Effect of Chronic Antipsychotic Treatment on Brain Structure: A Serial Magnetic Resonance Imaging Study with Ex Vivo and Postmortem Confirmation

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Background: There is increasing evidence that antipsychotic (APD) may affect brain structure directly. To examine this, we developed a rodent model that uses clinically relevant doses and serial magnetic resonance imaging (MRI), followed by postmortem histopathological analysis to study the effects of APD on brain structures.

Methods: Antipsychotic , haloperidol, and olanzapine were continuously administered to rats via osmotic minipumps to maintain clinic-like steady state levels for 8 weeks. Longitudinal in vivo MRI scanning (T_2-weighted) was carried out at baseline, 4 weeks, and 8 weeks, after which animals were perfused and their brains preserved for ex vivo MRI scanning. Region of interest analyses were performed on magnetic resonance images (both in vivo as well as ex vivo) along with postmortem stereology using the Cavalieri estimator probe.

Results: Chronic (8 weeks) exposure to both haloperidol and olanzapine resulted in significant decreases in whole-brain volume (6% to 8%) compared with vehicle-treated control subjects, driven mainly by a decrease in frontal cerebral cortex volume (8% to 12%). Hippocampal, corpus striatum, lateral ventricles, and corpus callosum volumes were not significantly different from control subjects, suggesting a differential effect of APD on the cortex. These results were corroborated by ex vivo MRI scans and decreased cortical volume was confirmed postmortem by stereology.

Conclusions: This is the first systematic whole-brain MRI study of the effects of APD, which highlights significant effects on the cortex. Although caution needs to be exerted when extrapolating results from animals to patients, the approach provides a tractable method for linking in vivo MRI findings to their histopathological origins.

Key Words: Brain structure, cortex, haloperidol, magnetic resonance imaging, olanzapine, schizophrenia, striatum

The advent of atypical antipsychotic (APD) in the 1990s created an impression they were safer than previous typical APD (1). Early intervention, use in bipolar disorders, polypharmacy, and increasing off-label use to treat children and adolescents for aggressive behavior have led to a dramatic increase in prescriptions in the last decade (2–6). Along with this increase in use, evidence from clinical and primate postmortem studies suggest chronic exposure to APD may be associated with a reduction in brain volume, particularly gray matter (7–10). While the data are not unequivocal (11,12), the increasing use of APD makes it critical that this issue is examined rigorously.

Postmortem brains from schizophrenic patients show significant structural abnormalities (7,13–18), with evidence for slight shrinkage (~5%) of the brain in terms of weight, length, and cortical volume (14,19,20) and for enlarged (~15%) ventricles (13,14,19–23). These studies come from patients with a long duration of illness and APD exposure; thus, distinguishing effect(s) of illness from APD becomes difficult. Interestingly, longitudinal studies suggest the degree of change in frontotemporal cortical gray matter is often associated with intensity or duration of APD treatment (24–29). However, the lack of longitudinally followed untreated patients as a control means it remains unclear whether this outcome is the effect of illness progression or APD treatment. Further, none of the human studies have linked the imaging changes to postmortem findings and therefore the relationship between imaging-related structural changes and postmortem findings remains unclear (7,15).

Animal studies have usually focused on a single APD (haloperidol [HAL]), often given at doses 10 times higher that the clinical dose and with inappropriate pharmacokinetics (7,30). The only rigorous postmortem study to date, using clinic-like plasma levels and long-term exposure (2.5 years) (8,31) demonstrated a ~10% reduction in total brain weight and volume following treatment with either HAL or olanzapine (OLZ) in primates. This study suggests reduced brain volume and parietal gray or white matter reduction, traditionally accorded to the illness, may be influenced by APD exposure. However, these single-point, cross-sectional, histopathological studies do not use whole-brain imaging methods, limiting cross-species comparison with clinical measurements.

To overcome these limitations, we have developed a rodent model using a clinically relevant drug exposure by matching D2 receptor occupancy with a method of continuous delivery using osmotic infusion pumps (30). A typical APD (HAL) and an atypical APD (OLZ) were administered chronically (8 weeks). Effects on brain volume were determined from longitudinal in vivo magnetic resonance imaging (MRI) scans acquired at baseline, 4 weeks, and 8 weeks. These measurements were corroborated by ex vivo MRI and postmortem histology.

Methods and Materials

Animals

Male Sprague-Dawley rats (Charles River UK, Ltd., Kent, United Kingdom), initial body weight 240 g to 250 g (9 weeks of age) were housed four per cage under a 12-hour light/dark cycle (7:00 AM lights on) with food and water available ad libitum. Room temperature was maintained at 21 ± 2°C and relative humidity at 55 ±

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10%. Animals were habituated for 7 days before experimental procedures. Animal experiments were carried out with local ethical approval and in accordance with the Home Office Animals (Scientific Procedures) Act, United Kingdom.

Experimental Design

A repeated measures design was employed in which vehicle (β-hydroxypropylcyclodextrin, 20% wt/vol, acidified by ascorbic acid to pH 6), HAL (2 mg/kg/day; Sigma-Aldrich, Gillingham, Dorset, United Kingdom), and OLZ (10 mg/kg/day; Biophore Pharmaceuticals Ltd, Hyderabad, Andra Pradesh, India) were administered using osmotic minipumps for 8 weeks (approximately 5 human years, considering 11.8 rat days equals 1 human year) (32). The doses of each APD were chosen based on previous D2 receptor occupancy studies in our laboratory (30); serum plasma levels achieved following chronic administration in this study reflect D2 occupancy in the range of 75% to 90% (30), similar to clinical exposure. The osmotic pump delivers at a steady rate in comparison with daily injections where drug levels fall to undetectable levels in 24 hours (half-life $<$ 2.5 hours in rats for most antipsychotics). Each treatment group comprised $n = 8$ animals. The MRI-safe osmotic minipumps (Alzet Model 2ML4, 28 days; Alzet, Cupertino, California) filled with drug or vehicle solutions were inserted subcutaneously on the back flank under isoflurane anesthesia (5% induction, 1.5% maintenance) and replaced once after 28 days. In vivo MRI scans were acquired at baseline, 4 weeks and 8 weeks after the start of APD treatment. Animals were then killed by cardiac perfusion (.9% saline followed by 4% paraformaldehyde) under terminal anesthesia (sodium pentobarbital, 60 mg/kg intraperitoneal). Brains preserved in the skull were then scanned ex vivo and rinsed with phosphate-buffered saline before scanning, to assess changes due to tissue fixation. Postmortem, brain volumes were measured using unbiased stereology on Nissl-stained tissue sections (see below). Dyskinetic behavior, i.e., vacuous chewing movements (VCMs), was assessed at baseline, 2 weeks, 4 weeks, and 8 weeks after the start of APD treatment. This involved a simple measurement of purposeless chewing jaw movements in a 2-minute period, outside the home cage as described previously (33). A blood sample was collected at termination for estimation of drug levels, done commercially using tandem mass spectrometry. Body weight was measured biweekly, starting before minipump implantation until termination.

Magnetic Resonance Image Acquisition

In vivo T2-weighted (T2W) magnetic resonance (MR) images were acquired under isoflurane anesthesia (5% induction, 1.5% maintenance) in random order during each session, using a 7.0T horizontal small bore magnet (Varian, Palo Alto, California) with custom-built head radiofrequency coil (David Herlihy, Imperial College London, United Kingdom) connected to a console running VnmrJ acquisition software (v2.3; Varian) (34). In vivo T2W images were acquired using a multiecho, multislice spin-echo pulse sequence: field of view = 35 mm × 35 mm; matrix = 192 × 192; repetition time = 4200 msec; echo time = 10, 20, 30, 40, 50, 60, 70, 80 msec; 4 averages, 54 minutes. Ex vivo T2W images were acquired using modified multiecho, multislice spin-echo pulse sequence: field of view = 30 mm × 30 mm; matrix = 256 × 256; repetition time = 4200 msec; echo time = 10, 20, 30, 40, 50, 60, 70, 80 msec; 8 averages, 2 hours 30 minutes. For both in vivo and ex vivo scans, 50 contiguous 500 μm-thick coronal slices were acquired to cover the entire brain of the animal. Before analysis, MR images were visually inspected for motion or intensity artefacts. One animal from the haloperidol group (8 weeks) was excluded from further analysis on this basis.

MR Image Analysis

From in vivo and ex vivo MR images, whole brain, intracranial, cortical, and subcortical structure (striatum, hippocampus, lateral ventricles, corpus callosum) volumes were delineated manually by two reviewers (A.C.V. and S.N.) on a slice-by-slice basis in the coronal plane using the region of interest (ROI) tool in ImageJ software (National Institutes of Health, Bethesda, Maryland; http://rsb.info.nih.gov/ij/), blinded to treatment. For each structure analyzed, ROI contours were traced in both brain hemispheres at low magnification followed by manual correction of borders at higher magnification (34). Sample ROI contours for each region are shown (Figure 1). Measurements of T2 relaxivity in the cortex and striatum were made from

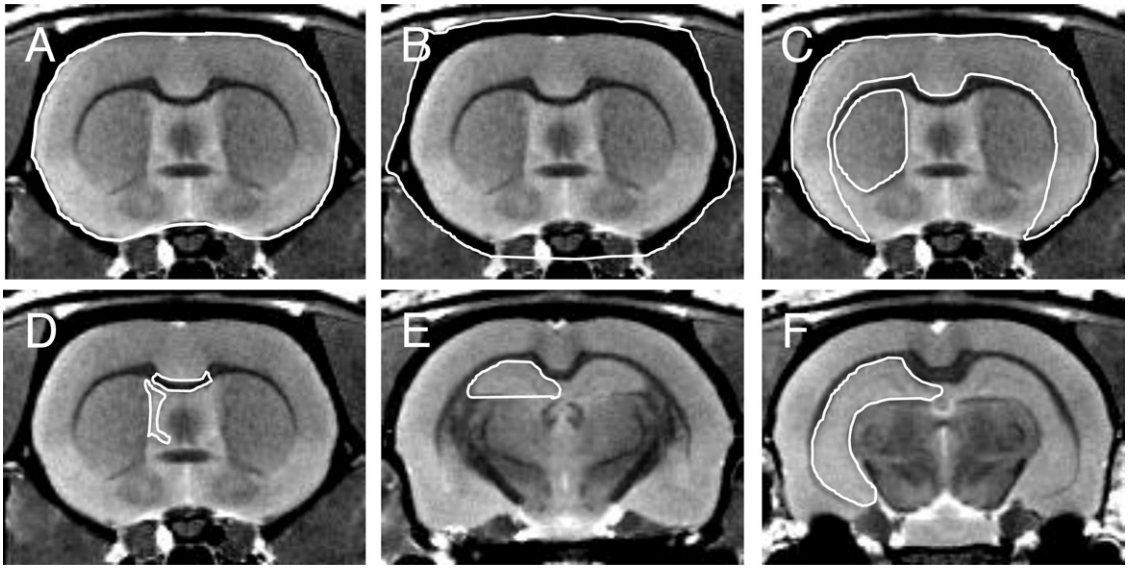

Figure 1. A representative set of ex vivo coronal T2-weighted images acquired from a control rat to illustrate region of interest contours used for manual outlining of **(A)** whole brain, **(B)** intracranial volume, **(C)** cerebral cortex and corpus striatum, **(D)** lateral ventricles and corpus callosum, and **(E)** dorsal and **(F)** ventral hippocampus on both in vivo and ex vivo images. Contour borders were defined as previously described (34) using a standard rodent brain atlas (35) and the anatomical criteria shown in Table S1 in Supplement 1.