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Alzheimer's Disease: Neural Stem Cell Therapy using 3x-Tg Mice

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Abstract

Through research in regenerative medicine, advancement has been shown to improve and treat diseases such as Alzheimer's disease. Neuronal stem cells specifically are further being explored for the correction of abnormalities in neuropathology. Alzheimer's disease is a neurodegenerative disorder that forms due to the presence of neuropathological lesions. This disease leads to dementia including learning and memory deficits. Neuronal stem cell transplantation is being used to improve cognition of those with AD. Current research on 3x-Tg mice shows promise in the improvement of cognition. Any stem cell therapy is a controversial topic that involves the conversation of ethics and future directions within society.

Keywords

Alzheimer's disease; Neural stem cells; Aggregated beta-amyloid protein; Neurofibrillary tangle; Synaptic density

Abbreviations

AD: Alzheimer's disease; Aß: Aggregated beta-amyloid protein; NFTs: Neurofibrillary tangles; NSC: Neural stem cells; MWM: Morris water maze; NOR: Novel object recognition

Introduction

Alzheimer's disease

The most common form of dementia to afflict humans is the neurodegenerative condition known as Alzheimer's Disease (AD). It was first described in 1906 by Alois Alzheimer following a postmortem study of his patient, Auguste D. Alzheimer's disease is now the most prevalent age-related neurodegenerative disorder. The incidence of AD is expected to more than double by the year 2030 [1]. The presence of neuropathological lesionsis observed through imaging technology. These include both amyloid plaques from aggregated beta-amyloid protein (Aß)and neurofibrillary tangles (NFTs), from abnormal tau proteins. These abnormalities in neuropathology can be observed prior to neurodegeneration. This prompts the hypothesis of an asymptomatic phase of AD with sustained cognitive function prior to the symptomatic phase of the disease [2]. Recent studies and brain imaging indicate neuronal and synaptic loss occur at this earlier stage as well. The hippocampus and entorhinal cortex, part of the medial temporal lobe, are the first areas to be affected. The atrophy of the medial temporal lobe is thought to predict the progression to AD. As the disease progresses, cortical areas are increasingly involved.

The earliest cognitive defect includes the impairment of episodic memory, an ability to recall events specific to a time and place [3]. Dementia syndrome comes about as cortical association areas are affected. Cognitive disruption is attributed to neuronal and synaptic density loss within different parts of the brain. Synaptic loss in AD more directly correlates with cognitive impairment than the presence of Aß and tau pathology [4-5]. The use of stem cells is a potential strategy for restoring memory and learning deficits by promoting regeneration, replacement or repair of damaged synapses.

Neural stem cells

Many adult tissues still retain cells that are self-renewing, multipotent stem cells which are able to generate into differentiated tissues. The adult brain is included in these tissues as it uses stem cells to create neurons and glia throughout life. Neural stem cells (NSC) are used to describe cells that (1) are able to generate neural tissue or derived from the nervous system, (2) can give rise to cells besides themselves through asymmetrical division and (3) have a capacity for self-renewal [6]. NSCs can give rise to three neural cell lineages, neurons, astrocytes and oligodendrocytes. Niches are a specialized microenvironment in certain parts of the brain that contain cells necessary for proliferation and differentiation of these NSCs. The surrounding cells affect behavior of NSCs through the production of soluble factors (growth factors, chemokines, neurotrophins) extracellular matrix molecules as well as membrane bound molecules [7] (Figure 1). This neurogenesis isn't widespread across the entire brain. Instead, it is localized largely to two germinal centers, the sub granular layer (SGL) of hippocampal dentate gyrus and the anterior subventricular zone (SVZ) of the lateral ventricles [8]. NSCs have proven effective in treating CNS injury, of both motor and cognitive impairment, as well as chronic neurodegeneration [9-10].

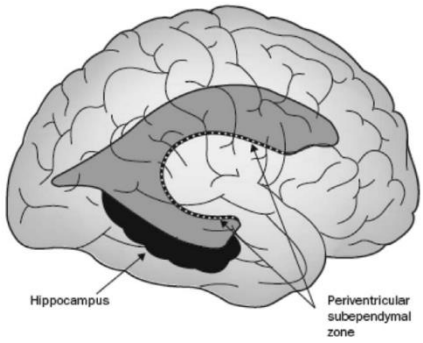

Figure 1: Location of neural stem cells within adult periventricular subependymal zone and hippocampus.

In vitro

Through dissection from of a region of an adult or fetal brain, isolation of neural stem cells can be completed. Usually, the tissue is disaggregated and then exposed to high concentration of mitogens on a substrate that allows binding. These mitogens include fibroblast growth factor-2 (FGF-2) or epidermal growth factor (EGF). Following proliferation, cells are induced to differentiation by removing mitogens andpresentation to other cells. This induces the develop of the cells into other lineages. Antibodies against specific antigens for each of the three cell lineages are used to differentiate between the cells when looking at imaging.

Transplantation in vivo

Grafted cells genetically marked are transplanted back into the brain. The fate of these grafted cells is seemingly more influenced by the environment they are placed into rather than their own intrinsic properties. Implanted NSCs have the ability to react to local signals and in damaged tissue they migrate to the site of the damage to replace depleted cells. Stem cells placed in the adult hippocampus can generate new neurons and glia usually found there [11].

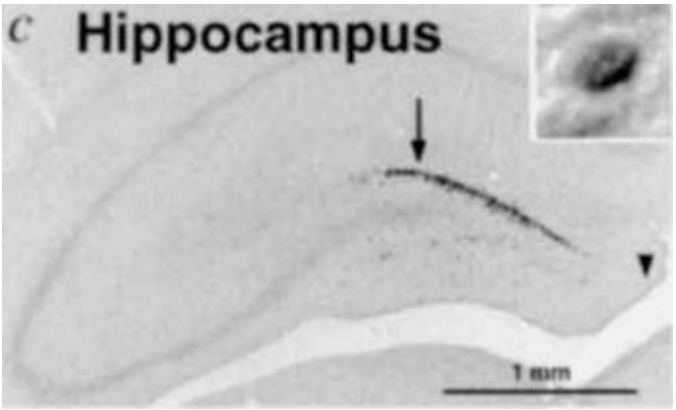

Figure 2: Example of a propagated and genetically marked adult hippocampal cell grafted to the hippocampus
Retrieved from https://www.nature.com/articles/383624a0
Laboratory of Genetics, The Salk Institute, 10010 North Torrey Pines Rd, La Jolla, California 92037-1099, USA [5].

3xTg Mice

3xTg mice contain the three mutations associated with familial AD; the human APP-Swedish mutation (KM670/671NL), tau mutation (P301L), and presenilin-1 mutation (M146V) and are maintained on a C57BL6/129 background [12]. Using transgenic mice species (PSEN1 containing the PS1M146V mutation) single-cell embryos from mice are injected with two human transgenes labelled APP with the Swedish mutation and MAPT along with the P30IL mutation.

Based on the transgenics involved the overexpressed transgenes are restricted to the central nervous system both including the hippocampus and cerebral cortex. No gross physical or behavioral abnormalities are displayed. The mice show plaque and tangle pathology that are characteristic of AD. The Aß deposition is progressive, similar to AD, and extracellular deposits are detected by six months. Changes of the tau protein in the hippocampus are observed later, around 12-15 months. Gliosis involving increased density of GFAP astrocytes is shown at around 7 months. Synaptic dysfunction is observed prior to plaque and tangles forming as has been discovered to occur in AD. Cognitive impairment can be seen by four months as a deficit in long-term retention [13].

Discussion

Neural stem cells are multipotent stem cells found in the brain that can differentiate into various lineages. Alzheimer's disease is characterized by multiple pathologies that affect different neuronal lineages across various brain regions. Accumulation of both Aß plaques and NFTs, which accompany gliosis and synaptic and neuronal loss, are distinctive of AD. These together cause a progressive loss of memory and cognitive function [14]. Evidence supports the processing of amyloid precursor protein (APP) initiates an amyloid cascade which causes extracellular deposition of Aß [15]. Aß leads to calcium dysregulation and oxidative stress to mature neurons leading to apoptosis of hippocampal neurons [8]. Stem cells produce neurotrophic and other neuroprotective factors that are lacking in patients with AD due to the apoptosis of neural cells. These factors promote the function and regeneration of endogenous neuronal circuitry. Stem cells may also migrate to areas of injury from the area of injection. It has been demonstrated that NSCs transplanted into rat brains can differentiate into neural cell lineages and significantly improve cognition. This approach involves differentiation of NSCs in response to the brain's microenvironment.

Research

Research to test the efficacy of NSCs using 3xTg-AD mice have shown promise for the use of human neuronal stem cells in AD treatment. AD cognitive dysfunction is most strongly correlated to hippocampal synaptic density which is demonstrated in recent studies. Measures of hippocampal dependent behavior are used to assess cognitive function pertaining to the hippocampus. Frequently used measures of hippocampal dependent behavior are the Morris water mazer (MWM) and the novel object recognition (NOR). MWM involves training the mice to swim to a platform. Trials are performed to test how long it takes the mouse to return to the previously viewed platform. Spatial memory retention can also be demonstrated based on testing the mice in a probe trial, 24 hours after the final

training session. NOR explores the innate preference mice have to recognize familiar objects placed into novel context. It also can be usedto demonstrate place dependent NOR through recognition of which of two identical objects have been moved [14].

A study done by the Department of Neurobiology and Behavior and Institute for Memory Impairments and Neurological Disorders at the University of California demonstrated the cognitive rescuing murine NSCs are capable of [14]. Murine NSCs are first analyzed to confirm capability of self-renewal and multipotency. The NSCs are delivered to the hippocampus of the 3xTg-AD mice as well as nonTg mice (control). Another group of 3xTg-AD and non-Tg mice are given a vehicle. A month following transplantation, mice were tested on two hippocampal dependent behavioral tasks, MWM and NOR. 3xTg-AD mice injected with NSCs demonstrated shorter latencies during MWM and probe testing, crossing the former location platform almost twice as often as vehicle injected transgenic mice. NSCs did not alter the performance of non-Tg mice. During the NOR, NSC injected mice significantly improved behavioral deficits over 3xTg-AD mice that were vehicle injected (Figure 3).

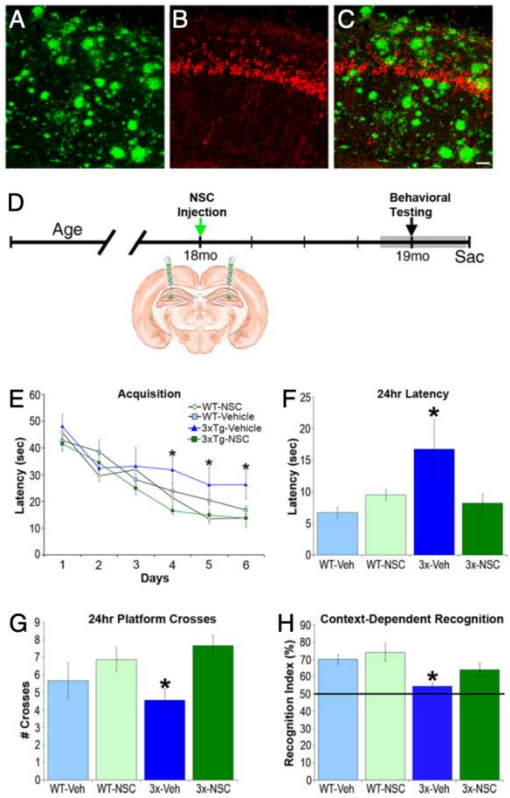

Figure 3: NSC transplantation improves AD cognitive dysfunction. 3xTg-AD mice exhibit plaques (A and C, green) and tangles (B, red) within the hippocampus. (D) 100,000 NSCs or vehicle control were injected and 4 weeks later tests were performed. (E) MWM training revealed NSC-injected mice exhibit significantly shorter escape latencies vs. vehicle injected transgenic mice. (F) During probe trial testing NSC-injected 3xTg-AD mice also showed significantly shorter latencies than vehicle-injected 3xTg-AD mice and perform equivalent to nonTg controls. (G) NSC injected mice crossed the former platform more often than control injected transgenics. (H) Context dependent recognition reveals vehicle injected 3xTg-AD mice are impaired and 3xTg-AD mice injected with NSCs exhibit significant recovery. Retrieved from the Department of Neurobiology and Behavior and Institute for

Memory Impairments and Neurological Disorders, University of California, Irvine, CA [6].

The migration and differentiation of NSCs was examined. Five weeks post injection of engrafted NSCs showed differentiation into neurons, astrocytes and oligodendrocytes. Only limited migration of NSCs to plaques was shown. This could mean that the plaques fail to elicit significant secretion of NSC attracting chemokines. Although lowering Aß and *tau* load are shown to rescue cognitive deficits in multiple other studies, NSC transplantation did not alter the pathology of Aß and *tau* presence [16-17]. Prior to this, few treatments have shown to restore cognition without fixing one of those pathologies (Figure 4).

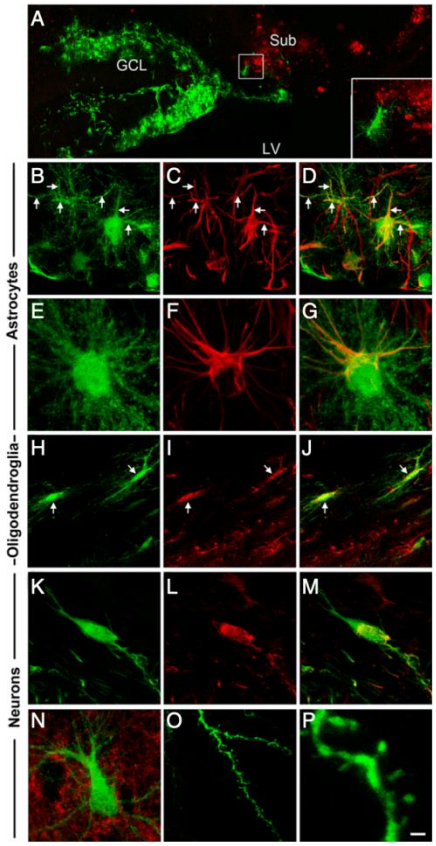

Figure 4: Engrafted neural stem cells differentiate into neurons, astrocytes and oligodendrocytes. (A) GEP-expressing NSCs (green), LV: lateral ventricle, Sub: subiculum. (B-G) majority of NSCs differentiated into astrocytes coexpressing GFAP (red). (H-J) Oligodendroglia markers (GalC; red). (K-M) Fewer cells adopted neuronal fate expressed through doublecortin (red). (N) neuronally differentiated NSCs surrounded by presynaptic terminals (synaptophysin, red). (O-P) dendritic spine architecture. Retrieved from the Department of Neurobiology and Behavior and Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, CA [6].

In other words, NSC transplantation rescued the cognitive performance in 3xTg-AD mice without altering either Aß or *tau* pathology. Synaptic density in the hippocampus was explored as an alternative to the means by which NSCs rescued cognition. The evidence through this study suggests that Aß oligomers impair cognition through the binding and altering of synaptic shape, composition and density [18]. The authors hypothesize that the NSCs compensate for toxic effects of those oligomers on the synaptic connectivity. Synaptic density was measured in the stratum radiatum of CA1 of the hippocampus and a 45% increase in synaptophysin protein levels were found in NSC injected mice.

Synaptophysin and synapsin are used as an indicator of synaptic integrity and are found significantly reduced in patients with AD [19]. Expression and responsiveness to neurotrophins is the primary mechanism for synaptic density alteration. BDNF in the hippocampus plays a central role in the synaptic remodeling which is associated with memory [20]. NSCs are known to express high levels of BDNF. The research demonstrated that NSC-derived BDNF enhances axonal outgrowth and alone is sufficient to improve memory performance. When BDNF was knocked down, the cognitive benefits previously seen in NSC delivery were abolished (Figure 5).

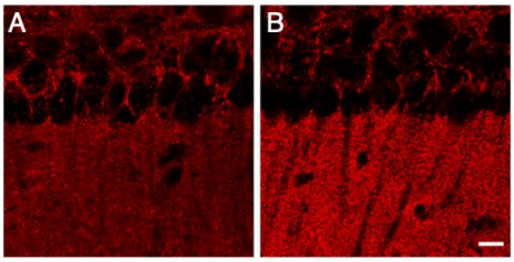

Figure 5: Neural stem cells increase synaptic density (A) NSC-injected 3xTg-AD mice (b) exhibit a 67% increase in synaptophysin immunoreactivity within the stratum radiatum of CA1 (red puncta). Retrieved from the Department of Neurobiology and Behavior and Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, CA [6].

Overall, the research demonstrated that NSCs cause beneficial rescue of cognition due to the elevation of hippocampal BDNF leading to increased synaptic density. NSCs were shown to differentiate into neurons, astrocytes and oligodendrocytes. These beneficial effects aren't provided by lowering the Aß or *tau* load but instead through a 'bystander effect". NSC transplantation can improve the function by providing missing or defective enzymes, modulating inflammation or providing trophic support [14].

A second study utilized two complementary transgenic models of Alzheimer's disease and neuronal loss. This paper also explored whether cognitive dysfunction could be rescued by human NSC transplantation in 3xTg mice. Again, the question was asked if NSC transplantation modulated Aß and *tau* pathology and/or synaptic growth and plasticity. 3xTg mice were used as well as a second group of mice created to mimic loss of hippocampal neurons. This transgenic mouse model was based on a tetracycline-off inducible system. Neuronal loss is restricted to CaMKIIa-expressing cells initiated with withdrawal of doxycycline from the mouse's diet. This led to expression of diphtheria toxin A(DT_A) chain within neurons. Withdrawal for 25 days resulted in significant reduction in neuronal markers in the hippocampal CA1 subfield as well as behavioral impairments. This transgenic group will be referred to as CaM/Tet-DTA. Both CaM/Tet-DT_A and 3xTg-AD mice were treated with NSCs or vehicle and then tested.

Beginning at 4 weeks post transplantation, MWM and NOR were used to assess hippocampal dependent learning and memory of each of the 4 groups. During the MWM trials of 3xTg-AD mice, there was no significant difference between those injected with a vehicle or NSCs. 3xTg-AD mice did perform significantly better during the probe trial 24 hours later. 3xTg-AD mice reached the platform twice as

fast and crossed the former platform almost three times as often as vehicle injected. Both context-dependent and place-dependent NOR tests were performed for the 3xTg-AD mice. During both NORs 3xTg-AD mice injected with NSCs spent significantly more time with the object placed into new context. Therefore, it was concluded, NSC transplantation improves 3xTG-AD cognitive function during three independent memory tasks. CaM/Tet-DT_A mice performed the NOR task. NSC transplanted mice in this group showed significant improvement for both context and place dependent NOR trials. Therefore, it was also concluded, NSC transplantation improves memory in the other model mimicking extensive loss of hippocampal neurons [21].

The survival, differentiation and migration of NSCs following 6 weeks transplantation was investigated. The immunosuppressant drugs modulate AD associated pathologies in both transgenic mouse models and led to great engraftment and survival of the human NSCs. NSCs were found mostly surrounding CA1 subfield of hippocampus and within overlying corpus callosum. The differentiation of NSCs largely depended on location and density of the engrafted cells. Markers were able to differentiate between immature neurons, astrocytes and oligodendrocytes [21] (Figure 6).

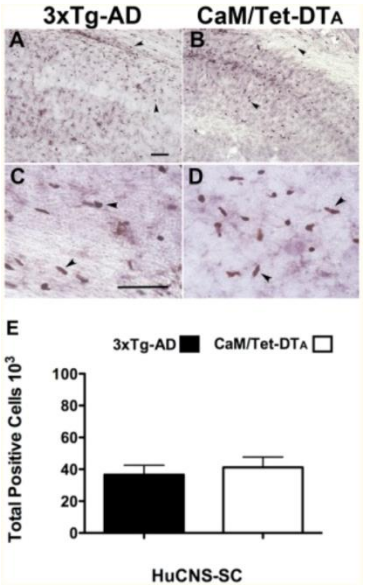

Figure 6: Survival and engraftment of human Neural Stem Cells due to immune suppression. (A) 6 weeks post-transplantation NSCs located within hippocampus overlying corpus callosum. (B) Similar degree of engraftment in CaM/Tet-DT_A mice. (C,D) Subfields A and B reveal NSCs positive for human nuclear antigen (brown). (E) Stereological assessment of NSCs engraftment demonstrates approximately 40,000 transplanted cells survive in both 3xTg-AD and CaM/Tet-DT_A

Aß and *tau* loads as well as the elevation of synaptic markers was examined. Both Aß and *tau* levels were found unaltered for both treated and vehicle mice. This is consistent with a hypothesis that NSC induced cognitive improvement occurs independently or downstream of the Aß pathology. Synaptophysin, synapsin and growth associated protein-43 (GAP-43) was investigated in all groups of mice. Levels of synaptophysin increased significantly by 47% in stratum radiatum of CA1 in NSC

transplanted mice. A 21% increase of presynaptic synapsin was observed in CaM/Tet-DT_A. GAP-43 is a protein localized to axons which facilitates synaptic maintenance and neurite growth. An increase of this was detected after induction of DTA in the CaM/Tet-DT_A group which suggests NSC transplantation can enhance axonal sprouting response to injury [21].

The transplantation of NSCs showed significant improvements for hippocampal dependent cognitive function in both of the transgenic mice models. Again, NSCs had no effect on Aß or tau pathology, suggesting a downstream mechanism of action. NSCs introducing increased levels of hippocampal synaptic connectivity is shown to mediate the cognitive impairments of both transgenic mice models. A final interesting discovery made by this research includes the short post-translation time from the injection of donor cells (4-6 weeks) [21]. This suggests that differentiation may not be necessary for efficacy but instead intrinsic properties of NSCs.

Conclusion

Alzheimer's disease is the leading cause of dementia and is increasing in incidence. Current therapies for AD attempt to slow the effects or improve quality of life but not to prevent or cure the disease. Stem cell therapy provides an advantage of targeting multiple mechanisms as opposed to a single-approach therapy. This offers possible mediation to replacement of lost cells, neuroprotection through trophic factor secretion, and modulation of inflammation [21].

NSCs have been shown to improve hippocampal dependent function in various studies using 3xTg-AD mice. It has been demonstrated that transplanted human NSCs are able to differentiate into neural cells and work to improve cognition. Aß and tau load have not been shown to be affected by the transplantation of NSCs. Instead, increased synaptic density is shown to restore cognition. NSCs produce soluble factors that improve synaptogenesis of endogenous NSCs which is hypothesized to increase the synaptic density. Furthering preclinical research of NSCs shows great promise to prevent and treat cognition loss associated with Alzheimer's disease.

Ethics

The use of stem cells is ethically questioned in regard to funding, regulations, procedures as well as safety. An adequate method of regulation and oversight to stem cell research is lacking in the United States. At both the state and federal level, determination of financial conflicts of interest needs to be explored. The debate on when a human life begins is one of the main causes of ethical debate in this field. Embryonic stem cells are one of the sources of transplanted stem cells. The use of these stem cells presents legal and ethical concerns due to the destruction of live embryos. There is also broad inexperience in the stem cell field regarding standardization of resources, processes, origin of stem cells, application methods and surgical procedures. The lack of experience and regulation leads to distrust of the field. Due to undecided upon variables, other limitations aren't addressed such as possible rejection of transplanted stem cells as well as the possibility of inducing tumor lineages [22].

Future Directions

NSCs provide a new method of treatment for patients that could potentially lead to preventing and curing AD. Current treatment focuses to only reduce the impairment and improve the lives of patients. Active adult neurogenesis creates a possibility to repair and prevent degenerative neuropathies. Better methods are needed for isolation and purification of NSCs in order to further research that looks to prevent and actively treat AD. Improved tracking of the migration of stem cells into already established neural circuits would allow for a greater understanding of the effect NSCs have on already existing and damaged tissue. Following the timeline of migration would allow us to investigate if the intrinsic properties of NSCs are the reason for cognition improvement or if differentiation is in fact necessary for their healing properties.

Due to the lack of regulation and acceptance of stem cell research, the entire field is limited. By creating regulation policies and financial plans for stem cell research, the field would be able to grow. Endogenous generation of new neurons using one's own NSCs could also be a potential mediation from transplanting stem cells which largely deals with ethical concerns. Long-term behavioral efficacy research is lacking in a largely new field. Many studies use short term cognitive tests on the animal subjects. Relating these tests to long term cognition improvement in humans is difficult. Research exploring long term effects as well as effects of multiple NSC treatments should be examined in the research of neural stem cells and Alzheimer's disease.

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