Cortical Effects of Chronic Haloperidol Administration in rats.

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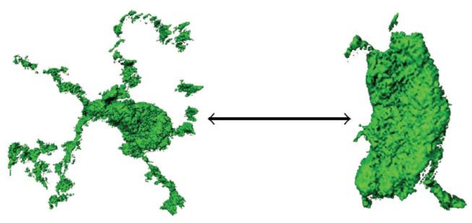
Background

Patients with schizophrenia are routinely prescribed antipsychotic medication. Magnetic resonance imaging (MRI) in newly medicated patients has demonstrates how, over a 5 year period, there is an average whole brain structural deficit of 8%, with regional losses of up to 20%, when compared to baseline [1]. A recent study investigating schizophrenic patients showed a regional increase in binding with the [C_{11}]PK11195 positron emission tomography (PET) radiotracer, which acts as a marker of neuroinflammation [2]. While these are changes since the start of medication, it is not possible to differentiate between changes arising from medication and those occurring with the disease progression. In the present study we dose naïve rats with haloperidol (Hal) to test the hypotheses that antipsychotic drugs induce brain volume loss and microglial activation. Recent studies have shown that microglial morphology is not a true representation of their level of activity, therfore in this investigation we use cell number as an indicator of inflammatory activity.



[3]

Aims

The Present study is designed to provide information about the influence of antipsychotic drugs on neuroinflammation and cerebral morphology.

- Do antipsychotics cause cerebral volume/mass/density changes?
- Is there an inflammatory component?

Methods

We used subcutaneous drug pellets to slowly release Hal over a two-week period in randomized mixed cages of control (n=9) and medicated (n=9) male Sprague Dawley rats. The drug pellets released a 0.05 mg/kg/day dose, control animals were implanted with a placebo pellet. The dose used is a comparatively low dose in terms of preclinical literature, with a similarly low D_2 receptor occupancy (~65%) when compared with clinical data (calculated from [4]).

Following perfusion, brains were dissected for post mortem analysis. The brain mass was measured and whole brain volumes were calculated using water displacement. Density was calculated from the mass and volume using the equation Density = Mass/Volume. The cortical tissue was processed for histological analysis, and changes in microglial cell number were determined to assess neuroinflammatory changes. Microglial cells were stained with Ionized calcium binding antibody 1 (Iba-1) and coverslipped using vectashield with an incorporated DAPI (nuclear) stain. Images were acquired using a Leica confocal microscope and images were analysed using Cell Profiler software (fig. 1) [5].

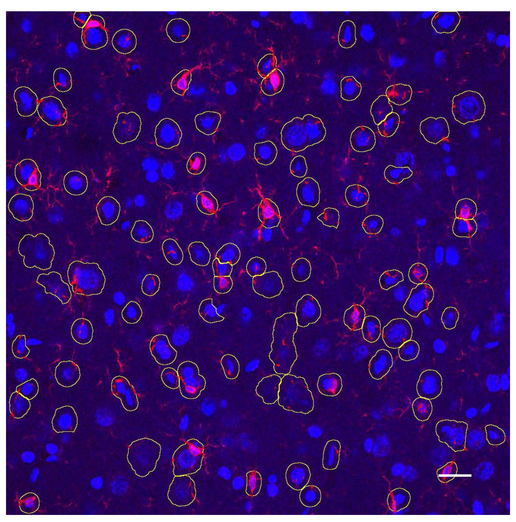


Figure 1: Colocalization of Iba-1 and DAPi neuclei were calculated to determine inflammatory changes associated with haloperidol treatment. Maximum projections were created from 3d stacked images. Scale bar = 50 µm.

Results 1

Here we show that there is a significant reduction in brain volume (fig. 2), mass (fig. 3) but not density (fig. 4) in Hal treated animals (1.58 cm^3 (±1.1 SEM), 2.25g (±0.029 SEM)) when compared with placebo controls (2.17 cm^3 (±1.5 SEM), 2.36g (±0.035 SEM)) ($p<0.05$, independent samples t-test). The body weight of animals did not significantly differ between the treatment groups (313g Placebo, 356g Hal $p>0.05$).

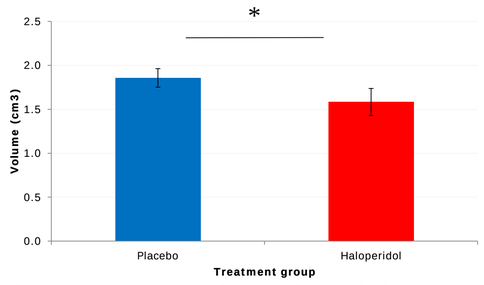


Figure 2: Mean cerebral volume following a two week treatment regime. Error bars +/- SEM p=0.011

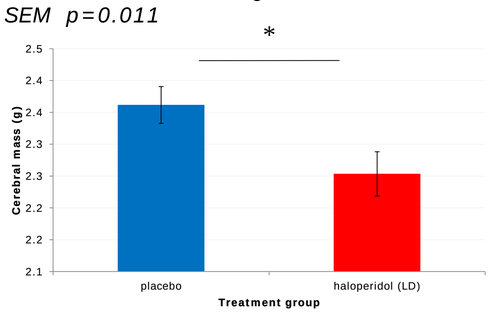


Figure 3: Mean cerebral mass. Error bars +/- SEM p=0.038

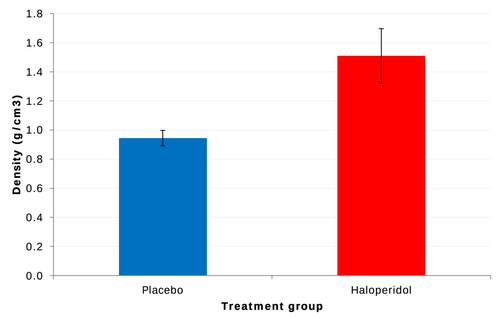


Figure 4: Mean cerebral density. Error bars +/- SEM p=0.063

Immunohistochemical quantification of colocalized Iba-1 and DAPI stains determined the number of microglial cells in the following regions of interest (ROIs); the prefrontal cortex (PFC), hippocampus (Hip) and ventral striatum (VS).

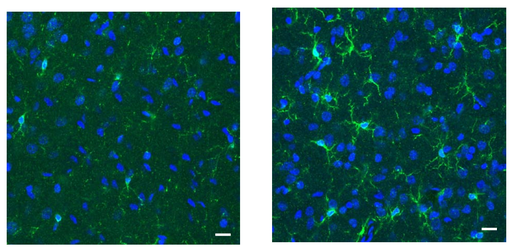
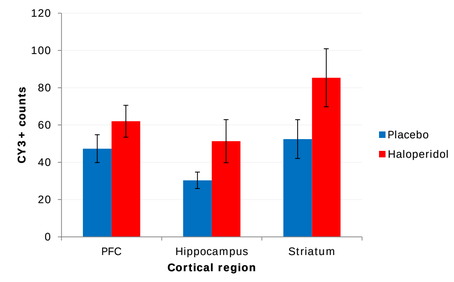


Figure 5: Iba-1/DAPI stained Prefrontal cortical ROIs (800 µm2 field of view in a 4 step z-plane stack) placebo (a) and haloperidol (b) (0.05 mg/kg/day). Scale bar = 50 µm.

Results 2

Further to global cerebral changes, we aimed to determine the inflammatory impact of low dose antipsychotic treatment. Using histological analysis we observed a consistent increase in microglial number (colocalized Cy3/DAPI nuclei) across the 3 ROIs in drug treated animals with significant trend ($p=0.07$).



	PFC (± SEM)	Hippocampus (± SEM)	Striatum (± SEM)
Placebo	47.3 (±7.4)	30.3 (± 4.4)	52.5 (± 10.4)
Haloperidol	62.0 (± 12.4)	51.3 (± 13.2)	85.3 (± 19.4)

Figure 6: Regional increases in cortical microglial cell counts.

Conclusion

The results we have seen here show how a low dose of a typical antipsychotic medication is able to produce significant changes in the brains of naïve rats. Further investigation is required to determine the full effects of these changes and the mechanism involved. An experiment investigating the effects of a higher dose regime is in progress and will elucidate the consequences further. While evaluating the results of this preliminary investigation, the impact of function needs consideration, as the changes we see here may not be detrimental to cognitive ability.

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References

1. Doorduin, J., de Vries, E.F.J., Willemsen, A.T.M., de Groot, J.C., Dierckx, R.A., and Klein, H.C. (2009). Neuroinflammation in Schizophrenia-Related Psychosis: A PET Study. Journal of Nuclear Medicine
2. Thompson, P.M., Vidal, C., Giedd, J.N., Gochman, P., Blumenthal, J., Nicolson, R., Toga, A.W., and Rapoport, J.L. (2001). Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proceedings of the National Academy of Sciences
3. Tremblay, M.-Ã.v., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The Role of Microglia in the Healthy Brain. The Journal of Neuroscience
4. Kapur, S., VanderSpek, S.C., Brownlee, B.A., and Nobrega, J.N. (2003). Antipsychotic Dosing in Preclinical Models Is Often Unrepresentative of the Clinical Condition: A Suggested Solution Based on in Vivo Occupancy. Journal of Pharmacology and Experimental Therapeutics
5. Kamentsky L, Jones TR, Fraser A, Bray M, Logan D, Madden K, Ljosa V, Rueden C, Harris GB, Eliceiri K, Carpenter AE (2011) Improved structure, function, and compatibility for CellProfiler: modular high-throughput image analysis software. Bioinformatics

No potential conflicts of interest