A T2 MR Study of Brain Development in a Valproic Acid Model of Autism

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Introduction: While neurobiological anomalies have been reported in autism spectrum disorders (ASD), the mechanisms underlying these neuroanatomical alterations is less known. Abnormal regulation of brain growth in ASD has consistently been demonstrated in human neuroimaging studies[1], suggesting that the processes governing apoptosis and synaptic pruning are highly implicated. In fact, accelerated early neuronal growth[2], followed by excessive synaptic pruning is hypothesized to underlie the aberrant cortical connectivity observed in ASD. The aim of this study was to investigate brain tissue changes in the valproic acid (VPA) animal model of ASD[3] as well as placental transfer[5] of VPA and its implications. *In vivo* T_2-relaxometry measurements were collected for both brain and placental analysis.

Methods: *Experimental Design:* Using a within-litter design, pregnant Long-Evans rats (n=3) were exposed orally to VPA (800mg/kg) – mixed with peanut butter – on gestational day (GA)12.5. Control dams (n=3) received peanut butter alone. Twenty-four Long-Evans male and female rats born to the VPA dams (n=12) and control dams (n=12) were utilized in this study. On postnatal (P) day 30, P35, P40, and P45, the offspring were imaged using a 4.7 T Oxford magnet (Oxford, UK). Rats were perfused on P60 and the brains were extracted for histological processing. All procedures were approved and in accordance with the Canadian Council of Animal Care and the University of Lethbridge Animal Welfare Committee. *MR Imaging:* The imaging protocol was comprised of: A) Localizer images (SE-TR/TE 700/16 ms, 0.2x0.2x1.5 mm^3), B) T_2 measurements (TE/ TR 21, 25, 35, 60, 90/2500 ms, 0.2x0.2x2 mm^3), and C) T_2-weighted images (TE/TR 22/3000ms, 0.2x0.2x2 mm^3). A similar sequence protocol with the respective parameters modified was used to image the pregnant dams at GA=16, 18 and 20 days: D) T_2 measurements (TE/ TR 16, 20, 30, 60, 90/2500 ms, 0.9x0.9x3mm^3), and E) T_2-weighted images (TE/TR 22/3000ms, 0.9x0.9x3mm^3). A region of interest (ROI) based analysis was performed for both studies and mean values were extracted from the T_2 maps with Image J (NIH, USA). A ROI corresponding to the prefrontal cortex (PFC; Figure 1B, 1C and 1D) was chosen for the pups while the abdomen placental ROIs were drawn on the central slice of all control and VPA dams (Figure 1A). T_2 values were calculated using Marquardt-Levenberg fitting routines from software written in our lab in IDL (ITT, USA). All images were co-registered.

Results: *T_2-Relaxometry:* There was a significant mean difference in the T_2 values from P30 to P45 in the VPA [12.157 ± 6.933] and control groups [25.616 ± 6.781], in the PFC (Figure 1C and 1D). This mean difference in T_2 values suggest that prenatal exposure to VPA altered the expected neuronal density in PFC. A clear trend of altered T_2 values was observed across all postnatal ages (Figure 1C, see significance). A parietal cortex ROI was also quantified, which produced a trend similar to the PFC.T2 values for the placental ROIs in the VPA dams were altered from controls for all GA's investigated (Figure 1A).

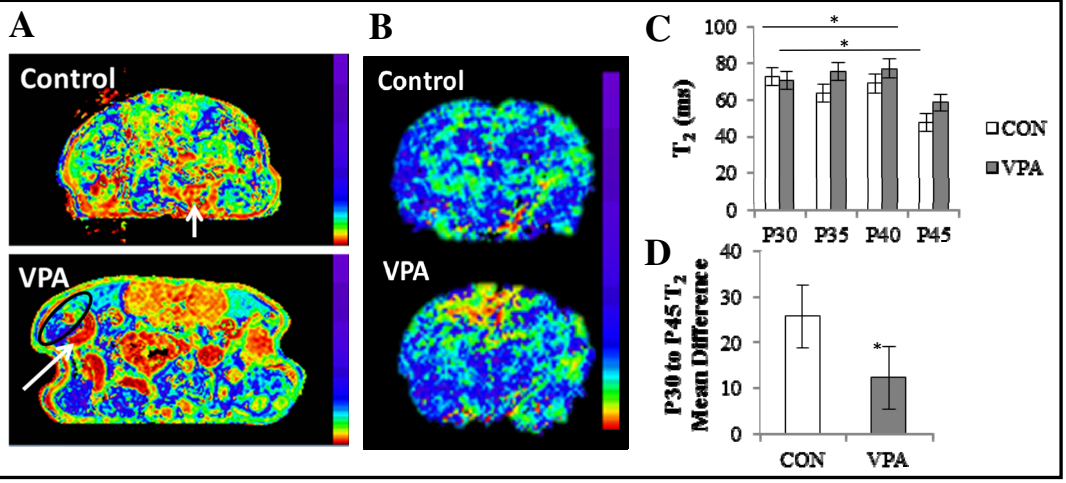

Figure 1.
A) T_2 maps of pregnant rats illustrating the clear differences in placental T_2 color gradient between VPA and control groups at G20. Arrows indicate placenta. Black oval indicates a fetus. **B)** T_2 maps of the pups at P30 brain illustrating the clear differences in the cortical T_2 color gradient. **C)** T_2 values for VPA and controls at P30, 35, 40, and 45 in the PFC. Values represent mean ± SEM. **D)** Mean difference in T_2 values from P30 to P45 for VPA and control groups.

Discussion: The results of the present study demonstrate that abnormalities in grey matter tissue density of the PFC in rats prenatally exposed to VPA can be detected using T_2-relaxometry. Both VPA and control animals exhibited significant decreases in T_2 values from P30 to P45, suggesting an increase in neuronal density during this adolescent period. While control animals showed significant increases in neuronal density from P30 to P45, as demonstrated by the mean difference in T_2 values, the effect among VPA animals was far less robust. That is, the neuronal density from P30 to P45 was altered in response to prenatal VPA exposure. Such changes may suggest that the developmental maturation among the VPA animals is altered, in comparison to control counterparts. This may reflect a delay in synaptic pruning, which is consistent with human ASD literature[4]. VPA transfer through placenta was evident in the T_2 maps. VPA crosses the placenta rapidly[5], and is found in larger concentrations in the umbilical cord and fetal villi, hence the more intense color gradient on the placental T_2 VPA maps.This outcome supports the evidence that VPA impacts fetal development pre and post-natally. Taken as a whole, T_2-relaxometry offers valuable insight into the neurobiological abnormalities induced by prenatal VPA exposure.

References:

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