V1a antagonism normalizes a social brain network in valproate rat model of autism revealed by functional MRI

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TARGET AUDIENCE: The presented data are of interest for scientists and clinicians involved in imaging and/or drug development with focus on neurodevelopmental disorders.

PURPOSE: Vasopressin, akin to oxytocin, is a neuropeptide thought to play an important role in regulating social behavior. Here, we investigated the role of vasopressin in the rat valproate (VPA) model of autism presenting autistic-like behaviors including social behavior deficits, increased repetitive behavior, sensory dysfunction and deficits in communication[1,2]. The aim was to identify a potential imaging endophenotype in the VPA rat model and, by making use of a potent, selective, brain-penetrant vasopressin receptor 1a (V1a) antagonist, to test the role of the V1a receptor in this particular animal model.

METHODS: The **animal model** used was previously described in detail[3]. Pregnant Wistar rats received a single intra-peritoneal dose of 600 mg/kg VPA (Sigma, UK) on gestational day 12.5 (E12.5). Control dams received an injection of dH_2O at E12.5. The study was conducted in male VPA-exposed offspring (VPA, n=8) with tail malformation and control animals (WT, n=21, WT & VPA w/o tail malformation). Group allocation was done by presence of a tail kink[4], a postnatal malformation used to confirm successful exposure to the teratogenic actions of VPA during gestation[5]. After assessment of baseline imaging (ASL-perfusion MRI, rs-fMRI) at post-natal day 60 (p60) animals were subjected to a 7-day **chronic treamtent** either with V_{1a} antagonist (RO, Hoffmann La-Roche, 90 mg/kg, daily, oral) or vehicle (VEH). At day 8, 24h after last dosing, animals underwent MRI follow-up assessment (ASL-Perfusion MRI). For imaging rats were sedated using a s.c. infusion of medetomidine (0.1mg/kg/h following a priming bolus of 0.2mg/kg). **MRI** was carried out on a Bruker BioSpec 9.4T system equipped with a 72 mm bird-cage resonator for excitation and a surface coil for reception. **Perfusion MRI** was obtained using a protocol described earlier[6]. Briefly, continuous arterial spin labelling (CASL) with centred-RARE readout (TR/TE = 3000ms/5.7ms, RARE factor = 32, FOV = 4cm x 4cm, 128 x 64 matrix, 1mm slice thickness, 8 slices, 2 averages, 2.5s labelling, 0.4s post labelling delay). For subsequent registration to an anatomical template with an associated atlas defining 62 regions of interest (ROIs), T_2-weighted anatomical RARE images were acquired. Perfusion values for each ROI were normalized slice-wise to brain-mean perfusion to derive region-specific values independent of inter-individual differences of the animals' global hemodynamic status. ROI-wise differences between the groups were tested for significant effects using univariate statistics (ANOVA, post-hoc). **Rs-fMRI** data were acquired using a T2*-weighted single shot gradient echo EPI sequence (TR/TEeff=2000/17.5ms; 128x128 matrix; 32x32mm² FOV; 20 coronal slices; 1mm thickness). Two scans of 165 EPI volumes were acquired per subject. EPI data were preprocessed in FSL v5.0 (steps: brain extraction, motion correction, high-pass filter >0.007 Hz, regression of motion parameters), and normalized to an in-house rat brain MRI template prior to spatially smoothing (0.5x0.5 mm²). Rs-fMRI time courses were extracted from 36 brain regions. We estimated functional connectivity (FC) by computing Pearson correlation coefficients of the rs-fMRI time courses, between all pairs of brain regions, resulting in a 36x36 covariance matrix, which was subsequently Fisher z-transformed for normality. Using two-sample paired t-tests, we investigated if there were any differences of the FC values between VPA and control WT rats.

RESULTS AND DISCUSSION: Compared to WT, offspring of VPA-exposed rats, assessed at P60, are characterized by a significantly altered regional perfusion in sensory and perirhinal cortex, superior and inferior colliculi, hippocampus, hypothalamus, ventral tegmental area (VTA) and dorsal striatum (Fig.1). Supplementary discriminant analysis in the feature space spanned by the ROIs corroborated group allocation based on the characteristic tail kink[5]. The main differences in FC between VPA and WT rats were observed between VTA and sensory cortices (decreased FC) as well as between motor and sensory cortices and several striatal regions (CPu, N.acc.), globus and ventral pallidum (GP, VP) and inferior and superior colliculus (increased FC) (Fig.2). One week of chronic treatment with V_{1a} antagonist RO normalized some of the perfusion difference present at baseline, specifically in dorsal striatum, VTA and superior colliculus in the VPA group with no compound effect in WT rats (Fig.3). The only other region which showed a significant difference between VEH and RO90 in the VPA group was the inferior colliculus. No effect of 7 day chronic treatment was observed in the hippocampus (data not shown). Due to the small differences in FC at baseline and the limited group size (VPA group split in VEH and RO) we did not include rs-fMRI in the follow-up study.

Taken together our data show that VPA rats present an imaging phenotype manifested as altered perfusion and connectivty in brain networks critically involved in motivation, reward and salience detection (striatum, VTA) as well as sensory processing (sensory and perirhinal cortex, colliculi). Deficient sensory processing, including hypersensitivity to sensory stimuli has been described as a hallmark of the VPA rat model[2,8] , and altered fMRI BOLD activation to primary sensory, but also to more complex social stimuli in VPA rats was recently desribed[9]. The finding of altered perfusion in the mesolimbic reward system (VTA and striatum) in VPA rats and its reversal by V_{1a} antagonsim is of high relevance in the context of the social motivation model, which integrates mesolimbic reward and social behavior network. This suggests that early impairments in the reward circuitry in autism reduces motivation for social experiences[10]. Altered striatal activity, on the other hand, has also been implicated in restricted repetitive behavior in OCD and autism.

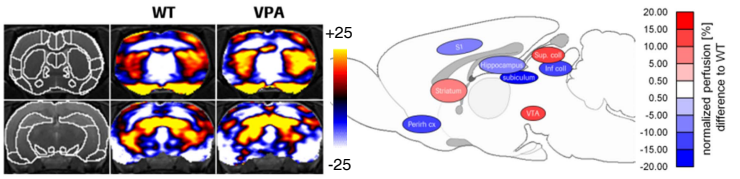

Fig. 1: VPA phenotype at baseline depicted as perfusion difference to WT. Left panel shows perfusion maps of WT and VPA at the level of the level of the striatum (increased in VPA) and subiculum (decreased in VPA) (Bregma -0.3mm and -5.3, respectively). Right panel shows perfusion differences on schematic sagittal representation of the rat brain.

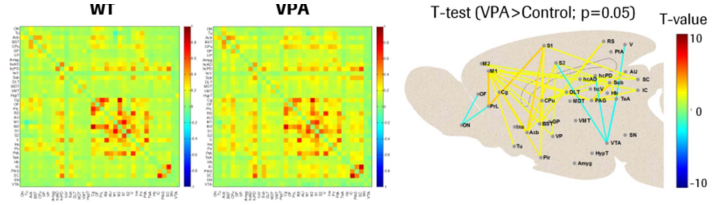

Fig. 2: FC matrix for Control WT and VPA rats and FC differences between the groups visualized on sagittal representation of the rat brain.

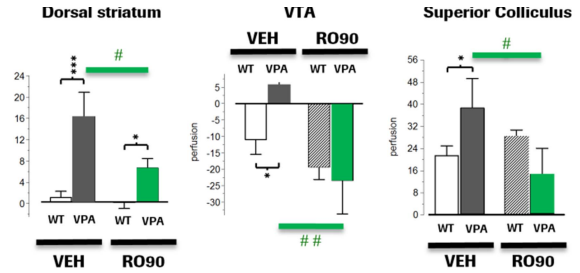

Fig. 3: Normalization of perfusion phenotype (WT vs VPA) in the dorsal striatum, VTA and superior colliculus after chronic treatment with RO90. * p<0.05, * * * p<0.001 WT vs VPA or # p <0.05, # # p <0.01 treatment effect

CONCLUSION: Our results demonstrate a functional phenotype in VPA rats with altered perfusion as a surrogate of disturbed neural activity in brain regions implicated in social behavior. Data from treatment study are indicative of an overactive vasopressin neurotransmission in the VPA model of autism and suggest that V1a antagonists have the potential to improve core symptoms of autism such as social interaction for which there is currently no drug treatment.

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