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

Anthony C. Vernon, Sridhar Natesan, Mike Modo, and Shitij Kapur

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

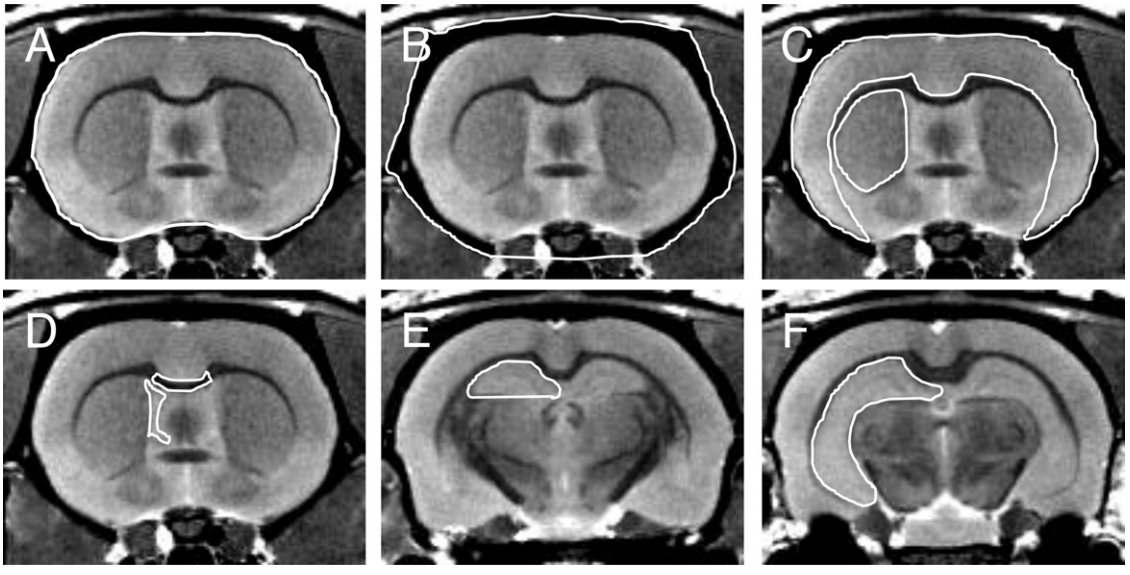

Figure 1. A representative set of ex vivo coronal T2-weighted images acquired from a control rat to illustrate region of interest contours used for manual outlining of **(A)** whole brain, **(B)** intracranial volume, **(C)** cerebral cortex and corpus striatum, **(D)** lateral ventricles and corpus 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.

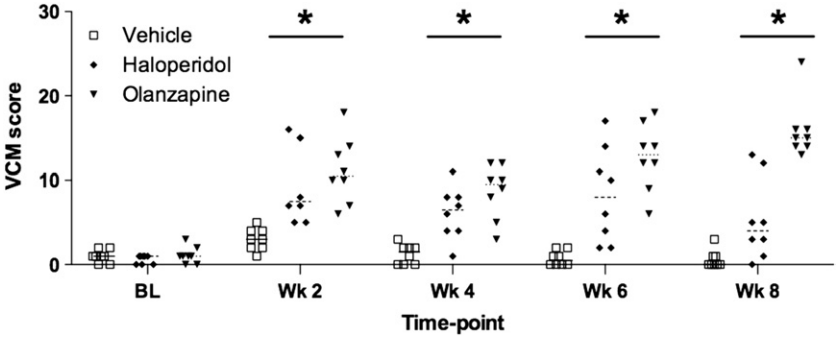

Figure 2. Chronic antipsychotic drug treatment induces stereotypical vacuous chewing movement behavior, which was apparent from 2 weeks of treatment onward and was maintained until 8 weeks. Data shown are individual vacuous chewing movement (VCM) scores (scatter plot with median) for control and antipsychotic drug-treated animals across time (*$p < .05$ haloperidol vs. vehicle and olanzapine vs. vehicle, respectively). BL, baseline; Wk, week.

quantitative T2 maps using an identical ROI-based approach (34). Clear anatomical landmarks and reference to the rodent brain atlas (35) were used to define ROI contours (Table S1 in Supplement 1). Volumes were calculated by multiplying the sum of the areas of a given structure on all slices measured by the slice thickness (500 μm). Intrarater and interrater reliability were assessed following repeated measurements using the intraclass correlation coefficient as previously described (36).

Postmortem Tissue Handling and Volume Measurements

Following ex vivo imaging, brains were removed and cryoprotected in buffered 30% sucrose for 48 hours before storage in tissue cryoprotection solution (25% glycerin [vol/vol] 30% ethylene glycol [vol/vol] in .2 mol/L phosphate buffer). Measurements of brain weight and volume were repeated at each step in the tissue processing, as described elsewhere (37). Serial coronal sections (40 μm, interval 1/12) were cut on a freezing microtome at −20°C and stored in tissue cryoprotection solution containing .05% sodium azide until further processing. Sections were washed in .1 mol/L phosphate buffer before mounting in series onto gelatin-coated slides and allowed to adhere by air drying before Nissl staining using cresyl fast violet solution (10% wt/vol; Sigma-Aldrich). A single observer (A.C.V.) blinded to experimental group by coding measured cortical and striatal volume using the Cavalieri estimator probe method. An Olympus microscope with charge-coupled device camera and XYZ motorized specimen stage (Olympus UK Ltd., Southend-on-Sea, Essex, United Kingdom) connected to a personal computer running Stereo investigator software v7.0 (MBF BioScience, Williston, Vermont) was used. Stereological analysis was carried out using systematic random sampling from a section interval of 1 in 12, choosing a random starting section for each series, over the entire brain corresponding to the number of slices measured on MR images. Briefly, a contour was drawn around the region corresponding to cortex or striatum in each section. A grid of points (150 × 150 μm) was superimposed over each section and all the points lying within the counter recorded automatically by the software. From these counts, using the Cavalieri method (38), the volume of each region was estimated as: $V = Ta\Sigma P_i$, where T is the mean slice thickness, a is the area per point, and P_i is the number of points hitting the marked region. Coefficients of error were calculated with values < .10 accepted (39).

Data and Statistical Analyses

Statistics were performed using SPSS 17.0 software (SPSS Inc., Chicago, Illinois). Longitudinal assessment of variables was performed using two-way repeated measures analysis of variance (ANOVA) with one between-subject factor (treatment) and one within-subject factor (time) followed by post hoc Bonferroni evaluation if criteria for statistical significance were met. Postmortem brain weight and volume changes and Cavalieri probe estimates of brain volume were analyzed using one-way ANOVA followed by Dunnett's post hoc procedure. Vacuous chewing movements because of APD treatment were analyzed using repeated measures nonparametric Friedman test followed by pairwise nonparametric comparisons using paired sample *t* test. Correlations between in vivo and ex vivo measurements were modeled using Pearson product moment or Spearman's rank correlation as appropriate. An α level of .05 was selected.

Results

Plasma Levels and Behavior

Administration of APD by osmotic pump achieved plasma levels (mean ± SD) of 20.58 ± 1.99 ng/mL for HAL and 60.13 ± 20.75 ng/mL for OLZ, respectively. The emergence of VCMs in both HAL and OLZ treated animals by 2 weeks confirmed animals were responding to the APD (Figure 2). However, there was poor correlation (Spearman's $\rho = .112$; ns) between drug plasma levels and VCM behavior at 8 weeks. The dosing regimen used in this study was tailored to capture clinical practice, i.e., OLZ with a median dose of 15 mg and HAL often in the range of 5 to 10 mg. The results may have been different if the minimal therapeutic dose (i.e., 10 mg of OLZ or 2–3 mg of HAL) were modeled. A detailed dose-response study may be helpful in the future. Control and APD-treated animals gained weight (Table 1); although, in the first 2 weeks, APD-treated animals gained less weight compared with the control group (Figure S1 in Supplement 1).

Intracranial and Whole-Brain Volumes

To establish the effect(s) of HAL and OLZ, whole-brain volume (WBV) was measured on T2W MR images. Potential confounding effects of animal growth can be identified by changes in intracranial volume. Although intracranial volume significantly increased with time (Table 2, Figure 3A), it did so comparably for all groups. Hence, there was no effect of APD treatment on growth (Figure 3A). To account for intra-animal variation in intracranial volume, their base-

Table 1. Change in Body Weight over Time in Vehicle- and Antipsychotic Drug-Treated Animals

Time Point	Body Weight (g) Vehicle	Haloperidol	Olanzapine
Baseline	271.1 ± 3.1	265.1 ± 3.3	269.8 ± 2.9
Week 2	312.3 ± 6.1	291.6 ± 6.8	291.8 ± 7.1
Week 4	342.6 ± 7.9	314.0 ± 7.8	323.8 ± 6.0
Week 6	354.4 ± 8.2	324.1 ± 9.3	328.8 ± 5.8
Week 8	353.0 ± 8.2	318.8 ± 9.1	324.4 ± 6.0

Data shown are body weight (mean ± SEM) for each treatment group at each time point.

Table 2. Summary of ANOVA for In Vivo MRI Quantified Brain Volumes

	Two-Way Repeated Measures ANOVA			
	Within Subjects		Between Groups	
Variable	Time	Time × Treatment Interaction	Treatment	Post Hoc Test (Bonferroni's Test for Multiple Comparisons)
Whole Brain Volume	$F(2,38) = 3.84; p < .5$	$F(4,38) = 3.47; p < .5$	$F(2,19) = 1.89; p > .5$	8 Wks $p < .5$ vehicle vs. haloperidol 8 Wks $p < .5$ vehicle vs. olanzapine 8 Wks $p > .5$ haloperidol vs. olanzapine
Lateral Ventricular Volume	$F(2,38) = 2.23; p > .5$	$F(4,38) = 2.91; p > .5$	$F(2,19) = .48; p > .5$	n.d.
Cerebral Cortex Volume	$F(2,38) = .21; p > .5$	$F(4,38) = 10.87; p < .5$	$F(2,19) = 2.33; p > .5$	8 Wks $p < .5$ vehicle vs. haloperidol 8 Wks $p < .5$ vehicle vs. olanzapine 8 Wks $p > .5$ haloperidol vs. olanzapine
Corpus Striatum Volume	$F(2,38) = 1.22; p > .5$	$F(4,38) = 1.23; p > .5$	$F(2,19) = 1.61; p > .5$	n.d.
Hippocampus Volume	$F(2,38) = .487; p > .5$	$F(4,38) = 1.45; p > .5$	$F(2,19) = .31; p > .5$	n.d.
Corpus Callosum Volume	$F(2,38) = .06; p > .5$	$F(4,38) = 4.03; p > .5$	$F(2,19) = 2.25; p > .5$	n.d.

Results of two-way repeated measures ANOVA statistics for longitudinal in vivo MRI volume measurements using intracranial volume as a covariate. APD treatment served as between-subject factor and time as within-subject factor.

ANOVA, analysis of variance; APD, antipsychotic drug; MRI, magnetic resonance imaging; n.d., not determined; Wks, weeks.

line measurement was used as a covariate for analyses of brain volumes.

Before administration of APD, there was no significant difference in WBV (Figure 3B). Chronic treatment (8 weeks) with HAL or OLZ resulted in significantly decreased WBV (Figure 3B), highlighted by a time × treatment interaction (Table 2). This mean percentage reduction in WBV was calculated to be −7.45% (HAL-vehicle) and −6.22% (OLZ-vehicle). There was no significant difference in this effect between HAL- and OLZ-treated animals. No significant effect of APD treatment on WBV was observed after 4 weeks treatment, suggesting long treatment duration is required for this effect. A significant correlation was observed between VCM behavior and WBV at 8 weeks (Spearman's $\rho = -.421; p < .05$).

Lateral Ventricles

There was no significant effect of time or APD treatment on lateral ventricle (LV) volume (Table 2, Figure 3C), as illustrated by representative MR images from each group (Figure 3D).

Cerebral Cortex and Subcortical Structures

To pinpoint the areas in the brain that account for smaller WBV observed after APD treatment, a detailed analysis of neuroanatomi-

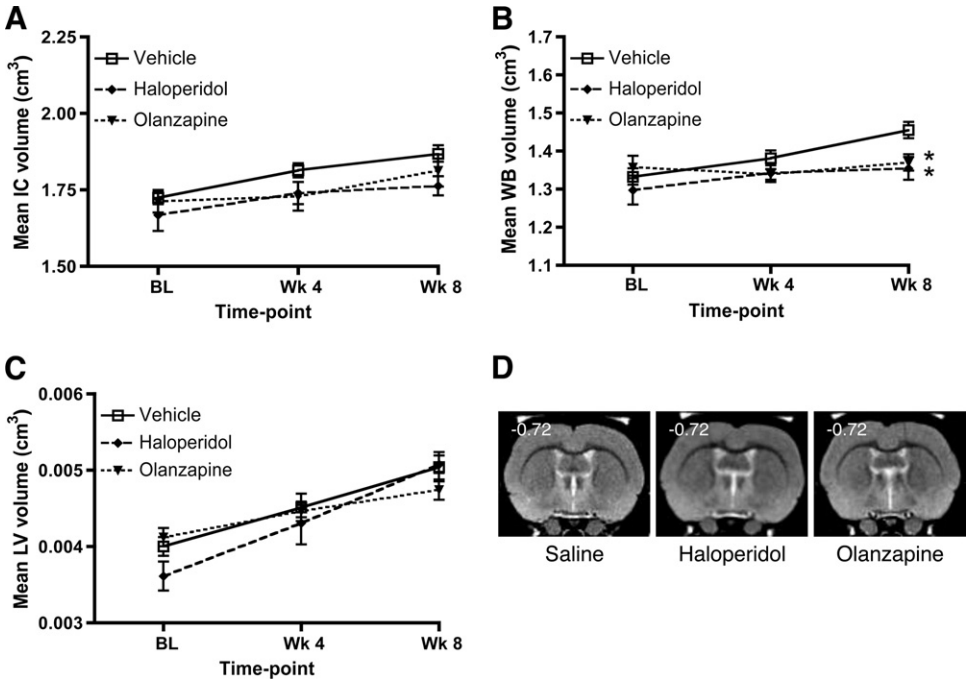

Figure 3. Chronic antipsychotic drug treatment results in a global decrease of in vivo whole-brain volume, with no effect on intracranial or lateral ventricle volumes. **(A)** Mean ± SEM intracranial volume in each group at baseline, 4 weeks, and 8 weeks of treatment. **(B)** Mean ± SEM whole-brain volume in each treatment group at baseline, 4 weeks, and 8 weeks of treatment (*$p < .05$ haloperidol vs. vehicle and olanzapine vs. vehicle, respectively). **(C)** Mean ± SEM lateral ventricular volume in each treatment group at baseline, 4 weeks, and 8 weeks of treatment. **(D)** Representative T2-weighted images from each treatment group at 8 weeks to illustrate the lack of lateral ventricular volume change (−.72, distance from bregma in mm). BL, baseline; IC, intracranial; LV, lateral ventricular; WB, whole brain; Wk, week.

Figure 4. Chronic antipsychotic drug treatment induces in vivo cortical volume decrease but no change in corpus striatum, hippocampal formation, or corpus callosum volume. **(A)** Mean ± SEM cortical volume in each treatment group at baseline, 4 weeks, and 8 weeks of treatment (*$p < .05$; **$p < .01$ haloperidol (HAL) vs. vehicle and olanzapine vs. vehicle, respectively). **(B)** Slice profile analysis of cortical volume in each treatment group suggests volume decreases in antipsychotic drug-treated animals are localized to frontal cortical regions (*$p < .05$; HAL vs. vehicle; δ $p < .05$; olanzapine vs. vehicle). **(C)** Mean ± SEM striatal volume in each treatment group at baseline, 4 weeks, and 8 weeks of treatment. Note the apparent striatal enlargement at 4 weeks, particularly in HAL-treated animals. **(D)** Mean ± SEM hippocampal volume in each treatment group at baseline, 4 weeks, and 8 weeks of treatment. **(E)** Mean ± SEM corpus callosum volume in each treatment group at baseline, 4 weeks, and 8 weeks of treatment. BL, baseline; CC, corpus callosum; CTX, cortical; HPC, hippocampal; STR, striatal; Wk, week.

cal structures was conducted. Cerebral cortex (CTX) volume was significantly reduced after chronic APD treatment (8 weeks) but not at any other time point (Table 2, Figure 4A). Both HAL-treated ($p < .01$) and OLZ-treated ($p < .05$) animals showed a significant reduction of CTX volume compared with vehicle-treated control animals (Figure 4A). No significant difference was observed between HAL- and OLZ-treated animals. The mean percentage reduction in CTX volume was calculated to be −11.99% (HAL-vehicle) and −8.08% (OLZ-vehicle), respectively. Slice profile analysis of cortical volume revealed highly significant effects overall for APD drug treatment [$F(2,420) = 121.8$; $p < .0001$] and slice position [$F(20,420) = 152.1$; $p < .0001$] but no interaction between APD treatment and slice position [$F(40,420) = .874$; $p =$ ns]. Post hoc testing confirmed that HAL- and OLZ-treated animals had significantly smaller slice volumes in the frontal cortex (Figure 4B). Notably, HAL treatment was associated with volume decreases in more widespread cortical areas, while OLZ-treated animals only showed changes in the frontal cortex. Changes in CTX volume were significantly correlated to VCM behavior at 8 weeks (Spearman's $\rho = -.522$; $p < .05$).

Longitudinal analysis of total corpus striatum (STR) volume (left + right hemisphere) revealed no effect of either time or APD treatment (Table 2). However, STR volume in HAL-treated, but not OLZ-treated, animals showed a trend toward an increase after 4 weeks of treatment, although this had normalized by 8 weeks (Figure 4C). No significant correlation was observed between VCM behavior and STR volume at 4 weeks (Spearman's $\rho = .328$; ns). However, when these data were stratified to exclude low responders (<5 VCM score), a trend toward correlation emerged (Spearman's $\rho = .447$; $p = .055$). Despite significant effects in the frontal cortex, there was no significant effect of APD treatment on hippocampal formation volume (Table 2, Figure 4D). To investigate whether white matter structures were affected equally to gray matter, we measured the volume of the corpus callosum. There was, however, no significant effect of APD exposure on this structure (Table 2, Figure 4E).

Comparison between in Vivo and Ex Vivo MR Imaging

To determine the effects of perfusion fixation on brain volumes following chronic APD exposure, we performed ex vivo high-resolution MRI on perfusion-fixed brains. Overall, there was a comparable reduction of brain volume after perfusion for each ROI in all treatment groups (Table 3). Importantly, good correspondence between in vivo and ex vivo MRI measurements for each brain region was highlighted by very significant correlations between volumes (Table 3). Further, the mean percentage reduction in WBV was comparable in vivo to ex vivo (HAL: −8.99% vs. −7.45%; OLZ −7.75% vs. −6.22%), as was the reduction in CTX volume (HAL: −8.73% vs. −11.99%; OLZ: −5.93% vs. −8.08%).

T2 Relaxivity Measurements

T2 relaxivity was measured in the CTX and STR (Table S2 in Supplement 1). Only a significant effect of time was evident in both the CTX [$F(2,42) = 10.11$; $p < .01$] and STR [$F(2,42) = 7.36$; $p < .01$]. Nevertheless, APD treatment did not influence tissue characteristics as measured by T2 relaxivity.

Postmortem Brain Weight and Volume Measurements

Fresh mean brain weights did not differ significantly between groups following dissection of the brains from the skull after perfusion and ex vivo imaging, although one-way ANOVA suggested a trend toward significant differences overall between groups [$F(2,21) = 2.941$; $p = .07$] (Figure S2 in Supplement 1). Similarly, there was no significant difference in brain volume, although,

Table 3. Comparison Between In Vivo MRI Scans and Perfused Brain Ex Vivo MRI Scans for Individual Brain Region Volumetric Data

Brain Region	Group	In Vivo MRI (cm^3)	Ex Vivo MRI (cm^3)	% Shrinkage	Correlation In Vivo to Ex Vivo
Whole Brain	Vehicle	1.451 ± .012	1.333 ± .027	8.12	.779[a]
	Haloperidol	1.324 ± .049	1.234 ± .049	6.76	
	Olanzapine	1.348 ± .022	1.238 ± .022	8.19	
Intracranial Volume	Vehicle	1.869 ± .026	1.653 ± .048	11.55	.539[a]
	Haloperidol	1.719 ± .051	1.616 ± .041	6.01	
	Olanzapine	1.813 ± .052	1.638 ± .032	9.69	
Cerebral Cortex	Vehicle	.398 ± .008	.366 ± .004	8.04	.851[a]
	Haloperidol	.346 ± .011	.333 ± .007	6.01	
	Olanzapine	.368 ± .004	.344 ± .009	6.62	
Corpus Striatum	Vehicle	.0530 ± .001	.0518 ± .001	2.26	.608[a]
	Haloperidol	.0544 ± .007	.0539 ± .001	.93	
	Olanzapine	.0528 ± .001	.0507 ± .001	3.97	
Hippocampus	Vehicle	.0548 ± .0008	.0527 ± .001	3.83	.669[a]
	Haloperidol	.0512 ± .0018	.0502 ± .004	1.99	
	Olanzapine	.0553 ± .0015	.0514 ± .0006	7.02	
Lateral Ventricles	Vehicle	.00503 ± .0002	.00500 ± .0003	.50	.649[a]
	Haloperidol	.00508 ± .0001	.00507 ± .0001	.26	
	Olanzapine	.00473 ± .0001	.00468 ± .0003	1.12	
Corpus Callosum	Vehicle	.0122 ± .0002	.0107 ± .0004	12.28	.418[b]
	Haloperidol	.0125 ± .005	.0097 ± .0005	22.41	
	Olanzapine	.0112 ± .006	.0103 ± .0002	7.40	

MRI, magnetic resonance imaging.
[a]Correlation significant at $p < .01$ level.
[b]Correlation significant at $p < .05$ level.

again, there was a trend toward significant differences overall between groups [$F(2,21) = 2.944$; $p = .07$]. Importantly, during the three phases of tissue processing, brain weight and volume, respectively, changed in a similar manner across the three exposure groups (Figure S2 in Supplement 1). Over time in storage, brain weight appeared to return to initial volumes, while brain volume did not (Figure S2 in Supplement 1).

Postmortem Measurement of Brain Volume Changes by the Cavalieri Estimator Probe Method

Postmortem volume analysis using the Cavalieri probe method revealed a significant overall difference in CTX volume between treatment groups [$F(2,21) = 7.574$; $p < .01$]. Post hoc testing confirmed a significant reduction in CTX volume of HAL-treated ($p < .05$) and OLZ-treated ($p < .05$) animals compared with vehicle-treated control animals (Figure 5A). For STR volume, no overall significant difference between groups was observed [$F(2,21) = 1.022$; ns] (Figure 5B). Volumes measured from in vivo MRI, ex vivo MRI, and these postmortem histological measurements were highly correlated and reinforced the same differences (Table 4).

Discussion

This is the first in vivo longitudinal study in rodents demonstrating that chronic (8 weeks) APD treatment results in altered brain morphology. We observed a decrease in WBV and CTX volume in rats chronically treated with therapeutically relevant doses of either HAL or OLZ (40,41). Exposure to either drug resulted in similar reductions in brain volumes, compared with vehicle-treated animals, the magnitude of these effects being 6% to 8% on WBV and 8% to 12% on CTX volume, respectively. The effects of APD were confined to the frontal cortex (prefrontal and cingulate cortex) and no effect was observed in the hippocampus. No significant effects of APD treatment were observed on the volume of the STR or LV. The volume of the corpus callosum was also unaffected, perhaps suggesting that APD effects are prominent in gray matter. We con-

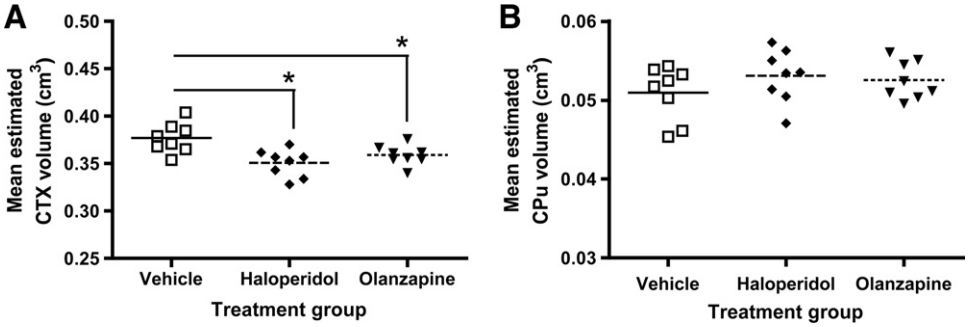

Figure 5. Postmortem confirmation of decreased cortical but not striatal volume in animals chronically treated with antipsychotic (APD) using the Cavalieri probe method. **(A)** Individual cortical volume (scatter plot with mean) in vehicle and APD-treated animals (*$p < .05$; haloperidol vs. vehicle and olanzapine vs. vehicle, respectively). **(B)** Individual striatal volume (scatter plot with mean) in vehicle and APD-treated animals. CTX, cerebral cortex; CPu, corpus striatum.

Table 4. Correlation Between In Vivo MRI, Ex Vivo MRI, and Postmortem Cavalieri Probe Measurements of Cortical and Striatal Volume of All Treatment Groups

Brain Region	In Vivo MRI—Postmortem Cavalieri Probe	Ex Vivo MRI—Postmortem Cavalieri Probe
Cerebral Cortex	.817[a]	.708[a]
Corpus Striatum	.538[a]	.830[a]

MRI, magnetic resonance imaging.
[a]Correlation significant at $p < .05$ level.

firmed our MRI findings postmortem using an unbiased stereology method. Significant correlations between in vivo, ex vivo, and postmortem findings validate our imaging findings.

The present results raise several important issues. First, our findings are consistent with previous postmortem studies in primates (8,31,37). These demonstrated that macaque monkeys chronically treated (2.5 years) with HAL or OLZ at dosages that produced a serum concentration equivalent to medicated patients showed an approximate 10% reduction in brain weight relative to monkeys receiving placebo. These changes were most robust in the frontal and parietal lobes (37), consistent with our findings in the rat cortex.

Second, the clinical literature suggests differential effects of typical and atypical APD on brain volumes (10). Striatal hypertrophy is commonly associated with typical APD, while atypical APD are associated with smaller striatal volumes (28,42–46). Importantly, previous chronic studies in rodents have lacked therapeutic equivalence, because animals were either dosed intermittently (daily injections) or not for sufficient duration; thus, it is difficult to make comparisons with the present study (7). We observed a trend toward striatal enlargement after 4 weeks in HAL-treated animals, but this normalized by 8 weeks, in contrast to extant data (47). This discrepancy may reflect a methodological difference in the analysis of brain volumes due to the use of adjusted volumes based on ratios between striatum and whole brain in this previous study (47). We observed no effect of OLZ treatment (8 weeks) on striatal volume. Potentially, striatal hypertrophy could therefore represent an early event following typical APD treatment (48); however, while some studies support this notion (49,50), others do not (51–53).

Third, HAL treatment (4 weeks) has been shown to increase hippocampal volume in rats (54). However, we did not observe any increase in HAL-treated animals, consistent with clinical data (55,56). Early volume changes during antipsychotic treatment may therefore reflect transient physiological changes associated with neuroplastic processes (57–61). Further studies are required to elucidate the relationship between acute APD treatment and brain volumes.

Importantly, our data are subject to limitations. First, the age of the rats at the initiation of treatment (10 weeks) corresponds to late adolescence in humans (32). Typically, the age of onset for schizophrenia is 15 to 18 years (adolescent) and 19 to 30 years of age (adult), respectively (62). Consequently, further experimentation in younger and older animals is required.

Second, it is not known if antipsychotic-induced structural brain changes relate to functional behavior. However, previous studies have shown chronic APD treatment (>75 days) results in disruption of spatial memory acquisition and retention in rats (63,64). Nevertheless, future studies are required to identify the functional consequences of APD-induced brain morphological changes.

Third, we used manual segmentation to measure brain volumes. This is a robust and widely used method of analysis (34,65) but it is labor-intensive, prone to bias, requires a priori hypotheses, and is relatively insensitive to subtle morphological change (66). We have limited potential bias through repeated measurements of the same structures at different times, by independent examiners blinded to animal treatment status. High intrarater and interrater reliability were achieved (>.9), suggesting robust segmentation of each brain structure. Application of voxel-based automated morphometric analysis techniques, for instance deformation based morphometry, may overcome the limitations of manual segmentation (66,67). However, voxel-based methods have their limitations and should be complemented by atlas-based methods (68), which yield quantitative measures of segmented brain regions.

Fourth, our experimental design does not address whether the APD-induced morphological changes are reversible, a question of significant theoretical and practical relevance. Drug withdrawal studies to address this possibility are currently underway.

Consistent with previous data (37), both HAL and OLZ resulted in similar effects on brain structure. A common mechanism to both these agents is antagonism of dopamine D2/3 receptors (1). Interestingly, D2 receptors are expressed in pyramidal cells within lamina V and VI in the rat prefrontal cortex (69), consistent with the location of volume change in the current study. Notably, blockade of D2/3 receptors results in increased turnover of dopamine in the acute and subacute phase (70,71), which may result in cytotoxic free radical generation and oxidative damage. Evidence from primate studies suggests this may be realized by a reduction in cortical glia cell number at the anatomical level (8,31); this is yet to be confirmed in rodent studies. Although a D2/3 receptor driven mechanism seems most plausible, a few issues need to be considered. The STR has the highest concentration of D2/3 receptors; thus, turnover-related oxidative stress would be expected to be highest therein. However, we found no volume change in the STR. It may be, therefore, that other mechanisms (delayed signaling via the Akt and glycogen synthase kinase 3 pathways) (72) or idiosyncratic mechanisms for each drug are responsible. Notably, APD-treated animals gained less weight in the first 2 weeks of drug treatment. These data may be interpreted as chronic APD treatment blunts somatic and perhaps brain growth, leading to lower brain volumes, as opposed to brain tissue loss, although incidentally this is observed only after 8 weeks. In the absence of a detailed post-mortem examination, however, we cannot exclude this possibility. Clearly, further analysis is required to unravel the neurobiology underlying APD-induced morphometric change.

Three main findings are commonly reported in schizophrenia: lateral ventricle and striatal hypertrophy and a robust reduction in cortical gray matter volume. Ventricular enlargement is the most robust finding in terms of brain morphological changes in schizophrenia and is probably less influenced by APD exposure (73–75). Our study found no effect of chronic APD treatment on LV, reiterating that this change is probably due to the disease process. Early striatal enlargement seems to be most clearly related to APD treatment, as it is seen only in drug-treated patients, particularly with typical APD, and is plausibly linked to D2 blockade (45,48,75,76). However, as stated earlier, striatal changes appear to be sensitive to timing after acute APD administration and may be reversible (45,48,75,76). Patients with schizophrenia show reductions in cortical gray matter volume and several studies raise the possibility that APD may exacerbate to this abnormality (25,28,76–79). Our data would be consistent with such a proposal. However, it needs to be pointed out that these studies were done in normal rats, while APD are given to patients with schizophrenia. The animals in the current study were healthy and did not capture the innate pathology of schizophrenia. Thus, while there is little reason to think animals modeling schizophrenia would not show this reduction, whether have an additive or synergistic effect with illness is unaddressed by this study. Further, as the mechanism of this effect remains unknown, until this effect is better understood or convincingly re-

futed, one should be very cautious in drawing clinical inferences. Lastly, this study had relatively small numbers and replication in a larger number of subjects will be an important advance.

Overall, the results indicate that chronic APD treatment in rodents leads to distinct morphological changes, primarily in the cerebral cortex. The confirmation of the finding in vivo, ex vivo, and validating it against postmortem data add certainty to the finding and offers a system to investigate the underlying neurobiology of apparent APD morphological changes in the brain.

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Supplementary material cited in this article is available online.

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