

Report on ISN CAEN Grant – Funmilayo Olopade, Nigeria (April, 2012 Batch)

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. Marina Bentivoglio of the Department of Neurological Sciences, University of Verona, Verona, Italy.

I was awarded the ISN CAEN grant on the 3rd of June, 2012 to enable me visit the host laboratory and carry out the project “Study of neuroinflammation in experimental hydrocephalus in rats and effects of α -tocopherol”. On consultation with my host, it was agreed that the visit should be divided into two periods of time. During the first visit, I would learn the procedures for proper preparation of tissues for light and electron microscopy and I would go back home to prepare the experimental animal model, then bring tissue specimen back to her laboratory for processing and analysis.

First visit

My first visit took place from 12th of November, 2012 to 28th December, 2012. On arrival at the laboratory, I was given a tour round it and introduced to the various researchers working there. A post-doctoral researcher was requested by my host to be responsible for supervision of my work on the procedures for immunohistochemistry and my training for preparation of tissues for electron microscopy. During the first two weeks, I learnt how to cut brain sections using the freezing microtome, immunohistochemical processing using free-floating brain sections, serial mounting of these sections on slides. I also practiced staining procedures, like Nissl staining for brain cytoarchitecture and FluoroJade-B for degenerating neurons.

For the next four weeks, I learnt the processes involved in the preparation of brain tissues for electron microscopy. Two adult rats were provided by the laboratory, under ethical permission. I practiced all the processing, from the transcardial perfusion of the rats under deep anesthesia,

brain removal and dissection, post fixation, dehydration and embedding in Epon. Specimens from one rat brain were prepared for routine electron microscopy and specimens from the second one for immuno-electron microscopy.

I learnt the procedure for sectioning the Epon blocks, both semi-thin and ultra-thin sections using both the glass knives and diamond knife as well as mounting on the grids for viewing on the electron microscope. Finally, I viewed all the sections on the transmission electron microscope and was able to appreciate details of cell ultrastructure (nuclei and cytoplasmic organelles in neurons and glia).

I returned to my home institution in Nigeria and started the animal experiments, with the plan to return to Prof. Bentivoglio's laboratory later in the year, with the brain samples to process for immunohistochemistry and electron microscopy.

Second visit

My second visit to the host laboratory took place from 28th September to 15th November, 2013. Due to the previous exposure, I was able to properly prepare my tissues at home before going to Italy.

Animal Experiments: The animal experiment was carried out at my home institution and the animal sacrifice and preservation of tissues were done as I have learnt during the first visit. Three-week old rats had induction of hydrocephalus by intracisternal injection of kaolin solution while the controls had a sham injection into the cisterna magna. Three subsets were sacrificed one, four and eight weeks post –induction by intracardial perfusion and the brains removed, post-fixed and samples taken to the host institution.

Preparation of tissues: Building on my previous experience in the host laboratory, I was able to carry out the preparation of the tissues for immunohistochemistry and electron microscopy under supervision. The samples for immunohistochemistry were sectioned with a freezing microtome and immunostaining done on floating sections for neuroinflammatory markers: anti-glia fibrillary acid (GFAP) for astrocytes, anti-cluster of differentiation 11b (CD11b) for microglia and anti-interleukin 1 β (IL-1 β) for interleukin-1 β -producing cells. Triple immunofluorescence labelling was also done to determine co-localization. I was taught stereological analysis using the

Image Pro-Plus 7.0; taking photomicrographs of the tissue sections and confocal microscopy. The samples for electron microscopy were embedded in epon, ultra-sectioned onto copper grids, counterstained and viewed on the transmission electron microscope (Carl Zeiss, Germany).

Benefits of the award

The results of the study carried out were presented at the 25th ISN biennial meeting in Cairns, Australia, 23rd to 27th August, 2015 and the manuscript is almost ready for submission to a peer-reviewed journal. This research visit has allowed me to have hands-on experience in electron microscopy and 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. Marina Bentivoglio and all the wonderful people in her laboratory who made my stay truly memorable.

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Pictures of myself in the laboratory at the University of Verona

