Moses B. Ekong (University of Uyo, Nigeria)
Report of the visit to the University of Verona, Italy
24 January – 28 May 2016

I wish to thank heartily the International Society for Neurochemistry (ISN), which, through the April 2015 Committee of Aid and Education in Neurochemistry (CAEN) (Category 1A Visit by the applicant to another laboratory) funded my travel and living expenses, making possible my stay in Verona. I am also very grateful to my host, Prof. Marina Bentivoglio, for the opportunity to work in her laboratory.

As presented in the application, the project was aimed at the study of **the effects on the brain of Calabash chalk** and, in particular, of its potential neurotoxic effects. Calabash chalk is a mixture of clay and chalks occurring in natural and artificial forms and commercially available. It is a vital geophagic material in Nigeria and some other sub-Saharan African countries used in traditional food preparation. It is consumed mostly by pregnant and post-partum women, as well as children for its pleasant taste or its anti-emetic effect

Scientific report

According to the work plan, the research was initially carried out in my institution (University of Uyo, Nigeria). As planned, I brought with me to Verona a high number of rat brains (n = 50), pups of rats treated orally during pregnancy with different doses (200, 600 800 mg/kg) of calabash chalk, and sacrificed at 30 days and 60 days of age. BrdU (5-bromo-2'-deoxyuridine) was also administered to the pregnant dams to investigate cell proliferation and migration in the brain of the pups.

Upon arrival at the airport in Verona, my samples were, however, detained for a few days because I did not have ethical/safety documentations. This was resolved when I obtained documents from the Chairman of the Ethics Committee of my institution (Dr Monday Akpanabiatu), and also through the assurances of my Italian host to the security agencies. This indicates the need for my institution to take ethical issues very seriously if we need to continue collaboration with the rest of the world.

In the lab I was trained in many approaches, starting from brain sectioning with a freezing microtome, histochemical (cresyl fast violet and myelin staining) and immunohistochemical procedures. I was also trained in image analysis using the Image Pro Plus software, and stereological cell count using the Stereoinvestigator software.

Techniques I could not get familiar with, due to time limitations include the use of the cryostat, electron microscopy, confocal and two-photon microscopy. Also there was no time to get training in Western blotting and RT-PCR analyses.

During my stay, I had used immunohistochemistry to visualize neurons with NeuN immunolabeling, and astrocytes with glial fibrillary acidic protein (GFAP). I worked hard to visualize microglial cells with different markers (CD11b and Iba-1), using also antigen-retrieval procedures, but I could not achieve microglia immunostaining.

The preliminary results I have obtained indicate the lack of major changes in the Nissl-stained sections from the calabash chalk-treated brains I could process, compared to matched controls. In addition, no hypomyelination was seen in the treated groups using Black Gold II histochemistry.

As for neurogenesis, preliminary results based on BrdU immunolabeling showed no difference in some brain regions, such as the retrosplenial granular cortex (Fig. 1), dentate gyrus, hippocampal CA1-3 fields, in the treated groups. More parameters including doublecortin (DCX), proliferating cell nuclear antigen (PCNA) immunolabelling were studied. Statistically significant differences were observed in some combinations, but not in all of them (Fig. 2), so that many more analyses are needed.

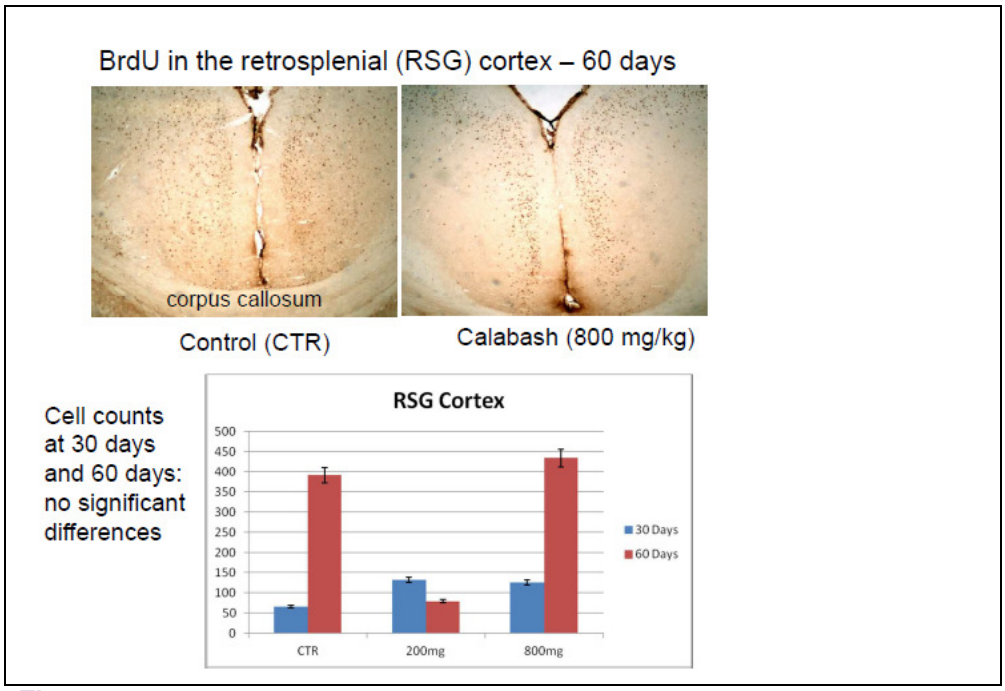


Fig. 1

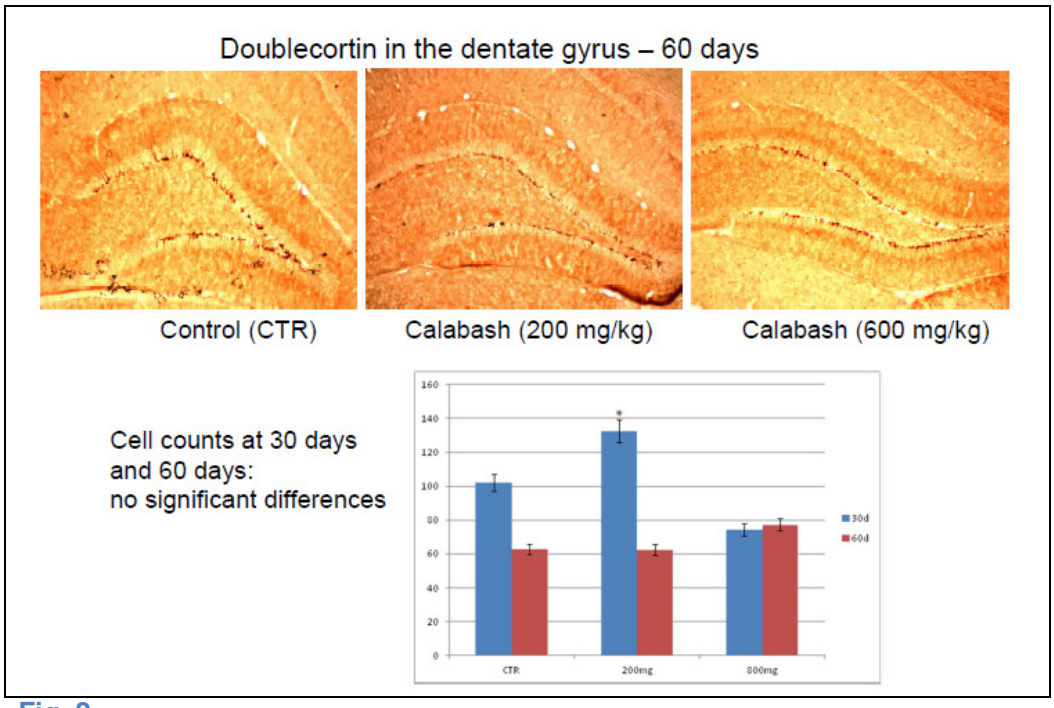


Fig. 2

Limitations:
My major limitation was time; there was insufficient time to analyze many brain areas, especially concerning parameters of neurogenesis (all the samples were left in the host lab). Therefore, more analyses on the brain samples left behind are required, which means it is expedient to return to the laboratory. This will involve obtaining a travel grant.

Funding:
I have implemented the ISN-CAEN funds (US $ 4000) with my personal funds, and the host lab invested several thousands of Euro in reagents.

Conclusion:
The results obtained until now did not point out toxic effects of calabash chalk in the developing brain. However, further analysis is needed to reach solidly grounded conclusions on an adequate number of samples (I have left the material in Verona).

Personal comments:

The stay in the lab was pleasant, and all the staff members (including the administrative personnel, and the personnel in charge of assistance for the staying permit in Verona) and junior co-workers were nice and helpful. I spent one month in a Guest Residence of the Medical School, and I was then assisted in hiring a student room close to the lab.

My photo with lab colleagues during my stay

Working in the Lab

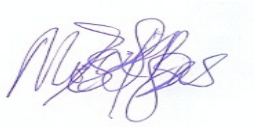
Working in the Lab

This training period has improved my research knowledge and skills, as well as my ability to supervise students at my University. It has also improved the collaboration between my research group and that of Prof. Bentivoglio's, which will hopefully enable continual exchange of ideas and research. When, at the end of my stay, Prof. Bentivoglio asked me if I had learned during my stay, my sincere answer was "it has been eye-opening".

Thank you once again for supporting me with funds for this research.

I remain at the disposal of the ISN-CAEN for any further information on this matter

Yours faithfully,



Dr. Moses B. Ekong