**Dynamic impact of morphine self-administration on brain structure and cellular density in male Wistar rats**

Cesar J. Carranza-Aguilar, David Medina-Sánchez, Ana D. Elizarrarás-Herrera, Mariana S. Serrano-Ramírez, Jalil Rasgado-Toledo, Diego Ángeles-Valdez, Luis Trujillo-Villarreal, Eduardo A. Garza-Villarreal.

Laboratorio de Neuropsiquiatría y Neurotoxicología Traslacional, Instituto de Neurobiología, UNAM campus Juriquilla, Qro., México.

**Introduction**: Opioid use disorder involves compulsive drug use despite negative consequences. Evidence suggests that chronic morphine administration induces changes in brain volume and neuroinflammation, but the interrelation of these effects remains unclear. The aim of this study was to investigate alterations in brain volume and the density of neurons and microglia in the brains of rats with morphine self-administration.

**Hypothesis**: We hypothesized that morphine self-administration could lead to changes in the volume of specific brain regions, potentially associated with mechanisms related to cell death and neuroinflammation, resulting in modifications in the density of various cellular types.

**Methods**: Male Wistar rats were habituated to operant conditioning chambers and underwent the implantation of jugular vein catheters connected to non-magnetic vascular access buttons. Two protocols were employed: 1) Morphine self-administration (0.01 mg/kg/infusion; n = 12) under a Fixed Ratio 1 (FR1) schedule for 20 days, and 2) Morphine self-administration (0.1 mg/kg/infusion; n = 12) following the FR1 schedule followed by a Progressive Ratio 9-4 (PR) schedule for 20 days. In vivo 3D magnetic resonance imaging (MRI) scans (sequence: TR = 30.76 ms, TE = 5 ms, rotation angle = 10°, slice thickness = 25.6 mm, FOV = 28.2 x 19 x 25.6 mm, and isometric voxel size = 160 µm) were conducted before treatment, during the FR1 and PR programs, as well as at the conclusion of each protocol. Immunofluorescence was employed to detect microglia (anti-Iba1) and neurons (anti-NeuN).

**Results**: We observed a gradual increment in morphine infusions during the FR1 program, which stabilized during the PR program. The morphine group exhibited a progressive increase in active lever pressing, while inactive lever pressing remained stable and similar to the control group. MRI scans revealed a significant ($p_{FDR} \leq 0.05$) reduction in brain volume induced by morphine across six distinct brain regions, including the insular cortex (t = -4.335). Simultaneously, an increase in volume was observed in 14 regions, including the brainstem (t = 0.045). Notably, the increase in brainstem volume occurred under the FR1 protocol with a low dose of morphine, while under the PR protocol with a higher dose, it was followed by a subsequent volume decrease. Morphine self-administration augmented the reactive microglia cell count ($p = 0.0035$) in the brainstem, whereas the number of neurons remained unchanged ($p = 0.38$). In the insular cortex, an increase in microglia ($p < 0.0001$) but a decrease in neurons ($p = 0.0123$) was observed.

**Conclusion**: The changes in brain volume induced by morphine self-administration are associated with alterations in neuronal populations and are influenced by microglial activation.

**Acknowledgments**:

We extend our gratitude to the following individuals and institutions: The university animal facility laboratory, for their exceptional technical assistance: José Martín García Servín, María A. Carbajo Mata, and Alejandra Castilla León. The Microscopy Unit, for their invaluable technical support: Elsa Nydia Hernández Ríos and Ericka A. de los Ríos Arellano. The Behavioral Analysis Unit, for their dedicated technical support: Deisy Gasca Martínez. The National Laboratory for Magnetic Resonance Imaging (LANIREM), for their crucial technical assistance: Dr. Juan Órtiz and Dr. Luis Concha. We also wish to express our appreciation to M. Mallar Chakravarty and Gabriel A. Devenyi from the Computational Brain Anatomy Lab (CoBrA Lab) (http://cobralab.ca/), CIC, Douglas Research Centre, Montreal, as well as Compute Canada (www.computecanada.ca). A special thanks to Mallar Chakravarty, Director of the Computational Neuroanatomy Laboratory at Douglas Research Centre, Montreal, Canada, who generously granted us access to computational tools through the Niagara Computing Cluster.

Work sponsored by UNAM PAPIIT IA202120 and UNAM PAPIIT IA201622.