The Role of Lipids in Neuronal Plasticity – Link to Autism Spectrum Disorders

Shalini Iyer[2], Ashby Kissoondoyal[1,3], and Dorota A. Crawford[1,2,3]

[1]School of Kinesiology and health sciences, [2]Department of Biology, [3]Neuroscience Graduate Diploma Program, York University, Toronto Canada

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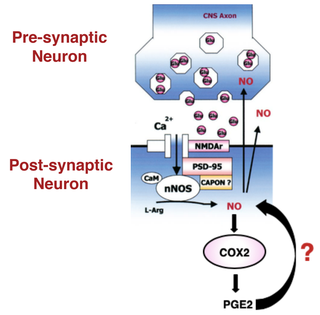
Abstract

Autism spectrum disorder (ASD) is a neurodevelopment disorder defined by deficits in social communication, restricted and repetitive behaviors. Interestingly, ASD is four times more likely in males. Recent research provides the link between abnormal lipid signaling in the brain and ASD. Lipids are critical for healthy brain development.

Introduction

There is increasing evidence that abnormal synaptic signalling and formation of synaptic connections in the brain may contribute to the pathology of neurodevelopmental disorders, such as Autism[1,2]. Neuronal morphology and nitric oxide (NO) production are indicators of synaptic plasticity in the brain[3,4]. Currently, there is insufficient literature on sex and developmental differences of synaptic formation in the healthy brain, which is critical for further understanding these differences in the pathological brain. Further, recent literature suggests that abnormal levels of prostaglandin E2 (PGE_2), the major bioactive lipid in the brain, may influence neuronal plasticity potentially through regulation of NO levels[5,6,7,8].



(Esplugues, 2002).

Objectives

The objective of my study is to obtain an understanding of synaptic connections in the healthy brain in-vivo and a preliminary understanding of abnormal synaptic signaling in-vitro.

Study 1: Are there age- and sex-dependent differences in neuronal morphology in the wildtype (WT) mouse cerebellum?

Study 2: Does PGE_2 levels influence NO production in neuroectodermal stem cells (NE-4C) stem cells?

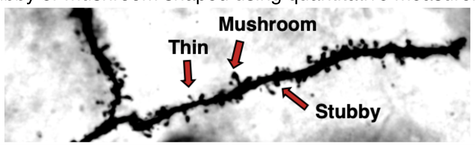
Methods

Study 1: Neuronal morphology in the healthy brain

Experimental Model: To determine sex- and stage-dependent differences on neuronal morphology, the cerebellum of 1296S WT mice were analyzed at two critical developmental stages, postnatal day 14 (P14) and postnatal day 25 (P25).

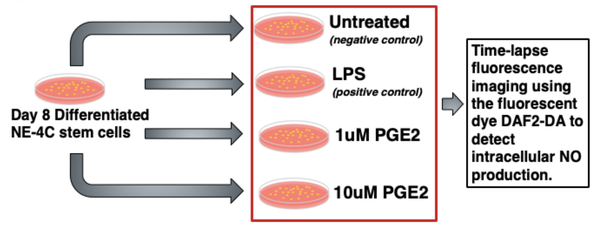
Golgi-cox staining → Confocal microscopy → Image analysis

Figure 1. The cerebellum of WT males and females at P14 and P25 were Golgi-cox stained and imaged using confocal microcopy. Dendrite length, thickness and spine density were quantified. Further, spines were classified as thin, stubby or mushroom shaped using quantitative measurements.



Study 2: The role of PGE_2 on intracellular NO

Experimental Model: In order to determine the influence of PGE_2 on NO production, neuroectodermal (NE-4C) stem cells were used.



Statistical Analysis: For study 1, a three-way ANOVA was conducted, followed by Tukey's post-hoc comparison. For study 2 a randomized block test was conducted, followed by Tukey's post-hoc comparison. *P<0.05, **P<0.01, ***P<0.001 was considered statistically significant.

Results

1 Sex and age influence dendrite length, diameter and spine density

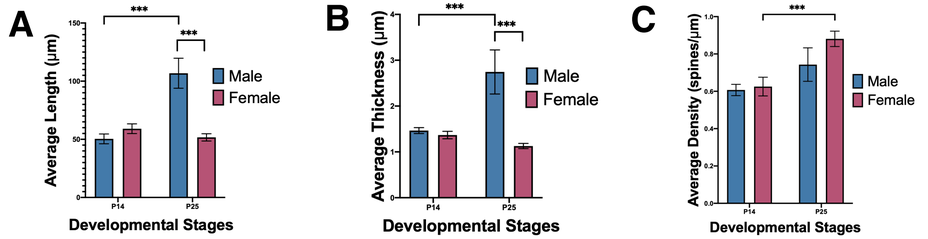


Figure 1. Sex differences in neuronal morphology. A) Dendrite length and B) thickness increase from P14 to P25, specifically in males and in comparison to P25 females. C) There is an increase in spine density from P14 to P25, specifically in females.

2 Sex and age influence spine morphology

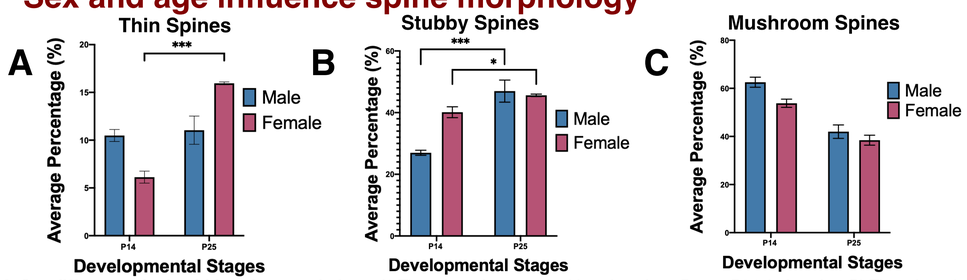


Figure 2. Sex differences in dendritic spine morphology. A) There is an increase in thin spines from P14 to P25, in females. B) There is an increase in Stubby spines from P14 to P25 in males and females. C) Developmental or sex differences were not observed in mushroom spines.

3 PGE_2 influences NO production in NE-4C cells

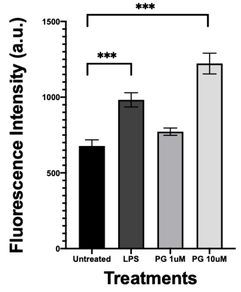


Figure 3. Average NO production in NE-4C cells. The cells were either untreated, incubated with LPS for 2 hours, PGE_2 for 30 minutes and imaged for 45 minutes using the fluorescent dye DAF-2DA. There is a significant increase in NO production in NE-4C cells exposed to 10µM PGE_2, compared to untreated and 1µM PGE_2 cells .

Discussion

Summary of main findings

Study 1:

- Sex has a stage-dependent effect on dendrite length, diameter, spine density and spine morphology

Study 2:

- PGE_2 has a dosage dependent effect on NO production in NE-4C stem cells

Model for PGE_2-NO in the Developing Brain

Abnormal PGE2 levels (Due to environmental factors) → Altered NO levels → Interferes with synaptic plasticity → Abnormal neuronal morphology → Influences synaptic connections → Linked to ASD

Future Studies

- To compare dendritic spine morphology in our mice models of Autism: (1) PGE_2-injected and (2) a knockout of the PGE_2 producing enzyme (COX2)
- To study if PGE_2 can influence NO levels in these mice models
- To examine age- and sex-dependent differences

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