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Effect of chronic haloperidol treatment on the rat anterior cingulate cortex: linking neuroimaging findings with neuropathology

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Purpose:

- Neuropathological and neuroimaging studies suggest reduced gray matter volume of the cerebral cortex in schizophrenia, including the anterior cingulate cortex (ACC)[1].
- It is unclear to what degree these changes are due to illness or antipsychotic medication intake. Notably, treatment with typical antipsychotics is associated with decreased ACC volume in first episode schizophrenia patients[2].
- We have previously demonstrated that chronic haloperidol treatment at clinically relevant concentrations decreases the total neocortical volume of rats[3,4]. This provides a powerful model system to link neuroimaging changes induced by antipsychotics with those at the cellular level *post-mortem* to identify biological mechanisms.
- Using this rodent model, we first investigated the effect of chronic haloperidol treatment on brain volume using operator-independent deformation based morphometry (DBM) to localise drug-induced brain volume changes. The strongest DBM signal(s) in the cortex were then confirmed using manual segmentation and *post-mortem* histopathology to link volumetric changes identified by MR imaging with changes at the cellular level.
- We hypothesise that chronic haloperidol treatment is associated with a specific decrease in ACC volume in the rat neocortex.

Methods:

Male Sprague-Dawley rats were chronically administered either vehicle (*n*=8) or haloperidol (HAL; 2 mg/kg/day; *n*=7) using subcutaneously implanted osmotic minipumps for a total of 8 weeks[3]. MRI scans were acquired *ex vivo* as previously described[3] and analyzed using whole brain and specific region-of-interest (cortex, hippocampus, striatum) DBM[5]. The resulting statistical maps were corrected for multiple comparisons using the false discovery rate (FDR) procedure (q=0.05). To confirm DBM results, major cortical sub-field volumes were analyzed *post-mortem* from Nissl stained sections (1 in 12, 40 µm thick) using unbiased stereology procedures (Cavalieri probe). Specific changes were further investigated by manual segmentation of *ex vivo* MR images and stereological estimates of volume and neuronal number/density (Optical fractionator). These data were analyzed using two-tailed students t-test (SPSS v.20; IBM)

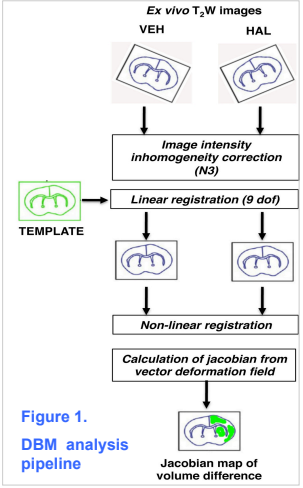
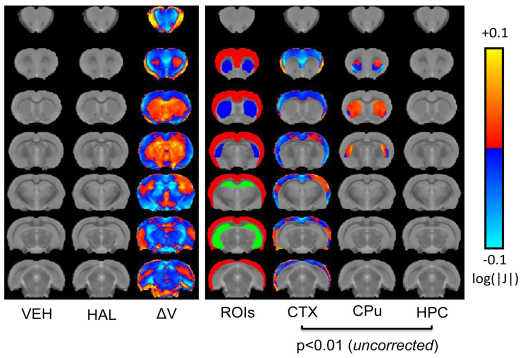


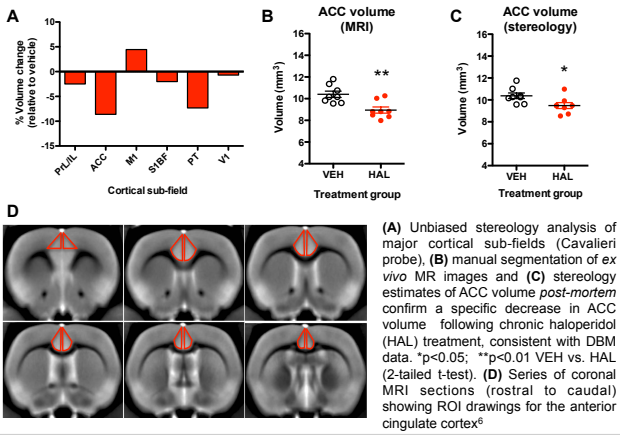
Figure 1. DBM analysis pipeline

Figure 2. Operator-independent DBM analysis reveals region specific brain volume changes induced by chronic haloperidol treatment



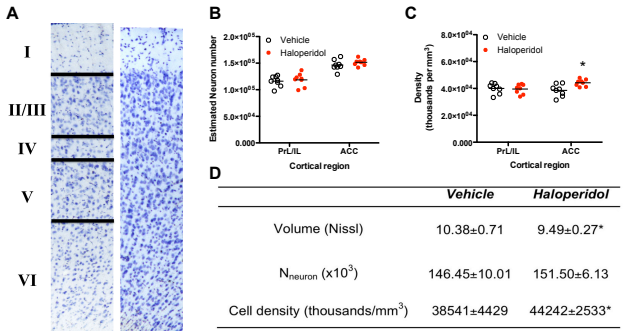
Regions of deficits (blue) and excesses (red) for brain tissue volume in animals treated chronically with either haloperidol (*n*=8; 2 mg/kg/day s.c.) compared to drug vehicle (*n*=8). Data shown are the raw Jacobian map of volume change (ΔV) and results of 9dof voxel-wise statistical analysis (*p*<0.01 uncorrected) using the ROI masks shown (CTX, cortex; CPu, caudate nucleus; HPC, hippocampus

Figure 3. Validation of DBM results: Manual segmentation and stereology identify a specific reduction in ACC volume following chronic haloperidol treatment



(A) Unbiased stereology analysis of major cortical sub-fields (Cavalieri probe), **(B)** manual segmentation of *ex vivo* MR images and **(C)** stereology estimates of ACC volume *post-mortem* confirm a specific decrease in ACC volume following chronic haloperidol (HAL) treatment, consistent with DBM data. *p<0.05; **p<0.01 VEH vs. HAL (2-tailed t-test). **(D)** Series of coronal MRI sections (rostral to caudal) showing ROI drawings for the anterior cingulate cortex[6]

Figure 4. Chronic haloperidol treatment has no significant effect on total neuronal number, but increases neuronal density in the ACC



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	Vehicle	*Haloperidol*
Volume (Nissl)	10.38±0.71	9.49±0.27*
N_{neuron} (x10^3)	146.45±10.01	151.50±6.13
Cell density (thousands/mm^3)	38541±4429	44242±2533*

(A) Representative Nissl stained sections from the ACC of VEH and HAL-treated animals. Laminar boundaries are indicated by roman numerals. Optical fractionator estimates of **(B)** the total number of Nissl stained neurons and **(C)** neuronal density per unit volume (Lamina I-VI) in the ACC and pre-limbic/infra-limbic (PrL/IL) cortex. (D) Summary table of neuronal density in the ACC. Note the increased density of neurons in the ACC of haloperidol treated group (*p<0.05; 2-tailed t-test).

Conclusions

- ✧ DBM analysis suggests region-specific changes in the volume of the rat brain following chronic haloperidol treatment.
- ✧ Manual segmentation and *post-mortem* histopathology confirm that chronic haloperidol treatment induces a decrease in cortical volume, localized in the ACC. This was not explained by a change in neuron number, but is associated with increased neuronal density, suggesting a loss of neuropil.
- ✧ These data are consistent with findings from first episode patients treated with typical APD[2,7]. However, ACC abnormalities also predate psychosis onset[1]. **Thus, typical antipsychotics may contribute to cortical volume changes, including the ACC, but cannot be the sole cause.**
- ✧ These data highlight the utility of this pre-clinical model system to investigate the neurobiology underlying antipsychotic medication induced changes in brain structure.

References: 1. Fornito et al. (2009). Schizophrenia Bulletin 35(5): 973-993; **2.** Dazzan et al. (2005) Neuropsychopharmacology 30: 765-744; **3.** Vernon et al. (2011) Biological Psychiatry; 69(10): 936-944; **4.** Vernon et al. (2012) Biological Psychiatry; 71(10): 855-863; **5.** Vernon et al. (2011) PLoS One; 6(2): e17269 **6.** Wolf et al. (2002) Brain Research Protocols 10: 41-46; **7.** Radua et al. (202) Neuroscience Biobehavioural Reviews http://dx.doi.org/10.1016/j.neubiorev.2012.07.012.

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