Characterization of microglia diversity through live cell image analysis

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Microglia are very sensitive to changes in the environment and respond through morphological transformation, phagocytosis and metabolism adaptations. The characterization of microglia heterogeneity is still a challenge since microglia can adopt multiple morphologies and it is not easy to categorize them, particularly in vitro. Though it is well-known that morphology is usually related to function, we are still unable to interpret the meaning of a change in shape. In order to depict microglia behavior in healthy and pathological conditions, we developed image analysis programs to quantify neuronal death, microglia morphologies and phagocytosis. Primary mice neuron-glial cultures, in which microglia express the tdTomato protein, were exposed to excitotoxic or excitotoxic+inflammatory challenges and analyzed 8h later in time-lapse acquired in a confocal microscope. Neuronal death was assessed by SYTOX staining of nucleic debris and phagocytosis through the engulfment of green SYTOX positive particles in red microglia. We identified 7 morphologies (amoeboïd, hypertrophic, fried-egg, bipolar and 3 “inflamed” morphologies) and found the morphometric features able to describe them. Through machine learning, we generated a classifier able to separate them and assign one of the 7 classes to microglia in sample images. In control or excitotoxicity-treated cultures, ameboid and hypertrophic morphologies were the most abundant and did not show changes in the distribution of the populations, or in phagocytosis. Conversely, excitotoxicity+inflammation decreased the amoeboid and hypertrophic populations, induced the appearance of inflamed morphologies and significantly increased the percentage of phagocytosing microglia. Our data suggest that in vitro accumulation of dead cells is not sufficient at least in our model to significantly modify microglia behavior at early time-points (up to 12h) and that inflammation is critical to promote phenotypical changes in microglia. The tools we generated can be useful to correlate microglia behavior with environmental changes and characterize the phenotype of disease-associated microglia.

Acknowledgment: This project is funded by a MICINN grant (PID2020-113202RB-I00) and has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 654248