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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 maldevelopment 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

Representative Regions of Interest

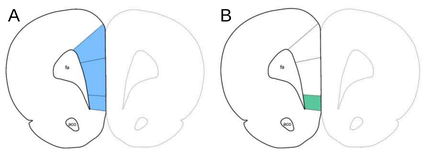

Figure 1: Coronal sections of the rat brain highlight the medial prefrontal cortex in blue (A) and the A25 region of the cingulate cortex in green (B).

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 saccharin (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.

Methods

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 cryostat, stained brain tissue was cut in the coronal plane at 100 µm 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.

Imaging and Isolating Dendritic Arbors

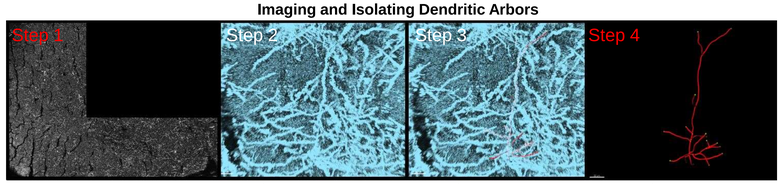

Figure 2: Images are shown representing the isolation of the dendritic arbors. Step 1 consists of obtaining the microscope imaging in 3D, followed by step 2 in which a neuron is identified in the region of interest. Utilizing the Imaris software, step 3 shows the 3D rendering of the dendritic arbor, following by step 4 in which the Sholl analysis is completed on the isolated neuron.

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

Medial Prefrontal Cortex Sholl Graphs

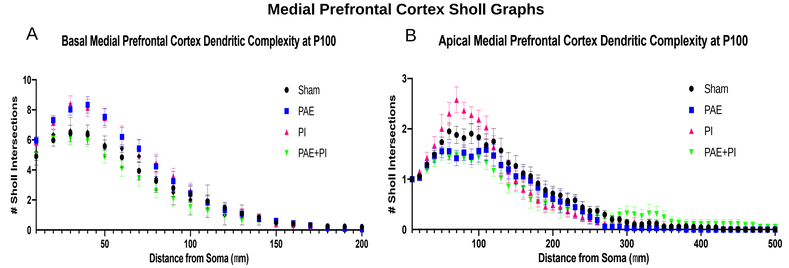

Figure 3: Proximal to the soma, basal dendrites of the mPFC in PAE and PI groups have greater dendritic complexity than Sham and PAE+PI groups (A). Apical dendrites of PI and Sham groups have greater complexity than PAE and PAE+PI groups proximal to the soma (B).

Results

mPFC: Proximal the soma, PAE and PI rats had greater basilar complexity than Sham and PAE+PI, while PI and Sham had greater apical complexity.

- Basal mPFC: Both PAE and PI groups had significantly more complex basal arbors than Sham rats between 10 and 80 microns from the soma.
- Apical mPFC: PI treated rats had significantly more intersections than sham between 70 and 80 microns, PAE between 50 and 110 microns, and PAE+PI between 50 and 120 microns from the soma. The Sham rats also had more complexity than PAE+PI from 60 to 100 microns and 120 to 140 microns from the soma.

A25 Cingulate Cortex Sholl Graphs

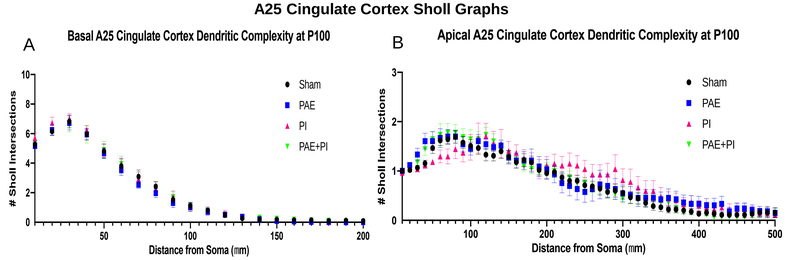

Figure 4: No significant difference in basal dendritic complexity in the A25 region (A). Apical dendrites of PAE and PAE+PI rats sporadically had greater complexity than PI and Sham rats proximal to the soma in the A25 cingulate cortex region (B).

A25: In several lengths near the soma, PAE rats had greater apical complexity than Sham and PI groups. PAE+PI rats only had significantly greater apical complexity than the PI group, but not significant when compared to Sham.

- Basal A25 region of the cingulate cortex: No significance between groups.
- Apical A25 region of the cingulate cortex: PAE rats had significantly more intersections than PI rats 40 microns from the soma ($p<0.05$). PAE rats had significantly more intersections than Sham rats at 40 ($p<0.05$) and 120 ($p<0.05$) microns from the soma. PAE+PI rats had significantly more intersections than the PI group at 50 ($p<0.05$), 70 ($p<0.05$), and 29 ($p<0.05$) microns from the soma.

Contextual Statistics: No statistically significant difference in the number of branch points or total length of dendritic material between groups in the apical or basal regions of the mPFC or A25 cingulate cortex.

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 suggests 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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