

Methods

Nissl staining and NeuN labelling

Nissl staining and NeuN labelling were performed to verify section quality using previously published techniques.¹ Briefly, sections were hydrated in 95% alcohol, stained with 0.5% cresyl violet, washed with 95% alcohol, submerged in xylene and mounted with Cytoseal™ 280. Nissl stained sections were viewed using a 10× objective. For cell counting analyses, 3 images per section were taken using a Nikon Eclipse 80i Microscope (Nikon Instruments Inc.) linked to Nikon NIS elements software, and the data from the 3 images were averaged. Cell counting was performed based on previously published techniques.² Neuronal and glial cell densities (number of cells per field) were determined by 2 independent individuals (H.K.K. and G.N. [acknowledged]), where neurons were distinguished from glial cells by the presence of cytoplasm, projections and a large nucleus.³ Inter-rater reliability was determined using Pearson correlation analysis, where $r = 0.78$ ($p < 0.001$) for neurons, and coefficient = 0.61 ($p < 0.001$) for glial cells. Acetone-fixed sections were labelled for NeuN (NeuN-Alexa Fluor® 488; Millipore; MAB377X; 1:100; overnight incubation at 4°C) using standard immunohistochemistry techniques detailed in the Methods section of the main article to verify the examiners' ability to distinguish neurons from glial cells. Three images per section were taken using a 10× objective with the same microscope. The number of neurons counted with Nissl staining and NeuN labelling was compared using the Pearson correlation coefficient, where $r = 0.82$ ($p < 0.001$), suggesting that the examiners were able to accurately distinguish neurons from glial cells in Nissl stained sections.

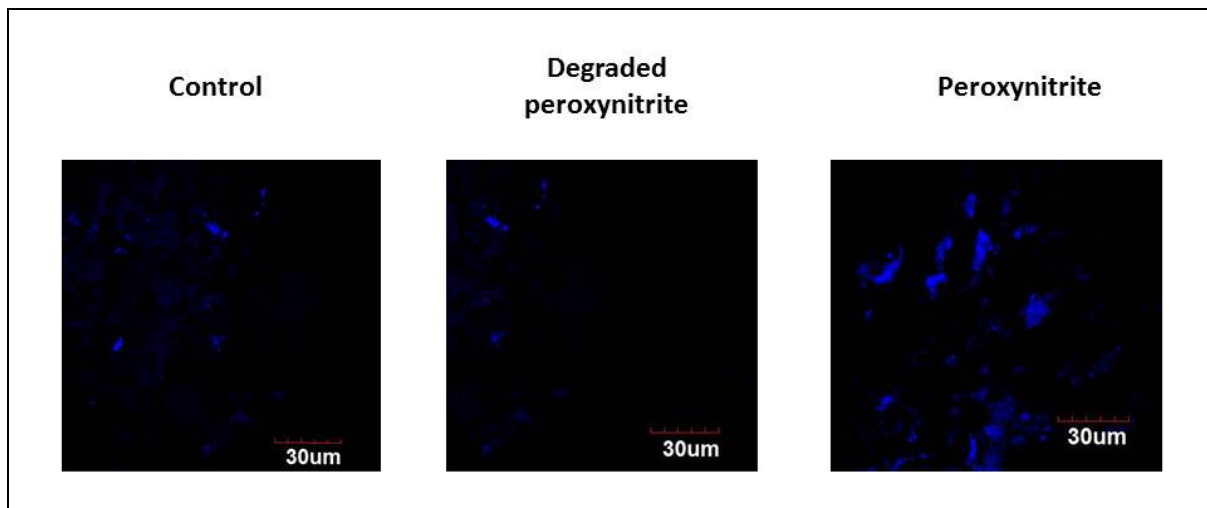


Figure S1: 3-nitrotyrosine (3NT) labelling (blue; Alexa350) following treatment with phosphate buffered saline (PBS) alone, peroxyntirite, or degraded peroxyntirite for 30 minutes. Images were taken with a confocal laser scanning microscope. Increase in 3NT labelling can be observed with peroxyntirite compared to PBS alone, but not with degraded peroxyntirite, indicating specificity of the anti-3NT antibody.

References

1. Brauch RA, Adnan El-Masri M, Parker JC, et al. Glial cell number and neuron/glial cell ratios in postmortem brains of bipolar individuals. *J Affect Disord* 2006;91:87-90.
2. Gittins R, Harrison PJ. Neuronal density, size and shape in the human anterior cingulate cortex: a comparison of Nissl and NeuN staining. *Brain Res Bull* 2004;63:155-60.
3. Sherwood CC, Stimpson CD, Raghanti MA, et al. Evolution of increased glia-neuron ratios in the human frontal cortex. *Proc Natl Acad Sci U S A* 2006;103:13606-11.