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Background

Alcohol consumption during pregnancy is a worldwide health concern due to exposure to the developing fetus, referred to as Prenatal Alcohol Exposure (PAE), which can cause abnormalities in fetal development, collectively termed Fetal Alcohol Spectrum Disorder (FASD). Nearly 12% of pregnant women reported drinking alcohol in the past 30 days, with ~4% of pregnant women reporting binge drinking in the United States. The impact of PAE is diffuse throughout the body but can severely alter brain development and result in neurocognitive deficits that persist throughout adulthood.

Certain areas of the brain, such as the prefrontal cortex, can be exquisitely sensitive to the impact of PAE. The prefrontal cortex is an area within the frontal lobe that has been found to be reduced in size following PAE and to have shortened orbito-frontal cortices. This area is of particular interest as regions of the frontal lobe are responsible for executive function, impulse control, and social behavior; areas in which deficits have been observed in FASD patients.

The etiology of PAE’s teratogenic effects primarily stem from alcohol’s direct contact with developing fetal body systems but secondary damage is done by inducing placental dysfunction, including placental insufficiency (PI). PI is often caused by maternal development of the placenta and can cause preeclampsia, intrauterine growth restriction (IUGR), cerebral palsy, and development of certain diseases in adulthood. Long term cognitive deficits can result from PI due to abnormalities in brain structure and white matter organization, with resultant alterations in cognitive, emotional and behavioral function.

While it is clear PI and its downstream effects have pathological consequences on neurodevelopment and that PI is concomitant with PAE, the combined effects of PI and PAE on the brain have not been explored.

Objectives

The objective of this study is to begin characterizing the combined effects of PAE and PI on the brain. To this end, changes in apical and basal dendritic complexity of pyramidal neurons in the medial prefrontal cortex (mPFC) and A25 region of the cingulate cortex were quantified by conducting a Sholl analysis and gathering other relevant statistics.

Methods

**Ethanol Exposure:** Pregnant Long-Evans rats voluntarily drank 5% ethanol or saccharine (Sacc) water from embryonic day 1 (E1) to E18, mimicking moderate PAE equivalent to the second trimester of fetal development in humans.

**Surgeries:** On E19, laparotomies were performed to model PI via in utero transient-systemic hypoxia ischemia. Pregnant dams were anesthetized with isoflurane and laparotomies were completed, during which aneurysm clips were placed to occlude both uterine arteries for sixty minutes. The clips were then removed, uterine horns returned to the abdominal cavity, and the incisions sutured close. Pups delivered normally and stayed with their dams until weaning.

**Golgi Staining:** On postnatal day 100 (P100), rats from Sacc+Sham (N=14-15), PAE+Sham (N=11-12), Sacc+PI (N=9-11), and PAE+PI (N=15-16) were deeply anesthetized with sodium pentobarbital and transcardially perfused with phosphate buffered saline (PBS). Brains were then promptly extracted and processed using a Golgi-Cox staining method. Utilizing a crystal, stained brain tissue was cut in the coronal plane at 100 mm and mounted for microscopy.

**Imaging and Analysis:** A Leica TCS SP8 Confocal microscope was used to create three-dimensional images of the mPFC and A25 region of the cingulate cortex. A three-dimensional Sholl analysis was conducted on the apical and basal dendritic arbors of three neurons in the mPFC and three neurons in the A25 region of the cingulate cortex per animal. This process is shown in Figure 2.

**Results**

A three-dimensional Sholl analysis quantifies dendritic complexity by counting the number of dendritic filaments that intersect spheres centered at the soma with radii increasing at ten-micron increments. Pyramidal neurons have two groups of dendrites, basal and apical, which carry distinct synaptic inputs. The basal dendrites are shorter and extend from the soma. The apical dendrite is a long single or bifurcated dendrite that eventually undergoes extensive branching to form what is called the apical tuft. In this study, the basal and apical dendritic arbors were analyzed separately. Sholl Results

**Conclusions**

- Moderate PAE+PI impacts neuronal development with resultant changes in neuronal complexity that is distinct from PAE or PI alone.
- PAE+PI reduces apical dendritic complexity in the mPFC and alters apical complexity in the A25 region of the cingulate cortex.
- The A25 region of the cingulate cortex was less affected by these prenatal insults than the larger mPFC region.
- While changes in dendritic structure in the medial prefrontal cortex suggest alterations in development following moderate PAE+PI, future studies are required to fully elucidate these effects, as well as how this may relate to functionality.

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