Aging Pathobiology and Therapeutics 2026; 8(3): 309-313 ***309***
DOI: 10.31491/APT.2026.09.235

Creative Commons 4.0

Development of an organotypic brain slice culture model for testing resilience to aging using tissues collected from geriatric mice

Addison Keely[a], Jackson Wezeman[a], Savanna Roberts[a], Sonali Bhana[a], Warren Ladiges[a, b,*]

[a] *Department of Comparative Medicine, School of Medicine, University of Washington, Seattle, WA, USA.*
[b] *Division of Gerontology and Geriatric Medicine, Department of Medicine, School of Medicine, University of Washington, Seattle, WA, USA.*

Abstract

Ex vivo organ slice cultures are attractive models to address specific biomedical research questions because they retain complex and dynamic three-dimensional architecture. In addition, they require only a few animals as tissue donors thus greatly decreasing the number needed for live animal studies. The model has been most successful using tissues from very young mice, but has generally not been considered for aging research because of the challenge of maintaining viability of tissues collected from older mice. The brain is a high-impact organ in aging and age-related disease research, so it was of interest to investigate the viability and related molecular and cellular characteristics of brain slice cultures (BSCs) derived from old mice. Coronal sections of 200 µm thickness were collected from the middle third of the brain of 21-month-old C57BL/6JN mice, cultured in a standard media concoction for seven days, exposed to chemical stressors glucose, hydrogen peroxide, lipopolysaccharide, or sodium hypochlorite for 24 to 48 hours, and then rinsed and cultured for another seven days. Results in non-treated BSCs showed cellular viability of greater than 80 percent with specific aging pathways relatively unaffected. Chemical stressors selectively targeted pathways of aging including DNA damage, autophagy, and inflammation. These preliminary observations suggest that BSCs derived from geriatric mice have potential for research in resilience to brain aging and age-related neurodegenerative conditions, and could serve as a prototype for developing organ slice cultures for other organs collected from old mice.

Keywords: Brain slice cultures, *ex vivo* model of brain aging, pathways of aging, chemical stressors, resilience to aging, geriatric mice

Introduction

Organ slice cultures are a promising yet under-utilized and under-appreciated model for research on aging. This *ex vivo* model consists of very thin slices of tissue that continue to function in a cell culture venue, and has potential for testing response to chemical stressors and gerotherapeutic drugs with minimal animals needed (as tissue donors), with low research costs compared to live animal studies. Organ slice cultures retain the complex and dynamic three-dimensional architecture allowing cellular interactions to occur [1]. These advantages provide the opportunity to model organ-specific characteristics of aging at the cellular and molecular level.

Of particular interest for neurodegenerative conditions is the ability to study the role of brain aging using a brain slice culture (BSC) model. Brain slice culturing of neonatal brain tissue has been used in numerous studies [2, 3] but there is concern that neonatal tissue may not typify brain aging observed in older mice. Therefore, BSCs derived from geriatric mice were tested to see if molecules involved in pathways of aging [4] were expressed, and if response to chemical stressors involved aging pathways as an ex vivo model to study resilience to aging, defined as the ability to recover from physical stressors [5].

* Corresponding author: Warren Ladiges
Mailing address: Department of Comparative Medicine, Division of Gerontology and Geriatric Medicine, Department of Medicine, School of Medicine, University of Washington, Seattle, WA, USA.
Email: wladiges@uw.edu

Received: 08 July 2026 / Revised: 03 August 2026
Accepted:07 September 2026 / Published:30 September 2026

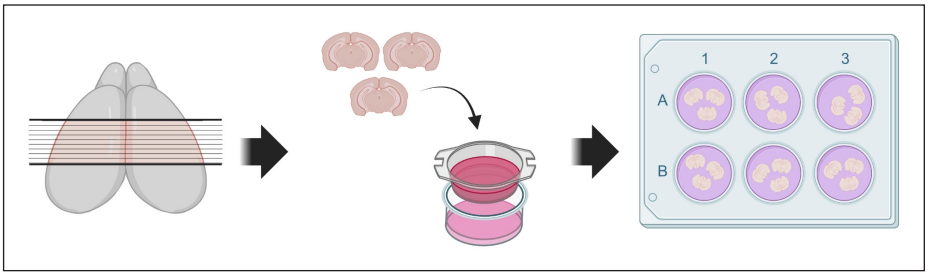


Figure 1. Prepared mouse brain was sliced into 200 µm thick coronal sections with a Vibratome. Collection of 21 total slices (4.2 mm rostral length) began after 3 mm of brain tissue had been removed. Sections were placed on the semi-permeable membrane of a removable cell culture insert in sequential wells of a 6-well plate with 1 mL of slice medium.

Methods

One C57BL/6JN mouse was obtained from the National Institute on Aging's Aged Rodent Colony and housed in the mouse vivarium at the University of Washington in Seattle, WA. Animal use was approved by the University of Washington IACUC. The mouse was euthanized at 21 months of age using cervical dislocation as the primary method. The brain was extracted onto a cold plate and the cerebellum and brainstem were removed. The remaining brain was immediately transferred to ice-cold 1× PBS (Gibco), then adhered to a vibratome plate with a drop of superglue and supported by sterile 1% agarose solution for cutting. Coronal slices of 200 µm were cut using a Vibratome 1500 Sectioning System. Collection of slices began after the first 3 mm of brain tissue were removed starting at the olfactory lobe, approximately where the hippocampus starts (Figure 1). Subsequent 200 µm slices were assigned to alternating treatment groups for representative tissue from the hippocampus in each treatment group. A total of 21 coronal slices were collected from one mouse over a 4.2 mm rostral length of brain. Three slices were placed in formalin to serve as control day 0. All remaining slices were placed on a 0.4 µm pore Nunc™ Polycarbonate cell culture insert in a 6-well plate (Thermo Scientific™) containing 1 mL of culture medium (50% MEM/HEPES (Gibco), 25% heat-inactivated horse serum (Gibco/Lifetech, Austria), 25% Hanks' solution (Gibco), 2 mM $NaHCO_3$ (Merck, Austria), 6.5 mg/mL glucose (Merck, Germany), 2 mM glutamine (Merck, Germany), pH 7.2, and 1% penicillin/streptomycin (Thermo Scientific)).

Brain slices were incubated at 37 °C and culture medium was changed every 2 to 3 days. One slice well was reserved as an untreated control, labeled control day 14. After seven days in culture, chemical stressors were added to all remaining wells: hydrogen peroxide (Thermo Scientific Cat. No: 426000010) at 150 µM for 48 hours [6], glucose (Fisher Chemical D-Glucose Anhydrous D16-3) at 25 mM for 48 hours [7], lipopolysaccharide (Sigma-Aldrich L4391) at 50 ng/mL for 48 hours (unpublished lab data), and sodium hypochlorite (NaOCl; Clorox) at 5 µM for 24 hours [8]. Media with chemical stressors were then removed and wells rinsed twice with 1× PBS before adding regular media and resuming culture conditions for the remaining seven days.

After 14 days, brain slices were transferred to cassettes and fixed in 10% neutral buffered formalin for 24 hours before being transferred to PBS for storage. Slices were embedded in type 6 paraffin wax (Fisher Scientific) according to standard embedding procedures. Histology slides were created from blocks using a microtome at 4 µm thickness on a charged glass slide.

Cresyl violet staining (Abcam ab246817) was used to visualize surviving neurons in the slices, based on stained Nissl substances in the endoplasmic reticulum. Staining was performed by rehydrating the slides in a 70 minute stepwise series as in immunohistochemistry (IHC) staining, then incubating slides in 1% cresyl violet stain solution for 5 minutes followed by dehydration in three washes of 100% ethanol then 10 minutes in xylene before adhering coverslips to slides with toluene permount. Slides were allowed to dry for 24 hours before imaging and analysis.

IHC was performed using kits from Abcam (HRP/DAB Rabbit Kit: ab64261, HRP/DAB Mouse Kit: ab64259) according to manufacturer protocols. Slides were rehydrated in a stepwise series running from xylene, 100% ethanol, 95% ethanol, 70% ethanol, and deionized water for a total running time of 70 minutes. Slides were then placed in a hot water bath with a citrate buffer antigen retrieval (pH 6.0, Sigma-Aldrich: C9999) for 20 minutes. Slides were allowed to cool for 10 minutes, and then rinsed in the wash buffer (1× TBST; Tris-buffered saline with Tween20) before a hydrogen peroxide buffer block was applied to

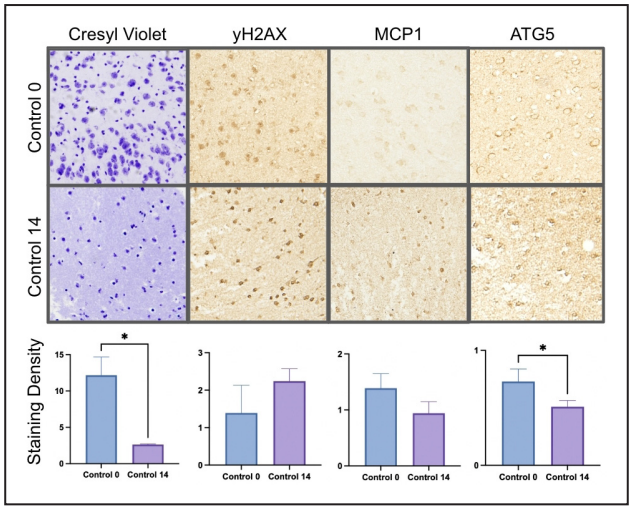


Figure 2. Digital images at 200× magnification showed identification of molecules representing pathways of aging in control day 0 slices compared to control day 14 slices. Cresyl violet staining was significantly lower in cultured BSCs ($P < 0.05$). There was no significant difference in γH2AX staining between groups ($P = 0.1$). MCP1 staining was slightly lower in Control 14 slices ($P = 0.07$). ATG5 staining was significantly higher in control day 0 slices ($P < 0.05$). $n = 3$ for each group.

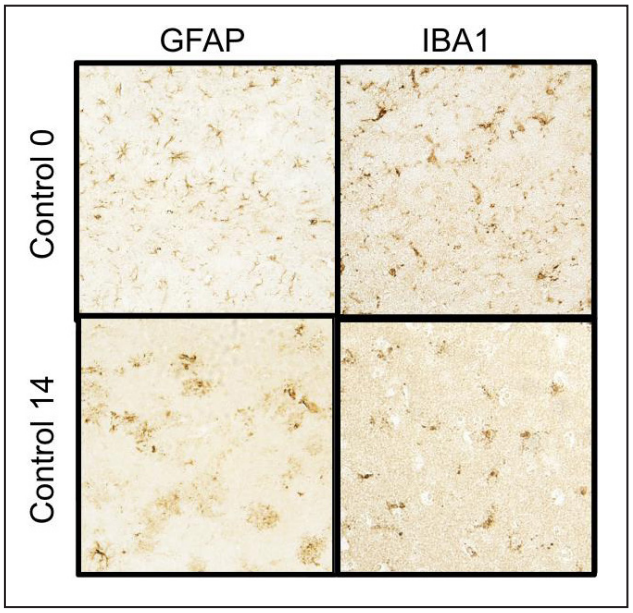


Figure 3. IHC on untreated control BSCs after 14 days in culture shows presence of astrocytes and microglia at 400× magnification.

the tissue. Slides were washed in the wash buffer for 10 minutes. A protein block was applied that reduces non-specific binding of the target antibody, and slides were then washed for 10 minutes as previously described. Antibody was diluted in the wash buffer according to recommended dilution (phosphorylated histone H2AX [γH2AX] ThermoFisher 10856-1-AP at 1:200 and CCL2/monocyte chemoattractant protein-1 [MCP1] Novus NBP1-07035 at 1:800, autophagy-related protein 5 [ATG5] 1:200, Novus NB110-53818, glial fibrillary acidic protein [GFAP] 1:1500 Invitrogen PA1-10019, ionized calcium-binding adaptor molecule 1 [IBA1] 1:1000, Abcam ab178846). Slides were incubated with the diluted antibody overnight in a 4 °C fridge. On the second day, the diluted antibody was washed, then slides incubated with biotinylated goat anti-mouse or anti-rabbit followed by streptavidin peroxidase, with washes after each. 3,3′-diaminobenzidine (DAB) was then applied in a fume hood for 2 minutes per slide. Slides were then washed with the wash buffer then deionized water for 15 minutes, then dehydrated in a reverse-series for 5 minutes per solution going from 70% ethanol, 95% ethanol, 100% ethanol, and finally xylene. Coverslips were adhered to the slides using toluene permount (Fisher). Slides were air dried for 24 hours before imaging.

Images were taken of all stained tissue at 20× with a Nikon DS-Fi3 camera on a Nikon Eclipse Ci microscope using NIS Elements software. Images were analyzed using ImageJ in conjunction with QuPath (version 0.3.2) for annotation, and the percentage of positively stained area in the tissue sections was determined. Unpaired two-tailed *t*-tests were performed to analyze the differences between means of control and treated groups. Shapiro-Wilk tests and Q–Q plots indicated no evidence of non-normality in the data sets. All data analysis was performed with GraphPad Prism version 10.4.2.

Results and discussion

Preliminary experiments showed cell viability greater than 90% after 14 days in culture using Trypsin-EDTA (Thermo Scientific) for tissue dissociation and Trypan blue (Thermo Scientific) stain for cell viability. Viability counts were performed using Accuris QuadCount™ automated cell counter. In addition, representative brain slices at 14 days were incubated with Trypan blue for three minutes then rinsed with PBS. Visual counts at 3× magnification showed cell survival at 80–90%. For neuron-specific assessment of viability, cresyl violet staining was used to show that neuronal density decreased by 23 percent indicating some neuronal degeneration and death, but the majority of neurons were still viable after 14 days in culture (Figure 2).

To see if any changes in expression of molecular markers of aging pathways occurred during the 14 day incubation period, IHC and digital imaging were carried out using antibodies optimized for mouse brain tissue. No significant changes occurred for γH2AX (Figure 2) suggesting culture techniques did not cause a significant amount of DNA damage. H2AX is phosphorylated to γH2AX, which triggers a double-stranded DNA damage repair response. Likewise, there was no significant difference in staining

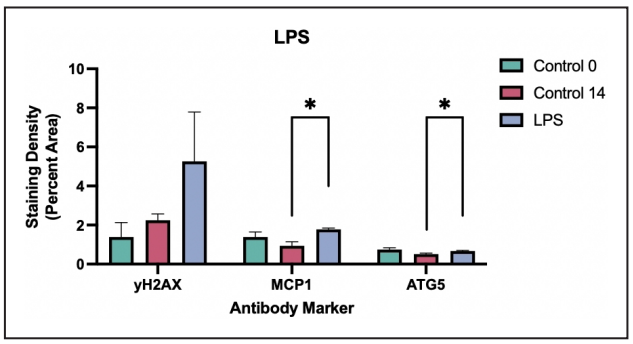


Figure 4. Brain slice cultures treated with LPS had significantly higher inflammation ($P < 0.05$) visualized through MCP1 staining, and autophagy ($P < 0.05$) visualized through ATG5 staining. Differences in DNA damage between LPS and control BSCs were not significant ($P = 0.07$).

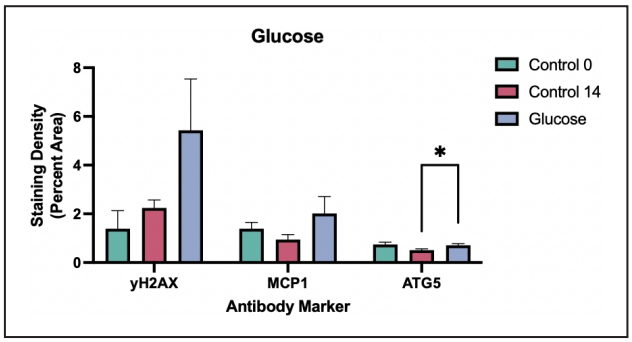


Figure 5. BSCs treated with high glucose had insignificantly higher averages in γH2AX staining for double-stranded DNA damage ($P = 0.06$) and MCP1 staining for inflammation ($P = 0.06$). ATG5 staining for autophagy was significantly higher in high glucose-treated BSCs ($P < 0.05$).

ANT PUBLISHING CORPORATION | All Rights Reserved

intensity for MCP1, a chemokine used as a biomarker for inflammation. However, IHC staining of ATG5, a protein involved in autophagy, showed a decrease in staining intensity in brain slices cultured for 14 days compared to non-cultured brain slices (day 0) suggesting culture conditions had some effect on the autophagy pathway.

It was also of interest to see if recognizable glial cells were present within the brain slices. IHC showed the presence of astrocytes and microglia at day 0 and after 14 days of culture (Figure 3).

The role of lipopolysaccharide (LPS) in inflammation pathways explains the increased staining intensity of MCP1 in treated BSCs (Figure 4). LPS is an endotoxin that triggers an immune response through toll-like receptor 4 (TLR4) [9], and is an established *in vitro* model of neuroinflammation. LPS also increased staining intensity of ATG5. Autophagy can protect the cell in response to stress [10] and is upregulated during inflammation by a variety of signals [11]. The observations raise the question of why increased autophagy was not sufficient to mitigate the LPS-induced increase in MCP1 expression, as both markers were significantly elevated in the treated group versus the day 14 control.

BSCs treated with high glucose levels showed a significant increase in ATG5 staining intensity but no differences in staining intensities for γH2AX or MCP1 (Figure 5). The relationship between high glucose levels and autophagy is complex with multiple involved pathways. Autophagy is upregulated in starvation conditions and suppressed during nutrient abundance to maintain energy homeostasis [10]. The shift from high glucose levels to normal glucose levels, as performed in this study, has previously been shown to induce a state of metabolic stress in microglia, leading to an increase in autophagy [12]. High glucose levels are expected to increase inflammation [13] and DNA breaks [14]. There was a trend for higher average staining of MCP1 and γH2AX though the results were not statistically significant. The glucose concentration used (25 mM) was chosen to optimize cellular stress and minimize cell death, and may have been insufficient to trigger these pathways.

BSCs treated with hydrogen peroxide showed an increased need for DNA repair (Figure 6). Phosphorylated H2AX (γH2AX) has a key role in the DNA repair process [15]. Hydrogen peroxide has been well established as a form of stress in *in vitro* studies to increase reactive oxygen species (ROS) presence [16]. Increased ROS levels in the brain cause oxidative stress and neuronal degeneration. The lack of significant differences in MCP1 and ATG5 suggests that the oxidative damage induced by hydrogen peroxide treatment was not severe enough to trigger significant increases in either pathway.

Treatment with NaOCl triggered a DNA damage response in BSCs, shown by increased levels of γH2AX (Figure 7), a marker for DNA repair signaling. NaOCl, widely used for its antimicrobial effects, works through oxidation, damaging proteins and other molecules in the cell [17]. The interaction between hypochlorite and DNA bases as well as the denaturation of secondary structure causes

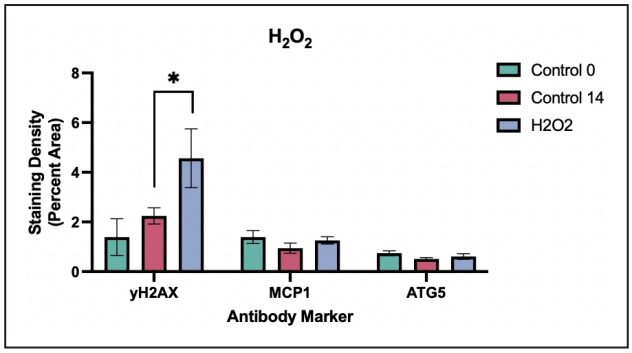


Figure 6. Mouse BSCs treated with hydrogen peroxide had significantly higher staining intensities of γH2AX ($P < 0.05$). Differences in MCP1 and ATG5 staining were not significant ($P = 0.1$ to $P = 0.2$).

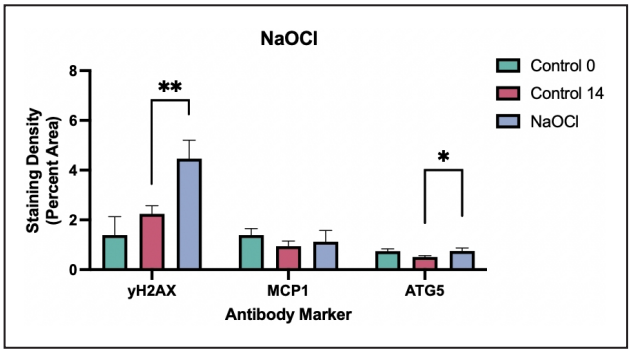


Figure 7. NaOCl-treated brain BSCs had significantly higher levels of γH2AX ($P < 0.01$) and ATG5 staining for autophagy ($P < 0.05$) compared to day 14 controls. The differences in inflammation, visualized through MCP1 staining, were not significant ($P = 0.5$).

DNA damage [18]. The dose and timing were chosen as a shorter time period compared to the other stressors due to the lack of cytotoxicity of NaOCl at the given dose for 24 hours [8]. Staining density was not directly compared between treatment groups in this study, making the time discrepancy statistically irrelevant. The NaOCl dose was low enough to preserve cell functions, as the γH2AX marker was significantly increased in treated BSCs compared to the day 14 controls. This suggests that DNA damage response pathways remained functional and were activated in response to DNA damage in the BSCs. NaOCl treatment also led to increased expression of ATG5. MCP1 was not affected.

Conclusions

In conclusion, preliminary evidence suggests that BSCs derived from old mice can retain viability for 14 days under enriched culture conditions. Culture conditions in general supported the ability of cells to express molecules involved in pathways of aging. The BSC model was able to respond to chemical stressors by triggering selected pathways of aging. Observations from this study suggest BSCs derived from geriatric mice have potential for research into brain aging and age-related neurodegenerative conditions, as well as serving as a model to test physi-

cal/chemical stressors for predicting resilience to aging. Resilience is observed in this model as a rapid return to baseline over the recovery period. In addition, BSCs are a prototype for developing organ slice cultures for other organs collected from older-aged mice.

Declarations

Author contributions: Made substantial contributions to conception and design of the study, generated the data, and prepared the manuscript: Keely A; performed data acquisition: Wezeman J, Roberts S, Bhana S; supervised the study, critically revised the manuscript, and provided administrative, technical, and material support: Ladiges W. All authors read and approved the final version of the manuscript.

Availability of data and materials: Not applicable.

Financial support and sponsorship: This study was supported by NIH grant R01 AG067193 (W Ladiges, PI). All authors acknowledge no competing interests.

Conflicts of interest: Warren Ladiges is a member of the editorial board of *Aging Pathobiology and Therapeutics*. The authors declare that they have no conflicts and were not involved in the journal's review or decision regarding this manuscript.

Ethical approval and informed consent: All animal procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Washington.

Consent for publication: Not applicable.

AI and AI-assisted tools statement: No AI tools were used in the preparation of this article.

References

1. Majorova D, Atkins E, Martineau H, Vokral I, Oosterhuis D, Olinga P, *et al*. Use of precision-cut tissue slices as a translational model to study host-pathogen interaction. *Front Vet Sci*, 2021, 8: 686088. [Crossref]
2. Gähwiler BH, Capogna M, Debanne D, McKinney RA, & Thompson SM. Organotypic slice cultures: a technique has come of age. *Trends Neurosci*, 1997, 20(10): 471-477. [Crossref]
3. Staal JA, Alexander SR, Liu Y, Dickson TD, & Vickers JC. Characterization of cortical neuronal and glial alterations during culture of organotypic whole brain slices from neonatal and mature mice. *PLoS One*, 2011, 6(7): e22040. [Crossref]
4. López-Otín C, Blasco M, Partridge L, Serrano M, & Kroemer G. Hallmarks of aging: an expanding universe. *Cell*, 2023, 186(2): 243-278. [Crossref]
5. Kirkland JL, Stout M, & Sierra F. Resilience in aging mice. *J Gerontol A Biol Sci Med Sci*, 2016, 71(11): 1407-1414. [Crossref]
6. Xin X, Gong T, & Hong Y. Hydrogen peroxide initiates oxidative stress and proteomic alterations in meningothelial cells. *Sci Rep*, 2022, 12(1): 14519. [Crossref]
7. Shi H, & Liu KJ. Effects of glucose concentration on redox status in rat primary cortical neurons under hypoxia. *Neurosci Lett*, 2006, 410(1): 57-61. [Crossref]
8. Hidalgo E, Bartolome R, & Dominguez C. Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. *Chem Biol Interact*, 2002, 139(3): 265-282. [Crossref]
9. Farhana A, & Khan YS. Biochemistry, lipopolysaccharide. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554414/
10. Levine B, Mizushima N, & Virgin H. Autophagy in immunity and inflammation. *Nature*, 2011, 469(7330): 323-335. [Crossref]
11. Messer JS. The cellular autophagy/apoptosis checkpoint during inflammation. *Cell Mol Life Sci*, 2017, 74(7): 1281-1296. [Crossref]
12. Hsieh CF, Liu C, Lee C, Yu L, & Wang J. Acute glucose fluctuation impacts microglial activity, leading to inflammatory activation or self-degradation. *Sci Rep*, 2019, 9(1): 840. [Crossref]
13. Nagai K, Fukushima T, Oike H, & Kobori M. High glucose increases the expression of proinflammatory cytokines and secretion of TNFα and β-hexosaminidase in human mast cells. *Eur J Pharmacol*, 2012, 687(1-3): 39-45. [Crossref]
14. Rahmoon MA, Elghaish R, Ibrahim A, Alaswad Z, Gad M, El-Khamisy S, *et al*. High glucose increases DNA damage and elevates the expression of multiple DDR genes. *Genes*, 2023, 14(1): 144. [Crossref]
15. Paull TT, Rogakou E, Yamazaki V, Kirchgessner C, Gellert M, & Bonner W. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol*, 2000, 10(15): 886-895. [Crossref]
16. Salim S. Oxidative stress and the central nervous system. *J Pharmacol Exp Ther*, 2017, 360(1): 201-205. [Crossref]
17. Winter J, Ilbert M, Graf P, Ozcelik D, & Jakob U. Bleach activates a redox-regulated chaperone by oxidative protein unfolding. *Cell*, 2008, 135(4): 691-701. [Crossref]
18. Osinnikova D, Moroshkina E, & Mokronosova E. Effect of sodium hypochlorite on nucleic acids of different primary and secondary structures. *J Phys Conf Ser*, 2019, 1400(3): 033001. [Crossref]

Cite this article as: Keely A, Wezeman J, Roberts S, Bhana S, & Ladiges W. Development of an organotypic brain slice culture model for testing resilience to aging using tissues collected from geriatric mice. *Aging Pathobiol Ther*, 2026, 8(3): 309-313. doi: 10.31491/APT.2026.09.235

ANT PUBLISHING CORPORATION All Rights Reserved