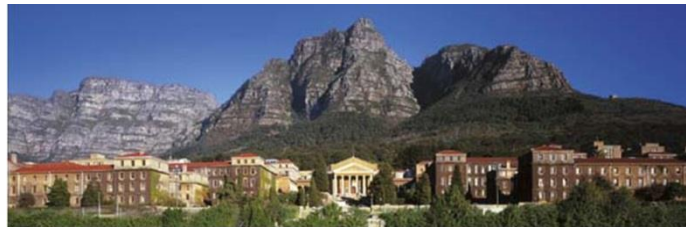# White matter integrity of the cerebellar peduncles as a mediator of effects of prenatal alcohol exposure on eyeblink conditioning

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❑ Eyeblink conditioning (EBC) is among the most sensitive endpoints affected in fetal alcohol spectrum disorder (FASD) [1,2].

❑ In EBC, the subject hears a tone, which is followed 550 ms later with an air puff to the eye. Over repeated trials, s/he learns to blink in response to the tone in order to avoid the aversive puff to the eye.

❑ The cerebellar peduncles, large bundles of myelinated nerve fibers that connect the cerebellum to the brainstem, constitute the principal white matter element of the EBC circuit [3].

❑ Diffusion tensor imaging (DTI), which yields a measure of white matter integrity [4], allows one to study anatomical links and fibre pathways between brain regions.

❑ The aim of the study was to assess white matter abnormalities in the cerebellar peduncles as a mediator of effects of prenatal alcohol exposure on EBC.

Methodology

- **Participants:** 54 children with FASD (11 FAS, 16 PFAS, 27 nonsyndromal heavily exposed (HE)), and 23 healthy controls (Ctl), mean age 10.1 ± 1.03 yrs, from the Cape Town Longitudinal Cohort Study [1].

- **Record of maternal alcohol consumption:** Timeline follow-back interviews [5] were conducted with the mother to record amount of alcohol consumed during pregnancy. Two interviews were administered during pregnancy and one at 1 month postpartum.

- **FASD participants:** Mothers consumed at least 14 standard drinks/week or engaged in binge drinking during pregnancy

- **Control participants:** Mothers abstained or drank<1 drink/month and did not binge drink during pregnancy.

- **FASD diagnosis:** All children were evaluated for FASD facial dysmorphology and growth at 5 years of age by three dysmorphologists.

- **Scanning protocol:** All children were imaged using DTI acquisitions with alternating phase encoding directions (i.e., anterior-posterior and posterior-anterior) on a 3T Allegra MRI. DTI was performed in 30 directions; 36 slices were acquired in an oblique axial orientation that included primarily infra-tentorial structures to provide high resolution cerebellar data. T2-weighted structural images were acquired with a matched spatial resolution and slice position.

- **Pre-processing:** Pre-processing included eddy current correction, which also includes correction for simple head movement, in FSL and susceptibility correction [6]. Outliers of each acquisition were examined by first calculating z-scores based on 25 and 75 percentile limits; any data points more than 3 standard deviations beyond the mean were discarded. The DTI acquisitions were averaged and FA images were generated. Unweighted B0)images were co-registered to their own T2-weighted structural image using linear and nonlinear co-registration algorithms in FSL. T2 images of controls were co-registered to the 'most representative' control image and then averaged to create a mean T2. All T2's were co-registered to this mean T2 image. The FA, MD images were warped using the same transforms to achieve intra- and inter-subject alignment. As a final step, co-registered FA's of all controls were averaged, whereafter individual FA and MD were co-registered to this mean FA.

- **Analyses:** The pre-processed FA's were masked manually to define the inferior, middle and superior cerebellar peduncles (FA threshold>0.2). Voxelwise group comparisons and correlations with alcohol exposure were performed in FSL. We report results that survive cluster size correction at $p<0.01$. Mean FA was determined in a 2x2x2 mm^3 ROI centred at the coordinate within each cluster
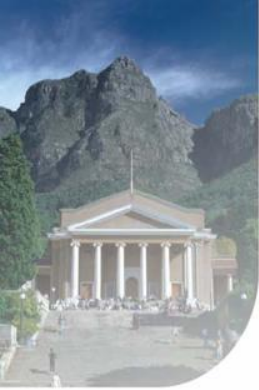
FA and MD Findings

FA = fractional anisotropy; MD = mean diffusivity

FA findings

Region (MNI peak coordinates)   r (AA/day)
L superior peduncle (-6, -43, -29)   -0.39***
R superior peduncle (7, -43, -28)   -0.39***
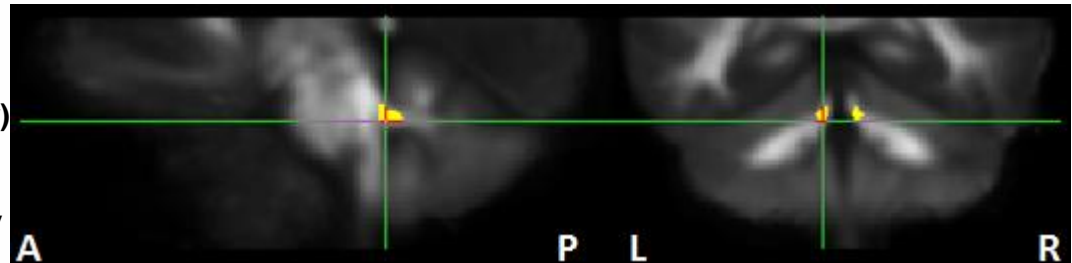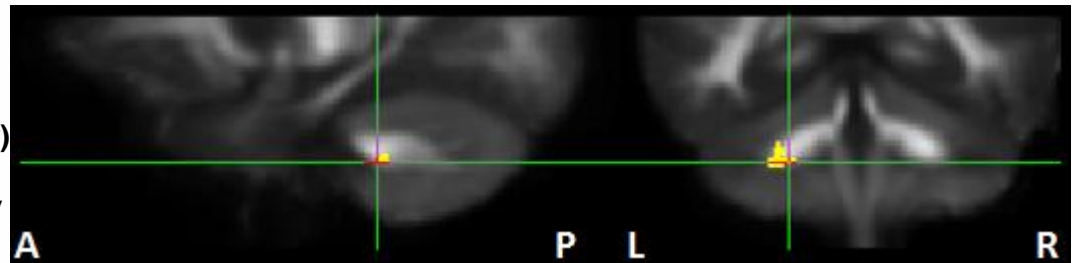AA/day=absolute alcohol per day during pregnancy
***$p<0.01$

Figure 1. Bilateral regions in the superior cerebellar peduncle (native space) where FA in children with FAS is lower than that in healthy controls. Cross-hairs indicate peak coordinates.

MD findings

Region (MNI peak coordinates)   r (AA/day)
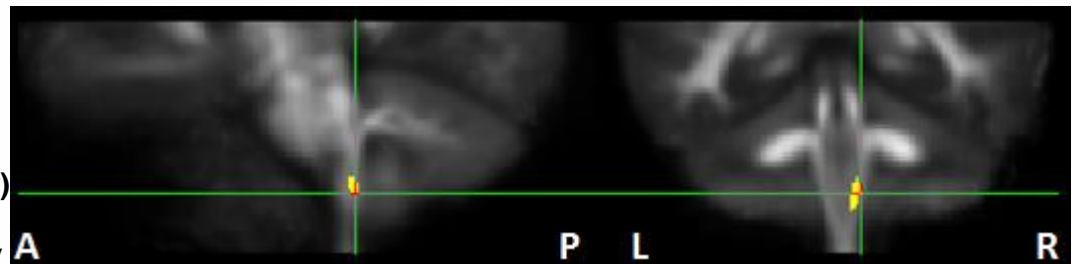L middle peduncle (-18,-35,-41)   0.37***
AA/day=absolute alcohol per day during pregnancy
***$p<0.01$

(a)

Region (MNI peak coordinates)   r (AA/day)
R inferior peduncle (5,-43,-57)   0.09
AA/day=absolute alcohol per day during pregnancy

(b)

Figure 2. Regions in the (a) left middle cerebellar peduncle (native space) where MD in the children with FAS or PFAS is higher than in healthy controls, and (b) in the right inferior cerebellar peduncle (native space) where MD in children with PFAS is higher than in healthy controls. Cross-hairs indicate peak coordinates.

Mediation of prenatal alcohol exposure on EBC

❑ Higher FA in the left and right superior peduncles was associated with poorer EBC performance at 5 years (r=0.39, p<0.001, and r=0.32, p<0.01).

❑ Higher MD in the left middle peduncle was associated with poorer EBC performance at 9-10 years (r=-0.28, p<0.05).

❑ Mediation of the effect on EBC was inferred for all three ROIs based on the significant reduction in the magnitude of the regression coefficients for exposure following the addition of the diffusion measures to the regression analyses.

FAS (yes/no) —————— -0.23[†] ——————→ Eyeblink conditioning (5 yr)

-0.34** 0.31*

FA (left superior peduncle)
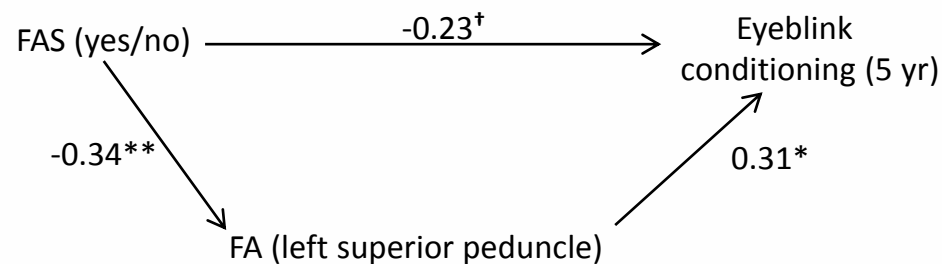
[†] p<0.10, *p<0.05, **p<0.01

Figure 3. Path model illustrating mediation of the effect of FAS status on eyeblink conditioning by lower FA in the left superior cerebellar peduncle

Conclusions and Acknowledgments

Conclusions

❑ Higher MD in left middle peduncles was found in children with FAS or PFAS, which confirmed our previous study on a different sample of Cape Town school-age children with FASD [7], providing evidence that the alcohol-related deficits in EBC may be mediated, in part, by microstructural deficits in this region.

❑ This study also provides evidence of lower FA in the superior peduncles specifically in the children with full FAS, which may account, in part, for their heightened risk for impairment in EBC performance.

❑ Consistent with what is known from animal models about the neurophysiology of EBC, the microstructural deficits in both regions of the cerebellar peduncles were related to poorer EBC performance.

❑ Moreover, data from mediation models provided statistical evidence that poorer microstructural integrity in the cerebellar peduncles may play an important role in the eyeblink conditioning deficit commonly seen in fetal alcohol-exposed children.

❑ Last but not least, significant correlations between DTI measures (FA and MD) and alcohol exposure in ROIs in these regions suggest dose-dependent impairment in these regions.

References

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