

Neurovascular coupling in brain aging and neurodegeneration: nitric oxide-mediated mechanisms and technological approaches.



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The coupling between neuronal activity and cerebral blood flow is essential for normal brain function and cognition. Yet the mechanisms behind this process - neurovascular coupling (NVC) - remain elusive, mainly due to difficulties to probe dynamically the functional and coordinated interaction between neurons and the vasculature *in vivo*. In this regard, imaging, epidemiological and pharmacotherapy studies support a critical contribution of cerebrovascular dysfunction and impaired NVC in the cognitive decline and neurodegeneration associated with Alzheimer's disease.

In this lecture, I will discuss a non-canonical mechanism by which neuronal-derived nitric oxide establishes a volume signaling communication between neurons and microvessels. Moreover, I will discuss the application of multimodal innovative technology to probe *in vivo* a spatial, temporal and amplitude association among vascular changes, oxygen tension and nitric oxide dynamics. Finally, I'll address the improvement of NVC via diet, particularly under conditions of compromised communication between active neurons and the microvasculature.

Relevant publications in the topic of neurometabolic regulation

Ana Ledo, Rui M. Barbosa, Greg A. Gerhardt, Enrique Cadenas, and João Laranjinha (2005) Concentration dynamics of nitric oxide in rat hippocampal sub regions evoked by stimulation of the NMDA glutamate receptor. *Proceedings of the National Academy of Sciences of the USA*, 102, 17483-17488.

Laranjinha, J. and Ledo, A (2007) Coordination of physiologic and toxic pathways in hippocampus by nitric oxide and mitochondria. *Frontiers in Bioscience*, 12, 1094-106.

Rui M. Barbosa, Cátia F. Marques, Ricardo M. Santos, Francois Pomerleau, Peter Huettl, Greg A. Gerhardt and João Laranjinha (2008). *In vivo* real time measurement of endogenous nitric oxide in anesthetized rat brain. *Methods in Enzymology*, 441, 351-367.

Ricardo M. Santos Cátia F. Lourenço, Ana P. Piedade, Rodney Andrews, François Pomerleau, Peter Huettl, Greg A. Gerhardt, João Laranjinha, Rui M. Barbosa (2008) A comparative study of carbon fiber-based microelectrodes for the measurement of nitric oxide in brain tissue. *Biosensors Bioelectronics*, 24, 704-709.

Nunes C., Almeida L. and Laranjinha J. (2008) 3,4-dihydroxyphenylacetic acid (DOPAC) modulates the toxicity induced by nitric oxide in PC-12 cells via mitochondrial dysfunctioning. *Neurotoxicology* 29, 998-1007.

Frade JG, Barbosa RM, Laranjinha J. (2009) Stimulation of NMDA and AMPA glutamate receptors elicits distinct concentration dynamics of nitric oxide in rat hippocampal slices. *Hippocampus*, 19, 603-611.

Rocha, BS, Gago, B., Barbosa R.M. and Laranjinha J (2010) Diffusion of nitric oxide through the gastric wall upon reduction of nitrite by red wine: physiological impact. *Nitric Oxide*, 22, 235-241.

Ledo, A, Rui Barbosa, Enrique Cadenas, and João Laranjinha (2010) Dynamic and interaction profiles of NO and O₂ in rat hippocampal slices. *Free Radic Biol Med*, 48, 1044-1050.

Lourenço CF, Santos R, Barbosa RM, Gerhardt G, Cadenas E and Laranjinha J. (2011) *In vivo* modulation of nitric oxide concentration dynamics upon glutamatergic neuronal activation in hippocampus. *Hippocampus*, 21, 622-630.

Santos RM, Lourenço CF, Pormealeu F, Huettl P, Gerhardt GA, Laranjinha J and Barbosa RM (2011) Brain nitric oxide inactivation is governed by the vasculature. *Antiox Redox Signal*, 14, 1011-1021.

Nunes C, Almeida, L, Barbosa RM and Laranjinha J (2011) Nitric oxide and DOPAC-induced cell death: from GSH depletion to mitochondrial energy crisis. *Mol. Cell. Neurosci.* 48, 94-103.

Ledo A, Barbosa RM and Laranjinha J (2012) Modulation of cellular respiration by endogenously produced nitric oxide in rat hippocampal slices. *Methods Mol Biol* 810, 73-88.

Santos RM, Lourenço CF, Ledo A, Barbosa RM, **Laranjinha J.** (2012) Nitric oxide inactivation mechanisms in the brain: role in bioenergetics and neurodegeneration. *Int J Cell Biol.* 2012, 391914.

Laranjinha J, Santos RM, Lourenço CF, Ledo A and Barbosa RM (2012) Nitric oxide signaling in the brain: translation of dynamics into respiration control and neurovascular coupling. *Ann N Y Acad Sci* 1259, 10-18.

Figueiras E, Oliveira R, Lourenço CF, Campos R, Humeau-Heurtier A, Barbosa RM, **Laranjinha J,** Requicha Ferreira LF, de Mul FF. (2013) Self-mixing microprobe for monitoring microvascular perfusion in rat brain. *Med Biol. Eng. Comput.* 51, 103-112.

Santos RM, Rodrigues MS, **Laranjinha J** and Barbosa RM (2013) Biomimetic sensor based on hemin/carbon nanotubes/chitosan modified microelectrode for nitric oxide measurement in the brain. *Biosensors and Bioelectronics* 44, 152-159.

Pereira C, Ferreira NR, Rocha BS, Barbosa RM, and **Laranjinha J** (2013) The redox interplay between nitrite and nitric oxide: from the gut to the brain. *Redox Biol.* 1, 276-284.

Ferreira NR, Santos RM, **Laranjinha J** and Barbosa RM (2013) Real time in vivo measurement of ascorbate in the brain using carbon nanotube-modified microelectrodes. *Electroanalysis* 25, 1757-1763.

Lourenço CF, Ferreira NR, Lukacova N, Barbosa RM and **Laranjinha J.** (2014) The pattern of glutamate-induced nitric oxide dynamics in vivo and its correlation with nNOS expression in rat hippocampus, cerebral cortex and striatum. *Brain Research* 1554, 1-11.

Laranjinha J (2014) Nutrition and ageing: from phenomenological observations to molecular mechanisms. Commentary. *Genes Nutr* 9, 407.

Lourenço CF, Santos RM, Barbosa RM, Cadenas E, Radi R and **Laranjinha J.** (2014) Neurovascular coupling in hippocampus is mediated via diffusion by neuronal-derived nitric oxide. *Free Radic Biol Med* 73C, 421-429.

Rocha BS, Nunes C, Pereira C, Barbosa RM, **Laranjinha J** (2014) A shortcut to wide-ranging biological actions of dietary polyphenols: modulation of the nitrate:nitrite:nitric oxide pathway in the gut. *Food Funct.* 5, 1646-52.

Ledo A, Lourenço CF, Caetano M, Barbosa RM and **Laranjinha J** (2015) Age-associated changes of nitric oxide concentration dynamics in the central nervous system of Fisher 344 rats. *Cell. Mol. Neurobiol.* 35, 33-44.

Santos RM, **Laranjinha J,** Barbosa RM and Sirota A (2015) Simultaneous measurement of cholinergic tone and neuronal network dynamics in vivo using a novel choline oxidase based electrochemical biosensor. *Biosens Bioelectron* 69C, 83-94.

Lourenço CF, Ledo A, Dias C, Barbosa RM, **Laranjinha J.** (2015) Neurovascular and neurometabolic derailment in aging and Alzheimer's disease. *Front Aging Neurosci.* 27, 7-103.

Ferreira NR, Lourenço C, Barbosa RM and **Laranjinha J** (2015) Coupling of ascorbate and

nitric oxide dynamics in vivo in the rat hippocampus upon glutamatergic neuronal stimulation: a novel functional interplay. *Brain Res Bull* 114, 13-19.

Dias C, Lourenço CM, Ferreiro E, Barbosa RM, Laranjinha J and Ledo A (2016) Age-dependent changes in the glutamate-nitric oxide pathway in the hippocampus of the triple transgenic model of Alzheimer's disease: implications for neurometabolic regulation. *Neurobiol Aging* (in press).

Curriculum vitae

JOÃO LARANJINHA

Personal Information

Place and birthdate: Teixoso - Covilhã; 17th May 1960

Country origin: Portugal

Married: two children

Address: Faculty of Pharmacy, University of Coimbra, Coimbra

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Academic Degrees

1984 - Graduated in Pharmaceutical Sciences (6 years degree), Faculty of Pharmacy, University of Coimbra, Portugal.

1987 - Proof of Pedagogic Aptitude and Scientific Capacity (academic degree equivalent to Master degree in Portugal), Faculty of Pharmacy, University of Coimbra, Portugal.

1996 - Ph.D., Biochemistry, Faculty of Pharmacy, University of Coimbra, Portugal.

2006 - "Habilitation in Biochemistry and tenure", University of Coimbra, Portugal.

Positions

1996-2005 Assistant-Professor of Biochemistry, Faculty of Pharmacy, University of Coimbra

1997-1998 Post-doctoral Research Fellow, Dept. Molecular Pharmacology & Toxicology, University Southern California, Los Angeles, USA

2005- Associate Professor, Faculty of Pharmacy, University of Coimbra.

- 2004- Group leader: Redox Biology and Brain Sensing, Center for Neurosciences and Cell Biology (CNC), Coimbra
- 2005 - Director of the *Center for Microelectrode Technology - Coimbra Labs Divison*. (University of Kentucky, Lexington, USA)
- 2005-2006 Secretary General of the General Assembly of Portuguese Biochemical Society
- 2007-2016 Member of the European Committee of the Society for Free Radical Research
- 2008- Member of the European Committee of the Society for Neurochemistry
- 2008-2012 Secretary General of the Portuguese Biochemical Society
- 7/2012 - 6/2013 Member of the New York Academy of Sciences
- 2012-2014 Member board of Directors of Center for Neurosciences and Cell Biology, Coimbra
- 2012- Coordinator of the Vivarium of the Center for Neurosciences, University of Coimbra
- 2013- Member of the Publication Committee of “Free Radical Biology and Medicine”
- 2013 Full Professor, Faculty of Pharmacy, University of Coimbra
- 2014- Vice President Assembly Faculty Pharmacy University of Coimbra
- 2014- Coordinator Group Biological and Bioanalytical Sciences, Faculty Pharmacy, University of Coimbra
- 2015- Member of the Advisory Board of Oxygen Club California
- 2015- President of the Portuguese Biochemical Society

Main Scientific Areas of Research

Redox regulation of cell functions with implications for aging and neurodegeneration. Molecular mechanisms inherent to neuromodulation and aging, that critically involve free radicals and oxidants, in particular those involving nitric oxide.

The nitrite:nitric oxide pathway as affected by dietary compounds (such as polyphenols) and ensued regulatory process with impact in in gastrointestinal and cardiovascular physiology.

Coordination of Externally Funded Research Projects

Photooxidation of Human Low Density Lipoproteins (LDL) by Sensitizers Used in Photodynamic Therapy. Inhibition by Phenolic Antioxidants and Effects on Cells. Portuguese Science and Tecnology Foundation. October 1998 - October 2001.

Nitric Oxide and Dopamine-mediated damage to mitochondria in neuronal degeneration. Portuguese Science and Technology Foundation. March 1999 - March 2001.

Dynamic interactions of phenolic compounds from wine and olive oil in plasma and lipoproteins with relevance for atherosclerosis prevention. Portuguese Science and Technology Foundation and European Funds for Regional Development. June 2002 - June 2006.

Real-time measurement of nitric oxide in hippocampal brain slices: modulation by tissue redox state with implications for neuronal degeneration. Portuguese Science and Technology Foundation and European Funds for Regional Development. March 2002 - October 2005.

New biological functions for wine polyphenols: cellular and anti-inflammatory actions via nitric oxide production from nitrite. Portuguese Science and Technology Foundation and European Funds for Regional Development. May 2007 - May 2010.

Neurovascular coupling between neuronal activity and blood flow in brain mediated by nitric oxide. Implications in Alzheimer disease. Portuguese Science and Technology Foundation. 2010-2013.

In vivo dynamics of nitric oxide in the brain: regulation of cellular respiration and implications for Alzheimer's disease and aging. Portuguese Science and Technology Foundation. 2010-2013.

Nitrate:Nitrite:Nitric oxide as a key pathway in gastrointestinal and cardiovascular physiology: uncovering new health properties of red wine and olive oil. Portuguese Science and Technology Foundation. 2011-2014.

Nitric oxide is a master regulator of brain energetic metabolism: resolving aerobic glycolysis. Portuguese Science and Technology Foundation. 2013-2015.

Prizes and Fellowships

1st. prize Merck Sharp & Dohme VII Portuguese Congress on Atherosclerosis, “*New biochemical mechanisms in atherosclerosis*” (1997)

Recipient of a Fellowship from Junta Nacional de Investigação Científica (Portuguese Government) for post-doc studies at the University Southern California (USA) (1997-8)

Recipient of a Fellowship from Luso-American Foundation for Development (Portugal) for post-doc studies at the University Southern California (USA) (1997-8)

Lifetime Honorary Member. The Oxygen Club of California (OCC) Award presented to João Laranjinha during the OCC 2015 meeting in Valencia (Spain) 24-26 June, 2015.

Science and Humanity Award. The Oxygen Club of California (OCC) Award presented to João Laranjinha during the OCC 2016 meeting at the University of Davis (USA) 4-6 May, 2016.

Editorial work/Reviewer

Guest Editor of *Toxicology*, volume **208**, No.2, 15 March 2005.

Guest Editor of *Genes and Nutrition* for a special issue on Nutrients and Aging (2014, vol 9).

Member of the advisory board of *Oxygen Club California (OCC)*. Starting 2009.

Member of the editorial board of:

Free Radical Research

Redox Biology

Free Radical Biology and Medicine

Archives of Biochemistry and Biophysics.

Reviewer work (Among others): Free Radical Biology and Medicine, Journal of Agricultural and Food Chemistry, Life Sciences, Current Drug Targets - Central Nervous System and Neurological Disorders, International Journal of Biological Sciences, Biochimica et Biophysica Acta, Chemico-Biological Interactions, Brain Research Bulletin, Medical Science Monitor, Food Chemistry, Neurochemical Research, Toxicology Letters, Electronic Journal of Biotechnology, Journal of Neuroscience Methods, Neurosciences Letters, Nutrition Research, Free Radical Research, Free Radical Biology and Medicine, Neurochemistry International, Brain Research, Cellular and Molecular Neurobiology, Molecular Medicine.