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Effects of early alcohol exposure on functional organization and microstructure of a visual tactile integrative circuit

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Abstract

Background—Children with fetal alcohol spectrum disorders (FASD) often have deficits associated with multisensory processing. Because ethanol disrupts activity-dependent neuronal plasticity, a process that is essential for refining connections during cortical development, we hypothesize that early alcohol exposure results in alterations in multisensory cortical networks, which could explain the multisensory processing deficits seen in FASD. Here, we use a gyrencephalic animal model to test the prediction that early alcohol exposure alters the functional connectivity and microstructural features of the rostral posterior parietal cortex (PPr), a visual-tactile integrative area.

Methods—Ferrets were exposed to moderate doses of ethanol during the brain growth spurt period. Functional connectivity and microstructural features were assessed using resting-state functional MRI and *ex vivo* diffusion kurtosis imaging (DKI) respectively when the animals reached juvenile age and adulthood respectively.

Results—While the whole brain volume was smaller in alcohol-treated animals, the relative size of the frontal brain area was larger when compared to control animals. Altered functional connectivity was observed in alcohol-treated animals, where increased connectivity was observed between PPr and the region that provides its major visual inputs (the caudal portion of the parietal cortex), but not with the region that provides its major somatosensory inputs (tertiary somatosensory cortex). DKI revealed reduced microstructural tissue complexity in all investigated sensory areas of alcohol-treated animals.

Conclusions—In this study, we observed alterations in cortical functional connectivity and microstructural integrity in a cortical area involved in multisensory processing in a ferret FASD

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model. These findings indicate an alteration in cortical networks that may be related to the multisensory processing deficiencies observed in FASD.

Keywords

fetal alcohol syndrome; multisensory systems; parietal cortex; resting-state functional connectivity; diffusion kurtosis

Introduction

Fetal alcohol spectrum disorders (FASD) are one of the most common causes of mental disability in the world. Despite efforts to increase public awareness about the risks of drinking during pregnancy, epidemiological studies do not indicate a decrease in FASD cases (May et al., 2014). Children with FASD often have deficits associated with multisensory processing. These include aversion to multiple sensory stimuli presented at the same time, attention deficits, poor visual-motor integration, delayed auditory processing and hypersensitivity to tactile stimulation (Carr et al., 2010, Coles et al., 2002). Multisensory processing, involving pooling and integration of various sensory inputs from different modalities is critical for perception, cognitive processing, and taking appropriate corrective actions. There is growing evidence that deficits in multisensory processing may contribute to abnormalities observed in FASD, including autistic-like behavior (Stevens et al., 2013, Franklin et al., 2008). For instance, FASD subjects often suffer from sensory stimulus overload and can be easily overwhelmed by sensory input from the presence of large groups, noise, touching from others, and visual distractions ((Substance Abuse and Mental Health Services Administration, 2014). The inability to process multimodal sensory stimuli may be the result of disrupted cortical circuitry and microstructural organization in multisensory areas. Ethanol is known to severely affect activity-dependent neuronal plasticity in the cortex, a process that is related to the refinement of synaptic connections and neuronal organization that occurs during the third trimester of human gestation. For instance, developmental alcohol exposure can permanently disrupt ocular dominance and barrel cortex plasticity in the visual and somatosensory cortices respectively (Rema and Ebner, 1999, Medina et al., 2003). Because multisensory integration relies on the correct microstructural organization of sensory areas and the accuracy of convergence of individual sensory systems on multisensory processing areas, disrupted neuronal plasticity from early alcohol exposure can lead to disrupted microstructural organization resulting in impaired multisensory integration that manifests as changes in functional connectivity.

Studies on children with FASD have demonstrated altered microstructural integrity within brain white matter, such as the body of the corpus callosum and white matter innervating bilateral medial frontal and occipital lobes (Fryer et al., 2009), and abnormal functional connectivity and global functional network organization (Fan et al., 2017, Wozniak et al., 2013). The altered functional connectivity was indicated to be associated with altered white matter microstructural alterations (Fan et al., 2017). However, to our knowledge, there are no reports that have focused on the microstructure and functional connectivity alterations related to sensory processing areas. Our group has developed a ferret model of FASD that shows disrupted cortical plasticity as well as aberrant functional architecture in the visual

cortex (Medina et al., 2003, Medina et al., 2005). Ferrets are particularly important for studying cortical processes due to their gyrencephalic brains - whose cerebral cortex is highly folded and convoluted with gyri and sulci. A multisensory area has been recently described in the ferret. The ferret rostral posterior parietal cortex (PPr) is a multisensory area that contains neurons activated by both visual and somatosensory stimulation (Foxworthy et al., 2013). It receives its main afferents from the caudal posterior parietal cortex (PPc, a visual associative area) and the tertiary somatosensory cortex (S3, a tactile associative area). PPr exhibits a pattern of connectivity similar to the human intraparietal sulcus (Foxworthy et al., 2013, Grefkes and Fink, 2005). Because alcohol exposure can affect cortical neuronal plasticity and disrupt the functional organization of the visual cortex (Medina et al., 2003, Medina et al., 2005), we hypothesize that the ferret PPr will exhibit aberrant functional connectivity and microstructural features after early ethanol exposure.

Brain functional and microstructural features can be assessed with *in vivo* neuroimaging techniques. One of the neuroimaging methods used to investigate brain functional networks is functional magnetic resonance imaging (fMRI) where the neural activity associated with a given task is measured by observing the changes in the blood oxygen level dependent (BOLD) signal (Friston, 1994). In recent years, there has been a growing interest in understanding regional brain interactions when a subject is not performing an explicit task using resting state fMRI (rsfMRI). rsfMRI signals are thought to arise from spontaneous low-frequency fluctuations in BOLD signal, the coherence of which determines tightly coupled brain regions in brain baseline functional systems (Deco et al., 2013, Fox and Raichle, 2007). Functional connectivity revealed by rsfMRI displays the basic brain functional organization that reflects the repeated history of co-activation patterns and therefore, indicates the process of plasticity (Guerra-Carrillo et al., 2014). Similarly, information on microstructural features can be obtained using diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI), which provides deep insights into the microstructure of the brain tissue. Both techniques rely on the diffusion of water protons in the tissue. Parameters frequently assessed include mean diffusivity (MD), which measures the average water diffusion within the brain tissue, fractional anisotropy (FA), which provides information on the degree of diffusion anisotropy existing within a given voxel and kurtosis in different directions (MK - mean kurtosis, AK - axial kurtosis, RK - radial kurtosis), which indicate the degree of diffusional heterogeneity and microstructural complexity (Alexander et al., 2007, Steven et al., 2014). Alterations in these parameters are associated with pathological or developmental process such as cytotoxic edema, axon degradation and myelination (Alexander et al., 2007, Steven et al., 2014, Cheung et al., 2009).

In the present study, we investigated brain functional and microstructural alterations with rsfMRI and *ex vivo* DKI on a ferret model of FASD. Specifically, we focused on functional connectivity alterations of PPr (with S3 and PPc) and the water diffusion feature alterations within all of these regions.

Methods

Animals

Ferret kits were ordered from Marshall Farms (North Rose, New York) and were given 3.5g/Kg i.p. ethanol, which leads to blood alcohol levels of approximately 250 mg/dl (Medina et al., 2003), or saline every other day between postnatal day (P) 10 to P30. Regarding cortical development, the timing of this exposure is similar to the third trimester of human gestation. We performed two sets of imaging experiments. The first experiment involved *in vivo* MR imaging of 8 control animals (4 males, 4 females) and 8 ethanol exposed animals (5 males, 3 females) on whom rsfMRI data was obtained. We also performed diffusion kurtosis imaging *ex vivo* on a second set of animals. This set consisted of 6 control animals (3 males, 3 females) and 6 ethanol animals (2 males, 4 females). All animal procedures conformed to NIH guidelines and were approved by the University of Maryland, Baltimore, Institutional Animal Care and Use Committee.

MR image acquisition

***In vivo* imaging**—All *in vivo* MR experiments were performed between P40-50 on a Bruker Biospec 7T 30-cm horizontal bore scanner (Bruker Biospin MRI GmbH, Germany) equipped with a BGA12S gradient system and interfaced to a Bruker Paravision 5.1 console. A Bruker 72-mm linear-volume coil was the transmitter and a Bruker ¹H four-element surface coil array served as the receiver. Light anesthesia was achieved with intraperitoneal injection of dexmedetomidine (0.03mg/kg) just before start of the imaging and 0.3% isoflurane during the imaging session. A MR compatible small-animal monitoring and gating system (SA Instruments, Inc., New York, USA) was used to monitor animal respiration rate and body temperature. The animal body temperature was maintained at 37–38.5°C using a warm water bath circulation.

Scout images in all three orthogonal planes (axial, mid-sagittal, and coronal) were obtained using rapid acquisition with fast low angle shot (FLASH) to localize the ferret brain. A fast shimming procedure (FASTMAP) was used to improve the B₀ homogeneity covering the brain. Anatomic images (repetition time/effective echo time (TR/TE_{eff}) = 4500/23.22ms) were obtained using a two-dimensional rapid acquisition with relaxation enhancement (RARE) sequence covering the entire brain. Imaging was performed over a 4.5 cm field of view (FOV) in the coronal plane with an in-plane resolution of 150μm using 26 slices at 1 mm thickness.

Resting state fMRI was acquired matching the anatomic images using a single shot, gradient-echo echo-planar imaging sequence (TR/TE = 1140/21.46ms) with a 4.5 cm field of view (FOV) and an in-plane resolution of 549 μm² using 26 slices at 1 mm thickness. Three separate rsfMRI sessions were acquired on each animal within the same imaging experiment. A total of 530 temporal volumes were obtained, resulting in a total scanning time of around 10 min for each dataset. During the rsfMRI experiment, the respiratory rate of the animal was monitored and maintained between 30–35 breaths/min.

Ex vivo imaging—For the diffusion kurtosis experiments, adult animals were transcardially perfused with saline followed by 4% paraformaldehyde. One half of the brain was used for ongoing electrophysiology studies. *Ex vivo* DKI data were acquired on the other half of the fixed brain using a single shot, spin-echo echo-planar imaging sequence. An encoding scheme of 64 gradient directions was used with the duration of each of the diffusion gradients being 4 ms with a temporal spacing of 20 ms between the two diffusion gradients. Two b-values (2000 s/mm² and 4000 s/mm²) were used for each direction following the acquisition of five images acquired at b = 0 s/mm². The DKI images were obtained using a single average at isotropic resolution of 250 μ m at a TR/TE of 500/31ms respectively.

Volume analysis

To obtain brain volumes, the anatomical images using the RARE sequence were first skull-stripped manually. Since the cerebellum and brain stem were not fully covered during the acquisition of the anatomical images, they were excluded from the volume analysis. To make sure brains were positioned in the same orientation for slice-by-slice comparison, skull-stripped brains were aligned with a representative male or female brain using 6 degree of freedom linear rigid-body transformation. Brain volume was then measured using MIPAV (Medical Image Processing, Analysis, and Visualization, <https://mipav.cit.nih.gov/>). Brain ventricles were manually extracted and excluded from global brain volume. Ventricle volumes of male and female ethanol treated animals were compared with male and female control groups respectively. No significant difference in the ventricle volume was observed between the two groups (data not shown). Brain volume was measured on a slice-by-slice basis on the 21 slices that were consistently acquired across animals as demonstrated in Fig 1A and between group comparisons were performed on each slice. P-values were corrected with false discovery rate (FDR) correction to maintain a false discovery rate of 0.05 (Benjamini and Hochberg, 1995). To assess the brain shape alterations in our animal model, the whole brain was divided into four sections – slice1–4 (occipital areas), slice5–9 (posterior parieto-temporal), slice10–15 (anterior parieto-temporal), slice16–21 (frontal areas), and volume ratio to the whole brain volume was calculated for each section. Both the male and female section volume ratio data were combined, prior to making between group comparisons.

Functional connectivity analysis

All rsfMRI image preprocessing and processing were conducted using SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>) and AFNI (<http://afni.nimh.nih.gov/afni>). The processing pipeline included slice timing correction, motion correction, alignment to the brain anatomical image of a representative control animal, orthogonalization of motion-derived parameters, spike censoring, band-pass filtering and smoothing. Specifically, the first 10 volumes were discarded from each dataset to avoid any transients associated with the MR signal reaching a steady-state. Slice timing correction and motion correction were performed in SPM12 with the 13th slice being the reference slice. rsfMRI images of each animal were aligned to the brain anatomical image of a representative animal based on tag marks put on representative areas (3dTagalignin AFNI). Next, the data underwent detrending of signal intensity drift, spike censoring, orthogonalization of six motion-derived parameters, band-

pass filtering (0.01–0.1Hz) and smoothing (FWHM = 2 mm) with 3dTproject function in AFNI.

Functional connectivity between PPr and PPc, PPr and S3 were estimated and compared between groups using the following steps. Regions of interest (ROIs) were manually placed on the left and right side of PPr, PPc, and S3. Each ROI contained at least 50 voxels. Correlation coefficients between the time course of each pairs of regions were transformed to connectivity z score using Fisher's transformation. Since no difference was observed between connectivity of left and right side, connectivity z scores from left and right side were combined prior to performing between group comparisons using a two-sample t-test.

Ex vivo diffusion kurtosis imaging analysis—DKI reconstruction of the diffusion data was performed on each voxel using in-house MATLAB program (Mathworks, Natick, MA) as described by Zhuo et al., 2015 (Zhuo et al., 2015) to generate MD, FA, MK, AK, and RK maps. Diffusion parameters were extracted from ROIs with the size of at least 100 voxels placed on the PPc, PPr, and S3 as shown in Fig 3A–C. Between groups comparisons were made using a two-sample t-test.

Results

Brain volume alterations

We observed a whole brain volume reduction in both male ($p = 0.003$, Cohen's $d = 3.11$) and female ($p = 0.001$, Cohen's $d = 4.97$) ethanol exposed animals (slice by slice volumes demonstrated in Fig 1B). To further investigate the effects of alcohol on brain volume we divided the brain in four sections: occipital (slice 1–4), posterior parieto-temporal (5–9), anterior parieto-temporal (10–15) and frontal (16–21). Group comparison between the control and ethanol exposed animals using the combined (both male and female) volume ratio revealed an 8% decrease in the occipital area volume ratio ($p = 0.024$) and a 3% increase in the frontal area volume ratio ($p = 0.015$) in the ethanol exposed group (Fig 1C), suggesting that volume alterations vary across different regions of the brain. These findings are in line with some human studies where whole brain volume reduction and disproportionate regional brain volume alterations were observed in alcohol exposed subjects (Wisniewski et al., 1983, Sowell et al., 2002, Li et al., 2008).

Functional connectivity alterations

Anatomical studies showed that PPc and S3 provide the major cortical inputs to PPr in the ferret (Foxworthy et al., 2013). Figure 2A shows the location of the ROIs in a coronal view and Figure 2B shows the position of these regions on a sagittal view of the ferret brain. As expected, we observed a strong functional connectivity between PPr-PPc and PPr-S3 in controls. Interestingly, ethanol exposed animals presented increased functional connectivity between PPr and PPc ($p = 0.045$) but not between PPr and S3 ($p = 0.726$) (Fig 2C), indicating that the visual portion of the visual-somatosensory system may be particularly sensitive to early ethanol exposure and may be the result of reduced synaptic pruning (Medina and Krahe, 2008, McCool, 2011), as previously suggested.

Diffusion feature alterations

No differences were observed with MD in all ROIs. Significantly increased FA was found only in PPc ($p = 0.022$) of ethanol exposed animals compared to control animals (Fig 3). All kurtosis parameters, MK, AK, RK, decreased significantly in PPr, PPc and S3 in the ethanol group (Fig 3). The widespread reduction in diffusion kurtosis parameters indicates less tissue complexity which occurs in conditions such as less cell packing and less maturation (Cheung et al., 2009).

Discussion

Our study reveals several features of altered brain development in ethanol exposed animals. We found that overall brain volume decreased in both male and female ethanol exposed animals. However, when looking into regional differences, we found that the relative volume of occipital area decreased and that of the frontal areas increased. Furthermore, we observed increased functional connectivity between PPr and PPc but not between PPr and S3. DKI results revealed increased FA in PPc while a widespread decrease in diffusion kurtosis was found in all regions. Overall the study provides evidence of disrupted cortical organization caused by developmental ethanol exposure resulting in both structural and functional deficiencies.

Brain volume reduction has been reported in various developmental brain disorders, e.g., attention deficit/hyperactivity disorder, Williams syndrome, and in Turner syndrome (Carmona et al., 2005, Chiang et al., 2007, Brown et al., 2002). Volume reductions were shown to be associated with behavioral impairments (Stevens and Haney-Caron, 2012, Chiang et al., 2007). As was observed in the present animal study, reduction of overall brain size of prenatal alcohol exposed subjects has been repeatedly reported in postmortem studies as well as in neuroimaging studies (Wisniewski et al., 1983, Archibald et al., 2001, Birch et al., 2016). In addition to the reduction of overall brain size, the absolute volumes of various brain cortical and subcortical areas were consistently reduced in humans with prenatal exposure to alcohol (Chen et al., 2012, Rajaprakash et al., 2014, Archibald et al., 2001). Despite of a whole brain volume reduction, each region can be differently affected. When regional volumes were normalized to whole brain volumes, it has been shown that some regions of the brain atrophy, while others increase in size or remain unchanged (Rajaprakash et al., 2014, Chen et al., 2012, Archibald et al., 2001, Sowell et al., 2002). We observed a relative decrease in the volume of occipital areas, consistent with previous studies where it was demonstrated that both absolute and relative occipital lobe volumes decreased in both prenatal alcohol exposed humans and early alcohol exposed animal models (Rajaprakash et al., 2014, Li et al., 2008, Mooney and Napper, 2005). A study with early ethanol exposed rats showed reduced neurons in the occipital cortex (Mooney and Napper, 2005), suggesting neuronal death as a possible cause for reduction of relative volume of occipital areas. The underlying mechanism for the disproportionate volume increase in our study is unclear. Pruning is an important process during normal neural development that eliminates unnecessary connections and strengthens important ones. The increased relative frontal volume may be a result of less pruning or excessive synapses (Sowell et al., 2004, Toga et al., 2006). Taken together with our findings, this suggests early alcohol exposure results in

total brain volume loss, and that such loss may be disproportionately spread across the whole brain.

rsfMRI is increasingly used for studying the development of basic brain functional organization. It has been demonstrated that brain networks that are involved in cognitive tasks can also be reliably identified while the brain is at 'rest' (Smith et al., 2009, Fox and Raichle, 2007). The spontaneous co-activation detected at resting-state is thought to reflect the existence of mono- or multi-synaptic connections between co-activated regions and therefore, can be considered an indicator of the existence of anatomical networks (Guerra-Carrillo et al., 2014). In the present study, we observed significantly increased functional connectivity between PPr and PPc in the ethanol exposed group. This hyper-connectivity can be a result of deficits in neocortical plasticity. The functional connectivity between PPr and S3 was not altered in our study. This may be due to the fact that the somatosensory cortex develops before the visual cortex and the timing of ethanol exposure used here might affect more the latter than the former.

To the best of our knowledge, this is the first DKI study investigating grey matter microstructures in animals exposed to ethanol. Recently published studies focus mostly on white matter using FA and MD from DTI. Alterations in MD reflect developmental or pathological changes in the brain tissue caused by changes in the diffusion characteristics of the intra- and extracellular water compartments, including restricted diffusion (e.g., cytotoxic edema, increased cell packing) and water exchange across permeable boundaries. Changes in the FA, on the other hand, are indicative of the structural integrity of the tissue (Yoshida et al., 2013). Reduced FA and increased MD were frequently observed in most white matter regions of subjects exposed to alcohol prenatally (Ma et al., 2005, Fryer et al., 2009), suggesting decreased white matter integrity and increased axonal degradation. In contrast, we observed significantly increased FA in PPc and A1. Although FA is usually indicative of microstructural integrity, higher FA does not necessarily suggest better brain function. Instead, increased FA in white matter has been thought to predict poor cognitive function in some developmental brain disorders such as Williams Syndrome (Hoeft et al., 2007). In accordance with our findings, a study on cortical gray matter microstructure after fetal ethanol exposure in rats also revealed higher FA at early postnatal ages (Leigland et al., 2013). This increased FA in the cortex may indicate lower morphological complexity of dendritic processes early after ethanol exposure, which has been observed in previous studies (Cui et al., 2010). As mentioned earlier, most imaging studies have focused on white matter changes and very little exists regarding changes in the gray matter following ethanol exposure. DKI is an innovative diffusion MRI technique that can provide more sensitive and comprehensive measures of both gray matter and white matter microstructural changes (Cheung et al., 2009, Falangola et al., 2008). The novel finding from the present DKI study is the widespread decrease in diffusion kurtosis in all of the tested regions. Traditional DTI is different from DKI in that the DTI parameters are derived based on the assumption that the diffusion of water follows a Gaussian distribution. However, given the complex nature of the brain's tissue, the water diffusion more likely follows a non-Gaussian distribution. Based on this assumption, diffusion kurtosis has been suggested as a possible imaging technique that more appropriately captures brain microstructural complexity (Shaw, 2010, Falangola et al., 2008). Moreover, in previous studies, diffusion kurtosis parameters have been shown

sensitive to increasing tissue complexity in developing gray matter (Paydar et al., 2014, Shi et al., 2016, Cheung et al., 2009). In the developmental period of both humans and rodents, age-related increases in white and gray matter diffusion kurtosis have been observed even at ages after myelination and axonal packing have peaked (Paydar et al., 2014, Cheung et al., 2009). The prolonged increase in diffusion kurtosis indicates an increased microstructural complexity resulting from axonal pruning and reorganization of myelin and synaptic refinement, cell packing and the addition of basal dendrites in gray matter which occurs even in late developmental stages – beyond 4 years old in human and from infancy to young adulthood in rats (Paydar et al., 2014, Cheung et al., 2009). Therefore, the widespread reduction of diffusion kurtosis in ethanol exposed animals may be a result of less synaptic refinement or decreased cell packing. Although this is the first study showing altered brain diffusion kurtosis after early ethanol exposure, lower MK has been reported in preterm infants and adolescents with attention-deficit hyperactivity disorder (Shi et al., 2016, Helpen et al., 2011) which is sometimes associated with prenatal alcohol exposure, and their neural alterations share some similarities (Bailey and Sokol, 2011, Burd, 2016).

The dose and treatment pattern in this study mimics multiple episodes of moderate to high levels of alcohol exposure in a fetus during the third trimester of gestation. Previously we have shown that the same paradigm of alcohol exposure leads to a disruption of the orientation tuning in individual neurons as well as a disorganization of the orientation selective columns in the ferret visual cortex (Medina et al., 2005). Moreover, we demonstrated that the exposure to alcohol during the period of brain growth spurt in mice affects contrast sensitivity but not spatial frequency acuity in visual evoked potentials and leads to a reduction in a and b- waves in electrocorticograms (Lantz et al., 2014). Additionally, alcohol-exposed mice presented alterations in cortical retinotopic maps. In non-human primates, moderate exposure to alcohol during late gestation (modeling third trimester exposure) reduced the soma size on magnocellular (M) but not parvocellular (P) neurons in the lateral geniculate nucleus (LGN) (Papia et al., 2010). Interestingly, the M-pathway is related to motion and spatial position, and an effect of alcohol in this stream may contribute to the visuo-spatial and visuomotor integration deficits seen in children with FASD (Doney et al., 2016, Gautam et al., 2015). In the current study, we observed altered functional connectivity between the PPC and PPr, one visual, and one visual-tactile integrative area. These findings suggest that the visual system abnormalities in FASD are not restricted to the primary visual cortex and the LGN, and can be observed even in visual associative areas.

The present study is the first MRI study on a ferret FASD model which should be viewed in the context of certain limitations. First, the amount and pattern of alcohol exposure used in this model may not represent all of the drinking situation in human. To get a comprehensive alteration profile within the visual-tactile integrative circuit after prenatal alcohol exposure, other exposure regimen such as daily mild exposure should be tested in future studies. Second, our study was unable to distinguish whether the brain loss was within the gray matter or white matter since the contrast between these two regions was suboptimal in the data for segmenting these two tissue types. Third, while the interpretations of the findings confirm previous reports, it should be noted that both rsfMRI and DKI are only indirect

methods to assess brain axonal and dendritic properties. Direct evidence from neural morphology studies is needed to confirm these findings.

Since neural development is a dynamic process future studies should build upon the findings presented here and look into the effects of developmental alcohol exposure on the developmental trajectory of brain structural and functional connectivity.

Conclusion

In this study, we observed alterations in cortical function and microstructure integrity in cortical areas involved in multisensory process with rsfMRI and DKI on a ferret FASD model. The findings indicate altered neuronal plasticity and neural maturation processes following early ethanol exposure that may lead to disruption in multisensory regions.

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Figure 1.

Brain volume decreased in both male and female ethanol exposed ferrets. The relative volume of frontal areas increased and occipital areas decreased in ethanol exposed ferrets.

A. Demonstration of slices; B. Slice volume; C. Relative regional volume of four brain sections: slice1–4 (occipital areas), slice5–9 (posterior parieto-temporal), slice10–15 (anterior parieto-temporal), slice16–21 (frontal areas). * $p < 0.05$, ** $p < 0.01$ between group difference (FDR corrected).

Figure 2.

Functional connectivity significantly increased between PPr and PPc in ethanol exposed ferrets. A. Demonstration of ROI locations a coronal view of the MRI images. B. Sagittal view of the ferret brain with localization of cortical regions and slices (I, II, III) used for rsfMRI studies; C. Functional connectivity between PPr-PPc and between PPr-S3. * $p < 0.05$; PPc= caudal portion of the posterior parietal cortex; PPr=rostral portion of the posterior parietal cortex; S3= tertiary somatosensory cortex; V1= primary visual cortex; A1= auditory cortex; S1=primary somatosensory cortex.

Figure 3.

Diffusion parameters in PPc(A), PPr (B) and S3 (C). Fractional anisotropy significantly increased in PPc ($p = 0.022$), while most kurtosis parameters significantly decreased in PPr (MK, $p = 0.011$, AK, $p = 0.024$, RK, $p = 0.012$), PPc (MK, $p = 0.010$, AK, $p = 0.011$, RK, $p = 0.013$), S3 (MK, $p = 0.007$, AK, $p = 0.015$, RK, $p = 0.005$). * $p < 0.05$, ** $p < 0.01$ between group difference.