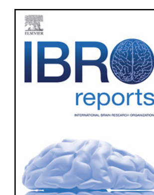
IBRO Reports 5 (2018) 110–115

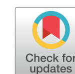
Contents lists available at ScienceDirect

IBRO Reports

journal homepage: www.elsevier.com/locate/ibror



Mild heat stress induces hormetic effects in protecting the primary culture of mouse prefrontal cerebrocortical neurons from neuropathological alterations



Narayan R. Mane[a], Kavita A. Gajare[b], Ashish A. Deshmukh[a,*]

[a] Cellular Stress Response Laboratory, Cell Biology Division, Department of Zoology, Shivaji University, Kolhapur, Maharashtra, 416 004, India
[b] Department of Zoology, The New College Kolhapur, Maharashtra, 416 012, India

ARTICLE INFO

Keywords:
Hormesis
Mild heat stress
Neurofibrillary tangles
Senile plaques
Lipofuscin granules
Nissl substance

ABSTRACT

Hormesis is a dose response phenomenon of cells and organisms to various types of stressors. Mild stress stimulates prosurvival pathways and makes the cells adaptive to stressful conditions. It is a widely used fundamental dose-response phenomenon in many biomedical and toxicological sciences, radiation biology, health science etc. Mild heat stress is an easily applicable hormetic agent that exerts consistent results. In the present investigations mouse cerebrocortical prefrontal neurons from E17 mouse embryos were grown in the laboratory on poly-L-lysine coated glass cover slips. The cells from the mild heat stressed group were subjected to a hyperthermic stress of 38 °C for 30 min every alternate day (i.e. mild heat stress was repeated after 48 h) up to the sixth day. After completion of twenty four hours of the final i.e. third exposure of the mild heat stress, the neurons were fixed for the cytochemical studies of neurofibrillary tangles, senile plaques, lipofuscin granules and Nissl substance. There was highly significant decrease in the neuropathological alterations (viz. deposition of Neurofibrillary tangles, deposition of senile plaques, accumulation of Lipofuscin granules) in the neurons from the mild heat stressed group as compared to control. Moreover, the Nissl substance was significantly preserved in the mild heat stressed group as compared to control. The results indicate that the applied mild heat stress (38 °C for 30 min) exerts beneficial effects on the prefrontal cerebrocortical neurons by slowing down the neuropathological alterations, suggesting the hormetic effect of the mild heat stress.

1. Introduction

Hormesis represents a phenomenon in which exposure to low dose of various stressors such as mild heat stress, toxicants, pesticides, radiation etc. induces adaptive responses in cells, tissues, organs and organisms (Calabrese and Baldwin, 2002). Organisms and their cellular components develop adaptive pathways to survive and reproduce in adverse and stressful conditions. These adaptive pathways are evolutionarily conserved and are stimulated by different stressors at a low dose. Many physiological, biological, chemical, as well as environmental factors are used as hormetic agents (Calabrese and Baldwin, 2002; Rattan, 2006). Exposure to mild stressors preconditions the cells and the organisms to tolerate and adapt to different stressful conditions of the higher intensity. This preconditioning is also a manifestation of hormesis. Cook and Calabrese (2006) believe that the concept of hormesis has immense benefits in healthcare sector. It will substantially contribute to improve the health of general public directly by creating the awareness and indirectly by using the concept of hormesis while framing the government policies related to healthcare sector. Various studies have reported that mild heat stress exerts beneficial effects on the neurons by improving the functional ability, neurite outgrowth, neuroprotection and neuronal survival in various neurodegenerative disorders such as Alzheimer's disease, Parkinson disease, Huntington's disease etc. (Snider and Choi, 1996; Mattson and Cheng, 2006; Umschweif et al., 2014). The hormetic effects can be studied as a preconditioning hormesis and a postconditioning hormesis (Calabrese et al., 2007; Calabrese, 2016a, 2016b). In the preconditioning hormesis, a prior exposure to an appropriate low dose of a toxic agent or stress (called as conditioning dose), reduces the adverse effects or the toxicity of a subsequent challenging dose of the same or different stressors (Calabrese et al., 2007; Calabrese, 2016a, 2016b). On the other hand, when the conditioning dose of a lower intensity is given after a challenging dose, the adverse effects of the challenging dose are reduced. This phenomenon is termed as postconditioning hormesis (Calabrese,

* Corresponding author.
E-mail address: aad_zoo@unishivaji.ac.in (A.A. Deshmukh).

https://doi.org/10.1016/j.ibror.2018.11.002
Received 22 September 2018; Accepted 4 November 2018
2451-8301/ © 2018 The Authors. Published by Elsevier Ltd on behalf of International Brain Research Organization. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

2016a, 2016b).

The neurons being terminally differentiated cannot rejuvenate by cell division. As a result, over a period of time, neurons exhibit degenerative alterations. These neuropathological alterations are 1. formation of neurofibrillary tangles that affects axonal transport, 2. deposition of senile plaques that interferes with the propagation of action potential, 3. accumulation of lipofuscin granules that increases the oxidative stress, protein crosslinking, lysosomal dysfunction etc. leading to neuronal death, 4. loss of Nissl substance that causes decreased production of neurotransmitters and affects the synaptic plasticity (David et al., 2005; Müller et al., 2010; Kanaan et al., 2011; DuBoff et al., 2012; Mondragón-Rodríguez et al., 2013; Revett et al., 2013; Cheng and Bai, 2018).

Hyperphosphorylation of microtubule-associated protein Tau by various kinases contributes to the formation of neurofibrillary tangles that affects axonal transport (Götz et al., 1995; Avila et al., 2012; Taylor et al., 2018). Senile plaques are extracellular depositions of amyloid beta (Aβ) peptides (Cras et al., 1991). These senile plaques are formed due to abnormal cleavage of amyloid precursor protein present in the cell membrane by α, β and γ secretases (Vassar et al., 1999; De Strooper and Annaert, 2000). Accumulation of Aβ peptides in the brain causes mitochondrial dysfunction, increased oxidative stress, abnormal neuroinflammatory responses, decreased neuroplasticity and increased tau phosphorylation that finally leads to neuronal death (Hsia et al., 1999; Walsh et al., 2002; Reddy and Beal, 2008).

Lipofuscin granules are brown-yellow, electron dense, autofluorescent granules that accumulate in the post-mitotic cells (Björkerud, 1964; Samorajski et al., 1965; Goyal, 1982; Mazzitello et al., 2009). The age related accumulation of lipofuscin granules leads to neurodegenerative diseases particularly caused by lysosomal dysfunction (Kiselyov et al., 2007). Nissl substance is present in the cyton. It comprises of ribonucleoprotein particles that gets typically decreased with aging. Loss of Nissl substance reduces the functional efficiency and synaptic plasticity of the neurons (Scarborough, 1938). The present investigation was designed to evaluate the effect of mild heat stress on these neuropathological alterations in the primary culture of mouse prefrontal cerebrocortical neurons.

2. Material and methods

2.1. Primary culture of prefrontal cerebrocortical neurons from E17 mouse embryos

The prefrontal cerebrocortical neurons from the brain of E17 mouse embryos were cultured on poly-L-lysine coated glass coverslips. All the experimental procedures were carried out with due permission of Institutional Animal Ethics Committee (1825/PO/EReBi/S/15/CPCSEA). The cerebral cortex was dissected from 17 day embryonic brain in chilled PBS (pH 7.4) containing 40 μg/ml gentamycin. The meninges were removed, the prefrontal cortex was separated from the rest of the cerebral cortex and transferred to a falcon tube containing Ca-Mg free Hank's Balanced Salt Solution (HBSS)(Himedia TL1108). The prefrontal cortices were finely minced and enzymatically dissociated using 0.25% trypsin (SAFC Bioscience SLBC9215) for 10 min at 37 °C. Trypsin activity was inhibited by adding Soyabean trypsin *inhibitor (Himedia TCL068)*. Neurons were dissociated by trituration using fire polished pipettes and the cell suspension was used for the study of viability by trypan blue dye exclusion method. 50,000 live cells/coverslip were seeded on the Poly-L-lysine (Sigma P1399) coated glass coverslips (18 mm). Four coverslips were placed in a 60 mm cell culture dish. The cells were incubated at 5% CO_2 and 37 °C for 30 min in the CO_2 incubator (New Brunswick an Eppendorf company, Galaxy 48R, 41,823) for adhesion. The cells were fed with neurobasal medium (Gibco, 15630-106) supplemented with 0.2% B-27 (Gibco, 17504-044), 40 μg/ml Gentamycin (Abbott), 25 mM HEPES (Himedia, TL1108) and 1% Glutamax (Gibco35050-061). The culture was maintained at 37 °C and 5% CO_2 in the CO_2 incubator. 2/3rd of the old culture medium was replaced by fresh medium on every third day.

2.2. Exposure to mild heat stress

The neurons were divided into two groups; a control group and the mild heat stressed group. In the control group, the neurons were incubated at 37 °C without any heat stress. For deciding the dose of mild heat stress i.e. temperature and exposure time, the neurons were subjected to varied temperatures from 37.5, to 40 °C at an increment of 0.5 °C. These trials were carried out for 30 min and 60 min duration of incubation. The results were biphasic (i.e. Non-linear) and the exposure to 38 °C for 30 min exerted beneficial effects in terms of cytoarchitectural alternations and hence this dose was used as an optimal dose. The neurons from the experimental group were subjected to hyperthermic stress of 38 °C for 30 min on 2nd, 4th and 6th day of seeding the cells.

2.3. Fixation of cells for the cytochemical staining

After completion of 24 h of the third mild heat stress, the cells from both the groups were fixed in 4% paraformaldehyde containing 4% sucrose, washed three times in chilled PBS. The cells were permeabilized by treating with 0.02% Triton X-100 for 15 min, washed three times in chilled PBS. These cells were further used for various cytochemical staining procedures.

2.4. Cytochemical staining of the neurofibrillary tangles

Neurofibrillary tangles were stained by Silver staining method described by Bielschowsky and Cobb's (1925). The fixed neurons were treated with pre-warmed 10% silver nitrate for 30 min (40 °C), washed three times with distilled water and treated with freshly prepared 10% ammonium silver nitrate solution for 30 min at 40 °C. Afterwards, the neurons were treated with the developer solution for one minute. Subsequently, the neurons were treated with 5% sodium thiosulphate solution and washed three times with distilled water, dehydrated in ascending grades of ethanol, cleared in xylene and mounted in DPX. The cells showing the neurofibrillary tangles were counted at 400X magnification.

2.5. Cytochemical staining of amyloid beta (senile plaque)

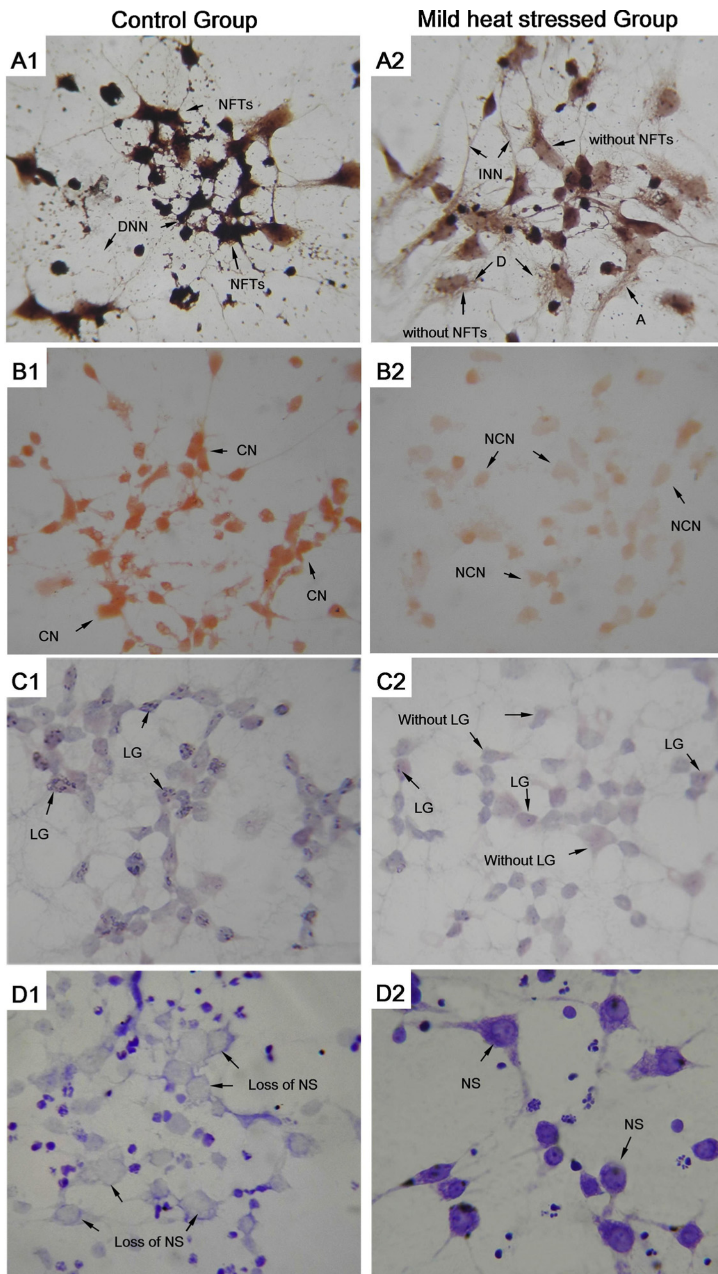
Staining of the senile plaque was carried out using Highman's Congo red method described by Puchtler et al. (1962). The fixed neurons were treated with 80% ethanol saturated sodium chloride for 30 min and stained by 0.5% Congo red for 30 min. The neurons were rapidly dehydrated in ascending grades of ethanol, cleared in xylene and mounted in DPX. The Congophilic neurons were counted at 400X magnification.

2.6. Cytochemical staining of lipofuscin granules

The lipofuscin granules were stained using Ziehl Neelson carbol fuchsin method as described by Troyer (1980). After fixation, the neurons were stained by 1% carbol fuchsin for 3 h at 60 °C, rinsed with distilled water and differentiated in acid alcohol (1% HCl in 70% ethanol) until excess stain was washed off. Subsequently, the neurons were counterstained with 0.5% toluidine blue for one min., washed in distilled water, dehydrated in ascending grades of ethanol, cleared in xylene and mounted in DPX. The neurons containing lipofuscin granules were counted and the numbers of lipofuscin granules per neuron were counted at 1000X magnification.

2.7. Cytochemical staining of Nissl substance

Nissl substance was stained by Nissl staining method of Powers and



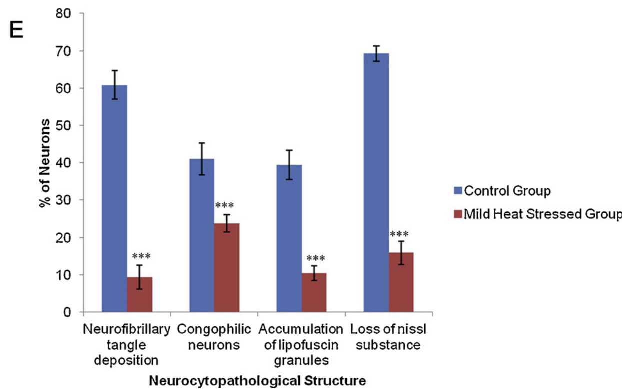


Fig. 1. Photomicrograph (×1000) of the neurocytopathological alternations in the control group and the mild heat stressed group. **A1 and A2:** Silver nitrate staining of Neurofibrillary tangles: more brown and black coloured deposition of neurofibrillary tangles and damaged neuronal network in the control group as compared to the mild heat stressed group. **B1 and B2:** Congo red staining of amyloid β; intense orange coloured staining of neurons (Congophilic neurons) in the control group, whereas in the mild heat stressed group neurons show less intense staining (Non-Congophilic neurons). **C1 and C2:** Ziehl Neelson carbol fuchsin staining of lipofuscin granules; high deposition of lipofuscin granules in the neurons from the control group (arrow in C1), whereas, the neurons from the mild heat stressed group are with less accumulation of lipofuscin granules (arrow in C2). **D1 and D2:** Cresyl violet acetate staining of Nissl substance; the neurons from the control group show weak violet coloured staining, whereas, the neurons from the mild heat stressed group are darkly stained. NFTS: Neurofibrillary tangles; DNN: Damaged neuronal network; INN: Intact neuronal network; CN: Congophilic neurons; NCN: Non-Congophilic neurons; LG: Lipofuscin granules; NS: Nissl substance. **E:** Graph depicts quantification of neurocytopathological alternations from the control group and the mild heat stressed group. The results were analyzed using Student's t-test. Data is represented as arithmetic mean ± SD *** $p < 0.001$ versus control.

Clark (1955). The fixed neurons were stained with 0.1% Cresyl violet acetate solution for 15 min, washed three times and rapidly dehydrated in ascending grades of ethanol, cleared in xylene and mounted in DPX. Nissl positive neurons were recorded at 400X magnification.

2.8. Statistical analysis

The observed data from the control group and the mild heat stressed group were expressed as arithmetic mean ± standard deviation and

the level of significance was calculated using unpaired Student's t-test.

3. Results

The results of the cytochemical studies of the neurons from the control group and the mild heat stressed group (age six days *in vitro*) are shown in Fig. 1.

60.83 ± 3.88% neurons from the control group exhibited deposition of NFTs, whereas, in the mild heat stress group 9.38 ± 3.16% neurons were showing NFTs deposition. In the control group, 41.0 ± 4.22% neurons exhibited Congophilic (beta-amyloid) material, whereas, in the mild heat stress group it was 23.72 ± 2.34%.

39.44 ± 3.98% neurons from the control group revealed deposition of lipofuscin granules, whereas, in the mild heat stress group 10.34 ± 1.96% neurons showed lipofuscin deposition. 69.23 ± 2.04% neurons from the control group exhibited loss of Nissl substance, whereas, in the mild heat stress group 15.88 ± 3.14% neurons showed loss of Nissl substance. In the mild heat stress group, neuropathological structures like NFTs, Congophilic neurons, lipofuscin granules were significantly ($p < 0.001$) decreased as compared to the control group and the Nissl substance was significantly ($p < 0.001$) preserved.

4. Discussion

In the present study, the neurons from the control group exhibited neuropathological alterations such as accumulation of neurofibrillary tangles, senile plaques, lipofuscin granules and the loss of Nissl substance. These alterations were significantly lower in the neurons from the mild heat stressed group. Hormesis is a biphasic dose-response phenomenon in which low dose of mild stressful condition exerts adaptive responses that elicits beneficial effects, whereas a higher dose of the stress causes deleterious changes and cell death (Calabrese and Baldwin, 2000). This was evidenced in the pilot trials during finalizing the exposure temperature and duration of exposure.

It is a well-known fact that the mild heat stress induces expression of many heat shock proteins (Kristensen et al., 2003; Banerjee Mustafi et al., 2009). These heat shock proteins act as molecular chaperones and prevent misfolding as well as aggregation of proteins thereby reducing the formation of neurofibrillary tangles and β-amyloid (Welch et al., 1993). Dou et al.(2003) demonstrated that increase in the expression of heat shock proteins causes increased solubility of tau and stable association of tau with microtubules. Therefore, in the present investigations significantly decreased deposition of NFTs in the heat stressed group could be because of expression of heat shock proteins as a response to mild heat stress given to these neurons on every alternate day.

Many *in vivo* and *in vitro* studies demonstrated that Congo red displays high affinity to amyloid peptide (Puchtler et al., 1962; Glenner et al., 1972). In the present study, there was a significant decrease in the Congophilic neurons in the mild heat stressed group as compared to the control group. This might be due to the expression of heat shock proteins. Muchowski et al. (2000) found that the Hsp70 with its co-chaperone Hsp40 inhibits aggregation of polyglutamine protein (polyQ) into insoluble amyloid like fibrils. Evans et al. (2006) demonstrated that the purified Hsp70 and Hsp40 when mixed with synthetic amyloid beta (1–42), inhibited the aggregation of synthetic amyloid beta (1–42) by binding to the amyloidogenic peptides. The present investigation demonstrates the effect of mild heat stress in reducing the aggregation of amyloid beta *in situ,* which might be due to the activation of heat shock responsive pathways thereby expressing the heat shock proteins.

Accumulation of lipofuscin granules within the neurons is the major indication of neuronal stress and aging related neuropathology (Dowson et al., 1995; Brunk and Terman, 2002). Lipofuscin granules contain polymorphic waste material including damaged mitochondria and worn out cell organelles accumulated in the lysosomes of postmitotic cells (Terman et al., 2007). In several studies, it was reported that accumulation of lipofuscin granules declines cellular and molecular functions of the cell including lysosomal degradation (Ivy et al., 1989), antioxidant defence (Beckman and Ames, 1998), and proteasomal activity (Sitte et al., 2000). Deposition of lipofuscin granules causes generation of intralysosomal reactive oxygen species (ROS) leading to oxidative cross-linking of lysosomal proteins (Brunk and Terman, 2002). In the present investigations there was a decline in the accumulation of lipofuscin granules in the neurons from the mild heat stressed group as compared control. This might be due to stimulation of degradative capacity of the lysosomes by the mild heat stress. The present results demonstrate that the mild heat stress improves the lysosomal efficiency of recycling and thus maintain the intracellular clean-up functions.

Nissl substance consists of ribonucleoprotein particles which are the prime sites of protein synthesis (Palay and Palade, 1955). In many physiological conditions and pathological disorders, Nissl substance disappears, known as chromatolysis (Nandy, 1968; Torvik, 1976; Wakayama, 1992). Martin (1999) reported that chromatolysis in the spinal motor neurons is the main neuropathological characteristic of Amyotrophic lateral sclerosis.

In the present study, neurons from the mild heat stress group exhibited intense staining with cresyl violet as compared to the control group. This indicates that mild heat stress might be involved in the preservation of Nissl substance. This might be due to the expression of molecular chaperones that take care of cells from incorrect folding of proteins. According to Verbeke et al. (2000), mild heat stress stimulates expression of heat shock proteins and other components of the stress response pathways.

Vogel et al.(1997) examined the effect of heat shock on primary rat neuronal culture. Moderate heat injury of 30 min exposure to 43 °C–44 °C resulted in delayed neurodegeneration. Snider and Choi (1996) investigated the effect of sublethal heat stress in murine cortical cell culture exposed to combined oxygen and glucose deprivation. In this study, the investigators found that a conditioning heat stress is able to attenuate both the excitotoxic and the apoptotic components of oxygen–glucose deprivation induced neuronal death *in vitro*. Rattan (1998) and Verbeke et al. (2000) observed that repeated mild heat stress prevents the onset of age-related alterations in human fibroblast undergoing aging *in vitro*. Rattan (2004) found that, exposure of serially passaged human fibroblast to 41 °C for 1 h twice a week throughout their replicative lifespan *in vitro* leads to beneficial antiaging effects such as maintenance of youthful morphology, increased antioxidant abilities, increased resistance to ethanol and H_2O_2. The present investigation suggests that the beneficial effects of mild heat stress on the primary culture of mouse prefrontal cortical neurons are due to the preservation of cytoarchitecture and reduction in the neurocytopathology.

According to Calabrease et al., (2007) preconditioning hormesis comprises of the administration of conditioning stimulation i.e. mild stress, which is followed by a challenging dose. In the present study, such challenging dose was not given. According to Calabrese et al. (2007); Van Wijk and Wiegant (2010); Calabrese (2013, 2016a, 2016b); in postconditioning hormesis, a challenging dose is given prior to the conditioning stimulation. In the present investigations, the control group was not subjected to any kind of mild heat stress, whereas, the neurons from the experimental group were subjected to repeated mild heat stress of 38 °C for 30 min at an interval of 48 h from the 2nd day up to the 6th day. Therefore, the study neither depicts the preconditioning hormesis nor the postconditioning hormesis. Repeated exposure to mild hyperthermic stress of 38 °C for 30 min might have stimulated the adaptive responses within the neurons.

The cytopathological alternations observed in the prefrontal cerebrocortical neuronal culture indicates gradual degenerative changes. Although, the seventh day of *in vitro* life is not an aging model in the true sense, however, the cytopathological alternations and their extent

suggest age related neurodegeneration. According to Rattan et al. (2004), the aging related changes are primarily the result of a failure of maintenance and repair mechanisms. Calabrese (2018) termed it as a background disease process. The exposure of neurons to repeated mild heat stress has preserved the cytoarchitecture of neurons to a healthy condition. This might be due to the stimulation of stress response pathways and strengthening the cellular adaptability i.e. homeodynamics (Verbeke et al., 2001; Rattan, 2014), overcompensation response (Calabrese and Baldwin, 2002) and according to Calabrese (2018) it may be reducing the background disease burden i.e. ‘subtraction to background’.

In conclusion, the present study demonstrates that the mild heat stress exerts beneficial effects to slow down the occurrence of cytopathological alterations in the mouse prefrontal cerebrocortical neurons and helps maintain the cytoarchitecture of these neurons to a healthier condition.

Conflict of interest

The authors declare no conflict of interest.

References

Avila, J., León-Espinosa, G., García, E., García-Escudero, V., Hernández, F., DeFelipe, J., 2012. Tau phosphorylation by GSK3 in different conditions. Int. J. Alzheimers Dis. 2012, 1–7.

Banerjee Mustafi, S., Chakraborty, P.K., Dey, R.S., Raha, S., 2009. Heat stress upregulates chaperone heat shock protein 70 and antioxidant manganese superoxide dismutase through reactive oxygen species (ROS), p38MAPK, and Akt. Cell Stress Chaperones 14, 579–589.

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Bielschowsky, M., Cobb, S., 1925. A method for intra-vital staining with silver ammonium oxide solution. Journal Fur Psychologie Und Neurologie 31, 301–304.

Björkerud, S., 1964. Studies of lipofuscin granules of human cardiac muscle: II. Chemical analysis of the isolated granules. Exp. Mol. Pathol. 3, 377–389.

Brunk, U.T., Terman, A., 2002. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. Free Radic. Biol. Med. 33, 611–619.

Calabrese, E.J., 2013. Biphasic dose responses in biology, toxicology and medicine: accounting for their generalizability and quantitative features. Environ Pollut 182, 452–460.

Calabrese, E.J., 2016a. Preconditioning is hormesis part I: documentation, dose-response features and mechanistic foundations. Pharmacol. Res. 110, 242–264.

Calabrese, E.J., 2016b. Preconditioning is hormesis part II: how the conditioning dose mediates protection: dose optimization within temporal and mechanistic frameworks. Pharmacol. Res. 110, 265–275.

Calabrese, E.J., 2018. Post-conditioning hormesis creates a “subtraction to background” disease process: biological, aging, and environmental risk assessment implications. J. Cell Commun. Signal. 12, 31–34.

Calabrese, E.J., Baldwin, L.A., 2000. The marginalization of hormesis. Hum. Exp. Toxicol. 19 (1), 32–40.

Calabrese, E.J., Baldwin, L.A., 2002. Defining hormesis. Hum. Exp. Toxicol. 21, 91–97.

Calabrese, E.J., et al., 2007. Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose–response framework. Toxicol. Appl. Pharmacol. 222, 122–128.

Cheng, Y., Bai, F., 2018. The association of tau with mitochondrial dysfunction in Alzheimer’s disease. Front. Neurosci. 12, 163.

Cook, R., Calabrese, E.J., 2006. The importance of hormesis to public health. Environ. Health Perspect. 114 (11), 1631–1635.

Cras, P., Kawai, M., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., Perry, G., 1991. Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. Proc Natl Acad Sci U S A 88, 7552–7556.

David, D.C., Hauptmann, S., Scherping, I., Schuessel, K., Keil, U., Rizzu, P., Ravid, R., Dröse, S., Brandt, U., Müller, W.E., Eckert, A., Götz, J., 2005. Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L tau transgenic mice. J. Biol. Chem. 280, 23802–23814.

De Strooper, B., Annaert, W., 2000. Proteolytic processing and cell biological functions of the amyloid precursor protein. J. Cell. Sci. 113 (Pt 11), 1857–1870.

Dou, F., Netzer, W.J., Tanemura, K., Li, F., Hartl, F.U., Takashima, A., Gouras, G.K., Greengard, P., Xu, H., 2003. Chaperones increase association of tau protein with microtubules. Proc. Natl. Acad. Sci. 100, 721–726.

Dowson, J.H., Mountjoy, C.Q., Cairns, M.R., 1995. Wilton-Cox H Alzheimer’s disease: distribution of changes in intraneuronal lipopigment in the frontal cortex. Dementia 6, 334–342.

DuBoff, B., Götz, J., Feany, M.B., 2012. Tau promotes neurodegeneration via DRP1 mislocalization in vivo. Neuron 75, 618–632.

Evans, C.G., Wisén, S., Gestwicki, J.E., 2006. Heat shock proteins 70 and 90 inhibit early stages of amyloid β-(1–42) aggregation *in vitro*. J. Biol. Chem. 281, 33182–33191.

Glenner, G.G., Page, D.L., Eanes, E.D., 1972. The relation of the properties of Congo red-stained amyloid fibrils to the β-conformation. J. Histochem. Cytochem. 20, 821–826.

Götz, J., Probst, A., Spillantini, M.G., Schäfer, T., Jakes, R., Bürki, K., Goedert, M., 1995. Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. EMBO J. 14, 1304–1313.

Goyal, V.K., 1982. Lipofuscin pigment accumulation in human brain during aging. Exp. Gerontol. 17, 481–487.

Hsia, A.Y., Masliah, E., McConlogue, L., Yu GQ, Tatsuno G., Hu, K., Kholodenko, D., Malenka, R.C., Nicoll, R.A., Mucke, L., 1999. Plaque-independent disruption of neural circuits in Alzheimer’s disease mouse models. Proc. Natl. Acad. Sci. U. S. A. 96, 3228–3233.

Ivy, G.O., Kanai, S., Ohta, M., Smith, G., Sato, Y., Kobayashi, M., Kitani, K., 1989. Lipofuscin-like substances accumulate rapidly in brain, retina and internal organs with cysteine protease inhibition. Adv. Exp. Med. Biol. 266, 31–45 discussion 45-7.

Kanaan, N.M., Morfini, G.A., LaPointe, N.E., Pigino, G.F., Patterson, K.R., Song, Y., Andreadis, A., Fu, Y., Brady, S.T., Binder, L.I., 2011. Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases. J. Neurosci. 31, 9858–9868.

Kiselyov, K., Jennigs, J.J., Rbaibi, Y., Chu, C.T., 2007. Autophagy, mitochondria and cell death in lysosomal storage diseases. Autophagy 3, 259–262.

Kristensen, T.N., Sørensen, J.G., Loeschcke, V., 2003. Mild heat stress at a young age in Drosophila melanogaster leads to increased Hsp70 synthesis after stress exposure later in life. J. Genet. 82, 89–94.

Martin, L.J., 1999. Neuronal death in amyotrophic lateral sclerosis is apoptosis: possible contribution of a programmed cell death mechanism. J. Neuropathol. Exp. Neurol. 58, 459–471.

Mattson, M.P., Cheng, A., 2006. Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses. Trends Neurosci. 29, 632–639.

Mazzitello, K.I., Arizmendi, C.M., Family, F., Grossniklaus, H.E., 2009. Formation and growth of lipofuscin in the retinal pigment epithelium cells. Phys. Rev. E 80, 051908.

Mondragón-Rodríguez, S., Perry, G., Zhu, X., Moreira, P.I., Acevedo-Aquino, M.C., Williams, S., 2013. Phosphorylation of tau protein as the link between oxidative stress, mitochondrial dysfunction, and connectivity failure: implications for Alzheimer’s disease. Oxid. Med. Cell. Longev. 2013, 1–6.

Muchowski, P.J., Schaffar, G., Sittler, A., Wanker, E.E., Hayer-Hartl, M.K., Hartl, F.U., 2000. Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. Proc. Natl. Acad. Sci. U. S. A. 97, 7841–7846.

Müller, W.E., Eckert, A., Kurz, C., Eckert, G.P., Leuner, K., 2010. Mitochondrial dysfunction: common final pathway in brain aging and Alzheimer’s disease—therapeutic aspects. Mol. Neurobiol. 41, 159–171.

Nandy, K., 1968. Histochemical study on chromatolytic neurons. Arch. Neurol. 18, 425–434.

Palay, S.L., Palade, G.E., 1955. The fine structure of neurons. J. Biophys. Biochem. Cytol. 1, 69–88.

Powers, M.M., Clark, G., 1955. An evaluation of cresyl echt violet acetate as a nissl stain. Stain Technol. 30, 83–88.

Puchtler, H., Sweat, F., Levine, M., 1962. On the binding of congo red by amyloid. J. Histochem. Cytochem. 10, 355–364.

Rattan, S., 1998. Repeated mild heat shock delays ageing in cultured human skin fibroblasts. IUBMB Life 45, 753–759.

Rattan, S.I.S., 2004. Mechanisms of hormesis through mild heat stress on human cells. Ann. N. Y. Acad. Sci. 1019, 554–558.

Rattan, S.I.S., 2006. Hormetic modulation of aging and longevity by mild heat stress. Dose Response 3, 533–546.

Rattan, S.I.S., 2014. Molecular gerontology: from homeodynamics to hormesis. Curr. Pharm. Des. 20, 3036–3039.

Rattan, S.I.S., Eskildsen-Helmond, Y.E.G., Beedholm, R., 2004. Molecular mechanisms of anti-aging hormetic effects of mild heat stress on human cells. Nonlinearity Biol. Toxicol. Med. 2, 105–116.

Reddy, P.H., Beal, M.F., 2008. Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer’s disease. Trends Mol. Med. 14, 45–53.

Revett, T.J., Baker, G.B., Jhamandas, J., Kar, S., 2013. Glutamate system, amyloid ß peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology. J. Psychiatry Neurosci. 38, 6–23.

Samorajski, T., Ordy, J.M., Keefe, J.R., 1965. The fine structure of lipofuscin age pigment in the nervous system of aged mice. J. Cell Biol. 26, 779–795.

Scarborough, E.M., 1938. Nissl granules in "fatigued" nerve cells. J. Physiol. 94, 184–185.

Sitte, N., Huber, M., Grune, T., Ladhoff, A., Doecke, W.D., Von Zglinicki, T., Davies, K.J., 2000. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. FASEB J. 14, 1490–1498.

Snider, B.J., Choi, D.W., 1996. Heat stress reduces glutamate toxicity in cultured neurons without hsp70 expression. Brain Res. 729, 273–276.

Taylor, L.M., McMillan, P.J., Liachko, N.F., Strovas, T.J., Ghetti, B., Bird, T.D., Keene, C.D., Kraemer, B.C., 2018. Pathological phosphorylation of tau and TDP-43 by TTBK1 and TTBK2 drives neurodegeneration. Mol. Neurodegener. 13, 7.

Terman, A., Gustafsson, B., Brunk, U., 2007. Autophagy, organelles and ageing. J. Pathol. 211, 134–143.

Torvik, A., 1976. Central chromatolysis and the axon reaction: a reappraisal. Neuropathol. Appl. Neurobiol. 2, 423–432.

Troyer, H., 1980. Pigments and minerals’. In Principles and techniques of histochemistry. Little Brown and Company. Boston 163–171.

Umschweif, G., Shabashov, D., Alexandrovich, A.G., Trembovler, V., Horowitz, M., Shohami, E., 2014. Neuroprotection after traumatic brain injury in heat-acclimated mice involves induced neurogenesis and activation of angiotensin receptor type 2 signaling. J. Cereb. Blood Flow Metab. 34, 1381–1390.

Van Wijk, R., Wiegant, F., 2010. Postconditioning hormesis and the homeopathic Similia principle: molecular aspects. Hum. Exp. Toxicol. 29, 561–565.

Vassar, R., et al., 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286, 735–741.

Verbeke, P., Clark, B.F., Rattan, S.I., 2000. Modulating cellular aging in vitro: hormetic effects of repeated mild heat stress on protein oxidation and glycation. Exp. Gerontol. 35, 787–794.

Verbeke, P., Clark, B.F., Rattan, S.I., 2001. Reduced levels of oxidized and glycoxidized proteins in human fibroblasts exposed to repeated mild heat shock during serial passaging in vitro. Free Radic. Biol. Med. 31, 1593–1602.

Vogel, P., Dux, E., Wiessner, C., 1997. Effect of heat shock on neuronal cultures: importance of protein synthesis and HSP72 induction for induced tolerance and survival. Metab. Brain Dis. 12, 203–217.

Wakayama, I., 1992. Morphometry of spinal motor neurons in amyotrophic lateral sclerosis with special reference to chromatolysis and intracytoplasmic inclusion bodies. Brain Res. 586, 12–18.

Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.

Welch, W.J., Gething, M.-J., Clarke, A.R., Viitanen, P., Lund, P., Haas, I.G., Georgopoulos, C., 1993. Heat shock proteins functioning as molecular chaperones: their roles in normal and stressed cells. Philos. Trans. R. Soc. B Biol. Sci. 339, 327–333.