Centre for Addiction and Mental Health
Centre de toxicomanie et de santé mentale

Final Protocol

TITLE: **Effects of Maintenance Treatment with Olanzapine vs. Placebo on Brain Structure**

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Project Summary

Recent animal and human neuroimaging studies suggest that antipsychotic medications are potentially toxic to cortical brain structures. However, the human studies have not been placebo-controlled and cannot establish causality. Also, some studies suggest that antipsychotics are actually protective against structural brain changes associated with untreated psychotic disorders. With the growing number of younger and older patients receiving antipsychotics, determining the overall effect of these medications on brain structure is a public health imperative. For ethical reasons, this question cannot be resolved in patients with schizophrenia. We are proposing to conduct a pre/post MRI/DTI neuroimaging study in the context of STOP PD I, a newly NIMH-funded randomized, placebo-controlled trial of olanzapine in the continuation/maintenance treatment of major depression with psychotic features ("psychotic depression", PD).

The main aims of this application are to assess brain changes in gray and white matter associated with olanzapine treatment. Using a mixed model regression analysis, we will compare changes in brain structure and connectivity over time in patients in whom olanzapine has been continued or discontinued. We hypothesize that, compared to placebo, continuing treatment with olanzapine will be associated with: cortical thinning throughout all lobes; increase in striatal volumes; reductions in white matter microstructure; and no change in surface area or hippocampal and amygdala volumes. Our application leverages the clinical infrastructure (i.e., screening, recruitment, treatment, and clinical monitoring) provided by STOP-PD II: 38 participants who achieve remission after 12 weeks of acute open treatment with sertraline plus olanzapine and remain stable over the following 8 weeks will be randomized to continuing olanzapine vs. being switched to placebo. These participants will undergo a first ("pre") MRI scan and will be rescanned ("post") at the completion of the 36-week double-blind trial or at the time of relapse. This study is innovative and unique in its use of a randomized placebo-controlled design combined with advanced neuroimaging techniques: high resolution MRI (including DTI), measurements of cortical thickness, cortical surface area, microstructural integrity of white matter tracts, and subcortical striatal and limbic morphometry. It will extend and advance conventional volumetric approaches that have been used in humans and animals to assess longitudinal effects of antipsychotics on brain structure, providing a more biologically meaningful assessment of both gray and white matter.
In addition, 30 healthy control will be scanned at one timepoint to compare the same outcome measures of cortical thickness, cortical surface area, microstructural integrity of white matter tracts and subcortical striatal and limbic morphometry with participants who are in sustained remission from psychotic depression (i.e. at their baseline scan). There is currently a paucity of neuroimaging research in psychotic depression, and the neurobiology of disease is not well-understood. By comparing individuals with psychotic depression to healthy controls using neuroimaging, we hope to identify neurobiological correlates of disease. In addition, we also plan to explore relationships among neuroimaging measures and cognitive performance, given that many people with psychotic depression have impairments in cognitive function.

PUBLIC HEALTH RELEVANCE: This study has been designed to impact clinical practice by providing the first placebo-controlled evidence regarding the potential effects on brain structure and connectivity of

antipsychotic maintenance treatment in patients with mood disorders. Our results will address the unresolved question of whether antipsychotics are neuroprotective or neurotoxic in these patients.

1. Objective and Specific Aims

1A. Objectives:

The "Sustaining Remission of Psychotic Depression" (STOP-PD II study) aims to assess the efficacy of olanzapine, in combination with sertraline, in preventing relapse of major depressive disorder with psychotic features ("psychotic depression", PD). This randomized placebo-controlled trial (RpCT) offers a unique opportunity to prospectively assess the effect of continuing antipsychotic treatment on brain structure. Using an advanced neuroimaging approach, we propose to measure the impact of olanzapine vs. placebo on cortical thickness, cortical surface area, microstructural integrity of white matter tracts, and subcortical striatal and limbic morphometry in patients with PD with sustained remission or relapse.

Untreated psychosis associated with schizophrenia or mood disorders may have "toxic" effects on the brain[1]: on balance, antipsychotic treatment, particularly atypical antipsychotics, may mitigate these effects[2,3]. However, recent animal[4, 5] and human[6] data suggest that antipsychotics themselves may be neurotoxic and associated with substantial reductions in cortical brain volumes. Thus, in the face of conflicting evidence, the overall impact of antipsychotics on brain structure in humans remains undetermined. This important question has not been directly addressed in patients with schizophrenia or bipolar disorder due to methodological and ethical challenges associated with randomizing them to long-term treatment with an antipsychotic vs. placebo[7]. In this context, we believe a unique opportunity arises from the combination of the STOP-PD II funded RpCT and the experience of our research team with newer neuroimaging approaches such as diffusion tensor imaging (DTI) for white matter[8,9], and cortical thickness and surface area analyses for cortical gray matter[10,11]. These neuroimaging techniques can detect submillimetric brain changes that occur with very short periods of intervention[12-15].

Conducting longitudinal neuroimaging studies in patients treated for a severe mental illness requires a substantial clinical infrastructure and it is typically extremely expensive. Thus, very few such studies have been published, and there is no such study to date in patients with PD. We propose to build on the already funded clinical infrastructure of STOP-PD II to obtain state-of-the-art neuroimaging measures in the study group at two time points in the trial. An advantage of our proposal is that we follow the clinical trial design of the STOP-PD II study and scan participants at the time of randomization to olanzapine vs. placebo and 36 weeks later (or at the time of relapse). We plan to conduct high resolution MRI imaging to comprehensively assess gray matter morphology and white matter connectivity at these two time points. The proposed study would be the first to examine effects of continuing treatment with antipsychotic vs. placebo on brain structure and connectivity using these neuroimaging approaches in humans with mood disorders.

1B. Specific aims:

Primary aim: Controlling for psychotic relapse, to compare brain changes over 36 weeks in participants who have been randomized to olanzapine vs. placebo, using gray matter morphology approaches (cortical thickness, surface area, and subcortical volumetry).

Secondary aim: Controlling for psychotic relapse, to compare brain changes over 36 weeks in participants who have been randomized to olanzapine vs. placebo, using DTI-based measures of white matter microstructure.

Exploratory Aim: Controlling for treatment, to compare brain changes in gray matter morphology and white matter microstructure in patients with PD who remain well for 36 weeks and those who experience a relapse.

Hypotheses:
(1a) Compared to placebo, continuing treatment with olanzapine will be associated with cortical thinning throughout all lobes, but no association with surface area change will be found.
(1b) Compared to placebo, continuing treatment with olanzapine will be associated with increases in striatal volumes, and no change in hippocampal and amygdala volumes.
(2) Compared to placebo, continuing treatment with olanzapine will be associated with reductions in white matter microstructure.

Innovation: This study is unique in its use of a randomized placebo-controlled design combined with advanced neuroimaging techniques. It will impact clinical practice by providing the first placebo-controlled evidence regarding the potential effects on brain structure and connectivity of antipsychotic maintenance treatment in human participants.

2. Background and Significance

Increasing Antipsychotic Usage in the United States:
Before the mid-1990s, the use of antipsychotics was largely reserved for adults with severe psychotic disorders. Since that time, atypical antipsychotics have been increasingly used not only for adult patients with schizophrenia but also children, the elderly, and patients with bipolar or depressive disorder[16]. Expenses for antipsychotics are now exceeded only by lipid lowering medications and proton pump inhibitors[17]. Among these, olanzapine still accounts for 25% of sales of atypical antipsychotics in the United States and 30% of sales worldwide[18]. These figures have remained relatively constant in recent years[18]. Antipsychotics have now become the most costly drug class for Medicaid programs, exceeding the runner-up (antidepressants) by a wide margin. Beyond concerns regarding cost, patients and advocates are raising questions about the balance between the unquestionable benefits of antipsychotics vs. their newly identified risks.

Antipsychotic Effects on the Brain: A Crucial Consideration for the Risk/Benefit Analysis of Maintenance Treatment in Non Schizophrenia Populations:
During the past decade, the primary risk/benefit analysis for antipsychotic medications has focused on weighing the risk of metabolic side effects with atypical antipsychotics (or neurologic side effects with typical antipsychotics) vs. their effectiveness in the treatment of psychosis or in mood stabilization. More recently, the increased risk of mortality with antipsychotics in the elderly has prompted a “black box” FDA warning for their use in patients with dementia. In younger and older patients with schizophrenia, antipsychotics remain the foundation of treatment, in part because it is believed that antipsychotics protect against the harmful effects of untreated psychosis on the brain[3]. Some studies have shown that atypical antipsychotics appear to have protective effects against cortical brain volume loss thought to be associated with schizophrenia[2]. However, more recent data using longitudinal neuroimaging challenge this assumption[5,6]. A large study published by Ho, Andreasen et al., in 2011 reported that the greatest predictor of both cortical gray matter volume and cortical white matter volume loss over time was antipsychotic_treatment, NOT illness duration, illness severity, or substance abuse[6]. Furthermore, recent animal[4,5] studies have correlated exposure to both typical and atypical

antipsychotics to cortical brain volume loss over time. These findings have attracted substantial attention and raised considerable concern[7].However, due to the natural design of the human longitudinal imaging studies in schizophrenia (i.e., the absence of a placebo arm) we cannot rule out that the observed changes were due to factors other than antipsychotics. For example, disease effects could be responsible for all or part of these brain changes. Patients with the greatest symptom burden often require the highest antipsychotic doses, experience the greatest brain volume changes, and are more likely to abuse substances such as cannabis and alcohol that have substantial impact on brain structure[19,20].While published studies have attempted to control for these variables, their findings are based on observed associations and cannot demonstrate causation. In order to more definitely address this question, a placebo-controlled trial is necessary to demonstrate causation.

Antipsychotic vs. Placebo Effects on the Brain: A Call to Action:

In their recent paper[6], Ho, Andreasen et al., conclude that their finding may lead to heightened concerns regarding potential brain volume changes associated with the sharp rise in atypical antipsychotic use in non schizophrenia psychiatric disorders. They state that "[their] study could have been strengthened by having control groups, e.g., schizophrenia patients assigned to deferred or no antipsychotic treatment […] however, ethical standards in human research prohibit such comparison groups." Even if such a study could be conducted and these results could be confirmed, antipsychotics would remain the mainstay of schizophrenia treatment until viable alternatives are developed. However, the issue is different for patients with mood disorders or other non-schizophrenia disorders (e.g., anxiety disorders, borderline personality disorders…). The long-term use of antipsychotics in these patients could be dramatically curtailed if a deleterious effect on brain structure was proven. In a recent editorial[7], David Lewis calls for a neuroimaging placebo-controlled trial in a nonschizophrenia population. Stating that identifying an association does not necessarily indicate a causal relationship, he proposes: "an alternative means to address this issue may be available due to the increasing trend toward prescription of antipsychotic medications in the treatment of mood disorders, in which disease related decrements in brain volume are less prominent and widespread than in schizophrenia […] a positive finding would suggest a conserved medication effect that is independent of diagnosis or underlying disease process." We agree and propose to conduct such a study building on the clinical infrastructure of the STOP-PD II randomized placebo-controlled trial recently funded by the NIMH.

Leveraging the NIMH-Funded Trial, STOP-PD II for this Neuroimaging Application:

In STOP-PD II, following open acute treatment (12 weeks) and stabilization (8 weeks) with a combination of sertraline and olanzapine, participants with psychotic depression (PD) who achieve sustained remission (or near sustained remission) are randomized to continuing olanzapine or switched to placebo under double-blind conditions. The primary aim of this trial is to weigh the benefits (i.e., protection from relapse) of 36 weeks of continuation/maintenance treatment with olanzapine vs. the associated risks of metabolic and neurologic adverse effects. In PD, antidepressants are required both for acute and continuation/maintenance treatment[21, 22]. In the initial STOP-PD study, our research group showed that combination of sertraline with olanzapine during the acute phase of treatment results in a remission rate of 42% in the intent-to-treat sample and 67% in the completer sample[23].However, it is not known whether antipsychotics need to be continued once an episode has responded acutely to a combined antidepressant-antipsychotic treatment. The recently launched STOP-PD II study will provide clinical information about the rates of relapse and metabolic and neurologic adverse effects to assist clinicians with this important decision. This funded trial offers a unique opportunity to also assess the

effects of the antipsychotic olanzapine vs. placebo on brain structure in vivo in a nonschizophrenia population. Obtaining MRI/DTI scans in STOP-PD II at the time of randomization and 36 weeks later at the time of trial completion (or at the time of relapse) would impact clinical practice: it would provide the first placebo-controlled evidence regarding the effects on brain structure and connectivity of antipsychotic maintenance therapy in patients with mood disorders. Our research team brings scientific expertise and skills in both the clinical study of PD and sophisticated brain imaging techniques that provide biologically meaningful measures.

Medication Effects on the Brain: Moving Past Volumetric Neuroimaging Approaches to Assess the Cerebral Cortex – Cortical Thickness, Surface Area, and Diffusion Tensor Tractography:

Recently developed tools to assess gray matter morphology and white matter tract integrity can provide data that are more biologically meaningful than traditional volumetric approaches. These tools provide a new approach to resolve the conflicting findings of harmful vs. protective effects of antipsychotics on the brain and start to illuminate potential mechanisms underlying observed brain changes.

Advanced Neuroimaging in Gray Matter:

Cortical volume is determined by two different dimensions of the cortical sheet – surface area and cortical thickness, and is a product of these two measures. Cortical thickness and surface area capture distinct evolutionary[24], genetic[25, 26], and cellular[27] processes, which are otherwise conflated in the single measure of cortical volume. Disease states[28], common genetic variants[29], and environmental modifications[30] can all have distinct consequences for cortical thickness and surface area. Therefore, it is essential to measure them separately as antipsychotics may differentially affect each measure. Cortical thickness in particular is quite dynamic across the lifespan and can be influenced by environmental/intervention factors over short periods of time (e.g., weeks).[31]On the other hand, surface area is largely determined early in life, and has not been reported to change in the same manner as cortical thickness[31]. Therefore, we anticipate that antipsychotic effects on cortical volume may be mediated primarily via changes in cortical thickness rather than surface area. Members of our team have been instrumental in developing surface area and cortical thickness mapping tools that can pinpoint differences at submillimetric resolution between study populations[10]. The cortical thickness measurements that we propose to employ are meaningful quantitatively (i.e., measurements are provided in mm) and are operator independent (i.e., no regions of interest (ROIs) are drawn, etc.). We have validated this technique[32, 33] through manual measurements and a population simulation; we have used this technique successfully in cross-sectional and longitudinal studies, in children, adults, and the elderly[34-36]. Therefore, we are confident of its use in this proposal, which includes longitudinal imaging in younger and older adults.

Advanced Neuroimaging in White Matter:

Traditional MRI-based volumetric measurements of white matter are limited by various factors including partial volume effects, the fact that white matter appears homogeneous on conventional MRI and that fibers connecting different brain regions cannot be appreciated [37]. Using diffusion tensor imaging (DTI) followed by whole brain tractography, we have reliably segmented and measured white matter tracts in their native space, an approach that is well-suited to the proposed longitudinal study [9]. Ho et al.,[6] found that white matter volume is even more susceptible than gray matter to the effects of antipsychotics. However, it is unclear what these observed changes in white matter volume represent. DTI based approaches can resolve substantial inconsistencies from studies that have used traditional white matter volumetric approaches[38]. DTI represents major advances over volumetric approaches because it takes advantage of the diffusion of water to infer properties of the white matter tissue in a

manner not possible with any other neuroimaging tool[39]. In healthy white matter tissue, water diffuses parallel to myelinated fibers, a property known as anisotropy[40]. In diseased white matter tissue, diffusion is often more isotropic, and the magnitude of diffusion (mean diffusivity) is greater within a given voxel. Fractional anisotropy (FA) and mean diffusivity (MD) provide complementary information about white matter tract properties by indexing directionality of diffusion and magnitude of diffusion, respectively[41]. Differences in directionality and magnitude of diffusion may be due to smaller diameter of axons, loss of coherence of axonal membranes, or changes in myelination[42]. Furthermore, with DTI, potential changes in diffusivity due to effects on axons (axial diffusivity) and myelin (radial diffusivity) [43, 44] can also be determined, providing a more nuanced biological understanding of the effects of antipsychotic medication on white matter.

Antipsychotic Effects in Cortical Gray Matter (Aim 1 -- Hypotheses 1a)

Several studies in animals and humans, primarily conducted 5-10 years ago showed atypical antipsychotics, in particular olanzapine, in a favourable light with respect to effects on brain structure. For instance, in rats, olanzapine, but not haloperidol, increased neurogenesis in striatum and prefrontal cortex[45]. However, continued olanzapine treatment did not increase the survival of newly generated cells. Similarly clozapine, but not haloperidol, increased the number of newly dividing cells in the dentate gyrus of the adult rat; again survival of newly generated cells was not promoted by either antipsychotic[46]. In rhesus monkeys, 6 months of antipsychotic exposure led to glial proliferation and hypertrophy of the cerebral cortex, especially prefrontally[47]. These neuroscience findings were congruent with clinical observations that olanzapine and other atypical antipsychotics appeared to provide greater benefits for cognitive and negative symptoms than haloperidol and other older antipsychotics[48]. Similarly, neuroimaging studies, particularly in the first episode of schizophrenia, reported that olanzapine provided more protective effect than haloperidol against ongoing deleterious brain changes observed during this phase of the illness. For instance, olanzapine, but not haloperidol prevented a disease-related trajectory of cortical loss in first episode patients within the first 12 months of treatment[2, 3]. Olanzapine was also shown to help restore functional connectivity in patients with schizophrenia[49]. Finally, higher cumulative intake of typical antipsychotics in patients with schizophrenia was associated with more pronounced cortical thinning, while higher cumulative intake of atypical antipsychotics was associated with less pronounced cortical thinning[50]. The authors of all of these studies concluded that atypical antipsychotics confer protection against disease-related effects on the brain.

In contrast, the largest study conducted to date[6], highlighted in earlier sections above, implicates duration and magnitude of antipsychotic medication exposure with brain volume loss in schizophrenia after trying to control for effects of illness duration, illness severity, and substance misuse. Higher antipsychotic exposure was associated with smaller gray matter volumes; this was observed for the 3 classes of antipsychotics (typical, nonclozapine atypical, and clozapine). Other evidence for harmful effects of antipsychotics on the brain also emerges from very recent animal studies where in vivo MRI findings were confirmed postmortem. In rats treated with haloperidol or olanzapine for 8 weeks at doses providing equivalent dopamine D2 receptor occupancy to that recommended in humans, 6% and 8% volume declines respectively for whole brain were found compared to control animals. These volume reductions were driven mainly by a decrease in frontal cerebral cortex volume (8% for haloperidol and 12% for olanzapine)[5]. Similarly, in macaque monkeys, antipsychotic exposure over 17-27 months caused substantial decreases in brain weight and volume (8% for haloperidol and 11% for olanzapine), with the most pronounced effects in frontal and parietal lobes[51].

In summary, while initial evidence seemed to support protective effects of olanzapine against potentially toxic effects of psychosis on the brain, recent well-conducted animal studies, and one large recent longitudinal neuroimaging study in patients with schizophrenia demonstrate cortical gray matter volume reductions with antipsychotic treatment. This conflicting evidence highlights the need for a placebo-controlled trial to resolve this question of high public health relevance: are antipsychotics neuroprotective or neurotoxic in humans?

Antipsychotic Effects on Subcortical Structures (Aim 1 – Hypothesis 1b)

While the most recent alarming data on effects of antipsychotics on brain structure pertains to the cortex, effects on specific subcortical structures, namely caudate, putamen, and globus pallidus have been shown consistently for nearly two decades. These structures have substantially greater volumes in patients with schizophrenia exposed to typical antipsychotics over long periods than healthy controls[52]. In another study, a switch from typical antipsychotics to olanzapine led to substantial reductions (i.e., 10% of volume) in basal ganglia structures nearly reverting to volumes comparable to those of healthy participants over 45 weeks[53]. These findings are confirmed in rodent models[4, 54]. However, the effects on hippocampus and associated limbic structures (e.g., amygdala) with antipsychotics are unknown[55, 56]. The 8-week study in rats of haloperidol vs. olanzapine discussed above showed no change in hippocampal volume[5]. The recent longitudinal imaging studies in schizophrenia did not report hippocampal volume measures[6, 50]. The hippocampus and amygdale are critically important to human behaviour and cognition across psychiatric disorders and the lifespan[57, 58]. Thus, it is imperative to have a more definitive understanding of the effects of antipsychotics on these limbic structures. Our research team has developed novel morphometric methods to measure subcortical volumes[59] that we will use in the proposed study. These methods will allow us to assess whether long-term antipsychotic exposure is associated not only with changes in volume but also deformation in subfields of the hippocampus.

Antipsychotic Effects in Cortical White Matter (Aim 2 -- Hypothesis 2)

Like in cortical gray matter, the effects of antipsychotics in cortical white matter are also unclear with conflicting protective and harmful effects having been reported. For example, quetiapine, has been shown in mice to direct the differentiation of neural progenitors to oligodendrocyte lineage, facilitate myelination, and prevent cortical demyelination and concomitant spatial working memory impairment induced by the neurotoxin cuprizone[60]. DTI imaging in these mice confirmed quetiapine protective effects in vivo[61]. In patients with schizophrenia, atypical antipsychotics, compared to typical antipsychotics, have been associated with increased intracortical myelin volume[62, 63]. An increase in anterior internal capsule volumes following a switch from typical antipsychotics to olanzapine has also been shown[64]. A very small DTI study reported that reductions in microstructural integrity of white matter associated with psychosis were reversed following antipsychotic treatment in 8 patients who experienced remission[65]. We are not aware of any other published DTI longitudinal data addressing the effects of antipsychotics in humans. Some cross-sectional studies (including our own)[8] have failed to identify a relationship between exposure to antipsychotics and white matter integrity. Questioning these reassuring animal and human results, other studies suggest that antipsychotics are associated with reduction in white matter volumes. For instance, administration of haloperidol or olanzapine causes a reduction in glial cell number in macaque monkeys[66]. This mechanism has been hypothesized as a mechanism for cortical volume reduction[67]. In the study by Ho et al.[6], progressive decrement in white matter volume was most evident among

patients who received higher dose and longer duration of antipsychotic treatment. This was true for all 3 classes of antipsychotics studied. Furthermore, there was a time x treatment interaction for the effects of antipsychotics on white matter: i.e., the longer the duration of treatment, the greater the decrement in white matter volume, suggesting white matter may be particularly susceptible to the effects of antipsychotics.
Therefore, while antipsychotics appear to be associated with changes in white matter volumes, the data are conflicting, and the biological meaning of the reported volume changes is unclear. Our use of DTI will address the limitations of the available studies, clarifying the effects of antipsychotics on microstructural integrity of white matter tracts, structures that are essential for effective brain function.

Focus on Olanzapine
The sharp rise of atypical antipsychotic usage in mood disorders and other non-schizophrenia disorders and the recent neuroimaging findings reviewed above, have prompted calls to investigate the effects of antipsychotics on brain structure in a placebo-controlled trial[7]. Our proposed placebo-controlled study, will determine whether maintenance treatment with olanzapine changes brain structures. Olanzapine is the atypical antipsychotic that is being studied in STOP-PD II. Along with haloperidol, it is the antipsychotic that has been the most studied in animal models and in small human neuroimaging studies. It has the most evidence supporting its protective effects for the brain. However, some recent studies[5, 51] suggest that olanzapine could be equally, or even more harmful than haloperidol to cortical brain volume, especially in frontal cortex. In the clinical world, the ongoing widespread use of olanzapine underscores the importance of the proposed study. Combined with the rigorous assessment of olanzapine's benefits in preventing relapse and its other risks (i.e., metabolic or neurologic risks) from STOP-PD II, our neuroimaging data will provide patients and clinicians with comprehensive evidence about the benefits and overall risks of olanzapine as a maintenance treatment in PD. These data can also be used to estimate the risks of olanzapine in other mood disorders (e.g., unipolar non-psychotic major depressive disorder, bipolar disorder) and in other nonschizophrenia disorders, for which olanzapine is commonly prescribed.

3. Innovation
The question of how maintenance antipsychotic treatment affects brain structure is timely and of great public health importance. It is also of great interest to clinicians, patients, and advocates. Our placebo-controlled study design, powerful MRI/ DTI acquisition, and advanced analytical approach position us uniquely to answer this question. We believe our study is innovative in several ways:

- To our knowledge, it will be the first prospective placebo-controlled longitudinal antipsychotic MRI/DTI neuroimaging study in any human population.
- The placebo-controlled design provides a unique opportunity to assess causality regarding the effect of olanzapine on brain structure, rather than association, as in all human studies published to date.
- Our use of gray matter morphology measures in the cortex (Aim 1) rather than a more traditional volumetric approach will allow us to disentangle potentially different effects of olanzapine on cortical thickness and surface area (Aims 1, hypothesis 1a). As reviewed above, the measurements of cortical thickness and surface area are more biologically meaningful than cortical gray matter volume (which is a product of cortical thickness and surface area). Cortical thickness and surface area are under different genetic and environmental control; examination of olanzapine effects on these specific brain measures will illuminate the mechanisms underlying these effects.

- Our subcortical volumetric approaches will allow us to determine the effects of olanzapine on volumes of structures where effects are not yet well-known, namely hippocampus, amygdala, and thalamus (Aim 1, hypothesis 1b). Furthermore, our advanced morphometric analysis will allow us to determine deformations within hippocampal subfields, which are not detectable with conventional volumetric approaches (see above).
- Except for one small uncontrolled study (N = 8), this will be the first study to use DTI to examine longitudinally the effects of antipsychotics on white matter microstructure in humans (Aim 2, hypothesis 2). Data on the association of antipsychotic exposure with white matter volumes have been inconsistent. Our use of DTI will provide a much more sensitive index of white matter microstructure within the major cortico-cortical and cortico-subcortical tracts of the brain. It may allow us to infer the effects of antipsychotics on axons and myelin.
- To date, the only longitudinal MRI studies of the effects of antipsychotics on brain structures have been conducted in patients with schizophrenia. Our results will have important implications for nonschizophrenia disorders, particularly mood disorders (major depressive disorder, bipolar disorder), for which antipsychotic use is on the rise.
- All relevant MRI studies have been conducted in younger adults with schizophrenia. In STOP-PD II, about 50% of the participants are expected to be over 60 years of age. Thus, our results will also have implications in this specific demographic group that has been understudied. This is important because older brains may have different vulnerability to the effects of antipsychotics (the same way age influences their metabolic effects).
- Our study will be conducted with a 3T MRI machine, while all human investigations of the effects of antipsychotic on brain structure to date have been conducted with 1.5T machines.

4. Research Design and Methods

Summary:

This neuroimaging study will utilize the clinical infrastructure and clinical design of the STOP-PD II study. STOP-PD was an NIMH-funded randomized placebo-controlled trial that assessed the efficacy and tolerability of olanzapine and sertraline in the acute treatment of PD. Following the publication of the results of this five year study (Meyers et al.[23]; see above), the NIMH has recently funded a follow-up study, STOP-PD II. Enrollment in STOP-PD II has recently started. We briefly summarize the design of the STOP-PD II trial since the design of our proposed neuroimaging study is specifically superimposed on the randomized placebo-controlled component of STOP-PD II. However, we do not comment on the scientific objectives of this ongoing funded study (e.g., assessing the clinical risks and benefits of continuation/maintenance treatment of olanzapine for PD or the pharmacogenetic determinants of clinical outcomes).

4A. Research Design:

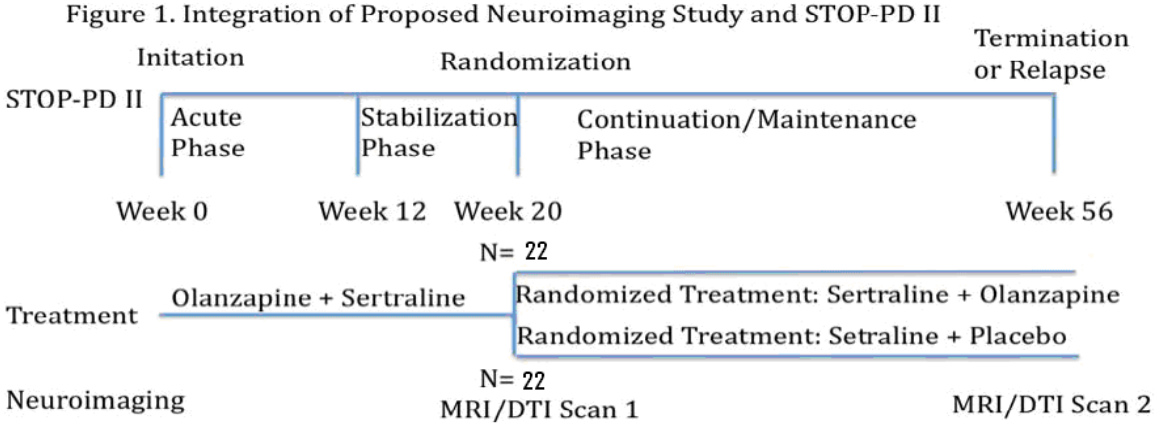
Summary: General Overview of the STOP-PD II Clinical Trial (see Figure 1)

98 patients (age 18-85 years) with non-bipolar major depression with psychotic features (delusions, with or without hallucinations) will be enrolled in STOP-PD II in Toronto. During the acute treatment phase (up to 12 weeks), these participants will receive a combination of open-label sertraline (target dose of 150 mg/day) and olanzapine (target dose of 15 mg/day). Participants who no longer have delusions and hallucinations and either are in full remission (defined as a 17-item Hamilton Depression Rating Scale (HAM-D)[68] score of ≤10 for 2

consecutive assessments) or in near remission (rated 'very much improved' or 'much improved' on the Clinical Global Impression (CGI) Scale[69], have a HAM-D score of 11-15, and have ≥50% reduction in the baseline HAM-D score during the last two assessments of the Acute Phase) will continue with open-label sertraline and olanzapine for an 8-week stabilization phase. Participants who continue to meet criteria for full-remission or near-remission and who are grossly cognitively intact (i.e., Mini-Mental State Examination (MMSE)[70] score ≥24) at the end of the stabilization phase will then enter the 36-week double-blind, placebo-controlled phase ('the RpCT'). All participants will take open-label sertraline for the duration of the RCT. After being randomized, participants will either continue olanzapine or be switched to placebo over four weeks under double-blind conditions. The continuation/maintenance phase lasts 36 weeks unless the participants meet criteria for relapse (i.e., a major depressive episode or Ham-D score ≥ 18 over two consecutive weekly visits; SCID and SADS criteria for psychosis over two consecutive weekly visits; or predefined criteria for significant clinical worsening [suicide, mania/hypomania, psychiatric hospitalization]). Participants will be assessed weekly during the first 8 weeks of the RCT and then every 4 weeks for the remaining duration of the trial (or more frequently if clinically needed). Participants who experience a relapse will be immediately referred to a psychiatrist at the research site for clinical management. 30 healthy controls (18-85 years) will also be enrolled and scanned.

In summary, over 38 months (January 2012 and February 2015), STOP-PD II will enroll 30 healthy controls and 98 Psychotic Depression (PD) participants in Toronto. Of these 98 PD participants about 50% of them will be below the age of 60; 44 participants will complete the stabilization phase and be randomized over 38 months (or 1.1 per month); 15% of those randomized to olanzapine and 35% of those randomized to placebo are expected to relapse.
Recruitment and flow in the neuroimaging study will mirror that of the STOP-PD II study: the first participant is expected to be randomized in the RpCT in the late spring 2012. Based on 44 participants expected to be randomized in STOP-PD II, and our estimate that 85-90% of them will enroll in the neuroimaging study, we project a total of 38 participants, yielding about 1 participant per month). 30 healthy controls will be recruited over 3 years, yielding about 1 partcipant per month.

Figure 1. Integration of Proposed Neuroimaging Study and STOP-PD II



4B. Methods:
Participant Recruitment and Flow in Neuroimaging Study (see Figure 2)

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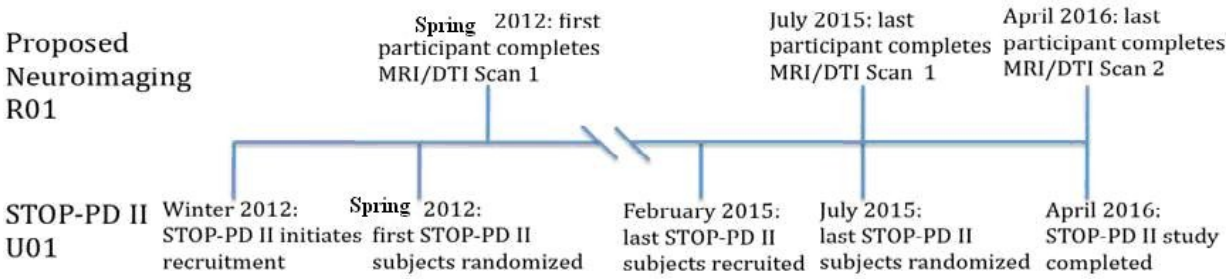
In the proposed neuroimaging study, PD participants will complete two or three MRI/DTI scans: one at the time of randomization ,the second one at the time of completion of the RpCT (either 36 weeks later or at the time of relapse) A third scan will be completed on a subset of participants who relapse during the RpCT or discontinue the RpCT but who continue to be treated with sertraline only or sertraline and olanzapine after they discontinue the protocol. These participants would be offered a third scan 36 weeks after the baseline scan. Healthy controls will complete only one baseline scan. Healthy controls will complete only one scan.

Based on our experience with ongoing MRI studies[8, 32, 71], we have estimated conservatively that 1- 2% of participants who are randomized in STOP-PD II will be ineligible for a MRI (e.g., because of a metal implant) and 5-10% will refuse to participate; this relatively low refusal rate is predicated on the fact that at the time of enrollment in the neuroimaging study, STOP-PD II participants will have been participating in the treatment protocol for 20 weeks, they will be fully engaged with the STOP-PD II investigators and research staff, and they will be clinically stable (and all of them will be competent to consent – see Human Participants section). Thus, we project conservatively that about 90% of participants randomized in STOP-PD II will participate in the neuroimaging study.

Figure 2. Timeline of Proposed Neuroimaging Study and STOP-PD II



4C. Rationale for Main Aspects of the Proposed Design:

The selection of the structures to be imaged and of the imaging techniques to be used is based on the animal and human data discussed in the Significance section. Here, we discuss the rationale for the timing of the scans and whether the duration between the two scans is sufficient to achieve the aims of this study.

Why are scans obtained only at baseline and completion of the randomized treatment phase?

We have made the decision of scanning participants twice (with the exception of the few participants who relapse or discontinue the protocol but who continue to treated with sertraline only or with stertaline and olanzapine; these participants will be offered a third scan) in order to capitalize on the STOP-PD II design. We considered scanning at other time points during the study, e.g., at study entry (Week 0) or at the completion of the acute phase (Week 12). However, after careful consideration, we have chosen to scan participants at the time of entry and either relapse and/or completion of the RpCT for the following reasons
:

1. We can obtain a true comparison of the effects of olanzapine vs. placebo on brain structure during the placebo-controlled phase (i.e., the RpCT).

2. Possible changes in brain structures observed during the acute treatment phase would likely not help with clinical decision making, as the risks of acute psychotic depression are so high that all guidelines recommend the use of an antipsychotic during the acute treatment phase: our study addresses the clinically meaningful question of the risks and benefits of long-term treatment with an antipsychotic in PD remitters who represent patients who are candidates for such long-term treatment.
3. The clinical investigators who have a long experience studying patients with PD have advised that many STOP-PD II participants who are acutely depressed and psychotic would not agree to participate in the neuroimaging study. The enrollment of a small subset of participants would yield a biased sample of participants with milder forms of PD who would not be representative of all patients with PD who are candidates for long-term antipsychotic treatment. Thus, we believe that the number and timing of the scans maximize enrollment, increase the clinical relevance of the results, and minimize the burden of participants.
4. Associations of the deleterious effects of olanzapine on the brain emerge from studies where there is sustained treatment in both humans and animals. Therefore, an examination of sustained effects of treatment on the brain using our placebo-controlled design can provide an answer to the question raised by those studies:
    a. In humans, recent work examining longitudinal brain changes in first episode schizophrenia patients treated with olanzapine compared to controls, suggests that olanzapine may be protective during the acute psychotic phase, i.e., over the first 3 months. It is once symptoms are under control that sustained treatment with olanzapine may carry the potential risk of adverse brain change.
    b. In mice, sustained, rather than acute treatment with olanzapine carried with it an 11% loss in brain volume, driven particularly by tissue loss in frontal brain regions.

Is the duration of olanzapine treatment long enough to detect effects on the brain? Can these effects be detected in a discontinuation study?

In this neuroimaging study, participants will be treated for 36 weeks with either olanzapine or placebo. Based on the literature regarding antipsychotic effects on the brain (see Significance section), we expect that this duration is more than sufficient to assess the hypothesized effect of treatment on brain structure. For instance, large effects of typical and atypical antipsychotics have been reported in participants scanned after 3, 6, and 9 months[3]. Therefore, 36 weeks should be long enough, particularly given that we propose to measure brain changes with much higher resolution (i.e., 3T MRI) and more sensitive neuroimaging analysis tools than what had been done in previous human antipsychotic studies (that have all used 1.5T MRI). The proposed neuroimaging approaches can demonstrate in vivo that the brain is exquisitely sensitive and highly plastic. Changes in brain structure can be measured within a few weeks even in populations where plasticity is thought to be reduced. For instance, MRI studies have shown that memory training for 8 weeks in older patients[72] and exercise training for 12 weeks in patients with schizophrenia[73] lead to significant alterations in brain structures. MRI/DTI has been used to document brain changes associated with interventions in healthy volunteers lasting only days to weeks: for instance, seven days of motor skill training in healthy controls can lead to gray matter changes in both cortex and striatum[74], while 40 hours of golf practice in middle aged golf novices[12] or 6 weeks of juggling have been associated with changes in white matter connectivity[14]. Similarly, two months of working memory training can impact structural connectivity[13]. Importantly, in these studies, when training stops and the

participants no longer engage in the activity, brain changes appear to be at least partially reversible[14]. Thus, even though all our participants will have been exposed to olanzapine for at least 20 weeks, we expect that 36 weeks will be more than long enough to measure different changes in both the gray and white matter of participants who are randomized to continue olanzapine and those who are switched to placebo.

5. Protection of Human Participants: leadership plan to protect the integrity of human subject data collected across multiple sites

Overview and Rationale:

The proposed neuroimaging study, "Effects of Maintenance Treatment of Olanzapine vs. Placebo on Brain Structure and Connectivity in Psychotic Depression", will focus on establishing under double-blind placebo controlled conditions the effects of continuation/maintenance therapy of olanzapine on the brain. This proposal will utilize the clinical infrastructure and design of the already NIH-funded four-site study "Sustaining Remission of Psychotic Depression" (Weill Medical College of Cornell University, overall and site PI Dr. Barnett Meyers University of Massachusetts Medical School, site PI: Dr. Anthony Rothschild, University of Pittsburgh, site PI: Dr. Ellen Whyte; University of Toronto, site PIs: Drs. Alastair Flint and Benoit Mulsant). This study also known as the Study Of the Pharmacotherapy of Psychotic Depression II (STOP-PD II) for the purpose of communications, presentations of research findings, and publications was funded based on the results of a previous U01 study involving the same sites: the initial STOP-PD study showed that a combination of sertraline with olanzapine during the acute phase of treatment results in a remission rate of 42% in the intent-to-treat sample and 67% in the completer sample[23]. STOP-PD II addresses the important question of the risks and benefits of discontinuing olanzapine once an episode of psychotic depression has fully responded to a combination of sertraline plus olanzapine using a randomized placebo controlled trial (RpCT).

The proposed neuroimaging study leverages the already funded clinical infrastructure of STOP-PD II (see Figure 1 below) that is ongoing in Toronto and already approved by CAMH and UHN REBs. The PI of the proposed neuroimaging study is Dr. Aristotle Voineskos, with Dr. Mulsant as co-PI, and Drs. Lerch and Chakravarty as co-investigators, bringing additional expertise in the application of biophysics and bioengineering to the statistical design and analysis of longitudinal neuroimaging studies. The proposed neuroimaging study will recruit STOP-PD II participants who have attained and sustained remission or near-remission during the acute and stabilization phase of open treatment with sertraline plus olanzapine, and who and are being randomized to 36 weeks of continuation/maintenance treatment with olanzapine vs. placebo (please see Figure 1 below). The potential risks associated with the discontinuation of olanzapine are part of participation in STOP-PD II. The proposed neuroimaging study adds minimal additional risks, i.e., the risks associated with completing two MRI scans at the time of randomization and completion of the RpCT (or at the time of relapse).

Functions, Roles, and Accountability:

Table 3 presents the functions and contributions of the site and associated accountability. Congruent with the proposed distribution of these roles, the Study Coordinator and Data Manager will be supervised by Drs. Voineskos and Mulsant. The study investigators will be responsible for: hiring, training, and supervising their research staff at their respective site; overseeing the recruitment and retention of participants and the quality of neuroimaging data acquisition; and ensuring that data are sent to the Toronto site in a secure and timely manner.

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Table 3: Functions and Roles

Function	Areas of Primary Responsibility
Subject recruitment & clinical management	▪ The study will enroll 44 STOP-PD II participants who have attained and sustained full or near-remission of their episode of psychotic depression. ▪ Dr. Flint will be responsible for recruitment, clinical management, and retention of these participants following the STOP-PD II protocol as summarized under Approach and below.
Acquisition of Neuroimaging Data	▪ 38 participants will be scanned at the time of randomization in STOP-PD II and completion of the RpCT 36 weeks late or at the time of relapse. A third scan will be completed on a subset of participants who relapse in the RpCT or discontinue it but who continue to be treated with sertraline only or with sertraline and olanzapine. These participansts would be offered a third scan 36 weeks after the basline scan. 30 healthy controls will be scanned at baseline. ▪ Dr. Voineskos and Dr. Chakravarty will be jointly responsible for ensuring high quality image acquisition, anonymization, and secure transfer of neuroimaging data.
Analysis of Neuroimaging Data; Overall Coordination; and Scientific Oversight	▪ Dr. Voineskos, as PI, will ensure that the neuroimaging protocols are implemented and conducted safely and with full integrity. ▪ He will have overall responsibility for the completeness, quality, and confidentiality of the neuroimaging data.

MRI Scanner Quality Assurance and Reliability

Quality Assurance (QA): CAMH MRI machines are operated by a full time physicist and MRI research technician. The site performs a rigorous quality assurance protocol on their phantom for their head coil, and all structural acquisition sequences.

MRI/DTI Scan/Rescan Reliability: As described in the Approach section, the proposed study will use acquisition sequences that have been tested extensively by General Electric (GE) for both T1-weighted and DTI acquisitions. Other investigators have done extensive testing examining scan-rescan reliability measures on dependent brain measures. When the same scanner, same acquisition, and same analysis method are used, within subject reliability is very high for cortical thickness and subcortical volumetric analyses (90, 91), and for white matter tract integrity measures. However, for DTI, segmentation of tracts in native space is necessary to achieve the best reliability possible (92). An analysis of CAMH scan/rescan reliability data demonstrates a less than 1% within subject difference for the majority of cortical thickness measures, surface area measures, subcortical volume measures, and white matter microstructural integrity measures. Actual values are shown in Tables 1 and 2 in the Approach section.

<u>Inclusion and Exclusion Criteria</u>
Human Participants' Involvement and Characteristics

This proposed study will recruit 38 participants 18 years or older with non-bipolar major depression with psychotic features (PD) for 'pre' and 'post' RpCT MRI/DTI scans. All participants will be recruited among the STOP-PD II participants who successfully complete the STOP-PD II acute and stabilization treatment phases and are eligible for the STOP-PD II RpCT. Immediately after having consented to participate in this RpCT; they will be invited to participate in the proposed neuroimaging study. Thus, all participants will meet eligibility criteria for STOP-PD II and for the STOP-PD II RpCT (i.e., they will have achieved sustained remission or near remission of their major depressive episode and complete resolution of the associated psychotic features). In addition, they will have no contra-indications for MRI scanning:

Eligibility Criteria for PD Participants-- Inclusion criteria:

1) Aged 18-85 years, inclusive;

2) Diagnosis: DSM-IV non-bipolar major depression with psychotic features, established through both a clinical interview by a research psychiatrist and the subsequent administration of the SCID-IV by a trained research clinician.

3) Having initially presented with score of ≥3 on the delusion severity item of the SADS ('delusion definitely present') and currently scoring 1 on this item ('definitely not delusional') consistent with a resolution of all delusions.

4) Having initially presented with score of ≥2 on any of the three conviction items of the DAS (the participant is certain a belief is true and does not change the belief in response to reality testing by the interviewer) and currently scoring 0 on these items consistent with a resolution of all delusions.

5) Having initially presented with a 17-item Ham-D score of ≥21 and currently either in full remission (defined as scores ≤10 for the last 2 consecutive assessments) or in near remission (defined as: (1) a HAM-D score of 11-15; (2) ≥50% reduction in HAM-D score compared to baseline HAM-D score; and (3) rated 'very much improved' or 'much improved' on the Clinical Global Impression [CGI]).

6) Cognitively intact as evidenced by a Mini-Mental State Examination (MMSE) score ≥ 24.

Eligibility Criteria for PD Participants-- Exclusion criteria:

7) Current or lifetime DSM-IV criteria for schizophrenia, other psychotic disorders (e.g., schizoaffective disorder, delusional disorder, brief psychotic disorder, and shared psychotic disorder), or mental retardation, or having presented meeting DSM-IV criteria for current brief psychotic disorder, body dysmorphic disorder, or obsessive-compulsive disorder.

8) Current or lifetime DSM-IV criteria for bipolar affective disorder.

9) Having presented with DSM-IV defined substance abuse or dependence, including alcohol, within the previous 3 months.

10) DSM-IV defined Alzheimer's dementia, vascular dementia, or dementia due to other medical conditions, or a history of clinically significant cognitive impairment prior to the index episode of depression, and/or having presented with a mean score of ≥4 on the 26-item IQCODE. STOP-PD II uses the IQCODE to screen for clinically significant cognitive decline that began prior to the index episode of PD. The IQCODE is not significantly confounded by age, education, or premorbid intelligence. Persons with a mean total IQCODE ≥4 are excluded from participation in STOP-PD II and thus also from this study; this cut score has been found to have a sensitivity of 84-93% and specificity of 88-94% in screening for dementia in general, psychiatric, and medical populations of older adults.

11) Type 1 diabetes mellitus (defined as insulin-dependent diabetes mellitus with onset < 35 years of age and/or diabetes mellitus that has been complicated by a prior documented episode of ketoacidosis).

12) Acute or unstable medical illnesses (e.g., delirium; metastatic cancer; unstable diabetes; decompensated cardiac, hepatic, renal or pulmonary disease; stroke; or myocardial infarction); abnormal serum free T4; abnormally low serum vitamin B12 or folic acid level; medical conditions and/or medications for which psychotic or depressive symptoms can be a direct manifestation (e.g., Cushing's disease, high-dose systemic corticosteroids, L-dopa).

13) Neurological disease associated with extrapyramidal signs and symptoms (e.g., Parkinson's disease); epilepsy, if the person has had one or more grand mal seizures in the past 18 months; history or physical signs of stroke; any diagnosis of a Central Nervous System (CNS) disorder.

14) Need for treatment with any psychotropic medications other than sertraline, olanzapine, or lorazepam; or with an anticonvulsant medication with mood-stabilizing properties (carbamazepine, lamotrigine, valproic acid) or need for treatment with ECT (e.g., imminent risk of suicide, refusing to eat or severe malnutrition, catatonic).

15) Current pregnancy or a plan to become pregnant during the duration of the study in woman of childbearing age; breast-feeding in woman with infants.

16) A clearly documented history of being unable to tolerate sertraline or olanzapine, including having had an untoward previous reaction to sertraline such as significant bradycardia (heart rate of <50 bpm) or development of the syndrome of inappropriate antidiuretic hormone secretion with a serum sodium of 129 mmol/L or below.

17) History of non-response of the index episode of PD to at least a 6-week trial of .150mg/day sertraline combined with .15mg/day olanzapine.

18) Any contraindication to MRI (e.g., pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body, claustrophobia or history of not being unable to tolerate a MRI scan).

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Rationale for Eligibility Criteria:

1-12 and 14-17: These are the eligibility criteria for the RpCT in STOP-PD II and thus all participants in the proposed neuroimaging study will also meet these eligibility criteria. Of note, MRI/DTI studies also use many of these criteria (e.g., excluding pregnant women).

13: STOP-PD II also excludes patients with medications or neurological illnesses (e.g., stroke, multiple sclerosis, Parkinson's disease) that could present as a Psychotic Depression. The proposed neuroimaging study would exclude participants with other more benign and rarer CNS disorders that would not have ruled-them out of STOP-PD II but that could interfere with assessment of changes on MRI scanning (e.g., Arnold-Chiari malformation, small meningioma).

18: Since this is a MRI/DTI study, STOP-PD II participants who have physical or psychological contra-indication to completing a MRI are excluded. Based on our experience in other MRI/DTI studies, we have estimated conservatively that 1-2% of participants who are randomized in STOP-PD II will be ineligible for a MRI. We have also estimated that 5-10% of those eligible will refuse to participate (see approach).

Eligibility Criteria for Healthy Controls - Inclusion criteria:

Male or female participants meeting all criteria listed below will be included in the study:
Age 18-85
No current or lifetime Axis-I psychiatric diagnosis on the DSM-IV/SCID except for adjustment disorder or a phobic disorder

Eligibility Criteria for Healthy Controls - Exclusion criteria:

Diagnosis of Mental Retardation (i.e. IQ<71)
Positive urine drug screen
Metal implants or a pace-maker that would preclude the MRI scan
History of head trauma resulting in loss of consciousness > 30 minutes that required medical attention
Unstable physical illness or significant neurological disorder including a seizure disorder
Recent stroke (within last year)

6. <u>Clinical Assessments and Measures</u>:

Clinical Measures: As part of STOP-PD II, PD participants will be characterized with: the Structured Clinical Interview for DSM-IV-TR (SCID-IV)[75], the 17-item HAM-D[68], the Delusion Assessment Scale[76], delusion and hallucination items of the SADS,[77] the Scale for Suicidal Ideation (SSI)[78], the CGI69, and the MMSE[70] . The 26-item Informant Questionnaire for Cognitive Decline (IQCODE)[79] will also be used to screen out patients with clinically significant cognitive decline that began prior to the index episode of PD (i.e., to avoid the inclusion of

patients in the early stage of dementia). Cognitive, anthropometric, metabolic, and genetic measures are also being obtained in STOP PD II and will be available for exploratory analyses.

The following measures will be added (all measures listed in gray will be added for controls, and only the WTAR and handedness scale will be added for PD participants, as the other measures in gray are already conducted in the parent clinical trial) :

Structured Clinical Interview for DSM-IV-TR (SCID-IV)[75] : The SCID is a semi-structured diagnostic interview designed to assist clinicians, researchers, and trainees in making reliable DSM-IV psychiatric diagnoses.

Cumulative Illness Rating Scale for Geriatrics (CIRS-G) [109] The CIRS-G is designed in measure the chronic medical illness burden in the elderly persons. This is a 13-category scale measuring elderly persons' cardiovascular-respiratory system, gastrointestinal system, genitourinary system, musculo-skeletal-integumentary system, neuropsychiatric system, and general system. For each of the 13 organ systems, the illness burden is rated on 5 point scale, as following, none, mild, moderate, severe, extremely severe. Total score is calculated by summing the score on individual category**.**

Wechsler Test of Adult Reading (WTAR). The WTAR is designed to assess pre-morbid level of intellectual functioning for individuals age 16 to 89 years (Wechsler D. Wechsler Test of Adult Reading. Harcourt Assessment, 2001).

Mental Status Exam (MMSE) The MMSE is a well known and widely used brief mental status test of cognitive impairment in elderly individuals. The test consists of 13 items, assessing orientation to place and time, learning and memory, construction ability, attention and calculation[110].

Scale to determine handedness: Hand Dominanace Questionnaire. The Hand Dominance questionnaire is a self administered 16 item questionnaire to determine handedness for various activities. Each item is rated on a 5 point scale ranging from “always left to always right.” The scale also assesses family hand dominance.

To assess cognitive function, we will use 2 tests from the Delis-Kaplan Executive Function Scale (D-KEFS)[106]; Delis et al, 2001) and 2 sub-tests from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)[107,108]

(1) The Color and Word Inteference , measuring behavioural inhibition. (DKEFS)
(2) Trails A and B, where trails B measures cognitive flexibility. (DKEFS)
(3) Immediate and Delayed recall tasks (RBANS)
(4) Coding (RBANS)

Other Measures

1. Demographic questionnaire

2. Current medications
3.. Height
4. Weight and vital signs (blood pressure, heart rate, and temperature)
5. Urine drug screen

Neuroimaging:
Within Subject Design: The design of this study is a within-subject longitudinal design. Within-subject mean change values in dependent measures of brain structure are the variables of interest, and it is these values that will be compared between the olanzapine and placebo groups. Regarding error that can occur due to a within-subject longitudinal design ("test-retest variability"), we have completed two power simulations from scan/rescan data using the acquisition protocols proposed for this study (see below) on two of the study's scanners. Thus, the measurement errors for our primary dependent variables of cortical thickness, surface area, subcortical volumes, and white matter tract diffusion measures can be estimated.
Acquisition: The CAMH MRI scanner is a fully research-dedicated 3 Tesla scanner and is operated by a well-organized team including a full-time MRI physicist, MRI scientist, and research technologist. At CAMH we are currently running high resolution T1-weighted and DTI scanning protocols (the key acquisitions for this proposal – sequences shown below). These acquisition parameters have been optimized for high quality T1-weighted and DTI data ensuring key parameters are consistent (voxel size, number of diffusion-weighted directions, etc.). Fast spin-echo T2-weighted scans will also be obtained to improve registration and segmentation. Myelin transfer ratio and FLAIR sequences using standard acquisitions already utlilized in the CAMH MR-Unit will help improve characterization of myelin and white matter lesions repectively. We will use GE-provided and tested acquisition parameters that have undergone rigorous reliability testing by GE, rigorous testing by the physicist with GE engineers, and are the protocols on which the QA has been tested.

We will also include a functional acquisition sequence. For resting state acquisition (i.e. subject is at rest) images will be acquired in the 2D axial plane, with an FOV=22 cm, slice thickness= 5mm, TR = 2000ms, with 31 slices, and a flip angle of 60. For task-based acquisitions, TR= 3000ms, TE=40ms, matrix size 256 by 256, flip angle 90, matrix 64x64, FOV=20cm, 36 slices. Our main tasks of interest will be based on presentation of images or video of others in order to determine whether participants can correctly asses the emotions of others and social situations.

We will also include a MRS acquisition sequence. Images will be acquired in the MRS oblique plane, with an FOV=24 cm, slice thickness= 20mm, TR = 1500 or 2000mm, with 1 slice. With the addition of these acquisitions, total scan time in the MRI scanner will be nearly one hour (i.e. 60 minutes).

In brief, QA data are obtained on the scanner on a daily basis using the phantom designed by GE. The data suggest that the scanner hardware is functioning in a highly reliable manner. Scanning only occurs when QA is met. Furthermore, the acquisitions and analysis methods that we are proposing to use demonstrate very high within-subject reliability from the acquisition all the way through the image processing to generation of the final quantitative brain measures (**please see Data Analysis section for image processing reliability measurements**). For DTI, it has been shown that greater than 30 non collinear diffusion-weighted acquisition

directions are necessary to generate highly reliable and robust measures of FA and mean diffusivity[80, 81]. All DTI acquisitions in this proposal are using 60 directions or greater.

We will obtain MRI data on a 3 Tesla GE Echospeed system research-dedicated scanner. This scanner permits maximum gradient amplitudes of 50 mT/m and is equipped with an eight-channel head coil that provides major improvement in signal to noise ratio (SNR) over the standard quadrature coil[82]. A T1-weighted will be acquired as a sagittal 3D FSPGR: (echo time (TE): 3 ms; repetition time (TR): 8.2 ms; time to inversion (TI): 650; flip angle 8, 24 cm; FOV, number of excitations (NEX) = 1 (0.9 mm x 0.9 mm x 0.9 mm, no gap). For DTI, we will include B0 distortion maps to correct for image artifacts, mainly geometric and image intensity distortions. We will use an echo planar imaging (EPI) DTI Tensor sequence with dual spin echo option to reduce eddy-current related distortions[83]. To reduce impact of EPI spatial distortion, we will use ASSET with a SENSE-factor of 2. We will acquire 60 gradient directions with b=1000, 5 baseline scans with b=0. Scan parameters are: TR 8800 ms; TE min; FOV 38 cm; 128x128 encoding steps; 2.9 mm isotropic voxels, no gap. Axial slices will be acquired parallel to the AC-PC line covering the whole brain. B0 field inhomogeneity maps will also be collected and calculated.

Image Analysis: We will use software programs (CIVET for cortical thickness and surface area, and SLICER for DTI-based measures) that have been specifically designed to work with all file formats produced by GE, Siemens and Philips scanners. We have substantial experience using the methods that we describe below for image processing and analysis[8-10, 59, 84-86].

Cortical Thickness Analysis: Following acquisition, all T1-weighted MRI data will be submitted to the CIVET pipeline (version 1.1.10). T1 images will be registered to the ICBM152 nonlinear sixth generation template with a 9-parameter linear transformation, inhomogeneity corrected[87] and tissue classified [88,89]. Deformable models will then be used to create white and gray matter surfaces for each hemisphere separately, resulting in 4 surfaces of 40,962 vertices each[90,91]. From these surfaces, the t-link metric will be derived for determining the distance between the white and gray surfaces[10]. Cortical thickness maps will be aligned across all scans using non-linear surface based registration[92, 93]. The thickness data will then be blurred using a 20-mm surface based diffusion blurring kernel[94] in preparation for statistical analyses. Unnormalized, native-space thickness values will be used owing to the poor correlation between cortical thickness and brain volume. Normalizing for global brain size when it has little pertinence to cortical thickness introduces noise and reduces power[95].

Surface Area Measures: Since it is unclear whether potential cortical volumetric changes are due to alterations in cortical thickness or surface area, we will ensure that we measure surface area in each cortical lobe using the CIVET pipeline. For the calculation of surface area, the middle cortical surface, which lies at the geometric center between the inner and outer cortical surfaces, is used for the calculation of the surface area[11] .The middle cortical surface lies at the geometric centre between the inner and outer cortical surfaces thus providing a relatively unbiased representation of sulcal vs. gyral regions. In contrast, the inner cortical surface model overrepresents sulcal regions, and the outer cortical surface model overrepresents gyral regions[96].

Subcortical volumes and morphology: These measures will be estimated using methods developed by our team[59, 84]. Automatically derived segmentations for the striatum, globus pallidus, and thalamus will be estimated

using our novel-multi atlas based segmentation procedure. This method takes a single atlas derived from serial histological data as input[97] and generates a template library from the data being analyzed[98]. Nonlinear registrations are then estimated in pair-wise fashion in order to filter errors due to sub-optimal registration and neuroanatomical differences. Finally a voxel-voting procedure is employed in order to prune labeled voxels that are not in agreement with the majority of the labels in the group. This method has been shown to be accurate and improves upon regular model-based segmentation. In addition, cytoarchitectonic boundaries are defined within the input atlas, thereby allowing for detailed delineation of structures in the final segmentations. The transformations that are used to estimate the final segmentations can also be averaged in order to provide information about the morphometry of each structure. By estimating the dot-product of the vector field from the final transformations and the normal to the surface representation (defined by a polygonal mesh) for each structure, a displacement along the normal can be estimated. Vertex-wise changes in displacement will then be analyzed to determine shape differences (using similar methods to cortical thickness vertex-wise statistics). We are expanding these techniques to measure subfields of the hippocampus and nuclei of the amygdala. In these segmentation procedures, structures will be defined on high-resolution T1 and T2 MRI data (300 micron isotropic voxels; derived in-house) that enable the visualization of specific subfields.

DTI Analysis: After preprocessing that involves eddy current correction, nonlinear EPI distortion correction (using previously acquired distortion maps), filtering, and tensor estimation (all part of the freely available 3D slicer software, www.slicer.org), we will use a deterministic whole brain tractography approach[99], in order to define and to analyze cortico-cortical and cortico-limbic fiber bundles. An eigenvector tracking algorithm based on the fourth order Runge-Kutta method for tracking white matter fibers will be used in this approach. A regularization scheme, where a small bias towards the previous tracking direction to the current tensor is added will be used. The linear anisotropy measure (CL) will be used for seeding and stopping thresholds, instead of FA. With respect to our clustering method, the use of CL is helpful because it lessens the effect of planar partial-volume regions where a fiber may jump from one structure to another. By reducing partial volume tractography errors, it improves the ability of the clustering to separate different structures[100]. For segmentation, our clustering method takes advantage of fiber shape, and groups fibers of similar appearance. Once the whole brain cluster model is produced, a trained operator combines the clusters that correspond to a fiber tract. Given the apparent propensity of antipsychotic medications to adversely affect cortical white matter, we will segment and measure major cortico-cortical and cortico-subcortical fiber bundles, including the uncinate fasciculus, cingulum bundle, inferior longitudinal fasciculus, inferior occipito-frontal fasciculus, arcuate fasciculus, thalamic radiations, and subdivisions of the corpus callosum. This method has been tested by our team and others on all the fiber tracts that will be examined in this study[9, 33]. We have demonstrated high reliability of this method for both spatial localization and diffusion based measures of white matter fiber tracts[9]. For each white matter tract we will calculate mean FA and mean diffusivity, and compare changes in these measures across time in the olanzapine and placebo groups. On an exploratory basis we will also measure axial diffusivity (sensitive to axonal membranes), and radial diffusivity (sensitive to myelin) [44] to understand more about the specific tissue compartments that may contribute to potential changes in mean diffusivity.

7. Sources of Materials

Data are obtained from MRI scans obtained solely for the purpose of this neuroimaging study and from clinical research assessments obtained as part of STOP-PD II. As in STOP-PD II, participant identity and information

will be confidential and protected. The CAMH Research Imaging Centre, where the MRI scans will be analyzed, has many ongoing research projects that involve medical patient information. The computer systems and facilities are designed to ensure the privacy of this information. Similarly, the clinical research data will be stored in locked facilities and subject confidentiality will be ensured. In STOP-PD II and in this study, all sources of materials will be kept strictly confidential through the use of a numerical identification coding system. Locked file storage of de-identified records of hard copy materials will also be employed. Only the investigators and key research staff supported by this project will have access to subject identities and linked research materials.

8. Potential Risks

Participants will be characterized and treated as part of STOP-PD II and closely monitored as per the protocol of this study. The procedures employed in STOP-PD II have been widely and safely used in other research protocols. There are, however, risks associated with participating in the STOP-PD II RpCT that have been outlined in detail in that proposal and in the consent forms approved by CAMH and UHN REBs. These risks include completing questionnaires and rating scales, risks directly related to the administration of sertraline and olanzapine, and the risks associated with a possible relapse of psychotic depression (including risks of suicide). However, these risks are dealt with directly within the STOP-PD II study and participating in the neuroimaging study does not modify these risks. There are some additional risks associated specifically with this additional neuroimaging study. As with any research study, there is always the risk of a potential breach of confidentiality. Other risks that are related to the MR imaging include discomfort due to anxiety associated with being confined in a scanner and potential injury if a participant has metal in his or her body that has not been identified. These risks are outlined in detail below:

General Risks of MRI/DTI: MRI is not associated with any known adverse effect except for people with metal or magnetic implants (such as metallic clips in the brain or cardiac pacemakers). Also, potential risks to pregnant women are not well-known (but pregnant women are excluded form this study). Some types of (home-made) tattoos can also heat up and cause discomfort. There are no other known risks of exposure to the magnetic fields used for MRI/DTI scans.

Individuals with implanted metallic foreign bodies are excluded because the strong magnetic field in the scanner could cause these bodies to change position, injuring participants. In order to assure that MR scanning is safe to undergo, participants will be screened by the study personnel and by personnel at the MR center prior to scanning. If an X-ray is required to rule out the presence of metallic fragments, the maximum dose to the involved body area will be 0.3 rems with minimal exposure to other body areas. Metal objects can also become projectile when placed near the magnetic field. This has been reported on a few occasions, but it is a very rare occurrence. Protection from magnetic objects can be safeguarded by the usual safety techniques that are practiced in MRI sessions such has having participants and researchers take all metal objects off of their person before entering the environment.

Another potential risk is psychological distress caused by being in the enclosed magnet bore. STOP-PD II participants who get severely anxious when in enclosed spaces or who have a history of not tolerating a MRI scan will not be included. Any participants who find that they become too anxious or uncomfortable during any part of the procedure will be immediately taken out of the scanner and excluded from further scanning.

Risks During Pregnancy: MRI studies of fetal brain and other tissues have been published. The risks during pregnancy are essentially unknown, but are probably of low likelihood. Several studies of prenatal MRI have now been published and the MRI techniques have not been demonstrated thus far to have risks. There have been no reports of adverse effects to the developing human fetus. However, since the risks have not adequately been assessed over a long period of time and especially for early pregnancy, we will not include pregnant or potentially pregnant women (who are already excluded from STOP-PD II anyway).

Risks of High Noise Levels: Noise levels in the magnet can be uncomfortable for some participants. All participants will be wearing earplugs through all MRI procedures. A microphone will be provided so that they may be able to communicate and stop the testing if they become too uncomfortable or anxious at any time.

Risks of Psychological Distress: Some participants can experience claustrophobia while in the scanner. Participants will have the opportunity to examine this space before the scanning starts. The study will be ended early if the space is a problem for them. No medications (e.g., benzodiazepines or tranquilizers) will be offered to them. This study will not offer alternative procedures for participants who choose not to participate in the neuroimaging study or who are excluded. In our experience of scanning a large number of participants with severe mental disorders (e.g., schizophrenia, bipolar disorder, major depressive disorder, Alzheimer disease), less than 2% of these participants have been unable to complete the scanning session. However, mild psychological distress must be considered to be a likelihood in these studies. Another potential source of distress could also come from delusional beliefs about the scanning. This has not occurred yet in our setting and we do not expect it will happen in STOP-PD II participants who are remitted, but it is something that we are aware of and for which we will monitor.

Risk of Incidental Findings: All MRI scans obtained for this neuroimaging study will be screened for incidental findings using standard MR research center policies: All MR images are viewed as they are being acquired by a trained MR technologist who is supervised by the MRI physicist and head of the MRI unit. If there are any suspicious findings, the study PI will also be informed. Anything of note or concern will be discussed with the participant, and if he or she provides release of information, findings will also be discussed with his or her physician. Upon further medical investigation, most incidental findings identified during neuroimaging studies turn out to be benign but initially (i.e., upon discovery), they are the source of anxiety. Thus, discovery of an incidental finding is considered by most neuroimaging researchers to be a potential risk of participation in this type of study. However, in some cases, it can also be a benefit because it may lead to an early beneficial medical intervention.

9. Adequacy of protection against risks

Protections against Risks:
Procedures to mitigate the potential risks associated with relapse of PD during the STOP-PD II RpCT include regular visits and structured assessments of depressive and psychotic symptoms, including suicidality: STOP-PD II participants are assessed weekly during the first 8 weeks of the RpCT and then every 4 weeks for the remainder of its duration (or more frequently if clinically needed). Those who experience a relapse will be immediately referred to a psychiatrist for clinical management. Thus, the risks of participating in STOP-PD II

and the mitigation of these risks will remain the responsibility of the STOP-PD II research teams. The investigators and research staff associated with the neuroimaging study will immediately contact the local STOP-PD II investigator if they identified clinical concerns (e.g., if a participant confided during his or her scanning session that he is feeling depressed and is considering hurting himself or herself). The rest of this section focuses on the risks associated with scanning.

Protection from MRI Risks:
General Considerations
Each participant is required to read and sign an informed consent form stating the risks of the MRI/DTI procedure. Each participant also completes a checklist to screen for metal implants, pregnancy, and other safety concerns. In addition to completing this MRI screening checklist, participants will also be asked verbally (and in a way to ensure confidentiality) by the experimenter about metal implants, surgical procedures, and the possibility of pregnancy. If the answers to any of these questions are not known or if the participant is uncertain about any answers, they will not be scanned. All participants are asked to empty pockets, remove any clothing with possible magnetic materials, and remove all jewellery before the scan.

Protection from High Noise Levels
Noise levels will be reduced, as participants will be given earplugs. These are used routinely in hospital MRI clinical settings. There is also a microphone in the bore of the magnet. Participants are instructed to talk to the experimenter if they feel uncomfortable for any reason during the testing procedures. If participants feel too uncomfortable or anxious they are immediately taken out of the scanner.

Protection from Anxiety or Psychological Distress
When we run MRI sessions, even with previously scanned participants, we do everything we can to make sure that the participant is not uncomfortable. Before the session, we inform the participant that they can end their participation at any time as follows: “Some people get claustrophobic or feel closed in when they are in the magnet. Often there is no way to tell ahead of time how you will feel. If you feel too anxious or uncomfortable at any time, for any reason, please let us know immediately and we will get you out of the scanner. You will still get reimbursed and it is no problem at all. There is a microphone in the scanner so that we can hear you.” When the participant is lying on the scanner table and is about to be put into the magnet we tell them “Now when I push this button you will go into the scanner, if you feel uncomfortable just tell us and we will get you out immediately.” After the participant is inside the bore of the magnet, we then ask them again how they are feeling before we go out of the room to run the scanner. We also check in with the participant (through our audio system) before and after each acquisition (e.g., between the localizer, MP-RAGE, DTI acquisitions) to make sure that they are doing well.

10. <u>Recruitment and Informed Consent Recruitment</u>
The RA of the STOP-PD II research team will be aware of all participants recruited into STOP-PD II and when these participants remit and enter the 8-week stabilization phase. He will inform the Study Coordinator and Dr. Voineskos’ RA who will reserve a scanning slot. Once STOP-PD II participants consent to the RpCT (see below), they will be invited to participate in the neuroimaging study. Control participants will be recruited through existing studies databases and referrals by word of mouth.

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Informed Consent:
PD may impair a person's capacity to consent to participate in a research study. Thus, in STOP-PD II, participants or their surrogate are asked to consent to the acute and stabilization treatment phase. Once STOP-PD II participants are in remission or near-remission, they are asked to reconsent to the RpCT. At this point, since participants are no longer either depressed or psychotic, surrogate consent is not authorized and only participants who are deemed to be competent to consent are invited to participate in the STOP-PD II RpCT. Thus, all STOP-PD II participants who will be invited to participate in the neuroimaging study will have achieved sustained remission or near remission and will be capable of giving informed consent. A separate consent form will be used for the neuroimaging study; this will be a single consent form asking for consent for both pre- and post-scan (i.e., the first scan at the initiation of the RpCT and the second scan at the time of termination or relapse, a third scan 36 weeks after the baseline scan on a subset of participants who relapse during the RpCT and are then treated with sertraline only or with sertraline and olanzapine after the relapse). Some STOP-PD II participants will not complete the 36 week RpCT due to relapse. These participants will already have given consent for the second and possibly third scan while they were competent to consent (during the stabilization phase). However, given their relapse, they may be no longer willing to complete the second scan or the third scan. Thus, only participants who assent will complete the second or the third scan . Of note, the power analysis has shown that the power remains adequate even if none of the participants who are expected to relapse complete the second scan (i.e., the power remains adequate even if 25% of participants do not complete the second scan).
The consent form will stipulate that participants' information will remain strictly confidential, with access limited to research staff, and, if indicated, CAMH REB. No one but selected study personnel will have access to the lists linking participant's names to code numbers. All information pertaining to participants will be coded to protect participants' identities and will be stored in locked cabinets in locked offices. Publications or presentations of findings will not include information that identifies participants. Once each participant is enrolled in the study, they will be assigned a unique study identification number that will be used for both imaging session. Identifying information (name, sex, age) will be stripped from the file name and the image file headers. All associations between participant study identification number and the participant name will be kept in a private key at the local site (to be used in the event of incidental findings). A centralized, secure, database of all variables will be kept in Toronto and will be managed by the Database Manager.

11. Potential Benefits

There will be no direct benefits to participants enrolling in this neuroimaging study, although there are direct benefits to their enrollment in STOP-PD II, including standardized open treatment to achieve remission of PD. The participants will contribute to evidence on whether antipsychotic medication affects brain structure. In turn, this knowledge will impact the way all patients with PD or other mood disorders are treated and the participants may benefit from such knowledge in the future (see next section).

12. Importance of Knowledge to be gained

As discussed in the Significance section, it is not known whether long-term treatment with antipsychotic medications adversely affects the brain. This issue is of tremendous importance. These medications are widely

prescribed and it is crucial to understand whether they are harmful to the brain. This neuroimaging study has been designed to impact clinical practice by providing the first placebo-controlled evidence regarding the potential effects on brain structures and connectivity of antipsychotic continuation/maintenance therapy in patients with mood disorders. Our results will address the unresolved question of whether antipsychotics are neuroprotective or neurotoxic in these patients. In our view, given the precautions that will be taken in this study, the potential benefits to participants and society outweigh the potential risks.

13. Data and Safety Monitoring Plan

Study investigators and the Study Coordinator will meet weekly to review accrued data, data confidentiality, adherence to protocol design, recruitment, and participant complaints. They will determine whether there has been any change in the benefit-to-risk ratio of the neuroimaging study. If an adverse event occurs during neuroimaging, it will be reported to the CAMH REB.If an unexpected adverse event occurs, the investigators will re-assess the risk/benefit ratio of the study; if deemed necessary, and they will submit any modifications to the CAMH REB for approval. At the time of REB annual renewal, Dr. Voineskos will submit to the CAMH REB the information about the frequency of the monitoring for adverse events and complaints, the dates that meetings took place, a summary of the cumulative adverse events and complaints, external factors or other relevant information that might have an impact on the safety or ethics of the study, final conclusion regarding changes to the anticipated risk/benefit ratio to study participation, and final recommendations related to the continuation, changing, or termination of the study.

14. Data Analysis

14A. Data Management and Integrity

The study investigators will be responsible for ensuring the reliability, completeness, integrity, and confidentiality of data. Dr. Voineskos will be responsible for data analysis and on a weekly basis will review the acquisition and transfer of neuroimaging data with the Study Coordinator and the Data Manager.

Following acquisition of neuroimaging scans, the Research Assistant (RA) will work with the MRI physicist (or technician that the physicist is supervising) to ensure anonymization of data: scans will be anonymized by using only the participant study IDs and the date and time of scanning. Once all data has reached the CAMH server, they will be checked to ensure that each file can be opened and is viewable. This will be done by the Data Manager under the supervision of Dr. Chakravarty. The Data Manager will then save on the server the raw data processed using the image processing stages previously described in the Approach section. The raw data will be immediately backed up on a second server in the CAMH Research Imaging Centre in case the first server crashes or becomes corrupted. Then, the data are immediately backed up on high capacity data cartridges. One cartridge is kept on site in a fire proof safe and the second copy is kept in a safe off site in the event of any major unforeseen physical disruption to the computer network or laboratory (e.g., flood, fire, etc.). CAMH servers are also backed up nightly. Once raw data are checked and backed up, they will be processed using the methods described in the Data Analysis section under Approach. Each image processing step will be tracked and its execution will be recorded within the study neuroimaging database. All derived measures (mean cortical thickness, structure volumes, surface area, mean FA, etc) will also populate the database automatically upon the completion of the pipeline execution. All imaging data, derived measures, and demographic data will be accessible via webportal (hosted at CAMH) through unique username and password credentials. Results will be published as group data without the use of characteristics that would identify individual participants.

As described above, each time a STOP-PD II subject consents to participate in this neuroimaging study, the STOP-PD II will notify the Study Coordinator and the project RA. In turn, the Study Coordinator will liaise with the STOP-PD II team to ensure that the participant's demographic and clinical data are uploaded to the secure ftp site managed by the Toronto Research imaging Center. These data will also be anonymized and stored in a separate database. With respect to the clinical data, the Cornell data management team has developed a set of standards and procedures for data entry and checking and for the documentation of data sets generated for statistical analysis. These methods assure the integrity of study data. All of the clinical and demographic data are carefully managed within the context of the STOP-PD II grant at Cornell. Currently the STOP-PD II Toronto site sends original copies of de-identified hard data to Cornell by overnight shipping every second week. STOP-PD II sites retain hard copies of these records. The hard data are stored in a locked filing cabinet stored in a locked office to further protect subject anonymity. Data auditing, entry and quality control are carried out at Cornell under the supervision of the data management team. The Cornell data management team has many years of experience in data management and coordination of multi-study, multisite research projects. They have developed an infrastructure that supports the design of and standardization of databases, data entry, data quality control, and preparation of data for statistical analysis. This system assures the quality of data entry, storage, verification, and validation, and the reporting of data from this study. This system will also support the tracking of participants and data at the individual sites. Regularly scheduled, and as needed, communications between the STOP-PD II Cornell team, the neuroimaging Data Manager, Study Coordinator and PI will clarify any inconsistencies and ambiguities in the data.

14B. Randomization will follow that of the STOP-PD II RpCT.

14C. Data Analytic Procedures:
Demographic characteristics between treatment groups will be compared using analysis of variance and two sample t-tests for continuous variables, and chi-squared test for categorical variables.
Hypothesis 1: At each vertex we will model the fixed effects of time and treatment group, while controlling for gender and age differences in cortical thickness. Mixed model regression will be used as it permits the inclusion of multiple measurements per person, and irregular intervals between measurements, thereby increasing statistical power. We will use nested random effects terms that will model within group and within subject dependence of observations. We will run all models with and without handedness, IQ, and socioeconomic status as main effects, and in interaction with the treatment group term to determine whether they need to be included in the final model. Assuming they do not need to be included, the final model, at each vertex for cortical thickness, for ith group's jth individual's kth time-point is modeled as:

$$\text{Cortical thickness}_{ijk} = \text{Intercept} + d_i + d_{ij} + ß_1(\text{sex}) + ß_2(\text{age}) + ß_3(\text{mean time}) + ß_4(\text{treatment group}) + ß_5(\text{mean time x treatment group}) + ß_6(\text{age x treatment group}) + e_{ijk}$$

d_i represents the fixed intercept and d_{ij} the random intercept (to account for within subject variability), ß represents regression coefficients, and e is the error term. Treatment group is a binary categorical variable with level olanzapine or placebo respectively. We have described analyses according to a linear model. However, it is possible that change in cortical thickness or surface area may follow a nonlinear pattern. We will test other fits

to the data (e.g., quadratic), to optimally fit our data. Results will be reported after application of a q = 0.05 false discovery rate threshold across P-values for all fixed-effects terms excluding the intercept. The analyses of surface area and subcortical structure will follow the exact same approach with appropriate covariates for each specific model (e.g., for analyses of subcortical structures, total brain volume is used as a covariate).

Hypothesis 2: For white matter tract integrity a similar model will be used as for the primary hypothesis. However, tract volume will serve as an additional variable modeled within the linear mixed model, as it may be correlated with tract FA or tract diffusion measures.

Exploratory Analyses: Clinical, cognitive, metabolic, anthropometric, pharmacokinetic, and genetic data are being collected by STOP-PD II. We will conduct several exploratory analyses including modeling olanzapine pharmacokinetic parameters in relation to brain structural changes, genetic predictors of these changes, and determine whether those with the worst clinical outcomes (i.e. relapse) present with the most changes (while controlling for treatment). Finally, we will determine whether older participants are more or less susceptible to brain structural change than the younger participants. (e.g, are there age x treatment interactions?).

14D. Statistical Power Analyses: The proposed study has more than adequate power for the primary and secondary hypotheses; it has not been powered for the exploratory analyses, for which no power calculations are presented.
Assumptions for Power Calculations (Hypotheses 1 and 2): The cortex is 3.5-5 mm thick at key neuroanatomic locations (e.g., anterior cingulate cortex, posterior cingulate cortex, superior temporal gyrus, entorhinal cortex, dorsolateral prefrontal cortex). The ‘intersubject standard deviation’ at these key regions is typically 0.23-0.29 mm, as estimated from our own samples of schizophrenia patients and healthy controls ranging from 18-85 years of age. These samples include patients with severe psychopathology and a very wide age range. Thus, we believe that this standard deviation is at least as high as what we will find in the proposed study and taking 7-8% S.D. of the mean is reasonable. Similarly we find approximately 8% standard intersubject deviation in our schizophrenia patients and healthy controls on DTI measures. For our primary analyses, based on the recent human and animal data, we conservatively hypothesize 3-5% difference in tissue loss in olanzapine vs. placebo over 36 weeks, corresponding for instance to the placebo group having fixed brain measures across time (i.e., 0% change in cortical thickness) and the olanzapine group experiencing a 3-5% loss in cortical thickness.

Within subject reliability testing for ‘within subject standard deviation’ for Hypotheses 1 and 2: In order to directly demonstrate within subject reliability of all of our dependent imaging measures (cortical thickness, surface area, subcortical volumes and white matter tract integrity), five healthy volunteers were scanned twice at a one week interval on the Toronto scanner. The acquisition protocols were the ones described above. Tables 1 and 2 show the Toronto measures, demonstrating high scan/rescan reliability and low within subject standard deviation.

Table 1: Highly reliable scan/rescan measures of gray matter morphometry in several regions.

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	Grey matter morphometry measures		
Measure	*Scan 1*	*Scan 2*	*% Difference*
Cingulate Thickness (mm)	3.559	3.549	0.3%
Cingulate Surface Area (mm²)	3328.264	3415.658	2.6%
Temporal Lobe Thickness (mm)	3.446	3.413	1.0%
Temporal Lobe Surface Area (mm²)	20253.180	20060.040	1.0%
Striatum Volume (mm³)	10037.040	10071.380	0.3%
Thalamus Volume (mm³)	6398.690	6339.360	0.9%

Most measures demonstrated a within-subject error of less than one percent, and all were less than three percent. These values agree with, or are an improvement on the literature with respect to scan-rescan error for dependent measures such as cortical thickness[101] or white matter integrity[102]. In particular for DTI based measures, where error is typically somewhat higher than for gray matter measures, our use of a 60 direction acquisition plus our use of a whole brain tractography clustering algorithm that is well suited to longitudinal studies due to the segmentation of discrete clusters of white matter tracts, that facilitates operator tract selection, ensures high reliability of the data[9, 33]. Our white matter segmentation approach occurs in the native space of each subject. DTI analysis approaches are otherwise easily subject to misregistration errors using voxel wise approaches[103, 104].

Table 2: Highly reliable scan/rescan measures of white matter integrity in five white matter tracts (CC = corpus callosum; Occ. = occipital)

	Fractional Anisotropy			Mean Diffusivity		
Tract	*Scan 1*	*Scan 2*	*% Difference*	*Scan 1*	*Scan 2*	*% Difference*
Uncinate	0.424	0.421	0.7%	0.00242	0.00242	0.0%
Inferior Longitudinal	0.469	0.464	1.0%	0.00238	0.00239	0.6%
Cingulum Bundle	0.459	0.456	0.7%	0.00244	0.00242	0.7%
Genu of CC	0.536	0.536	0.1%	0.00249	0.00250	0.5%
Inferior Fronto-Occ.	0.487	0.488	0.1%	0.00243	0.00242	0.1%

Power calculations (Hypotheses 1 and 2): One of our investigators, Dr. Lerch has published on power analysis simulations for neuroimaging data[10], including power analysis for detecting change in neuroimaging data over time between groups[105]. Here, we used a simulation approach for our power calculations in order to estimate the percentage change that is detectable at each brain structure at different alpha-corrected thresholds (with a focus on an alpha = 0.05, and an alpha = 0.01 for a more stringent threshold (Figure 3).
The parameters extracted to estimate statistical power for the analyses of each of the gray matter morphometry and white matter integrity measures comprise: Cortical thickness, surface area, volume, and FA value (*Baseline*), inter-subject SD ($\sigma_{population}$), within-subject SD ($\sigma_{subject}$), expected change (μ), N per group (n) number of scans ($N_{timepoints}$). The power estimation accounts for both the subject noise and the population noise:

$$Baseline \sim normal(\mu_{population}, \sigma_{population});\ TP_2 \sim Baseline + normal(\mu\left(\frac{\text{timepoint}-1}{N_{\text{timepoints}}-1}\right), \sigma_{subject})$$

where timepoint=2, $N_{timepoints}$=2, and TP_2 is the simulated value at the second timepoint. The percentage change (of cortical thickness, surface areas, volume, FA) was varied between 1-5% at steps of 0.5% in our simulations. We estimated the sources of population-based noise from the scan-rescan experiment described above. The

significance level from the simulated data was then calculated for each of the simulated changes. For each structure 1,000 simulations were performed.

The sample size proposed for this study was determined based on the recruitment and flow from the STOP-PD II study: 19 participants randomized to olanzapine will complete MRI/DTI scanning at baseline, 2-3 of whom are expected to relapse (based on the STOP-PD II expected relapse rate of 15% for olanzapine); of the 19 participants randomized to placebo, 6-7 are expected to relapse (based on a relapse rate of 35%). Based on the simulations described above, with 16 participants on olanzapine and 12 on placebo who maintain remission for 36 weeks, we estimate that we have a 80% power to detect a 3-5% group difference in structural brain change over time for all but one of our dependent measures at an alpha = 0.05. These group differences are comparable to our anticipated differences (see above).

15. Confidentiality

There is a potential risk of breach of confidentiality that is inherent in all research protocols. Procedures have been established, and will be followed, to minimize the risk of breach of confidentiality. All information obtained from participants will be kept as confidential as possible. Computer based files/ data will be entered into password-secured databases and paper-based files will be stored in a secure location. These data will only be accessible to personnel involved in the study and they will abide by confidentiality regulations of the REB. Subject confidentiality will be maintained by the use of a code number (not related to name, or date of birth) on all questionnaires and reports. A list of subject names will be kept in a separate locked cabinet with access only to study personnel authorized by the PI.

Participants will not be identified by name in any publication of research results. Results will be published as group data without the use of characteristics that would identify individual participants. All data pertaining to a subject's involvement in this study will be coded and stored in locked offices. This information will only be accessible to the research team. In unusual cases, a subject's research records may be released in response to a court order. If the research team learns that a subject or someone with whom the participants is involved is in serious danger or harm, an investigator will inform the appropriate agencies.

16. Costs and Payments

Reimbursement will be provided to cover costs of travel (TTC tokens) and *$65 per scan for time and effort.* If subject withdraws before the end of the study, they will be paid for the study visits that they participated in.

17. Qualifications of Principal Investigators

Aristotle. N. Voineskos, M.D., Ph.D., Koerner New Scientist. Head, Kimel Family Translational Imaging-Genetics Research Laboratory, Staff Psychiatrist, Geriatric Mental Health and Schizophrenia Programs CAMH and Assistant Professor of Psychiatry, University of Toronto. He has successfully conducted several neuroimaging studies in both healthy volunteers and patients with severe mental disorders (i.e., schizophrenia, major depressive disorder, bipolar disorder, and Alzheimer disease)

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Benoit H. Mulsant, M.D., Professor of Psychiatry and Clinical Director, Geriatric Mental Health Program, CAMH, has extensive experience implementing randomized clinical trials, including trials of patients with psychotic depression.

18. References