Comparative Analysis of Morphology in Morphine Self-Administration and LPS-Induced Reactive Microglia in a Wistar Rat Model.

David Medina S, C. Carranza-Aguilar, L. Trujillo Villarreal,J. Rasgado-Toledo, M. S. Serrano Rámirez, D. A. Elizarrarás Herrera, E. A. Garza Villarreal

Laboratory of Translational Neuropsychiatry and Neurotoxicology, Instituto de Neurobiología, Universidad Nacional Autónoma de México Campus Juriquilla, Querétaro

Introduction. Morphine is an opioid that has been proven to induce neuroinflammation, and the microglia have been recently implicated in opioid dependence and withdrawal. Therefore, evidence suggests that the morphine-induced neuroinflammation in regions implicated in opioid addiction as nucleus accumbens (NAc) and ventral tegmental area (VTA). Microglia could be suffering changes that would be related to the development of opioid addiction. Because of the complexity and the several reactive morphological types (ameboid or ramified), the morphology of the microglia is considered a spectrum. A better understanding of morphine induced microgliosis may contribute to the comprehension of the variability of cellular activity implicated in the opioid addiction.

Objective. Describe the morphological characteristics of the microglia of a model of self-administration of morphine in Wistar rats and compare it with LPS-induced reactive microglia.

Methods. For this, we used a morphine self-administration (0.1 mg/kg, MO+/LPS-, n=3), a neuroinflammation positive control (1 mg/kg unique dose, MO-/LPS+, n=3) and negative control (saline, MO-/LPS-, n=3). The intrajugular morphine self-administration was performed in a conditioning box with 2 phases: maintenance (FR1) and acquisition (PR 9-4) phases. For evaluation of the cellular density and morphological features of microglia in NAc and VTA, we used immunofluorescence staining using antibodies targeting the microglia marker (Iba-1). Subsequently, high-resolution images were acquired using a LSM 745 Confocal microscope. The image and statistical analysis was made with ImageJ2/Fiji and R language v.4.3.1, respectively.

Results. As a result, the M0+/LPS- group showed more reward infusions than the MO-/LPS- control. The morphological analysis has shown a difference between the cellular density in the treatments (p = 0.0004 Kruskal-Wallis), where MO+ and MO-/LPS+ showed more cellular density than the MO-/LPS- group (Dunn's Test. p = 0.0034, p = 0.0012 respectively). Also, the microglia morphology has qualitative differences between morphine and LPS groups. The LPS has more ramified and bushy phenotype correlating with a reactive microglia. Meanwhile the morphine has several phenotypes, such as primed, hypertrophied, bushy, rod and amoeboid.

Conclusions. The chronic exposure to morphine produces microgliosis, which is accompanied by heterogeneous microglial phenotypes. This suggests that the process appears to be more intricate or multifaceted.

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E-mail. davidmedshz@gmail.com