Techniques in Primary Microglia Cell Culture and Analysis

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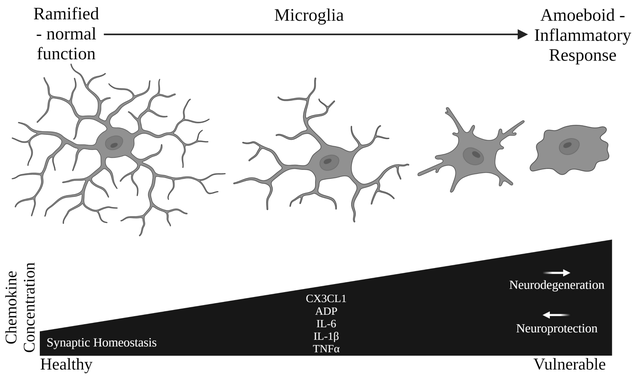
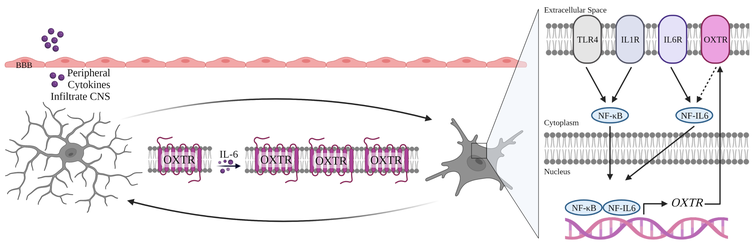
Introduction

Microglia are the immune cells of the central nervous system. They are involved in many important brain functions, from synaptic pruning to mounting immune responses, over the entire lifespan (Gonzalez and Hammock, 2022). Dysfunction of microglia has been implicated in disorders such as autism and even schizophrenia (Lenz and Nelson, 2018).



Adapted from Gonzalez and Hammock, 2022. Created with BioRender.com.

Oxytocin is a neuropeptide which is heavily involved in social bonding, including mother-offspring bonding and pair-bonding (Hammock, 2015). Multiple studies have revealed that microglia and oxytocin interact with each other such that each regulates the other in a variety of ways; oxytocin acts as an anti-inflammatory (Gonzalez and Hammock, 2022), and has been shown to be secreted by microglia in the presence of an endotoxin (Maejima et al., 2022).



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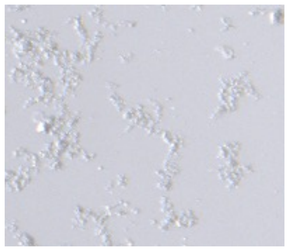
We are currently refining the following techniques for use in investigating the ability of oxytocin pretreatment to reduce the pro-inflammatory response of microglia to an endotoxin.

Primary Microglia Cell Culture

Primary cell culture is a method of growing cells directly from tissue. This results in cells which exhibit more normal physiology, and which typically behave more similarly to in vivo cells than cells taken from a secondary cell line. This experiment uses primary microglial cells, isolated from a mixed glial culture taken from the paraventricular nucleus of the hypothalamus (PVN) from brains of mice.

The main area which required troubleshooting in this process was understanding how to best plate cells at the required density, as both manually counting cells and using an automated particle counter presented challenges. Correct counting is necessary to avoid over- or underplating cells, both of which can have negative effects on cells and obtaining results. The main issue in both methods of counting was the presence of too much cellular debris in cultures which was the same size as our target cells. To fix this, we made changes to the use of the enzyme trypsin, to hopefully result in less debris during isolation. Additionally, changes to the method of dissection allowed for the presence of fewer large debris in cultures.

10x image of a mixed glial culture.



10x image of isolated microglia.

RNA Purification From Cells Grown in Monolayer

RNA purification is a technique used to collect the RNA present in a sample. For this experiment, we intend to extract mRNA from cultured cells of each treatment group to be used in the synthesis of complementary DNA (cDNA), which can then be analyzed through quantitative PCR.

Originally, we intended to use TRIzol reagent to extract the RNA from samples. However, we ran into issues with having sufficient RNA multiple times, likely because this protocol is best suited to RNA extraction from tissue samples as opposed to cultured cells, such as our microglia. Currently, we have had the most success in allowing each sample a freeze-thaw cycle after the protocol, before quantifying the RNA present. Another possible route to solve this issue is the use of a cells-to-cDNA kit, which does not require the additional step of RNA extraction.

Sample	RNA Concentration (ng/uL)
Water	2.972
PP	61.433
PL	6.955
OL	13.355
OP	9.278

PP: dPBS/dPBS (control group)

PL: dPBS/LPS

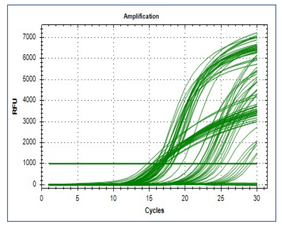
OL: oxytocin/LPS

OP: oxytocin/dPBS

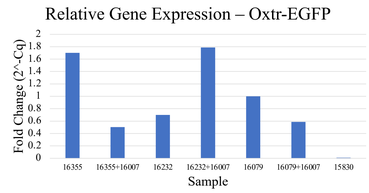
RNA concentration data from spectrophotometer (concentrations this low indicate no RNA present).

Quantitative PCR: Genotyping and mRNA Expression

Quantitative polymerase chain reaction (qPCR) is a technique used to quantify the amount of genetic material present. This experiment used qPCR in two capacities: first, to genotype mice and find males which were double-positive for the Oxtr-EGFP gene. Second, qPCR was used with cDNA generated from RNA extraction to quantify changes in protein expression, specifically for oxytocin (Oxt), oxytocin receptor (Oxtr), vasopressin receptor (Avpr1a), interleukin-6 (IL-6), and arginase-1 (Arg-1).



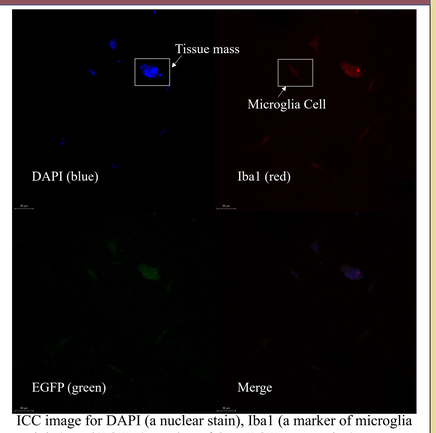
A qPCR amplification curve.



Relative gene expression – a fold change > 1 indicates upregulation; fold change < 1 indicates downregulation.

Immunocytochemistry

Immunocytochemistry is a technique used to qualitatively assess the expression of proteins by cultured cells through the use of fluorescently marked antibodies. With a fluorescence microscope, it is possible to image cells and these proteins. This experiment specifically examined Iba1, green fluorescent protein, and the nucleus of the cell. However, we encountered non-specific binding in all images; to address this, we are in the process of researching different blocking solutions and fixation methods.



ICC image for DAPI (a nuclear stain), Iba1 (a marker of microglia activity), and EGFP (a marker of Oxtr-EGFP expression).

References

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