

Lithium Prevents Telomere Shortening in Cortical Neurons in Amyloid-Beta Induced Toxicity

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Short Report

Keywords: telomere, lithium, cortical neurons, amyloid-beta, Alzheimer's disease

Posted Date: March 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-260558/v1>

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Abstract

Background

There is consistent evidence of the potential benefits of lithium attenuating mechanisms of neurodegeneration, including those related to the pathophysiology of Alzheimer's disease (AD), and facilitating neurotrophic and protective responses, including maintenance of telomere length. The aim was to investigate the protective effect of the pre-treatment with lithium on amyloid-beta ($A\beta$)-induced toxicity and telomere length in neurons.

Methods

Cortical neurons were treated with lithium chloride at therapeutic and subtherapeutic concentrations (2mM, 0.2mM and 0.02mM) for seven days. Amyloid toxicity was induced 24 hours before the end of lithium treatment.

Results

Lithium resulted in 120% (2mM), 180% (0.2mM) and 140% (0.02mM) increments in telomere length as compared to untreated controls. Incubation with $A\beta_{1-42}$ was associated with significant reductions in MTT uptake (33%) and telomere length (83%) as compared to controls.

Conclusions

Lithium prevented loss of culture viability and telomere shortening in neuronal cultures challenged with $A\beta$ fibrils.

Introduction

Telomeres are terminal DNA regions that lose 50-150pb fragments at each cycle of cell division. As a consequence, telomeres work as a 'biological clock', avoiding the endless proliferation of cells and establishing the replicative senescence (Alberts et al. 2014). Telomere shortening may also occur as a consequence of oxidative stress in the brain, along with protein oxidation and lipid peroxidation (Spilsbury et al. 2015; HUANG et al. 2016). Evidence of telomere shortening has been reported in age-associated diseases, including Alzheimer's (AD) and related neurodegenerative disorders (Boccardi et al. 2015).

Pharmacological compounds that hinder or attenuate telomere shortening by oxidative stress and other mechanisms, therefore preserving cell function and survival of neuronal cells, may be helpful in the treatment or prevention of age-related neurodegenerative disorders. There is a substantial body of evidence from experimental and clinical models suggesting that lithium, has additional neurotrophic and protective properties (Forlenza et al. 2014; De-Paula et al. 2016c); these properties may be relevant to

disease modification in certain neuropsychiatric and neurodegenerative diseases (Nunes et al. 2013), and AD (Forlenza et al. 2011; De-Paula et al. 2016c).

Therefore, the overall effect of lithium may have specific disease-modifying properties in AD by tackling core pathophysiologic mechanisms (Forlenza et al. 2011, 2019), and also deliver a myriad of unspecific yet potentially relevant clinical benefits by its multiple effects on cellular function, including the regulation of telomere homeostasis. In a recent study conducted in our group, using a triple-transgenic mouse model of AD, we were able to provide evidence of a dose-dependent, tissue-specific effect of long-term lithium treatment on the maintenance of telomeres in the presence of AD pathology (Cardillo et al. 2018a). In the present study, we address the protective effect of chronic lithium treatment on A β -induced telomere shortening in cultured cortical neurons.

Materials And Methods

Compliance with Ethical Standards

All animal experiments were approved by the local Ethics Committee (CAPPesq n $^{\circ}$ 1293/09) in accordance with Directive 2018/63/EU, committee of University of Sao Paulo Medical School, Brazil, under the protocol number n $^{\circ}$ 1293/09. All national guidelines were taken into consideration.

Primary cultures of cortical neurons:

Primary neuronal cultures were prepared according to the (De-Paula et al. 2015, 2016c, a) method. Treatment with beta-amyloid peptide (A β ₁₋₄₂) was done for 24 hours from the 9th to the 10th DIC, as described elsewhere (Apel et al. 2009). To assess the effect of lithium on telomere length in amyloid-challenged cultures, we used working concentration of 5 μ M of the A β ₁₋₄₂ peptide, previously incubated to turn into fibrillary state (Apel et al. 2009). After ten days in culture, neuronal viability was microscopically ascertained prior to harvesting the cultured cells upon completion of the incubations.

Assessment of cell viability:

The viability of neuronal cultures was quantitatively assessed by the MTT method [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)], which estimates the percentage of living cells in a given substrate compared to an experimental control. Briefly, 50 μ l of MTT solution (5 mg/ml in PBS) was added to each well (1 \times 10⁵ cells/ml) and multi-well plates were incubated for 3h at 37 $^{\circ}$ C and 5% CO₂. Then, 500 μ l of 10% sodium-dodecyl sulfate (SDS) in 0.01N HCl was added. After overnight incubation, the absorbance was measured by spectrophotometry at 570 nm.

DNA extraction:

We used the AllPrepDNA/RNA mini kit (QIAGEN) following the manufacturer's guidelines. The samples were quantified by spectrophotometry with the NanoDrop apparatus (ThermoFisher Scientific), and the samples were diluted to reach the final concentration of 25ng/ μ L of DNA. *Amplification primers:*The

complete protocol of primer sequence (36B4, control gene, and telomere region), dilutions and reaction time are described in the **Supplementary material 1. Statistical analysis:** An independent-sample Student's t-test was used to determine the difference between MTT mean values in treatment conditions compared to respective controls. The analysis of telomere size was done according to the $2^{-\Delta\Delta CT}$ method adapted (Jendrzejewski et al. 2011), comparing (triplicates samples) the target region (telomeres) with the control region (36B4). Finally, we obtained the $2^{-\Delta\Delta CT}$ defining the control condition ratio as 100% to compare the telomere size with other experimental conditions.

Results

Lithium treatment was associated with increased viability of cultured cortical neurons, as indicated by MTT assays. This effect was dose-dependent, although statistical significance was only achieved with the middle dose of lithium chloride (0.2mM), in which case there was a 34% increase in MTT uptake (**Figure 1**). Treatment with $A\beta_{1-42}$ (5 μ M) significantly impaired neuronal viability. This effect was partially (0.02mM, increased 32%) or totally (0.2mM and 2mM increased 57% and 65% respectively) reverted by the pre-treatment of cultures with lithium chloride for seven days, all such effects reaching statistical significance compared to the effect of $A\beta_{1-42}$ alone.

Figure 2 illustrates the analysis of telomere length in primary cultures of cortical neurons treated with lithium chloride (0.02mM; 0.2mM and 2mM) for seven days (DIC 4 through 11) and challenged with fibrillary $A\beta_{1-42}$, for 24 hours at the 11th DIC, compared to controls. Telomere length was significantly increased as a consequence of lithium treatment alone, with increments of 140%, 180% and 120% (respectively), as compared to the untreated control. The incubation of neurons with 5 μ M $A\beta_{1-42}$ resulted in a statistically significant, 46% reduction in telomere length (compared to untreated control). This effect was totally reverted by pre-treatment with lithium chloride, with final telomere lengths of greater magnitude than the one registered in non-disturbed neurons (controls).

Discussion

In the present study, we show that fibrillary amyloid-beta caused a statistically significant decrease in cell viability and telomere length in primary cortical neurons; these effects were partially or totally reverted by the pre-treatment of cultures with lithium chloride for seven days (chronic treatment). Our data reinforce the evidence supporting the multi-modal neuroprotective effects of lithium against amyloid toxicity, as reported in distinct experimental models (Cuello et al. 2019), and further suggest that the overall neurotoxic effect of $A\beta_{1-42}$, as depicted by a decrease in culture viability, is accompanied by impairments in telomere homeostasis. In this regard, lithium not only prevented amyloid-induced telomere shortening, but also was able to promote telomere elongation in spite of the presence of $A\beta_{1-42}$. This effect was observed at all working concentrations of lithium, i.e., ranging from micromolar to therapeutic levels.

Abnormal telomere shortening leads to premature senescence, cell arrest and loss of physiological functions (Boccardi et al. 2015). Amyloid toxicity has been associated with telomere shortening mostly

via mechanisms related to inflammation, oxidative stress and DNA degradation (Cai et al. 2013). However, a study by (Wang et al. 2015) showed that intracellular A β co-localizes in telomeres, inducing cell senescence and telomere shortening independently of the aforementioned mechanisms, supporting that the inhibition of telomerase activity may be an additional factor related to A β -induced cytotoxicity.

Telomerase, a key enzyme in the maintenance of telomere integrity, is regulated by its own catalytic subunit, the telomerase reverse transcriptase (TERT). Zhang et al. showed that the transcription of the *hTERT* gene is positively regulated by components of the Wnt/ β -catenin pathway, leading to its increased expression. Whilst glycogen synthase-kinase 3-beta (GSK3 β) phosphorylates β -catenin, leading to its degradation by the ubiquitin-proteasome pathway (Zhang et al. 2012a), its inhibition by lithium yields the retention of β -catenin and further downstream effects on the transcriptional activity of the *hTERT* gene (Cardillo et al. 2018b). Previous studies from our group and others indicate that lithium modulates multiple intracellular signaling cascades, with well-established effects such as inhibition of GSK3 β (Boccardi et al. 2015); activation of Wnt/beta-catenin signaling (Zhang et al. 2012b; De-Paula et al. 2020); increase in the synthesis and release of neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF) (De-Paula et al. 2016b); inhibition of apoptosis (Zhang et al. 2012b). Lithium also increases metabolic efficiency and respiratory rate in mitochondria (Jakobsson et al. 2017) and modulates inflammatory response (De-Paula et al. 2016c) and oxidative stress (Quiroz et al. 2010). Therefore, multiple biological effects of lithium may converge to the modulation of telomere homeostasis, in addition to the up-regulation of other neurotrophic/protective responses.

Our data in primary cortical neurons suggest that chronic lithium treatment alone may lead to a substantial increase in telomere length (4- to 7-fold, dose-dependent increments), although this effect failed to reach statistical significance, in view of the high variability of results. This effect would presumably reach statistical significance by increasing the number of replicates, which is unfortunately not available at the present time and represents a limitation of the study. In summary, our study shows that pre-treatment with lithium protected neurons against telomere shortening induced by A β ₁₋₄₂, restoring parameters similar to (or even higher than) baseline measures. Such effect was also observed with low working concentrations of lithium (0.02mM and 0.2mM), corroborating the notion that subtherapeutic or even lower (micromolar) concentrations of lithium may be effective in the modulation of biological responses (Forlenza et al. 2012; Cardillo et al. 2018b). In this regard, the present set of data suggests that the maintenance of telomere length is an additional mechanism by which lithium exerts neuroprotection, attenuating A β toxicity, with potentially relevant implications for the treatment and prevention of cognitive decline and dementia in AD.

Declarations

Funding:

This work was supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant No. 466625/2014-6; National Institute for Biomarker Research in Neuropsychiatry (INBION), Grants

Nos. 14/50873-3, 2016/01302-9, 2017/14418-8, 2018/08621-8 (FAPESP), 88887.463672/2019-00 PNPd/CAPES (Programa Nacional de Pós-Doutorado/Capes) and 465412/2014-9 (CNPq); Associação Beneficente Alzira Denise Hertzog da Silva (ABADHS).

Acknowledgments and Funding This work was supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant No. 466625/2014-6; National Institute for Biomarker Research in Neuropsychiatry (INBION), Grants Nos. 14/50873-3, 2016/01302-9 (FAPESP) and 465412/2014-9 (CNPq); Associação Beneficente Alzira Denise Hertzog da Silva (ABADHS). **Conflict of Interest**

All authors have no conflict of interest and are in accordance with the publication

Availability of data and material

The raw data for the realization of the average will be made available if requested by the authors.

Author's Contribution:

Rafael Martins Themoteo: Performed all cell cultures, data analysis and preparation of the manuscript.

Vanessa de Jesus Rodrigues de Paula: Performed all cell cultures, data analysis and preparation of the manuscript.

Nicole Kemberly Ribeiro Rocha: Performed all cell viability tests, data analysis and preparation of manuscript.

Helena Brentani: Data analysis and preparation of the manuscript.

Orestes Vicente Forlenza: Data analysis and preparation of the manuscript

References

1. Alberts B, Johnson A, Lewis J, et al (2014) The Initiation and completion of DNA Replication in Chromosomes. In: Molecular Biology of the Cell, 6th edn. W. W. Norton & Company, pp 254–266
2. Apel C, Forlenza O V, de Paula VJR, et al (2009) The neuroprotective effect of dental pulp cells in models of Alzheimer's and Parkinson's disease. J Neural Transm 116:71–8. <https://doi.org/10.1007/s00702-008-0135-3>
3. Boccardi V, Pelini L, Ercolani S, et al (2015) From cellular senescence to Alzheimer's disease: The role of telomere shortening. Ageing Res. Rev. 22:1–8
4. Cai Z, Yan L-J, Ratka A (2013) Telomere shortening and Alzheimer's disease. Neuromolecular Med 15:25–48. <https://doi.org/10.1007/s12017-012-8207-9>
5. Cardillo G de M, De-Paula V de JR, Ikenaga EH, et al (2018a) Chronic Lithium Treatment Increases Telomere Length in Parietal Cortex and Hippocampus of Triple-Transgenic Alzheimer's Disease Mice. J Alzheimers Dis 63:93–101. <https://doi.org/10.3233/JAD-170838>

6. Cardillo G de M, De-Paula V de JR, Ikenaga EH, et al (2018b) Chronic Lithium Treatment Increases Telomere Length in Parietal Cortex and Hippocampus of Triple-Transgenic Alzheimer's Disease Mice. *J Alzheimer's Dis* 63:93–101. <https://doi.org/10.3233/JAD-170838>
7. Cuello AC, Hall H, Do Carmo S (2019) Experimental Pharmacology in Transgenic Rodent Models of Alzheimer's Disease. *Front Pharmacol* 10:. <https://doi.org/10.3389/fphar.2019.00189>
8. De-Paula VDJ, Kerr DS, De Carvalho MPF, et al (2015) Long-term lithium treatment increases cPLA2 and iPLA2 activity in cultured cortical and hippocampal neurons. *Molecules* 20:19878–19885. <https://doi.org/10.3390/molecules201119663>
9. De-Paula VJ, dos Santos CCC, Luque MCA, et al (2020) Acute and chronic lithium treatment increases Wnt/ β -catenin transcripts in cortical and hippocampal tissue at therapeutic concentrations in mice. *Metab Brain Dis* 36:. <https://doi.org/10.1007/s11011-020-00638-8>
10. De-Paula VJ, Gattaz WF, Forlenza O V. (2016a) Long-term lithium treatment increases intracellular and extracellular brain-derived neurotrophic factor (BDNF) in cortical and hippocampal neurons at subtherapeutic concentrations. *Bipolar Disord* 18:692–695. <https://doi.org/10.1111/bdi.12449>
11. De-Paula VJ, Gattaz WF, Forlenza O V (2016b) Long-term lithium treatment increases intracellular and extracellular brain-derived neurotrophic factor (BDNF) in cortical and hippocampal neurons at subtherapeutic concentrations. *Bipolar Disord* 18:692–695. <https://doi.org/10.1111/bdi.12449>
12. De-Paula VJ, Kerr DS, Scola G, et al (2016c) Lithium Distinctly Modulates the Secretion of Pro- and Anti- Inflammatory Interleukins in Co-Cultures of Neurons and Glial Cells at Therapeutic and Sub-Therapeutic Concentrations. *Curr Alzheimer Res* 13:848–52
13. Forlenza O V., De-Paula VJR, Diniz BSO (2014) Neuroprotective Effects of Lithium: Implications for the Treatment of Alzheimer's Disease and Related Neurodegenerative Disorders. *ACS Chem Neurosci* 5:443–450. <https://doi.org/10.1021/cn5000309>
14. Forlenza O V., de Paula VJ, Machado-Vieira R, et al (2012) Does Lithium Prevent Alzheimer's Disease? *Drugs Aging* 29:335–342. <https://doi.org/10.2165/11599180-000000000-00000>
15. Forlenza O V., Radanovic M, Talib LL, Gattaz WF (2019) Clinical and biological effects of long-term lithium treatment in older adults with amnesic mild cognitive impairment: Randomised clinical trial. *Br J Psychiatry* 215:668–674. <https://doi.org/10.1192/bjp.2019.76>
16. Forlenza O V, Diniz BS, Radanovic M, et al (2011) Disease-modifying properties of long-term lithium treatment for amnesic mild cognitive impairment: randomised controlled trial. *Br J Psychiatry* 198:351–6
17. HUANG W-J, ZHANG X, CHEN W-W (2016) Role of oxidative stress in Alzheimer's disease. *Biomed Reports* 4:519–522. <https://doi.org/10.3892/br.2016.630>
18. Jakobsson E, Argüello-Miranda O, Chiu SW, et al (2017) Towards a Unified Understanding of Lithium Action in Basic Biology and its Significance for Applied Biology. *J. Membr. Biol.* 250:587–604
19. Jendrzewski J, Tomsic J, Lozanski G, et al (2011) Telomere length and telomerase reverse transcriptase gene copy number in patients with papillary thyroid carcinoma. *J Clin Endocrinol Metab* 96:. <https://doi.org/10.1210/jc.2011-1643>

20. Nunes MA, Viel TA, Buck HS (2013) Microdose lithium treatment stabilized cognitive impairment in patients with Alzheimer's disease. *Curr Alzheimer Res* 10:104–7
21. Quiroz JA, MacHado-Vieira R, Zarate CA, Manji HK (2010) Novel insights into lithium's mechanism of action: Neurotrophic and neuroprotective effects. *Neuropsychobiology* 62:50–60
22. Spilisbury A, Miwa S, Attems J, Saretzki X (2015) The Role of Telomerase Protein TERT in Alzheimer's Disease and in Tau-Related Pathology In Vitro. <https://doi.org/10.1523/JNEUROSCI.2925-14.2015>
23. Wang J, Zhao C, Zhao A, et al (2015) New insights in amyloid beta interactions with human telomerase. *J Am Chem Soc* 137:1213–1219. <https://doi.org/10.1021/ja511030s>
24. Zhang Y, Toh L, Lau P, Wang X (2012a) Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/ β -catenin pathway in human cancer. *J Biol Chem* 287:32494–511. <https://doi.org/10.1074/jbc.M112.368282>
25. Zhang Y, Toh L, Lau P, Wang X (2012b) Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/ β -catenin pathway in human cancer. *J Biol Chem* 287:32494–511

Figures

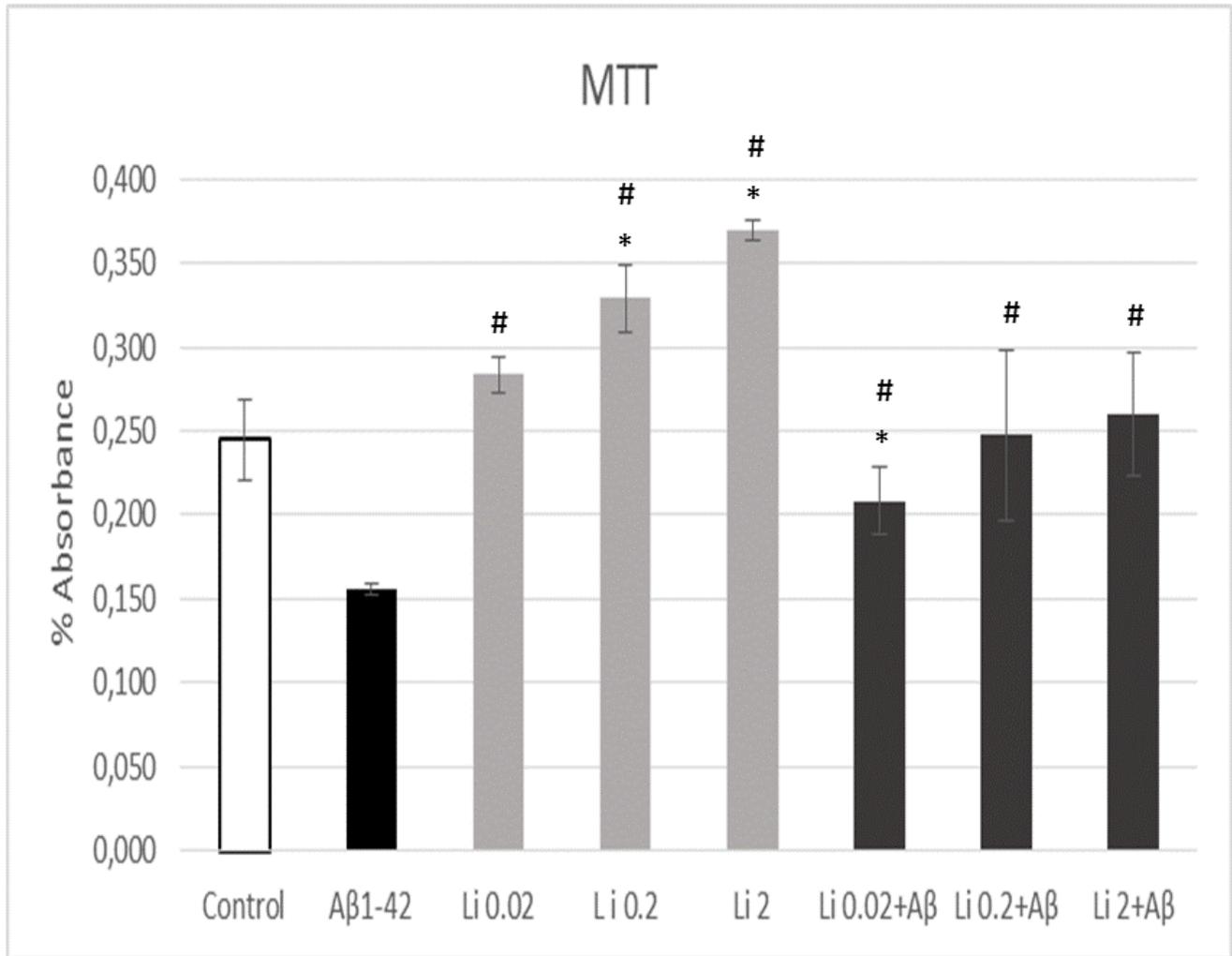


Figure 1

Graph representative of MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] absorbance in primary cultures of rat cortical neurons treated with different concentrations of lithium (0.02mM; 0.2mM and 2mM) and neurons co-treated with lithium chloride and 5µM Aβ1-42. The experimental controls are depicted in the leftmost (white) bar indicative of untreated neurons (effect set at 100%), and the black bar represents the positive control for Aβ1-42 toxicity. Light gray bars indicate the effect of lithium treatment alone on MTT uptake, as compared to untreated controls. Dark gray bars refer to the effect of lithium on neurons pre-treated with lithium and challenged with fibrillary Aβ1-42 (independent samples T-tests * p<0.05 vs. control, #p=0.05 vs. Aβ1-42).

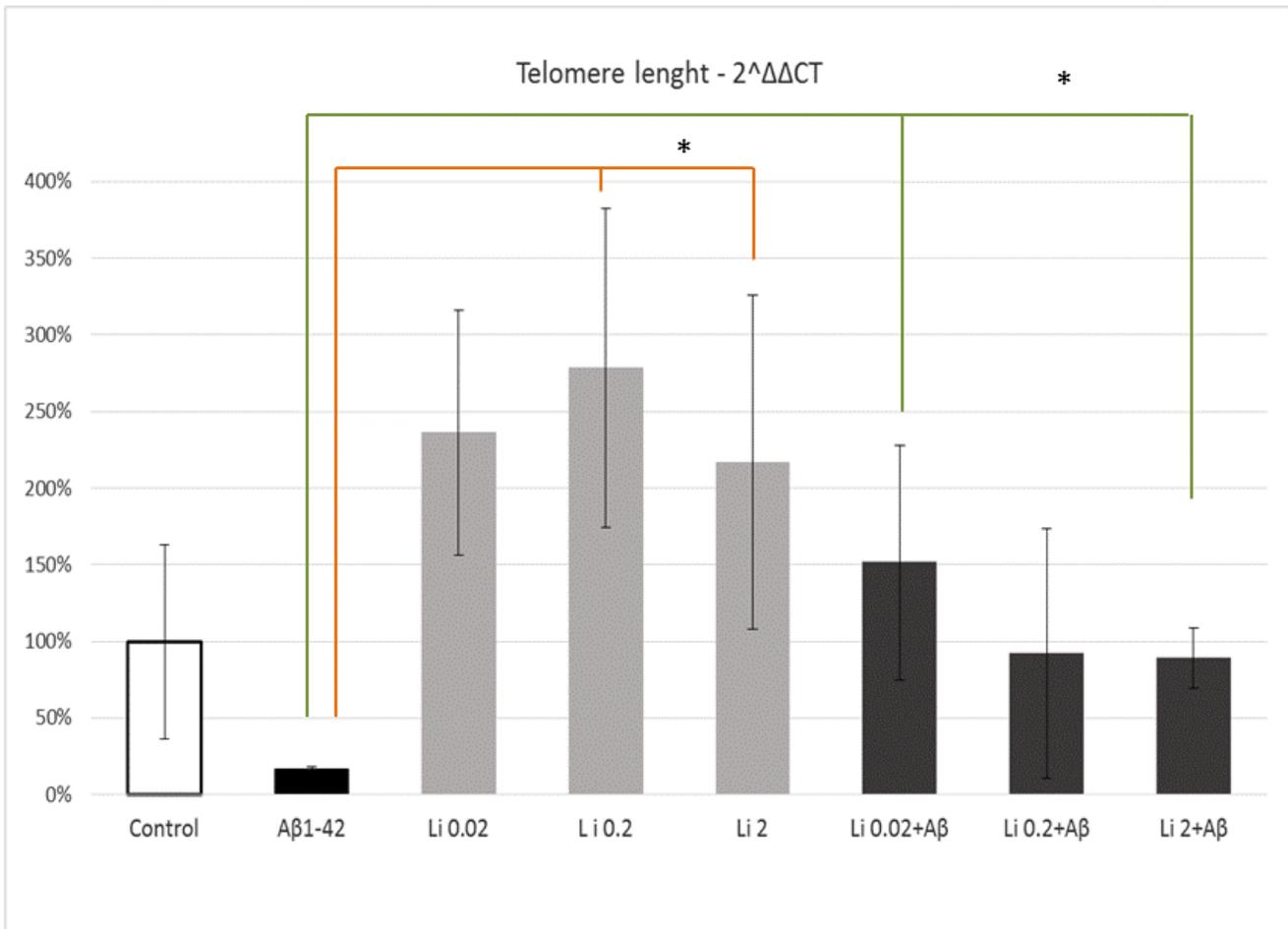


Figure 2

Estimates of telomere length in primary cultures of rat cortical neurons treated with different concentrations of lithium (0.02mM; 0.2mM and 2mM) and neurons co-treated with lithium chloride and Aβ1-42 (5μM). The experimental controls are depicted by the leftmost (white) bar, indicative of untreated neurons, and the black bar represents the positive control for Aβ1-42 toxicity (83% reduction in telomere length compared to untreated controls). Light gray bars indicate the effect of lithium treatment alone on telomere length as compared to untreated controls. Dark gray bars refer to the effect of lithium in neurons challenged with fibrillary Aβ1-42, as compared to the positive control, showing 136% and 179% increase in telomere length in neurons pre-treated with 0.02mM and 0.2mM lithium, respectively. $\Delta\Delta CT$, # $p < 0.05$ vs. control, * $p = 0.05$ vs. Aβ1-42.

Supplementary Files

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