**Report on ISN CAEN Grant (December, 2015) by Oluwabusayo Racheal Folarin, Nigeria**

The Chair

CAEN of ISN

REPORT ON RESEARCH VISIT TO ANOTHER LABORATORY – CATEGORY 1A.

This report is hereby submitted with all sense of gratitude to the Committee for Aid and Education in Neurochemistry (CAEN) of the International Society for Neurochemistry (ISN) upon my successful utilization of the award to visit another laboratory. My host was Prof. James R. Connor of the Department of Neurosurgery Penn State College of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, USA.

I was awarded the ISN CAEN grant on the 8th of February, 2016 to enable me visit the host laboratory and carry out the project "Study of Neuroinflammation, Tau and Amyloid Neurodegeneration in Brains of Mice Chronically Exposed to Vanadium: Progressive Changes after Vanadium Withdrawal.

**Visit: 20th June 2016- 5th October 2016.**

My visit took place from 20th of June, 2016 to 5th of October, 2016**.** On arrival at the Department of Neurosurgery, Penn State Hershey on the 20th of June 2016, I was taken through routine health, animal handling and safety training. Prior to the commencement of my research work plan, I was taken round the laboratory to familiarize with the various sections (the store, cell culture room, study room, cold room, the microscopy room, the seminar room, animal house and the radioactive room). All reagents (chemicals and antibodies) used for the project during my stay were provided by my host.

Under supervision, sagittal sections of paraffin embedded brains were made on a rotary microtome. Cut sections from the Control, Vanadium treated and Withdrawal mice brain were hydrated and ablated by Lacer Ablation Inductively Coupled Plasma Mass Spectrometry method (LA-ICP-MS) to reveal the microspatial distribution of vanadium element within anatomical regions of mouse brains. The samples for immunohistochemistry were sectioned from different animal groups (Control, Vanadium treated and Withdrawal) and immunolabelled with neuroinflammatory and neuronal markers: anti-glial fibrillary acid (GFAP) for astrocytes: anti-iba 1 for microglia cells: Anti-Neu-N for neurons and Anti-Neu-N immunofluorescence with Dapi for nuclear marker. I learnt how to handle paraffinized brain

sections for immuno-histochemical methods including various analytical techniques such as Fluorometric TUNEL System assay(G3250), Lacer Ablation Inductively Coupled Plasma Mass Spectrometry method (LA-ICP-MS) a new invention in Neuroscience used for quantifying metals in the brain was also exploited, advanced microscopy using the bright field and fluorescent microscopes equipped with digital camera for doubled Immuno-labelling was appropriately learnt, I also practiced staining procedures, like Nissl staining for brain cytoarchitecture and luxol Fast Blue for myelinated fibres. I was taught stereological analysis using the Image Pro-Plus 7.0; taking photomicrographs of the tissue sections and confocal microscopy.

**Benefits of the award**

My visit to an established laboratory (Professor Connor) has facilitated my interest and enhanced my knowledge on Vanadium and neurotoxicity and the next area of my work to exploit after this current research study. The abstract from this work has been accepted and will be presented by me at the13$^{TH}$International Meeting Society of Neuroscientists of Africa (SONA) Entebbe, Uganda 11$^{th}$ to 14th June, 2017, the manuscript already has been submitted to Frontiers in Neuroanatomy. This research visit allowed me to have hands-on experience in immuno-histochemistry and an idea of what Lacer Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) really is and its significance in neuroscience, this experience has contributed significantly to the completion of my PhD programme. I therefore, express my sincere appreciation to the CAEN and ISN for the grant and the significant and indelible mark this visit has made on my sense of research. Finally, I wish to express my profound gratitude to my host, Prof. James Connor who extended my stay at his own expense while in USA in order for me to complete my research work when I was running out of time. I also appreciate all the wonderful people in his laboratory who made my stay truly memorable.

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**A Grouped photograph of myself and the members of Professor Connor's Laboratory on wed 28th of September, 2016 during the farewell party organized for my departure to my home country Nigeria.**

**Some of the scientific results from my work**

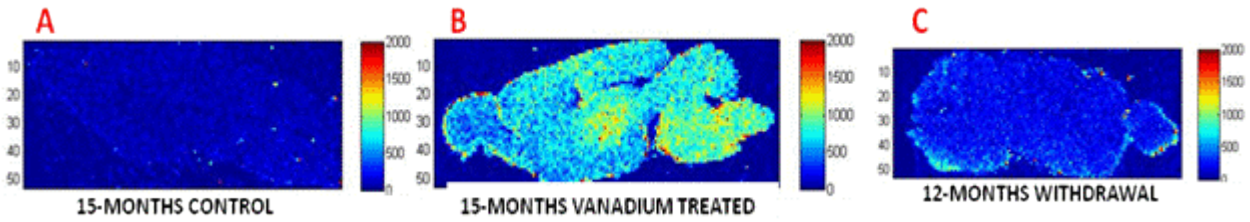


**Fig 1:** Laser ablation-inductively coupled plasma-mass spectrometry (LA – ICP – MS) was used to reveal the microspatial distribution of vanadium metal within anatomical regions of mice brain after chronic exposure at 15months of treatment .A: Control showed no vanadium uptake with zero intensity, B: Experimental showed vanadium uptake across the BBB and distributed in several brain regions, C: Withdrawal showed marked elimination of vanadium metal from the brain after withdrawal from treatment.

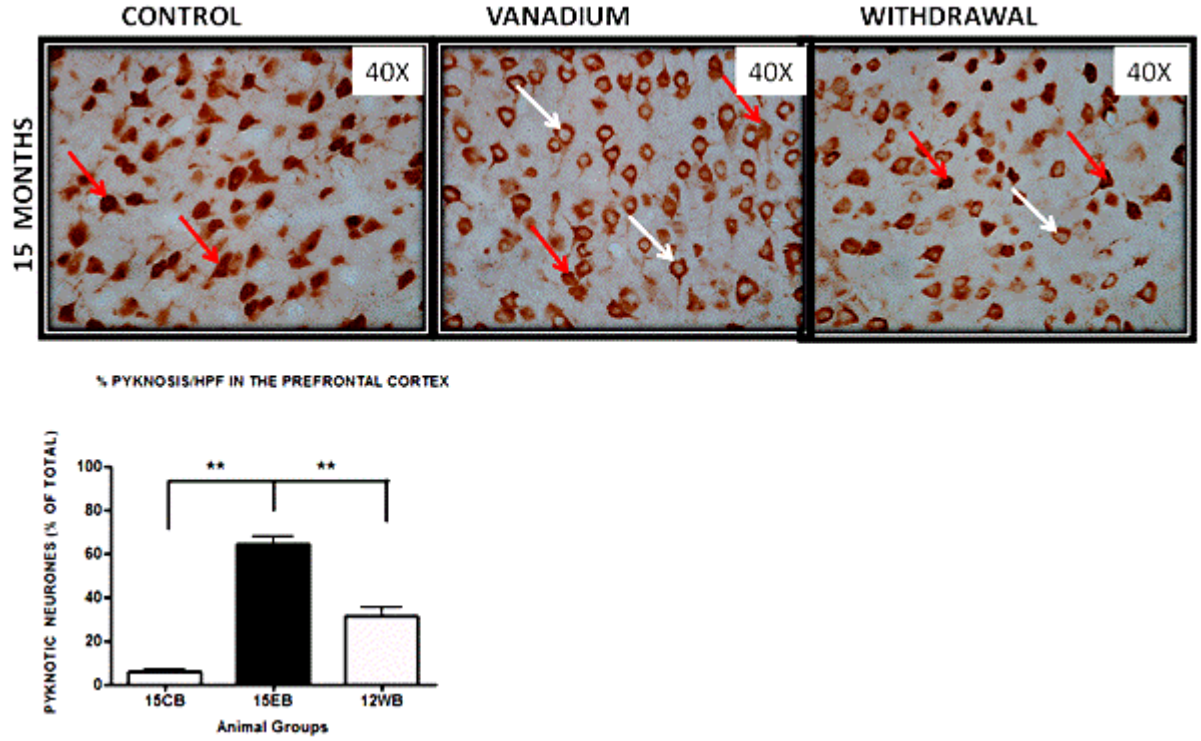


**Fig 2:** NeuN immuno-histochemistry revealed the cytotoxic effect of Vanadium on the pyramidal cells of the prefrontal cortex after chronic exposure .The cortical pyramidal cells showed morphological alterations including pyknosis, cell clustering ,loss of layering pattern and cytoplasmic vacoulation in the vanadium exposed groups(see white arrows ) relative to the control with normal neuronal morphology( see red arrows ). The withdrawal group showed reversal effect relative to vanadium exposed groups. Stereological analysis showed that the mean % pyknosis of vanadium exposed groups were significantly (** $P < 0.001$) elevated relative to the control brain while the withdrawal groups were significantly (** $P < 0.001$) less than vanadium exposed groups. Mag: x400 inset: x600.