⁵³⁾ intracellular transporters⁽⁵⁴⁾ and lipoprotein receptors.^(49,55) Within the hippocampus, mRNA encoding the HMG-CoA synthase and low-density lipoprotein receptor is restricted to the pyramidal cell layer.⁽³⁹⁾ It will be interesting to see whether the neuronal dependence on glia-derived cholesterol varies in a region-specific manner as well.

Regulation of the cholesterol shuttle

The idea that neurons depend on an import of cholesterol raises the question how this exchange is regulated. Obviously, neurons like all other cells must strictly control their cholesterol content, since too much or too little of this component is lethal.⁽⁵⁶⁾ In general, cells maintain the cholesterol level by an intriguing mechanism that involves sterol-sensing elements in membranes and transcription factors controlling the expression of cholesterol synthesizing enzymes and lipoprotein

receptors⁽⁵⁷⁾ (Fig. 1). Surprisingly, it is not known whether this pathway is implemented in neurons. Ong et al. showed recently that hippocampal and cortical neurons express a key element of this pathway called sterol regulatory element binding protein (SREBP),⁽⁵³⁾ but its function in neurons remains to be established.

Does synaptic plasticity alter cholesterol homeostasis?

The link between cholesterol and synaptogenesis suggests that neurons require external cholesterol for activity-dependent structural changes. This idea provokes the exciting question whether electrical activity influences cholesterol homeostasis. Two non-exclusive scenarios can be envisioned. First, electrical activity may activate the cholesterol-regulating pathway in neurons and increase selectively their capacity for lipoprotein uptake without activating cholesterol synthesis. Second, electrical activity may enhance cholesterol production and lipoprotein release in astrocytes (Fig. 1). There is good evidence that astrocytes can sense the level of synaptic activity by neurotransmitter receptors.⁽⁵⁸⁾ Interestingly, transporter-mediated glutamate uptake in astrocytes stimulates glycolysis thus coupling synaptic activity and energy metabolism.^(40,41) This pathway may also influence cholesterol synthesis and lipoprotein release in astrocytes thereby helping neurons to cope with the activity-induced need for cholesterol. As a first step to determine the validity of these considerations, it should be examined whether structural plasticity leads to a local cholesterol deficit in neurons and induces lipoprotein transfer from nearby astrocytes.

If electrical activity modulates cholesterol homeostasis in neurons, one would expect that an impairment of cholesterol synthesis or lipoprotein transport diminishes synaptic plasticity, and possibly learning and memory. Pharmacological inhibition of HMG-CoA reductase eliminated the late phase of long-term potentiation in hippocampal slices,⁽⁵⁹⁾ but it remains unclear whether this is due to reduced synthesis of isoprenoids or of cholesterol. Removal of cholesterol from hippocampal slices by cyclodextrin abolished tetanic potentiation of evoked synaptic responses,⁽⁶⁰⁾ but this effect may have been due to changes in the biophysical properties of the plasma membrane. A recent study revealed that eyeblink conditioning, a form of associative learning that involves synaptogenesis,⁽⁶¹⁾ is sensitive to cholesterol inhibitors.⁽⁶²⁾ Together, these results call for more studies on the possible link between cholesterol homeostasis and synaptic plasticity.

How do neurons get rid of excess cholesterol?

If neurons import cholesterol from glia, they need to protect themselves from overload. This could be accomplished by the release of surplus cholesterol or by a feedback mechanism that reduces lipoprotein import (Fig. 1). In general, cells

dispose of cholesterol via different pathways including ApoA1-containing high-density lipoproteins.^(5,6) So far, it is not clear, whether neurons employ similar mechanisms. Neurons express the ABCA1 transporter, which transfers cholesterol to high-density lipoprotein (HDL) particles,⁽⁶³⁾ and HDL-like particles containing apolipoprotein A1 (ApoA1) are present in CSF. However, ApoA1 is supposed to be synthesized outside the brain.⁽⁴⁾

An alternative pathway is suggested by recent evidence for a net flux of 24-hydroxy-cholesterol from brain to blood.⁽⁶⁴⁾ The enzyme that synthesizes this cholesterol derivate, a specific isoform of cytochrome P450, is expressed by neurons but not by astrocytes or oligodendrocytes⁽⁶⁵⁾ suggesting that neurons get rid of excess cholesterol by converting it to oxysterol (Fig. 1). In addition, the component may reduce cholesterol production and lipoprotein release in nearby astrocytes (Fig. 1). It is well known that oxysterols inhibit cholesterol synthesis in cultured cells.⁽⁶⁶⁾ Interestingly, a recent paper showed that activation of the liver X receptor, which binds oxysterols, leads to increase in cholesterol release from glial cells without affecting ApoE expression.⁽⁶⁶⁾ This prompts the idea that neuron-derived oxysterols may induce the release of cholesterol from glial cells in specific lipoproteins that cannot be taken up by neurons thereby reducing their cholesterol intake.

Obviously, a whole battery of experiments is required to attack exciting questions such as how neurons regulate their sterol content, whether they signal their need for cholesterol to astrocytes and whether electrical activity influences cholesterol homeostasis.

Do neurons need astrocyte-derived cholesterol in vivo?

Among the questions that have been raised in this article, this is probably the most pressing. Unfortunately, the existing literature does not provide an answer. On the one hand, genetic or pharmacological elimination of cholesterol synthesis in mammals is teratogenic and causes massive CNS defects.^(67–69) On the other hand, mice deficient in ApoE show no major developmental defects in the CNS,^(4,5,70) although cultured astrocytes from ApoE^{-/-} mice do not release cholesterol.⁽⁷¹⁾ Finally, mice lacking specific lipoprotein receptor subtypes either die during early development,^(72,73) show defects that are independent from lipoprotein uptake⁽⁷⁴⁾ or develop normally.⁽⁴⁷⁾ Evidently, these observations do not reveal whether neurons depend on glia-derived cholesterol. The fact that brain development proceeds normally in the absence of specific apolipoproteins or lipoprotein receptors may be due to activation of redundant pathways. Consequently, a rigorous test of the proposed shuttle hypothesis in vivo requires new animal models, where cholesterol synthesis or lipoprotein release can be modified in a cell-type-specific and temporally controlled manner. The fact that

animal species and strains differ in their cholesterol and lipoprotein metabolism⁽⁷⁵⁾ cautions that the cholesterol shuttle needs to be studied on different genetic backgrounds.

Implications for neurodegenerative diseases

The idea that neurons depend constitutively on astrocyte-derived cholesterol postulates that any interference with cholesterol delivery leads to neurodegeneration. The cholesterol shuttle involves several steps, each of which may be perturbed by injury or disease. These include production and secretion of cholesterol-rich lipoproteins by astrocytes, intercellular lipoprotein transport and, finally, their uptake and processing by neurons. A deficit in cholesterol may first cause degeneration of synapses, axons and dendrites, which are unable to synthesize cholesterol, and then lead to cell death. The damage in specific brain areas may vary depending on the neuronal need for glia-derived cholesterol with some regions showing massive degeneration and others remaining largely unaffected.

In the following, I will illustrate consequences of these scenarios by three exemplary diseases. First, according to the shuttle hypothesis an impaired cholesterol production by astrocytes should lead to neurodegeneration. Smith-Lemli-Opitz/RSH syndrome (OMIM #270400) is an autosomal recessive disorder affecting one in 20,000 to 40,000 births, which causes mental retardation and malformations in practically every tissue. The disease is caused by mutations in 7-dehydrocholesterol reductase, the enzyme that catalyzes the final step in cholesterol synthesis.^(67,69,76) It is possible that the enzyme is only present in astrocytes and that its inactivation causes neuronal damage indirectly by impairing cholesterol production by astrocytes. So far it is not clear, however, which cells in the brain express this enzyme.

Second, the shuttle hypothesis predicts that any interference with lipoprotein release, transport or uptake may cause neurodegeneration. Alzheimer's disease (OMIM #104300),⁽⁷⁷⁾ a devastating dementia whose late-onset form affects 50% of people above age 85, may be caused, at least in part, by an impaired delivery of cholesterol from astrocytes to neurons. The notorious culprits, β -amyloid and the ApoE4 isoform, may lower the efficacy of lipoprotein-mediated cholesterol transfer or disturb cholesterol homeostasis in neurons^(78–81) and glia.⁽⁸²⁾ Possible links between cholesterol and Alzheimer's disease have been discussed previously.^(6,83–86)

Third, the shuttle hypothesis predicts that an impaired neuronal ability to take up and process lipoproteins should cause degeneration. Support for this idea comes from studies on Niemann-Pick type C disease (OMIM #257220). This autosomal recessive lysosomal storage disorder occurs in about one of 100,000 births and leads to progressive neurodegeneration and premature death.⁽⁸⁷⁾ The defect has been traced to mutations in the Niemann-Pick protein C1

(NPC1), which is involved in intracellular processing of endocytotically acquired cholesterol.⁽⁸⁸⁾ The fact that a defect in NPC1 causes loss of axons and dendrites and neuronal cell death^(89,90) provides strong support for the hypothesis that neurons in vivo depend on cholesterol from an external source. Interestingly, neurotrophins fail to activate trk receptors and to induce neurite outgrowth in cultured neurons with a defective NPC1⁽⁹¹⁾ suggesting that external cholesterol is required to establish functional neurotrophin signaling pathways.⁽⁸⁴⁾ Finally, it would be interesting to know whether defective NPC1 also impairs cholesterol secretion by astrocytes, whose processes contain NPC1.⁽⁹²⁾ NPC1 disruption induces cholesterol accumulation^(93,94) and changes the apolipoprotein pattern⁽⁹⁵⁾ in astrocytes.

Taken together, the cholesterol shuttle hypothesis may provide an explanation why disorders of cholesterol homeostasis cause neurodegeneration. Notably, it may also prompt studies on the role of cholesterol in neurodegenerative processes that have not been linked to this lipid. An important prerequisite to define connections between cholesterol and brain disorders will be the ability to monitor activity- or disease-induced changes in brain cholesterol. Since such changes are probably restricted to small regions and do not show in a global parameter like the CSF concentration, their detection may require new experimental approaches like "molecular imaging".⁽⁹⁶⁾

Conclusion

In this article, I propose a new form of metabolic cooperation between neurons and astrocytes that allows neurons to specialize in electrical signaling. According to my hypothesis, neurons reduce or even abandon the expensive synthesis of cholesterol and import the component from astrocytes. This idea provokes numerous questions about cholesterol homeostasis in neurons and glia that need to be addressed by future studies. Given the complexity of cholesterol and lipoprotein metabolism and of neuron–glia interactions, these studies will require new animal models and techniques to manipulate and monitor cholesterol in the brain. These experiments may provide new insight into the still poorly understood cholesterol metabolism in the brain and its involvement in neurodegenerative processes.

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