

Research in Neurotoxicology and Stem Cell Biology

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Dept. of Neurology

ACTIVE BASIC RESEARCH GRANTS

PI: J. Sanchez-Ramos

Source: **VA Merit Review Grant**

Title of Project (*and/or Subproject*): **Oyradicals
and Macromolecular Damage in Aging Brain**

Dates of Approved/Proposed Project: 04/01/2003 to
10/31/2008

Annual Direct Costs / Percent Effort: 20% effort
\$120,000 (current year)

This project aims to elucidate the role of oxidative DNA damage and repair during the process of aging in vulnerable brain regions such as the nigro-striatal system.

ACTIVE BASIC RESEARCH GRANTS

PI: Juan R. Sanchez-Ramos

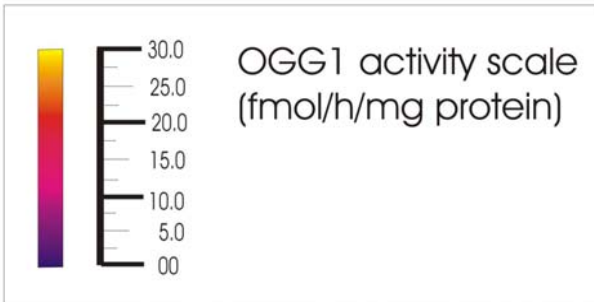
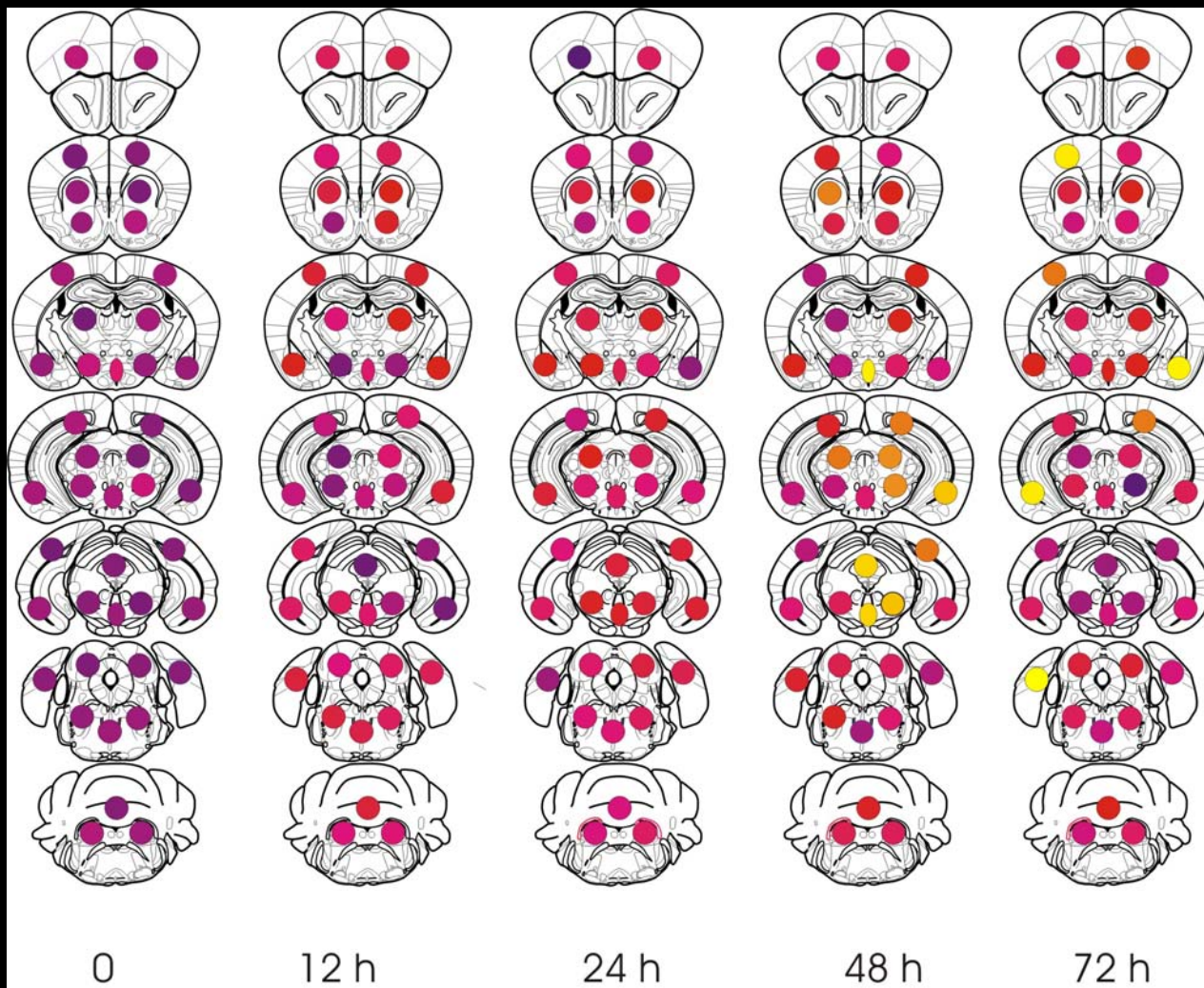
Source: Dept of Defense Grant # DAMD17-03-1-0501

Title of Project (*and/or Subproject*): **The Brain's DNA Response to Neurotoxicants**

Dates of Approved/Proposed Project: 07/01/2003 to 12/31/2006

Annual Direct Costs / Percent Effort: 10% effort; \$237,000 current year

This project will measure regional and cellular differences in the brain's DNA repair response to two neurotoxins known to interfere with mitochondrial function (ochratoxin-A and MPTP). We will focus on the DNA response of DA neurons in two compartments of the midbrain, the SN pars compacta and the ventral tegmental area. We will test whether pre-treatment with agents that modulate the DNA repair response will provide protection for DA neurons of the SN.



ACTIVE BASIC RESEARCH GRANTS

PI: J. Sanchez-Ramos

Source: Florida Alzheimer's Research Center Grant

Generation of New Neurons in Adult Hippocampus by Overexpression of NeuroD1

1. To determine whether enhanced neuronal differentiation of NSC occurs *in vivo*, we will micro-inject the lente viral construct **pTZV-CMV-NeuroD-eGFP** into normal mouse brain hippocampus or into lesioned hippocampus
2. To induce differentiation of neural stem/progenitor cells (NSC), prepared from adult hippocampus in culture dishes, into an exclusively neuronal cell type by overexpression of *hNeuroD1*. To improve transfection efficiency beyond that achieved with our *hNeuroD1/GFP* plasmid, we will create and utilize a lente viral vector that encodes *hNeuroD1/GFP* **pTZV-CMV-NeuroD-eGFP**

In vitro transfections of Adult Hippocampal NSC with the lentiviral vector encoding NeuroD1

1. Dissection Hippocampus



2. Disaggregate, plate in proliferation media, generate neurospheres



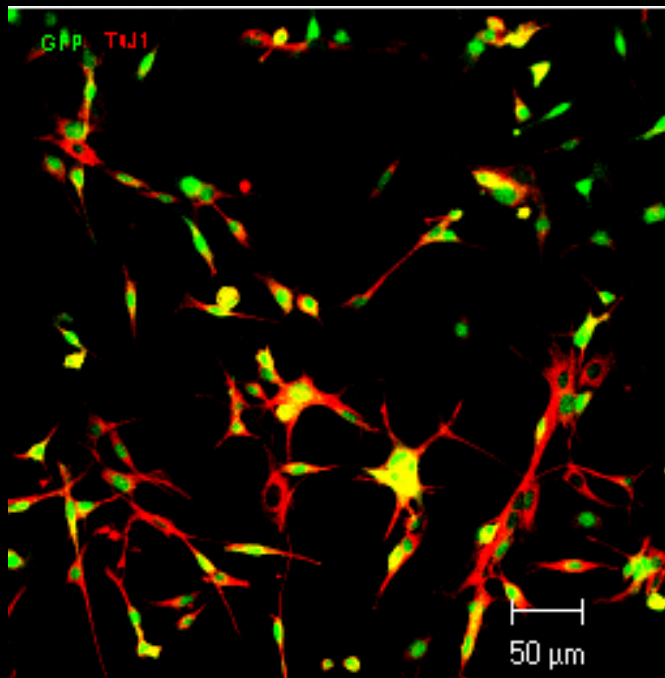
3. Transfect (24 hrs) pTZV-CMV-eGFP or pTZV-CMV-*NeuroD*-eGFP



4. Dissociate, plate in differentiation media (for 3 – 7 d)



5. Immunocytochemistry: View co-expression of GFP-NeuroD and neural stem-progenitor markers with fluorescence confocal microscopy



ACTIVE CLINICAL STUDIES

Investigator: J. Sanchez-Ramos

Source: Huntington Study Group and Amarin Pharmaceuticals, Inc.

Title of Project: **A Multi-Center, Double Blind, Randomized, Parallel Group, Placebo-Controlled Trial of (ethyl-EPA), Myraxion in Patients with Mild to Moderate Huntington's Disease ("TREND")**

Dates of Approved/Proposed Project 11/01/05 to 12/1/06

Annual Direct Costs / Percent Effort: \$74,000; 5% effort;

PENDING GRANTS

Impact of Mycotoxins on Neural Stem/Progenitor Cells of Adult Brain

Funding Agency: NIH (NINDS) or DOD

Humans exposed to “toxic molds” have been reported to suffer significant and measurable cognitive deficits involving several domains, including memory, learning, attention, processing speed, and executive functions . In light of the critical role played by hippocampus in cognitive function, and the importance of neurogenesis in this structure throughout life, the impact of mycotoxins on hippocampal NSC is highly relevant from both molecular pathogenetic and clinical perspectives.

Impact of Mycotoxins on Neural Stem/Progenitor Cells of Adult Brain

Aims:

- 1. To study the toxic effects of ochratoxin-A (OTA) on adult neural progenitor cells in vitro. Dependent variables: proliferative capacity of NSC and differentiation potential (determination of cellular phenotypes derived from NSC after OTA)**
- 2. To determine the role of oxidative DNA damage and repair in NSC in the proliferative phase and during the differentiation phase .**
- 3. To assess the effects of OTA on hippocampal neurogenesis *in vivo* in adult mice.**
- 4. To correlate effects of OTA on neurogenesis with deficits in hippocampal-based learning.**

PENDING

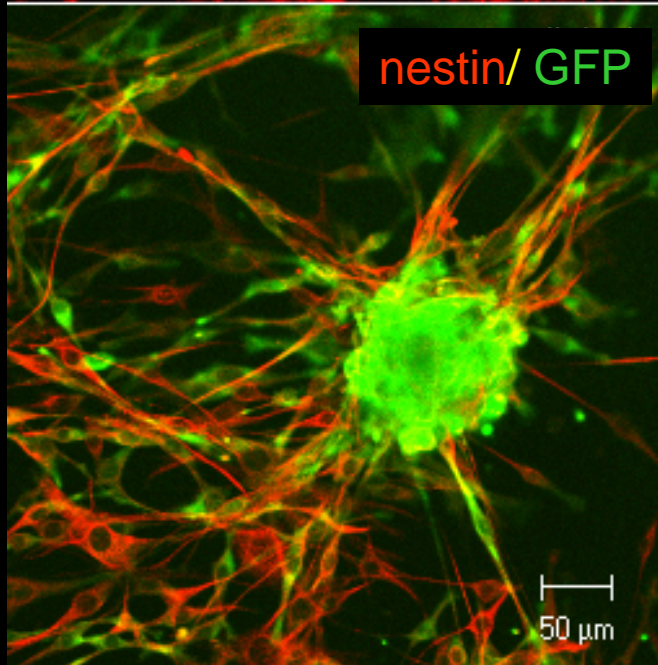
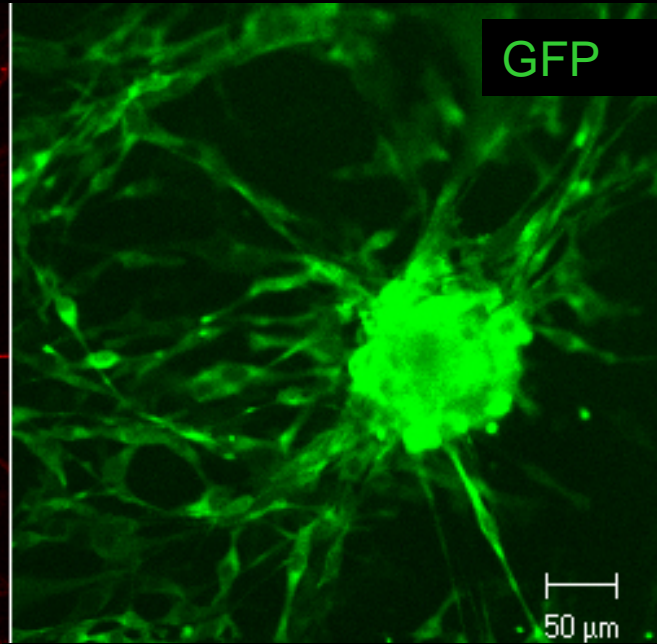
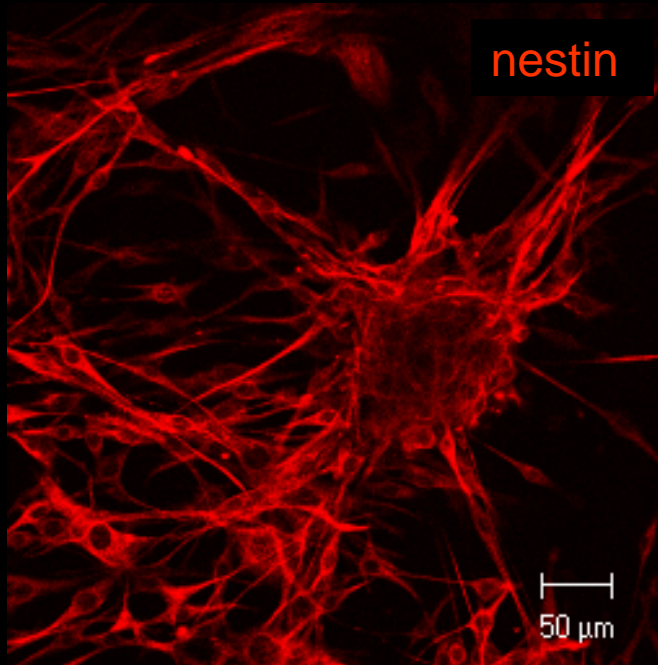
Co-PIs: J. Sanchez-Ramos and S. Song

Project Title: Response of Brain and Bone Marrow -Derived Neural Progenitors to Focal Brain Injury

Agency: VA Merit Review

1. To study the phenotype and functional activity of neurons differentiated from adult neural stem/progenitor cells and bone marrow-derived neural progenitors *in vitro*
2. To study the repair response to micro-stimulation of hippocampus (role of neural progenitors and marrow derived cells)

BMSC prepared from tg GFP mouse express Nestin



DMEM + FCS10% for first 5 passages;
On passage 5, Plated on
bacteriological dish (no attachment) for
4 days, then spheroid colonies were
dissociated and replated on polylysine
coated plates and grown in media
containing EGF+bFGF for 6 days

