ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology
C -Life Sciences and Biotechnology

Estuscience – Life, 2025, 14 (2) pp. 66-72, DOI: 10.18036/estubtdc.1703277

RESEARCH ARTICLE

11β-HSD1 REGULATES GLUT1 EXPRESSION IN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

Burak BERBER [1, 2 *] , **Hülya SİVAS** [2]

[1] Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425, USA
burakberber@eskisehir.edu.tr - 0000-0001-5136-5323

[2] Department of Biology, Faculty of Science, Eskisehir Technical University, Eskisehir 26555, Turkey
hzeytino@anadolu.edu.tr - 0000-0002-8570-83283

Abstract

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) locally regenerates active glucocorticoids and has been linked to metabolic dysfunction and neurodegeneration. In this study, we aimed to investigate whether 11β-HSD1, an enzyme known to modulate glucocorticoid activity and metabolic homeostasis, plays a regulatory role in the expression of glucose transporter-1 (GLUT1) in human brain microvascular endothelial cells (HBEC5-i), which are critical for maintaining blood–brain barrier integrity and cerebral energy balance. HBEC5-i were transduced with a GFP-tagged pLKO.1 lentiviral vector (MOI 10) encoding an shRNA against 11β-HSD1 or with a non-targeting control. Transduction efficiency was confirmed by GFP fluorescence and knockdown was validated by immunoblotting. Protein abundance of GLUT1, CPT1A, PFKFB3 and GSK3α/β was quantified by western blotting. 11β-HSD1 knockdown reduced GLUT1 and GSK3α/β while CPT1A and PFKFB3 remained unchanged. While the association between 11β-HSD1 and energy metabolism is well-documented, the precise molecular mechanisms governing this relationship remain incompletely understood. Our study is the first to explore this interaction specifically in HBEC5-i, providing foundational insights that not only elucidate the metabolic roles of 11β-HSD1 in this unique cellular context but also pave the way for future research aimed at uncovering the downstream signaling pathways and therapeutic potential of targeting 11β-HSD1 in cerebrovascular disorders.

Keywords

11β-HSD1,
GLUT1,
Brain microvascular endothelial cells,
Energy metabolism,
Blood–brain barrier

Time Scale of Article

Received :21 May 2025
Accepted : 04 July 2025
Online date :25 July 2025

1. INTRODUCTION

The endothelium is a complex and diverse organ in the human body, consisting of multiple subtypes of endothelial cells that differ in their characteristics, function, and location. These endothelial cells can be categorized into arterial, venous, microvascular, and lymphatic types, each with different roles and varying oxygen levels [1]. For example, pulmonary microvascular endothelial cells differ significantly from pulmonary arterial endothelial cells in their oxygen and glucose consumption and intracellular ATP levels [2]. This difference in metabolic activity may be attributed to the different oxygen levels in their respective environments. Furthermore, brain microvascular endothelial cells possess more mitochondria than peripheral endothelial cells, which suggests an increased oxidative metabolism in these cells [3]. However, more research is needed to confirm this hypothesis. Overall, the endothelium is a highly heterogeneous and complex organ, and understanding the differences between its various subtypes is essential for developing treatments for diseases and disorders that affect the endothelium.

---

*Corresponding Author: burakberber@eskisehir.edu.tr

Endothelial cells, cells lining the inner surface of blood vessels, play a crucial role in maintaining vascular homeostasis, which is critical for proper brain function. These cells provide 90% of their energy through glycolysis under normal conditions. However, recent studies have shown that quiescent endothelial cells perform FAO in basal metabolism to maintain tight junctions, redox homeostasis, and glucose transport [4]. This function is paramount in ensuring brain health and energy homeostasis, particularly in association with neurodegenerative diseases related to aging and diet. Detailed metabolic studies have revealed that endothelial cells require high glycolysis and FAO during angiogenesis, forming new blood vessels from pre-existing ones. During angiogenesis, endothelial cells switch from hypometabolism to hypermetabolism, which is essential for understanding the metabolic changes in the vascular structure that deteriorate with aging and pathophysiological neurodegenerative diseases such as TBI (traumatic brain injury) and CTE (chronic traumatic encephalopathy).

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an NADPH-dependent enzyme that catalyzes the conversion of inactive glucocorticoids (cortisone in humans) to their active forms (cortisol), exerting significant influence over local glucocorticoid availability within tissues [5],[6]. This enzyme is broadly expressed across various tissues, including the liver, adipose tissue, skeletal muscle, and the central nervous system, where it modulates glucose metabolism, inflammation, and cellular stress responses [7], [8]. In endothelial cells, which line the interior surface of blood vessels, 11β-HSD1 has been implicated in regulating vascular homeostasis and metabolic functions. Recent studies have highlighted that 11β-HSD1 may influence endothelial barrier integrity, reactive oxygen species (ROS) production, and endothelial cell proliferation [9]. Although the impact of 11β-HSD1 on metabolic pathways has been well-characterized in other cell types, its specific role in brain endothelial cells, which maintain the critical blood-brain barrier (BBB), remains largely unexplored. Understanding how 11β-HSD1 modulates glucose transport and metabolic signaling in these cells is essential for elucidating its contribution to cerebrovascular health and disease.

Brossaud, et al., (2023) revealed that 11β-HSD1 inhibition prevented hippocampal-related memory deficiency in diabetic juvenile rats [8]. In a study conducted with PET scan analysis, BMI (from lean to obese) and age factors measured 11β-HSD1 expression in the brain. The increase in BMI index and aging shows that 11β-HSD1 expression increases in the brain. Excessive amounts of cortisol in persistency CSF were detected in the first week after TBI, and researchers reported that this may be due to BBB disruption. In addition, cortisol, a glucocorticoid steroid hormone, is primarily responsible for stimulating gluconeogenesis in the liver and promoting adipocyte differentiation and maturation. Prolonged excess cortisol leads to visceral adiposity, insulin resistance, hyperglycemia, memory dysfunction, cognition impairment, and more severe Alzheimer's Disease [9].

We experimented with investigating the response of HBEC5-i when we silenced the expression of the 11β-HSD1 enzyme using pLKO lentivirus. After collecting the cells, we examined the protein expression of the resistant cells obtained after the puromycin selection process. Although the remaining cells did not proliferate, they survived for ten days. We investigated the changes in protein expressions that occurred due to suppressing 11β-HSD1 expression. Our study revealed that the expression of GLUT1, GSK3α and GSK3β in HBEC5-i's was significantly reduced. However, there was no significant change in the expressions of CTP1A and PFKFB3. These results suggest that 11β-HSD1 may regulate glycolysis and glycogen synthesis, but we need to clarify its mechanism further.

2. MATERIALS AND METHODS

2.1. Human Brain Microvascular Endothelial Cell (HBEC-5i) Culture

Human brain microvascular endothelial cells (HBEC-5i; ATCC® CRL-3245™), derived from male donor brain microvessels and immortalized via SV40 large T antigen, were cultured in T75 flasks precoated with 0.2% (w/v) gelatin (porcine Type A; Sigma-Aldrich). Cells were maintained in a 1:1 mixture of endothelial cell growth medium (VEC Technologies, Rensselaer, NY, USA) and Medium 199 (Corning, Manassas, VA, USA). The base medium included 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Invitrogen) unless otherwise specified. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere and passaged upon reaching 80–90% confluence using standard trypsinization protocols. For in vitro treatment studies, HBEC-5i cells were seeded into gelatin-coated multi-well plates.

2.2. Silencing of 11β-HSD1 expression by lentiviral vector in HBEC5-i

The lentivirus pLKO.1-puro-CMV-tGFP (from Sigma Aldrich's MISSION) with Clone ID TRCN0000028065 and sequence 'CGAGCTATAATATGGACAGAT' was used to silence 11B-HSD1 expression in BMVECs. The transfection was done using polybrene. The cells were seeded 24 hours prior to transfection in a 24 well plate, with $6\text{-}8 \times 10^{-4}$ cells per well. Various groups were established, including negative control (uninfected cells), positive control (GFP-lentivirus (MISSION, pLKO.1-puro Control), Blank and lentivirus injection) to monitor puromycin selection. We made sure to observe 30-40% confluency during the process. The multiplicity of infection (MOI), defined as the number of virus particles per cell, was calculated as 10 for lentiviral injection.

2.3. Western Blotting

Equal amounts of total protein (9 µg per lane) were resolved by SDS-PAGE using 4–12% Bis-Tris polyacrylamide gels and subsequently transferred to nitrocellulose membranes. Membranes were blocked for 1 hour at room temperature in 5% nonfat dry milk diluted in Tris-buffered saline with 0.1% Tween-20 (TBST), followed by overnight incubation at 4 °C with primary antibodies diluted in 5% bovine serum albumin (BSA) in TBST. Target antibodies were used at a 1:2,000 dilution; β-actin (loading control) was incubated separately at a 1:10,000 dilution. All antibodies purchased from Cell Signaling company except 11β-HSD1 antibody. Codes represents antibodies product number of cell signaling company. GSK3α-β (D5C5Z), PFKFB3 (13123), CPT1A (D3B3), 11β-HSD1 (NBP1-32027, Novus). Following primary antibody incubation, membranes were washed with TBST and probed for 1 hour at room temperature with species-specific horseradish peroxidase (HRP)–conjugated secondary antibodies (1:5,000 dilution). Prestained molecular weight markers were run in parallel to confirm protein band sizes. Signal detection was performed using enhanced chemiluminescence (ECL) reagents, and membranes were imaged using the Amersham Imager 680 system (GE Healthcare Biosciences, Marlborough, MA, USA). Relative protein expressions were quantified by densitometric analysis in ImageJ software and normalized to β-actin expression for each lane.

2.4. Statistics

All data were analyzed using GraphPad Prism (v10.0; GraphPad Software, San Diego, CA, USA). For multi-group comparisons, one-way ANOVA with appropriate post hoc corrections (Tukey's) were used.

3. RESULTS

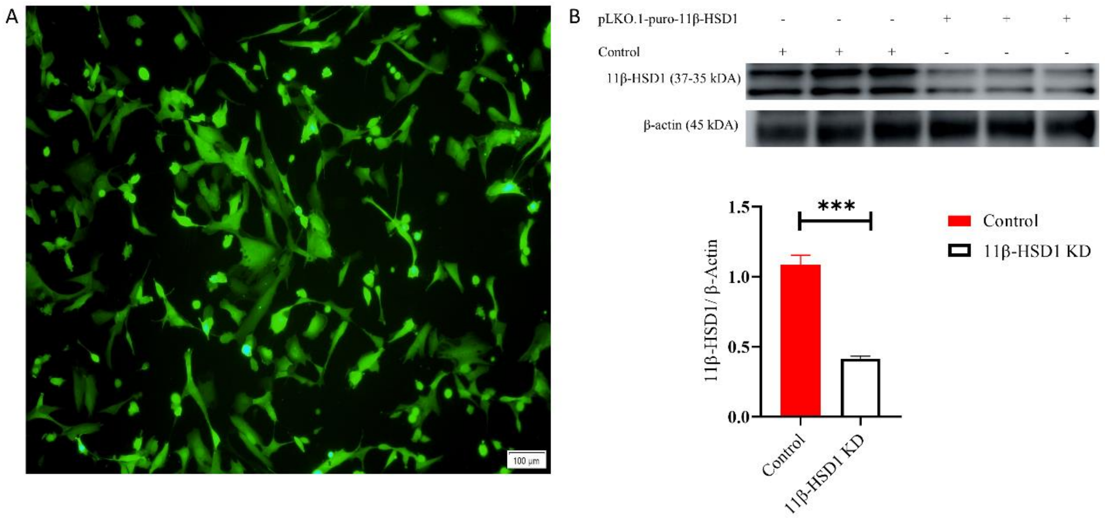

Figure 1. Efficient 11β-HSD1 knock-down in HBEC5-i cells.

GFP fluorescence imaging showing successful lentiviral transduction of HBEC5-i cells with pLKO.1-puro-CMV-tGFP-11β-HSD1 construct. Puromycin selection (2 µg/mL) was applied for 10 days. (Figure 1A) Western blot analysis confirming the efficient knockdown (KD) of 11β-HSD1 protein expression in HBEC5-i cells. β-Actin served as the control. Data are representative of three independent experiments (Figure 1B) (*p<0.05).

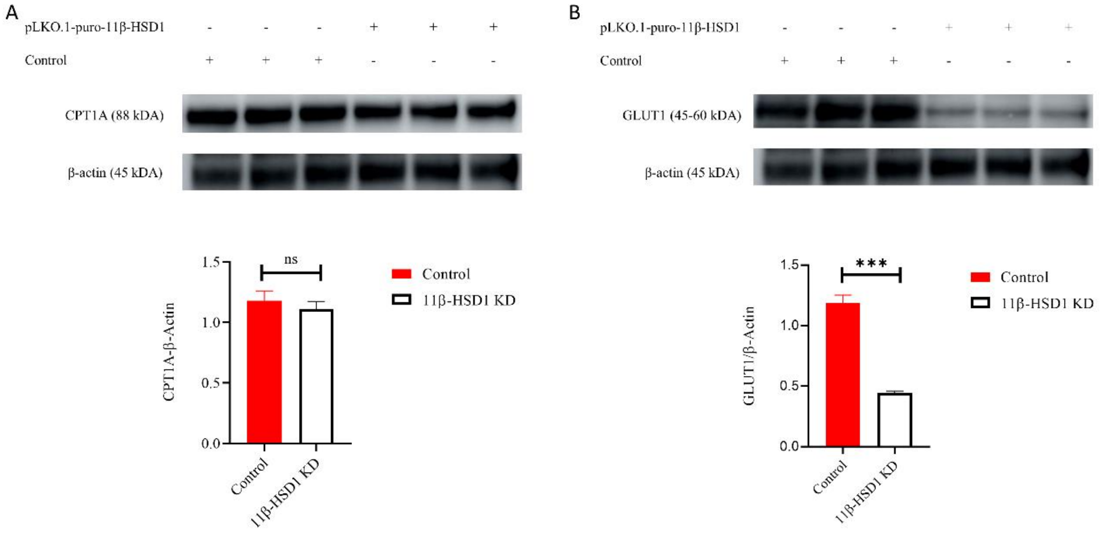

Figure 2.11β-HSD1 Knockdown reduces GLUT1 expression but not change CPT1A expression in HBEC5-i Cells.

Western blot analysis demonstrated that knockdown of 11β-HSD1 in HBEC5-i cells resulted in a significant reduction in GLUT1 protein levels compared to the control group (p<0.001) (Figure 1B). As

GLUT1 is the principal glucose transporter at the blood-brain barrier, its downregulation indicates impaired glucose uptake capacity in endothelial cells.

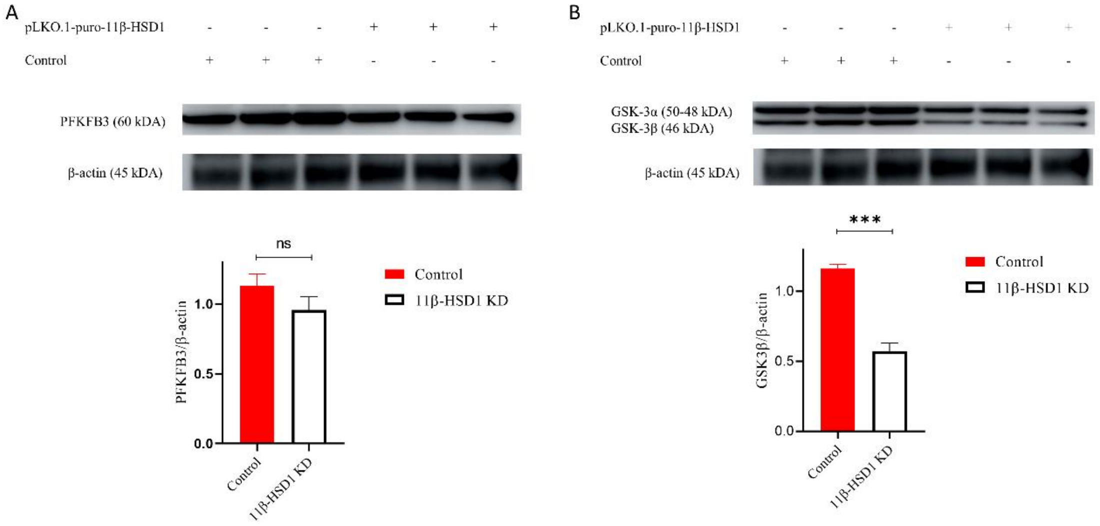

Figure 3. GSK-3α and GSK-3β expression is reduced following 11β-HSD1 knockdown. The expression level of PFKFB3 remains unchanged.

Further analysis revealed that 11β-HSD1 silencing led to a significant reduction in GSK3β protein expression ($p<0.01$), while GSK3α expression showed a decreasing trend but did not reach statistical significance ($p>0.05$) (Figure 3B). Notably, the reduction in GSK3β expression was more pronounced compared to GSK3α (Figure 3B). Notably, the decrease in GSK3β expression was more pronounced compared to GSK3α. Given the roles GSK3 in regulating glycogen synthesis and cell survival pathways, these changes suggest metabolic alterations associated with impaired glucose handling in endothelial cells. No significant differences were observed in PFKFB3 and CPT1A expression levels between 11β-HSD1 knockdown cells and controls ($p>0.05$) (Figure 1A and Figure 3A). This finding shows that protein-level expression of key regulators of fatty-acid oxidation and glycolysis remained largely unchanged, implying that 11β-HSD1 exerts a targeted influence on glucose transport rather than broadly reshaping cellular energy metabolism.

4. DISCUSSION

The findings from this study demonstrate that the suppression of 11β-HSD1 expression significantly alters the metabolic profile of human brain microvascular endothelial cells (HBEC5-i). Most notably, the downregulation of GLUT1, a key glucose transporter in endothelial cells, highlights a potential regulatory role of 11β-HSD1 in glucose uptake and cellular energy metabolism[10]. GLUT1 is essential for maintaining cerebral glucose homeostasis, and its decreased expression in 11β-HSD1 knockdown (KD) cells suggests that 11β-HSD1 positively influences glucose transport in brain endothelial cells. This supports the hypothesis that 11β-HSD1 may modulate glycolytic flux via regulation of GLUT1 expression, especially under conditions requiring increased energy demand such as neuroinflammation or injury.

The reduction of GSK3α and GSK3β further suggests a broader role for 11β-HSD1 in metabolic regulation. GSK3 is known to regulate glycogen synthesis and multiple signaling pathways related to cellular survival and inflammation [11]. The more pronounced decrease in GSK3β expression may indicate isoform-specific sensitivity to metabolic stress in HBEC5-i. Interestingly, no significant changes were observed in CPT1A or PFKFB3 expression. CPT1A, a key enzyme in fatty acid oxidation, and PFKFB3, a regulator of glycolysis, are typically involved in endothelial cell metabolism during angiogenic activity [12]. Their unchanged levels imply that 11β-HSD1 might primarily impact glucose transport mechanisms rather than directly modulating glycolysis or fatty acid oxidation pathways at the transcriptional or translational level.

The survival of 11β-HSD1 KD cells, despite reduced proliferative capacity, indicates that metabolic adaptation mechanisms may partially compensate for the loss of 11β-HSD1 activity. These cells might rely on alternative energy pathways or residual glycolytic activity for maintenance. Our findings align with previous reports implicating 11β-HSD1 in metabolic disorders, cognitive decline, and neurodegeneration. Elevated cortisol levels, regulated by 11β-HSD1, have been associated with blood-brain barrier dysfunction and increased neuroinflammatory signaling [9]. Thus, the observed metabolic alterations in endothelial cells may represent an early molecular event in the pathogenesis of neurological diseases, such as Alzheimer's disease and traumatic brain injury [8],[13].

Limitations of this study include the lack of transcriptomic validation and functional assays such as glucose uptake measurements or cell viability tests post-GLUT1 downregulation. Additionally, mechanistic insights into how 11β-HSD1 regulates GLUT1 at the molecular level remain to be elucidated. Future studies should aim to clarify the signaling cascades involved, explore the interplay with other metabolic regulators, and assess the *in vivo* relevance of our findings. Investigating whether pharmacological inhibition of 11β-HSD1 alters blood-brain barrier integrity and glucose transport in animal models could provide translational insights.

In summary, our study identifies 11β-HSD1 as a regulator of GLUT1 expression in human brain microvascular endothelial cells, implicating this enzyme as a key metabolic modulator in the cerebrovascular system.

CONFLICT OF INTERESTS

The authors stated that there are no conflicts of interest regarding the publication of this article.

ACKNOWLEDGEMENTS

This study was supported by the TÜBİTAK BİDEB 2214-A International Research Fellowship Program. With the help of this fellowship, I was able to utilize the research infrastructure of the Medical University of South Carolina (MUSC). I would like to express my sincere gratitude to Associate Prof. Dr. Önder Albayram and Prof. Dr. Adviye Ergül for their valuable support. Special thanks are also due to MUSC for granting access to their research facilities.

CRediT AUTHOR STATEMENT

Burak Berber: Conceptualization, Investigation, Methodology, Writing – Original Draft, Formal analysis**, Hülya Sivas:** Writing – Review & Editing, Supervision

REFERENCES

[1] Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. Circ Res. 2015;116(7):1231-1244. doi:10.1161/CIRCRESAHA.116.302855

[2] Parra-Bonilla G, Alvarez DF, Al-Mehdi AB, Alexeyev M, Stevens T. Critical role for lactate dehydrogenase A in aerobic glycolysis that sustains pulmonary microvascular endothelial cell proliferation. Am J Physiol Lung Cell Mol Physiol. 2010;299(4):L513-L522. doi:10.1152/ajplung.00274.2009

[3] Tang X, Luo YX, Chen HZ, Liu DP. Mitochondria, endothelial cell function, and vascular diseases. Front Physiol. 2014;5:175. Published 2014 May 6. doi:10.3389/fphys.2014.00175

[4] Kalucka J, Missiaen R, Georgiadou M, et al. Metabolic control of the cell cycle. *Cell Cycle*. 2015;14(21):3379-3388. doi:10.1080/15384101.2015.1090068

[5] Seckl JR, Walker BR. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. Endocrinology. 2001;142(4):1371-1376. doi:10.1210/endo.142.4.8114

[6] Chapman K, Holmes M, Seckl J. 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev. 2013;93(3):1139-1206. doi:10.1152/physrev.00020.2012

[7] Stimson RH, Walker BR. The role and regulation of 11β-hydroxysteroid dehydrogenase type 1 in obesity and the metabolic syndrome. Horm Mol Biol Clin Investig. 2013;15(2):37-48. doi:10.1515/hmbci-2013-0015

[8] Brossaud J, Bosch-Bouju C, Marissal-Arvy N, et al. Memory deficits in a juvenile rat model of type 1 diabetes are due to excess 11β-HSD1 activity, which is upregulated by high glucose concentrations rather than insulin deficiency. Diabetologia. 2023;66(9):1735-1747. doi:10.1007/s00125-023-05942-3

[9] Wyrwoll CS, Holmes MC, Seckl JR. 11β-hydroxysteroid dehydrogenases and the brain: from zero to hero, a decade of progress. Front Neuroendocrinol. 2011;32(3):265-286. doi:10.1016/j.yfrne.2010.12.001

[10] Boado RJ, Pardridge WM. Measurement of blood-brain barrier GLUT1 glucose transporter and actin mRNA by a quantitative polymerase chain reaction assay. J Neurochem. 1994;62(6):2085-2090. doi:10.1046/j.1471-4159.1994.62062085.x

[11] Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci. 2003;116(Pt 7):1175-1186. doi:10.1242/jcs.00384

[12] De Bock K, Georgiadou M, Schoors S, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. Cell. 2013;154(3):651-663. doi:10.1016/j.cell.2013.06.037

[13] Sotiropoulos I, Catania C, Pinto LG, et al. Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits. J Neurosci. 2011;31(21):7840-7847. doi:10.1523/JNEUROSCI.0730-11.2011