STUDY IDENTIFICATION

Project title	Dopamine buffering capacity measured by phMRI as a novel biomarker of disease progression in PD
Short title	Measuring Parkinson's Disease Progression
IRB	Washington University Human Research Protection Office
IRB title	Dopamine buffering capacity measured by phMRI as a novel biomarker of disease progression in PD
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Protocol version (revision date)	Version 20171024 (October 24, 2017)

OBJECTIVES

This application introduces a novel pharmacodynamic brain imaging method for objectively quantifying disease severity in Parkinson disease (PD). The method is levodopa pharmacological fMRI rapid quantitative pharmacodynamic imaging, or "bucket mapping" for short.

The novel method is based on the well-known clinical observation that the benefit from a dose of levodopa wears off more quickly as PD progresses. A well-known phenomenon in PD treatment is that early in the course of disease, a small dose of levodopa provides benefit for many hours. The body responds as if the levodopa in the plasma filled a reservoir and then slowly leaked out to produce benefit. With disease progression, even though the same amount of levodopa circulates in the blood, the benefit wears off much faster, as if the reservoir had become leakier. This wearing off of benefit has been quantified by a mathematical model that postulates a reservoir (central effect compartment) whose concentration of levodopa directly determines the clinical benefit. The buffering capacity in this model can be characterized by a single number, the effect site rate constant k_e. This constant, k_e, can be computed from serial measurements of plasma concentration and clinical status (like UPDRS scores or tapping speed). On average, patients with more severe disease and longer disease duration have a larger ("leakier") k_e when modeled this way (Fig. 1) [1-6]. Dopamine buffering capacity as measured by k_e also correlates significantly with nigrostriatal denervation as measured by DOPA uptake (FDOPA PET) [7] or dopamine transporter imaging (FP-CIT SPECT) [8]. Unfortunately, clinical measurements are influenced by confounding factors such as patient fatigue and motivation. A direct, objective brain measure of response to LD may reduce this added variance.

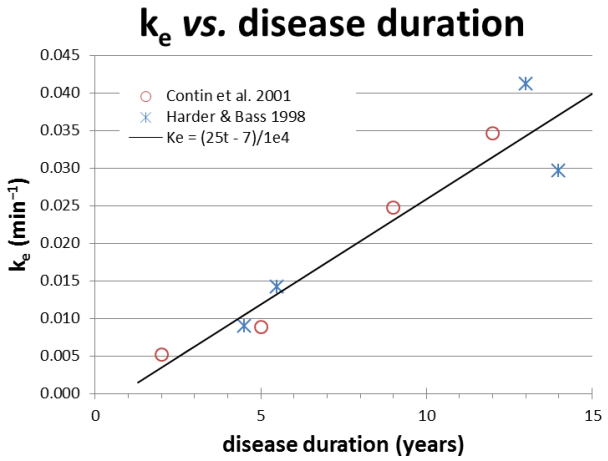

Figure 1. Across groups of PD patients, k_e is a surrogate for disease duration

The effect of levodopa in humans and other primates has been measured using regional cerebral blood flow (rCBF) to reflect regional brain activity [9-14]. Crucially, levodopa has ***no*** direct vascular effects when given with adequate carbidopa pretreatment (we use 200mg p.o. an hour before levodopa) [9-11]. Levodopa's regional CBF effects mirror its regional effects on glucose metabolism (studied with 2DG autoradiography or FDG PET) and are prominent in pons and midbrain, thalamus, middle frontal gyrus, insula, putamen and cingulate cortex [10,12].

The expected rCBF response in midbrain to the rapid i.v. infusion, based on levodopa pharmacokinetics measured in PD [15] and published mean pharmaco-dynamic parameters [3,5], are quite distinct (Fig. 2), suggesting that even with some imperfection in the rCBF signal, we can reasonably expect to derive an

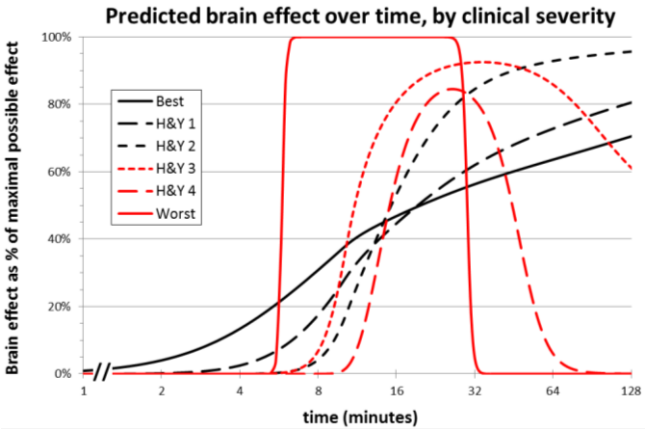

Figure 2. Patients from different Hoehn and Yahr stages of PD are predicted to show different time courses of brain activity (unpublished data).

accurate k_e for a brain region that responds to exogenous levodopa with a dose-response curve anywhere close to that of clinical response. Midbrain, for instance, has a robust rCBF response to single, clinically sensible doses of levodopa [9-12,14].

This study's goal is to validate "bucket mapping" as an objective, quantitative measure of disease severity in PD. Success would position the new method for use as a surrogate endpoint in clinical studies of putative disease-modifying therapies for PD, potentially speeding up such trials dramatically.

Specific Aims

1. Test whether dopamine buffering capacity in midbrain and posterior putamen, measured by levodopa phMRI, reflects clinical disease severity (disease duration, off-period motor function and clinical asymmetry) in 20 PD patients covering a range of current symptom severity and disease duration.
2. Test whether regional k_e values are affected by clinical treatment (6 weeks of clinically dosed carbidopa-levodopa (CD-LD) in 10 previously untreated PD patients).

Secondary Hypothesis

In Parkinson's disease (PD), while motor disturbances are the predominant and most readily recognized symptoms, various non-motor symptoms frequently co-occur. Cognitive functions such as working memory and executive control are on average worse in PD, although with substantial variability across patients. This variability highlights the need for ongoing studies exploring the nature of cognitive deficits in PD and their relationship to disease severity and progression. This study utilizes phMRI-measured dopamine buffering capacity in putative motor regions as a model of motor disease severity. We propose extending this approach to include measures of cognitive performance utilizing the NIH Toolbox [22]. The NIH Toolbox cognitive measures were chosen because they can be completed relatively quickly (important to minimize patient discomfort when assessed in the medication off state), because it incorporates tasks that directly assess functions known to be impaired in PD, and because of its recognized validity [23]. The hypothesis is that the extent of DA buffering capacity loss in pertinent regions (such as the caudate nucleus) will explain a significant fraction of the variance in specific cognitive abilities.

Secondary Aim

3. Test whether dopamine buffering capacity in caudate reflects severity of cognitive dysfunction.

STUDY DESIGN

Overall study timeline

The study is planned for complete enrollment within 3 years.

Overview of each subject's participation

After thorough clinical assessment on a screening visit, subjects return on a separate day for levodopa phMRI. Clinical severity ratings are repeated. Regional CBF data from pre-specified brain VOIs (volumes of interest) are collected continually with ASL fMRI before levodopa, during a 10-minute i.v. loading dose, and then repeatedly over the next approximately 90 minutes.

Levodopa-naïve patients in Aim 2 will have all scan day procedures repeated identically on a second scan day after 6 ± 1 weeks of clinically dosed treatment with CD-LD.

Screening visit

1. Potential subjects will review a written informed consent document with the investigator or designee and will have opportunity to resolve questions or concerns. All procedures below are performed only after written documentation of consent.
2. We will collect the following measures at screening:
    a. Basic demographic information
    b. MRI safety checklist
    c. Handedness (Edinburgh Handedness Inventory)
    d. PD symptom and treatment history including:
        i. onset, laterality, dystonia, dyskinesia, psychosis
        ii. past therapies (pharmacological, surgical and behavioral *e.g.* "Big and Loud"), their effects positive and negative, and current treatment
    e. MMSE
    f. Beck Depression Inventory
    g. Michigan Alcoholism Screening Test
    h. UPDRS Part II (past week activities of daily living)
    i. NIH Toolbox cognitive measures (approx. 31 minutes—see table below)
3. Dr. Black will review the history with the subject, perform a neurological and psychiatric examination, and document his review of the inclusion and exclusion criteria. For patients in Aim 2, Dr. Black will discuss the risks and benefits of about 6 weeks of treatment with oral CD-LD and will document the clinical appropriateness.
4. Subjects who have not previously had an MRI of the head or neck will be exposed to a mock scanner.
5. Introduce subject to tapping speed measures and practice until comfortable

Cognitive measures from the NIH Toolbox			
Task - Cognitive Domain	**Duration (minutes)**	**Screening Day (ON)**	**MRI Day (OFF)**
Picture sequencing - Episodic memory	7	✓	
Flanker task - Inhibitory control and attention	3	✓	✓
List Sorting - Working memory	7	✓	✓
Picture vocabulary - Language	4	✓	
Oral reading recognition - Language	3	✓	
Dimensional change card sort - Executive function	4	✓	✓
Pattern comparison - Processing speed	3	✓	✓
Approximate total duration (minutes)	**31**	**31**	**17**

MRI scan day

1. Subjects will refrain from antiparkinsonian medications, oral protein intake, and caffeine after midnight the morning of the scan day.
2. Review of compliance with medication, food and caffeine restrictions
3. Notify PI of any substantive changes in health since the screening visit
4. Urine pregnancy test in any woman of child-bearing potential (*i.e.* not postmenopausal or surgically sterile)
5. Carbidopa 200mg p.o. (8 × 25mg Lodosyn® tablets or FDA-approved generic equivalent) at least 1 hour before levodopa infusion begins
6. Record vital signs
7. Tapping speed with each hand separately (60 seconds sequentially depressing two keys 20cm apart with audible and tactile feedback; record the lower score of the 2 counters)
8. Tapping speed with the more affected hand (60 seconds sequentially depressing two keys 2cm apart with the index finger) using a device equivalent to an MRI-compatible button device.
9. An i.v. line is placed in each upper extremity (preferably antecubital; alternatively a hand vein), one for infusing levodopa solution and one for repeated blood samples. Saline lock. Usually the more affected side (by "off" tapping speed) will be connected to the small-bore light-resistant tubing for the levodopa infusion, and a clear extension tubing will be connected to the less affected side for later blood samples.
10. "Practical off" UPDRS Part III (motor exam)
11. Visual analog scales (VAS) to rate current affective state, PD symptoms and side effects (nausea/vomiting, sleepiness, dizziness or lightheadedness, and overall feeling poorly or well)
12. NIH Toolbox cognitive measures (approx. 17 minutes—see table above)
13. Blood sample for [LD] (baseline levodopa concentration)
14. Enter MRI room
    a. Headphones or ear plugs are worn to dampen the noise of the scanner and for communication between the experimenter and subject.
    b. Padding behind the head, thermoplastic mask, and/or nylon tape are used to restrict head movement.
    c. Structural MRI scans (3D TSE and MPRAGE) before or after the ASL scans, or at least one hour after the start of the levodopa infusion
15. During the phMRI scans:
    a. Ensure that at least 50 minutes have passed since carbidopa dose before starting ASL
    b. ASL before i.v. levodopa for 10 minutes, subject fixating a crosshair
    c. ASL during i.v. levodopa loading dose, subject fixating a crosshair
    d. ASL repeatedly during maintenance levodopa infusion, subject fixating a crosshair
    e. Breaks during the maintenance phase—preferably at least 30 minutes after start of LD infusion—as needed for subject comfort
    f. Tapping speed with the more affected hand is repeated in the scanner using an MRI-compatible button device once during the pre-levodopa scan block, once within 1 minute of the end of the loading dose, and again within 2 minutes of the following times measured from the start of the loading dose: 0:20, 0:30, and every 15 minutes thereafter.

g. Blood samples within 1 minute of 0:05:00, 0:10:30, 0:12:00, and 0:15:00, within 2 minutes of 0:19 and 0:24, and within 3 minutes of 0:30, 0:45, 1:00, 1:30.

16. After the MRI session:
    a. tapping speed with each hand separately (60 seconds sequentially depressing two keys 20cm apart with audible and tactile feedback; record the lower score of the 2 counters)
    b. Tapping speed with the more affected hand (60 seconds sequentially depressing two keys 2cm apart with the index finger) using a device equivalent to an MRI-compatible button device.
    c. UPDRS part III
    d. Final blood sample
    e. Stop LD infusion
    f. VAS ratings
    g. Remove i.v. lines
    h. Record any benefit or side effect the patient noticed since the start of the infusion
    i. Provide a cab voucher if subject feels sleepy and had planned to drive home.
17. For Aim 2 subjects: Dispense supply of CD/LD tablets, confirm contact information, and confirm next study date.

Levodopa treatment between MR sessions (for subjects in Aim 2)

See "Treatments and Dosage," a few pages below. This treatment is intended to match routine clinical practice. The initial treatment of PD varies among experts; CD/LD is a common option, and the most common one in the PI's group practice, but other options include an MAO-A inhibitor, a dopamine agonist, or CD/LD combined with a COMT inhibitor. In other words, the 6-week initial treatment of Aim 2 subjects in this study is not off-label or experimental in the usual sense, but is standardized to a single treatment to reduce variability for the second phMRI scan day.

With that exception, all procedures in this study are done for research purposes.

POTENTIAL RISKS

We have recently summarized the world literature on safety of i.v. levodopa and no SAEs are expected [16].

(*The language below, in this section, is addressed to the patient, as in an informed consent document.*)

You may experience one or more of the risks indicated below from being in this study. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study. Some risks described in this consent document, if severe, may cause death. You will be told of any new information that may affect your willingness to participate in this study. Dr. Black will answer any questions you have about these risks.

Levodopa

Likely:

- Temporary nausea
- Temporary sleepiness

Less likely:

- Temporary light-headedness (from lower blood pressure).
- Temporary vomiting
- Involuntary movements, hallucinations (seeing or hearing things that are not really there), delusions (being convinced of things that are objectively false), mental confusion, or impulse control disorders (like excessive gambling or change in sex drive). If any of these do occur, they are temporary.

Rare:

- There is a rare possibility of an allergic reaction, which could be mild (such as itching or rash) or severe (such as swelling of the throat, causing difficulty in breathing).
- There are no other known serious risks of only 1-3 doses of i.v. levodopa. However, i.v. administration of levodopa is considered experimental and there may be unexpected side effects. While none have been encountered to date at Washington University, and we know of none occurring elsewhere, unexpected side effects could potentially be serious or even fatal.

Carbidopa

Rare:

- There is a rare possibility of an allergic reaction, which could be mild (such as itching or rash) or severe (such as swelling of the throat, causing difficulty in breathing).

Being in the MRI scanner

Likely:

- Some people get muscle aches and pains from lying on their back. This will be minimized by providing cushions at pressure points and beneath the knees as desired.
- The MRI makes a loud knocking sound during scans. Earplugs or headphones will be used to dampen the sound of the MRI procedure.
- The MRI scanner produces a loud hammering noise, which has caused hearing loss in a very small number of patients. You will be given earplugs or headphones to reduce this risk and to dampen the sound of the MRI procedure.
- Lying in the scanner may be boring or fatiguing, and it may be challenging to stay awake.

Less likely:

- Lying in the MRI scanner may elicit feelings of claustrophobia (discomfort in a small or enclosed space). During the procedure, you will be able to talk with the MRI staff through a speaker system. You can tell them to stop the scan at any time.
- Some patients experience slight tingling or tapping sensations in the arms and legs (mild peripheral neuromuscular stimulation) during the MRI scans. If you notice this and it is uncomfortable, we can reposition you to try to reduce the effect, or you can choose to stop the study.

Rare:

- If you have a device such as a pacemaker, deep brain stimulation (DBS) device, bone hardware, metal in your body (such as shrapnel or surgically implanted items), or device placed in your uterus there may be additional risks. We will review what device you have and

inform you of these risks. **If you have any of these items in your body, participation in the study could cause serious harm and you may not be able to participate in the study. Therefore, it is very important that you notify Dr. Black if you have any of these items in your body.** In general, these risks could be:
    - heating or movement of the device
    - device malfunction
    - damage to the tissue that surrounds the device.
- If you have a skin tattoo, including cosmetic tattoos (eye-liner, lip-liner) you could experience the following:
    - irritation, swelling or heating in the area of the tattoos
    - in rare instances a primary or secondary burn.
    - If you have a tattoo we will offer you a cold, wet washcloth to put over the tattoo to reduce this risk.

Placing an IV catheter and drawing blood

Likely:

- You may have a bruise at the IV site.

Less likely:

- The blood draw may cause bleeding, bruising, or pain. Some people become dizzy or feel faint when blood is drawn or when the IV catheter is placed.

Rare:

- There is a slight risk of infection. We will attempt to reduce that risk by cleaning the skin and using appropriate care.

Other study procedures

Likely:

- The questionnaires and interviews may be slightly boring, fatiguing or challenging.
- Because you are asked to avoid caffeine and protein on the morning of the scan, you may notice fatigue, sleepiness, or hunger.
- If you routinely take anti-parkinsonian medications, going without them before the MRI scan on the MRI day may worsen the severity of your symptoms until you get back on your usual medication regimen.
- If you regularly drink coffee, tea or similar products in the morning, you may have a headache on the morning of the study from skipping caffeine.

Rare:

- The questions that you are asked during this study could make you feel uncomfortable. If any question makes you feel uncomfortable, you may choose not to answer it.
- Confidential information about you may be accidentally disclosed. However, we think the risk of accidental disclosure is small. The information we gather during the course of the study is coded only by a study number and is kept separately from your name, address, etc. Please see the Confidentiality section of the consent form for more information.

Pregnancy

If you are a woman capable of becoming pregnant, we will ask you to have a pregnancy test before beginning this study. You must use effective birth control methods and try not to become pregnant while participating in this study. If you become pregnant, there may be unknown risks to your unborn child, or risks to your unborn child that we did not anticipate. There may be long-term effects of the treatment being studied that could increase the risk of harm to an unborn child. You must tell the doctor if your birth control method fails while you are on the study. If you believe or know you have become pregnant while participating in this research study, please contact the research team member identified at the top of this document as soon as possible. Please discuss with the research team how long you need to wait before becoming pregnant after completing the treatment or procedures on this study.

If you are a sexually active male it is important that your partner not become pregnant during your participation in this study. There may be unknown risks to the unborn child or risks we did not anticipate. You and your partner must agree to use birth control if you want to take part in this study. If you believe or know that your partner has become pregnant during your participation in this study, please contact the research team member identified at the top of this document as soon as possible.

LOCATIONS / SITES OF STUDY

Data collection: Washington University School of Medicine

Data analysis: Washington University School of Medicine

STUDY DURATION

Anticipated duration of entire research activity per patient:

- Aim 2: Three days over the course of 1½ to 3 months*
- Patients not in Aim 2: Two days, which can occur up to a few weeks apart*

* One additional make-up scan day will be allowed for each subject, with his/her consent, in the event of technical problems that prevent completion of the scan day procedures. MRI equipment failure or a broken infusion pump are examples of such technical problems.

Anticipated duration of entire research activity:

Three years

SAMPLE SIZE

Aim 1: N = 20

Aim 2: N = 10 (up to 2 subjects can overlap between groups)

The N's above refer to subjects who are eligible after screening procedures and who continue in the study. Additional subjects may be screened to reach this number of eligible subjects.

Power considerations: For a first study of a method, there is no information available to confidently

predict variance of the key outcome measures and provide a formal power analysis. We have chosen the sample sizes here pragmatically, to allow recruitment and scanning at a steady, practically achievable rate. We note that the sample size of 20 for Aim 1 provides power >80% to find a moderately strong positive correlation ($r = 0.5$) while maintaining a low false positive rate α=0.05. Power exceeds 99% to find a very strong correlation that would explain half the variance in disease severity ($r = 0.7$).

Additional levodopa-naïve subjects would not help Aim 1 substantially, as a Pearson correlation analysis is not appropriate if a large fraction of subjects are lumped at one end of the range of disease duration or severity. Therefore we plan to limit to 2 the number of subjects included in both Aims. However, if severity or duration vary substantially among the never-treated subjects, we may include more than two of them in the Aim 1 analysis. The number of subjects for Aim 2 is largely pragmatic, given the difficulty of recruitment and the additional cost with 2 scans per subject.

INCLUSION CRITERIA

There are two groups of subjects. All have idiopathic Parkinson disease. For the first group, we will attempt to sample a broad range of severity and disease duration. The second group will not be treated currently with levodopa, so most subjects in this group will be early in the disease process.

Inclusion criteria for all subjects:

- Age 40-79 at screening
- Meet accepted diagnostic criteria for Parkinson disease

EXCLUSION CRITERIA

Exclusion criteria for all subjects:

- Pregnancy
- Metal in the head or eye, or other contraindication to MRI
- Deep brain stimulator (DBS)
- Patients taking a dopamine antagonist or dopamine partial agonist
- Claustrophobia
- Significant neurologic disease other than PD (exceptions would include febrile seizures or uncomplicated migraine)
- Head trauma with loss of consciousness for more than 5 minutes
- Severe or unstable systemic illness
- MMSE < 24
- History of a primary psychotic disorder
- Current severe major depressive episode (clinical diagnosis after psychiatric interview)
- Current alcohol use disorder (clinical diagnosis after screening with the Michigan Alcoholism Screening Test)
- Current psychosis
- Subjects who feel that going without nicotine for 3-4 hours would be uncomfortable
- Currently taking an extended-release formulation of a dopamine agonist

Additional exclusion criteria for Aim 2 subjects:

- Taking levodopa currently or in the past month, or ever for more than a month
- Taking a dopamine agonist currently

CONCOMITANT MEDICATIONS

Required:

- none

Allowed:

- Most drugs that do not prominently affect the brain or constrict blood vessels
- Loratadine and other non-sedating antihistamines
- Benzodiazepines or "Z drugs" with short half-life used in the evening only
- Medications taken only as needed (less than daily), except those listed below

PROHIBITED MEDICATIONS

Daily use of any of the following medications:

- medications containing caffeine or theophylline
- benzodiazepines with long half-lives (e.g. clonazepam), except that a single dose at bedtime for treatment of REM sleep behavior disorder may be allowed at the discretion of the PI
- centrally-active antihistamines or anticholinergics (e.g. Benadryl®)
- orally administered nasal decongestants

If taken in the 24 hours prior to the MRI scans:

- p.r.n. benzodiazepines with long half-lives, p.r.n. Benadryl®, illicit drugs

If taken on the morning of the MRI scans:

- antiparkinsonian medications
- stimulants, nasal decongestants, caffeine, nicotine, alcohol

TREATMENTS AND DOSAGE

MRI Scan day

Treatments:

- Carbidopa (Lodosyn® 25mg tablets), or FDA-approved generic equivalent
- Levodopa 2mg/ml in normal saline, as described in previous IND communications, supplied by the Barnes-Jewish Hospital Research Pharmacy service

Dose:

Carbidopa: 200mg = 8 × 25mg tablets by mouth at least 1 hour before starting the levodopa infusion

Levodopa solution: based on age and body mass as follows:

Loading dose: 0.6426mg / kg / (10 min.)

Maintenance rate: 2.882 mg/kg/min. × 10^{-5} × (140 year − age)/year

This is the "final dose" in Table 1 [15]. The total levodopa dose for a 60-year-old 70kg subject over a 2.5-hour infusion is 68mg; an oral dose of 80 mg would provide the same total absorbed dose, though

over a slower time scale [17]. We have used this method with the 200mg carbidopa in previous studies and reported on its safety [13,18].

Oral treatment between scan days, in the Aim 2 group

Treatment:

- Carbidopa/levodopa 25/100mg tablets (Sinemet® or FDA-approved equivalent)

Dose:

Individualized for each subject based on response (benefits and side effects). Generally the starting dose will be ½ tablet by mouth twice daily, with dose increases or reductions based on contacts with the subjects as needed. Supplemental carbidopa will be prescribed for patients with prominent non-CNS side effects who have inadequate motor response (or are expected to if the dose is lowered). The plan will be not to change the dose, except for urgent clinical need, during the week prior to the second MRI scan day. See Appendix.

ANALYSIS PLAN/APPROACH/METHODOLOGY

Outcome measures

There are no outcome measures relevant to clinical care. This is not a treatment study.

Data analysis

Neuroimaging data analysis will begin with routine data preprocessing steps. ASL images will be converted to rCBF images using our previously described methods [19], weighted inversely for head motion similar to the approach of [20]. A Bayesian parameter estimation [21] procedure, with the addition of a hysteresis parameter k_e as described in Objectives above, will then be applied to repeated serum levodopa concentrations, and time–CBF curves from the volumes of interest (VOIs) listed below to estimate k_e for each VOI.

Thalamus and striatal nuclei (putamen, caudate, nucleus accumbens) will be defined from each participant's structural MR image(s) using the method of ref. [22], or if needed by FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). Alternatively, the structures can be defined manually from the mean group atlas-aligned MPRAGE image. VOIs will be defined independent of any clinical data.

Primary VOIs:

1. Midbrain (MB). This VOI will be defined from the group difference image comparing the mean rCBF image from approximately 15-40' after the start of the LD infusion to the mean pre-infusion baseline rCBF image. The 15-40' time frame is expected to reflect activation from LD in nearly all subjects (see Fig. 2). The same time frame will be used in all subjects. The VOI will be masked to exclude voxels outside the brain. The VOI may extend into pons; comparisons to published results from 2DG and PET studies will guide any questions about defining the borders of the VOI.
2. Posterior putamen VOIs from each side of the brain (LpP, RpP). On each axial slice, a line normal to the sagittal plane and tangent to the fornix posteriorly will divide the anterior and posterior putamen, as in ref. [23]. The nucleus accumbens is excluded.

Secondary VOIs:

3. Left and right caudate VOIs (LC, RC).
4. Left and right anterior putamen (LaP, RaP) as defined above. The nucleus accumbens is excluded.
5. Left and right nucleus accumbens (LNA, RNA).
6. Thalamus (Th).
7. Each voxel in the brain.

The primary analysis will be a correlation across all Aim 1 subjects of the k_e's for a VOI to clinical measures of disease severity and duration. Duration of illness will be measured from the first symptom, as recorded on the screening day. Standard 20cm tapping speed in the L and R hand and hemi-UPDRS part III motor scores, both measured in the standard off condition, will capture the increased variance provided by clinical asymmetry and will be correlated with contralateral posterior putamen k_e. Midbrain k_e will be compared to total "off" UPDRS part III scores and tapping speed in the more affected hand. Specifically:

The primary comparisons for Aim 1 will be simple Pearson correlations:

1. between the off-period 20cm tapping speed in the more affected hand and the midbrain k_e, and
2. between the off-period total UPDRS part III motor score and the midbrain k_e.

Secondary comparisons for Aim 2 will be correlations:

3. between "off" 20cm tapping speed on each side and contralateral posterior putamen k_e, and
4. "off" hemi-UPDRS part III motor scores on each side and contralateral posterior putamen k_e.

For #3 and #4, we will calculate the r values using repeated measures correlation [24], with side as the repeated measure, rather than as $2n$ independent data points. However, we are interested in between-subject correlation as well as the within-subject r_{rm} that the rmcorr package focuses on, so we will also report that p value (corresponding to the first row in Table 3 of ref. [24]).

For Aim 2, the k_e's for each VOI will be compared before and after 6 weeks of treatment in a repeated measures ANOVA analysis. Here the key comparison is whether k_e changes with time (before vs after treatment) in any VOI, controlling for side (more affected side vs less affected side). Our hypothesis is that this change with time is at or near 0, so our answer will be framed in terms of 95% confidence intervals for change. Any VOIs identified from the Aim 1 SPM analysis will also be tested.

Secondary analyses will correlate k_e's from secondary VOIs to all measures of disease severity (off tapping speed, off UPDRS III, disease duration) and to cognitive test scores (NIH Toolbox task scores adjusted for age), using the off-period scores for measures done on the scan day. Voxelwise analyses will use SPM with an initial threshold of $t \geq 3.0$ and statistical inference at the cluster level with a false discovery rate of 0.05. Another secondary analysis will use the k_e computed from the button box tapping speeds from the more affected hand and the levodopa concentrations, independent of the perfusion MRI data ("tapping k_e"). We will test whether the tapping k_e correlates with the k_e from the midbrain or contralateral posterior putamen, or other VOIs.

Note that we expect that k_e for some VOIs will correlate better than for other VOIs with measures of disease severity or duration. This conclusion is based on the expectation that a specific clinical effect may be better associated with dopamine buffering capacity in a specific (set of) brain region(s).

Therefore we expect that the k_e-vs-clinical slope may differ significantly from zero only for a subset of the VOIs; in other words, we do not expect the slopes to be parallel across VOIs, and therefore will not include all VOIs in a single ANCOVA with a factor for VOIs. However, any errors in estimating pharmacokinetic parameters (i.e., in reconstructing the continuous function $C_p(t)$, plasma levodopa concentration as a function of time for $t \geq 0$) occur at the subject level. Such errors will affect all k_e estimates for that subject (*i.e.*, across all VOIs). Therefore we cannot assume independence across VOIs, within subject, for the measured estimates of k_e.

Visual analog scale (VAS) measures of side effects will be analyzed as repeated measures ANOVAs. Other variables (demographics, clinical measures, NIH Toolbox scores, etc.) will be summarized or compared as appropriate using standard statistical methods.

Additional analysis details

We will examine interactions of the key analyses with subject demographics or disease variables such as age or presence of dyskinesias.

We will use a p=.05 threshold for significance tests. All the correlation hypotheses are one-sided, i.e. k_e will increase with increasing disease severity or duration (e.g., positive correlation for UPDRS scores or duration, as in Fig. 1 of the attached protocol; negative correlation for tapping speed). We will not correct for multiple comparisons among the 4 numbered primary analyses named above. The secondary analyses described above involve a number of comparisons but are exploratory. We will correct individual voxelwise analyses for multiple comparisons using SPM, as described above.

For the primary analyses (correlations of k_e and disease severity or duration), outliers will be determined at the level of the individual variables before computing the correlations. If the distribution of k_e values or of disease severity markers clearly deviates from a normal distribution on visual inspection because of outlying values, after transformation if appropriate, we will use a nonparametric analysis such as a Spearman correlation.

Subjects without "off" tapping score or UPDRS will be excluded from the pertinent analyses.

Subjects without a k_e value, due to missing perfusion MRI data or missing [LD] data, will be excluded from the primary analyses. However, subjects with a few missing [LD] data points that do not prevent a good fit to the PK model will be included; an example may be a subject with missing [LD] values only between (say) 30 and 90 minutes after the infusion starts.

SAFETY MONITORING

We have recently summarized the world literature on safety of *i.v.* levodopa and no SAEs are expected [16].

The Data Safety Monitoring Plan for this protocol will consist of the following measures:

- The PI will monitor for unanticipated problems, life-threatening events and deaths.
- We will report adverse events to the Washington University Human Research Protection Office (HRPO), and in annual reports to the sponsor and the FDA.

If any SAE occurs that is judged to be probably or definitely related to participation in the study, enrollment will stop until restarting is approved by the HRPO.

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APPENDIX

Treatment parameters for dosing CD-LD between scan visits in the untreated PD group

Initiation of treatment

- Explain that the purpose is to get them to a dose that causes clear, short-term improvement in PD symptoms during the next few weeks, but that has tolerable side effects (if any).
- Discuss possible benefit by reviewing their current typical PD motor symptoms.
- Discuss common side effects, including non-CNS (nausea, drop in blood pressure upon standing) and CNS (sleepiness, dyskinesias, rarely hallucinations) side effects.
- Give the patient a copy of the "Carbidopa-levodopa for Parkinson's disease" handout.
- Ask them to keep track of any missed doses and of any observations or questions for us (e.g. on a 3x5 card or on a smart phone note).
- Confirm contact information and schedule a phone call within the next week.
- Give patients your and my contact information and encourage them to call with any concerns about the medication.

Start treatment with CD-LD 25/100mg tablets, one half tablet by mouth b.i.d. (morning and early afternoon).

Questions at each scheduled follow-up contact

- What symptoms are better?
- What symptoms are not?
- If not expressed spontaneously, inquire about each of these: tremor, stiffness, slowness, speech and gait
- If there is any benefit:
    - How long does it take to "kick in"?
    - How long does the benefit last?
- Any side effects?
- If not expressed spontaneously, inquire about the non-CNS and CNS side effects listed above.
- Are you satisfied with your treatment? Do you have any questions for me?
- (After 4 weeks) About how many tablets do you have left? [Enough until Scan Day 2?]

Dosing

1. If there is a CNS side effect other than mild, tolerable sleepiness, or for any questions, discuss with the PI.
2. Proceed based on the category below that best describes the patient's current situation:
    a. **Still has bothersome PD symptoms, no or minimal side effects:** If patient has been on current dose for less than 5 days, no change. If patient has been on current dose for at least 5 days, increase dose by one level (see dosing table below).
    b. **Still has bothersome PD symptoms but also has non-CNS side effects:** If mild side effects and taking less than 100mg carbidopa per day, increase dose by one level. Otherwise add carbidopa 50mg p.o. q a.m., ½ hour before first CD-LD dose.

c. **Still has bothersome PD symptoms but also has CNS side effects:** If patient had clear benefit at the previous dose, return to previous dose. Otherwise consult with PI.
d. **Clear improvement and no remaining bothersome PD symptoms:** Continue drug at current dose schedule.

Dosing table for CD-LD 25-100mg tablets p.o.		
Level	**Dose schedule**	**Total daily dose LD (mg)**
1	0.5 tablets b.i.d.	100
2	0.5 tablets t.i.d. (morning, approximately noon, approximately 5pm)	150
3	▪ if patient has had NO wearing off between b.i.d. doses: 1 tablet b.i.d.; ▪ if patient has had wearing off between b.i.d. doses: 1 tablet q a.m. plus 0.5 tablet at noon and at 5pm	200
4	1 tablet t.i.d.	300
5	1.5 tablets t.i.d.	450
6	2 tablets t.i.d.	600
7	2.5 tablets t.i.d.	750