

ISN-CAEN Category C grant
Final report

Luis Bernardo Tovar y Romo, Ph.D.

Department of Molecular Neuropathology

Instituto de Fisiología Celular

Universidad Nacional Autónoma de México

<http://www.ifc.unam.mx/investigadores/bernardo-tovar-romo>

This report is presented in continuation of a progress report that I submitted earlier this year in April.

Upon arrival to the Instituto de Fisiología Celular, I was given the laboratory AL-305 that was previously unoccupied. The laboratory space comprised a main area for bench work, a dark room, an office area for students and an office for the PI. During this year, we adapted all these spaces to the laboratory needs of our experimental work, which included the construction of a cell culture room and adapting one corner of the main lab area for surgical experiments that meet all the safety requirements for the use of volatile anesthesia. Four months ago the remodeling work was concluded and we are currently performing experiments in our own space.

With special financial support from the Direction of Postgraduate Programs and the Scientific Research Coordination of the Universidad Nacional Autónoma de México (UNAM), I acquired the equipment for the in vivo surgery experiments that includes a stereotaxic frame that fits mice and rats, an isoflurane regulator, a scavenger for volatile anesthesia and a surgery stereoscopic microscope, and the equipment for the cell culture room that includes a CO₂ incubator for mammalian cells, a level II biosafety cabinet and a dissection stereoscopic microscope. With this support, I also acquire small pieces of laboratory equipment such as a pHmeter and an analytical balance. I also received a grant from the International Brain Research Organization which allowed me to acquire a laser-Doppler monitor for our brain stroke experiments.

Six students have joined my laboratory; one of them is currently enrolled the Postgraduate (Master's) Program in Biochemistry of UNAM, one undergraduate student is working on his dissertation to obtain a degree in Biology, another undergraduate student is working on his dissertation to obtain a degree in Chemistry and Pharmacobiology and two more undergraduate students are doing a 2-year research internship to obtain their degree in Biology. The sixth student is preparing a project to apply to the Doctorate Program in Biomedical Sciences at UNAM next year. In addition, this summer we had a visiting MD student from another Mexican university participating in a summer research program

sponsored by the Mexican Academy of Sciences. Importantly, UNAM is the largest university in Mexico, serving students from all regions of our country, in my laboratory students come from 5 different Mexican States.

We started to work on our experimental model for stroke in vivo with the surgical implantation of an intraluminal filament inserted through the common carotid artery that occludes the middle cerebral artery. We have the experimental surgical procedure in place along with the laser Doppler analyses for assessing cortical blood flow (Fig. 1).

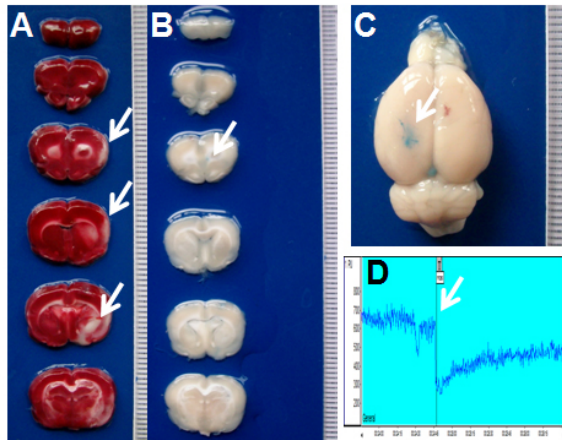


Fig 1. Experimental middle cerebral artery occlusion (MCAO) in the rat. **A)** 2-mm brain sections from a rat subjected to experimental stroke stained with TTC, which forms a red precipitate in the metabolically active tissue. Arrows point to infarct zones depicted as white unstained tissue. **B)** Blood brain barrier disruption following stroke. Arrow points to a site of BBB leakage indicated by the presence of Evans blue. **C)** Edema formation and BBB disruption following MCAO. Arrow points to the site of BBB leakage in the ipsilateral side to MCAO. **D)** Laser-Doppler flowmetry showing the moment of brain blood perfusion drop due to the insertion of the occluding filament.

We also established and validated the protocol for primary neuronal cultures from cortex and hippocampus, and we began the experiments to study the intracellular signaling pathways involved in neuronal death and survival (Fig. 2).

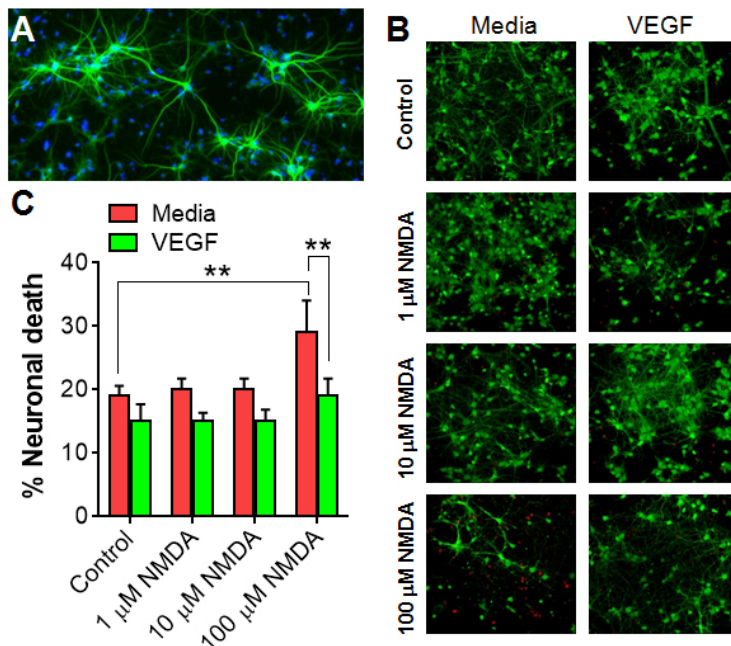


Fig. 2. In vitro model of neuronal toxicity with rat primary cortical neurons. **A)** Micrograph of cortical neurons immunostained with microtubule-associated protein 2 (green) and Hoechst 33342 (blue). **B)** Representative micrographs of cortical neurons treated with different concentrations of N-methyl-D-aspartate (NMDA) as an inducer of neuronal death and rescued by the co-administration of recombinant vascular endothelial growth factor (VEGF). Live cells are labeled in green, dead cells are labeled in red. **C)** Quantifications of the percentage of neuronal death

We began the experiments for culturing primary rat brain microvascular endothelial cells (Fig. 3), from which we will isolate trophic factors that might be involved in the endogenous response of neuronal survival after stroke. We expect to have the first preliminary results on the release of these trophic factors and the responses they trigger by the end of the year.

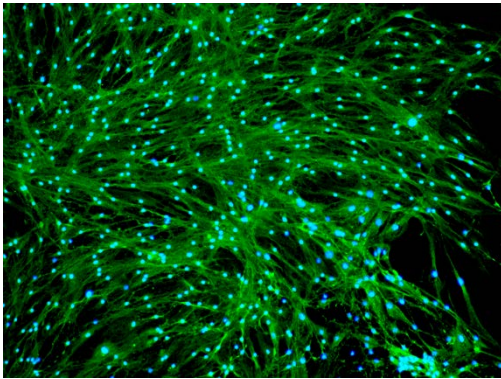


Fig. 3. Brain microvascular endothelial cells. Cells are isolated from adult rats and cultured in vitro for several days. Representative micrograph of endothelial cells immunostained for the endothelial cell marker platelet endothelial cell adhesion molecule (PECAM-1; green) and the nuclei marker Hoechst 33342 (blue).

During this year, I submitted 5 grant applications; 3 of them to the National Council for Science and Technology (CONACYT). The first one of these was submitted to the Basic Sciences Program to obtain funds for my research project on molecular mechanisms of neuronal recovery after stroke; this grant has already been approved and will fund my research for the next 3 years. The second grant application was submitted to the Program for Special Support for the Acquisition of Scientific Infrastructure, to finish furnishing the laboratory. The third one was submitted to the Study Section on Health Research and Social Security. The fourth grant application was submitted to the Pfizer Scientific Institute (Mexico) to support a research project on the effects of dexamethasone on the expression of trophic factors and the modulation of cerebral edema in patients diagnosed with glioblastoma that I plan carry out in collaboration with researchers at the National Institute of Neurology and Neurosurgery (Mexico). The fifth project was recently submitted to the Program for Research and Technological Innovations Projects (PAPIIT) of UNAM and the result of this will be out by the end of the year. In all these grant applications I am the principal investigator.

The money received from the ISN-CAEN Category C grant was spent on laboratory supplies and reagents to carry out experimental work, it was mostly used to buy: cell media, supplements, antibodies, general reagents, microscope slides and media, microsurgery instruments, anesthesia, animals and their housing expenses, labware and laboratory instruments such as micropipettes. These materials have been mostly employed in setting up the neuronal and endothelial primary cell cultures, the establishment of the surgical procedures for the in vivo stroke model and the immuno-detection of markers of cell type and neuronal damage and death.

Lab pictures

Exterior



Main area



Office area



Tissue culture room outside



Tissue culture room inside



Surgery area

