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Human/Nonhuman Primate AC-PC Ratio - Considerations for Translational Brain Measurements

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Abstract

This comparative magnetic resonance imaging (MRI) analysis evaluated the ratio of AC-PC (anterior commissure to posterior commissure) distance measures in selected groups of humans and nonhuman primates (NHPs). An understanding of the basis of this ratio between primate species may allow more accurate translation of NHP stereotactic targeting measurements to upcoming human trials. MRI datasets of adult humans [n=21], and juvenile and adult NHPs (Macaca fascicularis [n=40], and Macaca mulatta [n=32]), were evaluated in a mid-sagittal plane to obtain the AC-PC distance measure for each examined subject. Two trained evaluators, blinded to each other's results, carried out three separate measurements of the AC-PC length for each subject. Each observer carried out measurements of the entire dataset [n=93] before repeating the measurements two additional times. Previous dataset measures were not available for review at the time of subsequent measures. Inter- and intra-observer variabilities were not statistically significant. Minimal intraspecies variation was found in the AC-PC measurement of our human and NHP groups. We found significant interspecies differences, however, more between humans and NHPs, and less between the NHP groups. Regression analysis confirms the strong linear relationship of AC-PC distance based primarily on species in our study groups. Human/NHP AC-PC ratios varied between 2.1 to 2.3 based on the compared NHP species groups. We conclude that the scale differences in brain measurements between NHPs and humans described in this study allows improved translation of stereotactic targeting coordinates in future human clinical trials, which may lead to improved efficacy and safety.

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Keywords

AC-PC ratio; human; MRI; non-human primate; Parkinson's disease; stereotactic surgery; translational study

1.0 INTRODUCTION

The most common stereotactic targets for the treatment of Parkinson's disease (PD) with deep brain stimulation (DBS) are the subthalamic nucleus (STN) and the globus pallidus internus (GPi) (Rezai et al., 2008). Most recently, gene therapy has also been used to target the STN for the treatment of PD (Kaplitt et al., 2007). Because the STN has a small volume, and resides in a well-defined anatomical location, modern stereotactic targeting for DBS (or gene therapy) requires high resolution magnetic resonance imaging (MRI) for localization, with or without target confirmation via electrophysiological recording and/or stimulation (Ondo and Bronte-Steward, 2005; Starr et al., 2002; Starr et al., 2009). On the other hand, while larger subcortical structures such as putamen are easily visualized with MRI and even computed tomography (CT) scans, the larger and less distinct the target region, the more difficulty deciding, with visual cues alone, the optimal site for infusion cannula placement for delivery of therapeutics. While functional neurosurgeons have defined the optimal sites for DBS implantation within the STN (Benabid et al., 1994; Limousin et al., 1995; Starr, 2002; Theodosopoulos et al., 2003), no such optimal targets have been clinically defined within the human putamen for therapeutic infusions. With preclinical (Bankiewicz et al., 2006; Grondin et al., 2002; Sanchez-Pernaute et al., 2001) and clinical data (Eberling et al., 2008; Gill et al., 2003; Lang et al., 2006; Slevin et al., 2007) suggesting that delivery of therapeutics within putamenal foci can be effective in the treatment of PD, defining these optimal target sites in humans begins to gain priority.

A recent preclinical study (Yin et al., 2009a) utilizing gene therapy, delivered via real-time convective delivery (RCD) (Fiandaca et al., 2009) to the putamen of parkinsonian nonhuman primates (NHP), has suggested that there may be an optimal site for convection-enhanced delivery (CED) (Bankiewicz et al., 2000; Bobo et al., 1994) within this large subcortical structure. Reproducibly targeting such a locus allows CED to provide the largest volume of distribution (Vd) of the therapeutic agent within the putamen, while minimizing leakage into surrounding structures (Yin et al., 2009a). Since there is currently no known anatomical or electrophysiological correlate to this optimal putamenal CED infusion locus, site confirmation strategies used for STN DBS, such as high resolution MRI, and neural recording/stimulation strategies, do not appear helpful in targeting putamenal infusions. Successful putamenal targeting, therefore, requires an understanding of the optimal target volume and demands a delivery modality that features precision and reproducibility. While high-resolution MRI will certainly allow excellent visualization of the human putamen and other subcortical brain structures, the best site for placement of a CED cannula within the human putamen requires translation of stereotactic targeting information from recent NHP studies (Yin et al., 2010; Yin et al., 2009a), and an improved understanding of the scale differences between the human and NHP brains.

We have recently described the volumetric differences in striatal brain structures that exist between humans and NHPs (Yin et al., 2009b). In this article we investigate a novel method for comparing linear distance measurements within the brains of humans and NHPs using MRI. MRI-based brain atlases, utilizing data from multiple subjects, are now more commonly available for humans (Daniluk et al., 2010; Hasan et al., 2010) and NHPs (McLaren et al., 2009; Vincent et al., 2007). While the scope of this analysis is not meant to be an exhaustive definition and comparison of linear distances within the brains of humans

and NHPs, and does not pretend to comprehensively analyze all possible variables affecting these measurements, we present a strategy, based on our available data, for developing a distance measurement ratio (Human/NHP), which may be useful in translating preclinical stereotactic measurements for use in future human clinical trials targeting putamen for the treatment of PD. For this purpose, we elected to measure a well-known brain distance, the AC-PC (anterior commissure to posterior commissure) line, which is easily determined on mid-sagittal MRI, and is commonly utilized by functional neurosurgeons and incorporated in the planning algorithms of the majority of commercially available image-guided stereotactic systems. The Human/NHP AC-PC ratio allows us to quickly translate NHP stereotactic coordinates into comparable ones in the human.

2.0 MATERIALS AND METHODS

2.1 Human Subjects

Measurement data from normal human and PD individuals was derived from MRIs conducted as part of a prospective, open-label Phase I clinical trial examining the safety and tolerability of putamenal gene transfer in patients with PD at the University of California San Francisco (UCSF). The study was reviewed and approved by the Recombinant DNA Advisory Committee of the National Institutes of Health, the United States Food and Drug Administration, and the Institutional Review Board of UCSF. All patients underwent extensive pre-operative screening and counseling, and provided written informed consent prior to entry into the study, as well as prior to any surgical procedures. In this study, our human group featured twenty-one patients (*see* Table 1). Eleven patients diagnosed with PD were all at Stages III to IV on the Hoehn and Yahr scale (4 males and 7 females), and had classic clinical features of PD, with no evidence of any other neurological disease. Age data from ten individuals ranged from 61 to 71 years (mean of 66.1 years), with the eleventh subject's age not available. Ten normal individuals (4 males and 6 females) had no neurological disease or structural lesions involving the basal ganglia. The normal patients' ages ranged from 64 to 88 years (mean of 73.4 years).

2.2 Non-Human Primate Subjects

Our NHP groups (*see* Table 1) included forty Cynomolgus (*Macaca fascicularis*) and thirty-two Rhesus (*Macaca mulatta*) monkeys. The NHPs were housed separately in home cages in temperature-controlled rooms and exposed to 12-h light/dark cycle. They were fed twice daily in amounts appropriate for the size and age of the animals, and water was freely available. The diet was supplemented with fruit or vegetables daily. Furthermore, small pieces of fruit, cereal, or other treats were provided as part of the environmental enrichment program. Experimentation was performed according to the National Institutes of Health guidelines and to the protocols approved by the Institutional Animal Care and Use Committee at UCSF (San Francisco, CA) and at Wincon TheraCells Biotechnologies Co. Ltd. (Nanning, China). The Cynomolgus group featured 28 males and 12 females, with available ages in 38 subjects ranging from 5 to 12 years (mean of 8 years), and weights ranging from 3.1 to 11.5 kg (mean of 7.0 kg). The Rhesus group included 26 males and 6 females, with ages ranging from 5 to 25 years (mean of 16.3 years), and weights ranging from 4.0–16.8 kg (mean of 10.7 kg). Eight of the Rhesus macaques had undergone MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) lesions (Bankiewicz et al., 1986) 1–2 years prior to their MRI imaging studies, all of whom were males, with ages ranging from 5 to 8 years (mean of 6.9 years), and weighing 7.4–16.8 kg (mean of 12.3 kg). The remaining 24 Rhesus macaques did not receive MPTP lesions, 18 were males and 6 were females, ranging in age from 5 to 25 years (mean of 19.5 years), and weighing 4 to 14.5 kg (mean of 10.2 kg). The Cynomolgus monkeys had not been treated with MPTP prior to their brain MRIs.

2.3 Magnetic resonance image (MRI)

2.3.1 MR images of normal human subjects—were acquired on a 1.5T Siemens Magnetom Avanto (Siemens AG, Munich, Germany) scanner, with a rigid head coil. Three-dimensional rapid gradient echo (MPRAGE) images were obtained with repetition time (TR) = 2110 ms, echo time (TE) = 3.6 ms, and a flip angle of 15°. The number of excitations (NEX) = 1 (repeated 3 times), matrix = 240 × 240, field of view (FOV) = 240 × 240 × 240, and the slice thickness = 1 mm. These parameters resulted in a 1-mm³ voxel volume. The scanning time was approximately 9 min.

2.3.2 MR images in Parkinson's disease patients—were acquired on a 1.5T Philips Intera scanner (Philips Medical Systems, Best, The Netherlands) with a rigid head coil. Axial inversion recovery (IR) sequences were obtained with TR = 3000 ms, TE = 40 ms, and an inversion time (TI) = 200 ms. The NEX = 3, matrix = 304 × 195, FOV = 260 × 222 and the slice thickness = 2 mm. These parameters resulted in a voxel size of 0.86 × 1.14 mm. Scanning time was approximately 12 minutes.

2.3.3 MR images of NHPs—were acquired on a 1.5-T Sigma LX scanner (GE Medical Systems, Waukesha, WI) with a 5-inch surface coil on the animal's head, parallel to the floor. NHPs were sedated with a mixture of ketamine (Ketaset, 7 mg/kg, IM) and xylazine (Rompun, 3 mg/kg, IM). An intravenous line was established. After sedation, each animal was placed in a MRI-compatible stereotactic head frame. The ear-bar and eye-bar measurements were recorded. MRI data was then obtained and animals were allowed to recover under close observation until able to right themselves in their home cages. Spoiled gradient echo (SPGR) images were T1-weighted and obtained with a spoil grass sequence, a TR = 2170 ms, a TE = 3.8 ms, and a flip angle of 15°. The NEX = 4, matrix = 256 × 192, FOV = 16 cm × 12 cm, slice thickness = 1 mm. These parameters resulted in a 0.391 mm³ voxel volume. Scanning time was approximately 20 min.

2.4 Measurement of AC-PC line in humans and NHPs

Mid-sagittal brain MRIs were designated for measurement in each subject. Our measuring protocol consisted of defining the center of the AC and PC on the mid-sagittal image (see Figures 1 and 2), using the Schaltenbrand modification (Schaltenbrand and Bailey, 1959) of the Talairach reference (Talairach et al., 1957; Talairach and Tournoux, 1988). Using a cursor to designate the center of each structure, a distance measurement was obtained directly from the image software. The measurements were made on an Apple Macintosh G4 computer with OsiriX® Medical Image Software (v2.5.1) (OsiriX Foundation, Geneva, Switzerland). OsiriX software reads all data specifications from DICOM (digital imaging and communications in medicine) formatted MR images obtained via local picture archiving and communication system (PACS). The distances from AC to PC were manually defined, and then calculated by the precalibrated software. All the distances were measured in the same manner on the MRI sections. Two separate observers, trained in taking measurements on MRI images and using the same measuring protocol, measured AC-PC distances from each of the 93 subjects. Each observer collected three separate AC-PC measures from each of the 93 subjects, with the measures obtained separated by time and without having previous measures for comparisons. The two observers collected their three separate 93 measurement datasheets, which were then submitted for analysis by a separate investigator.

2.5 Statistical Analysis

Experimental subject data was grouped according to species. Individual observers' measurements (three for each case) were also grouped so that inter- and intra-observer reliability analysis could be performed. Reliability analysis was used to determine the

significance of variability of the observers' measurements. Descriptive statistics were obtained for each of the three evaluated species and comparative tabular and graphic representations were formulated. Linear regression analysis of combined data of all three species was used to define which independent factors primarily influenced the dependent AC-PC measure in our subjects. SPSS Statistics Gradpack17.0 for the Mac (2008, SPSS Inc., Chicago, Illinois) was used for the statistical analysis of the data. Statistical significance for all tests was indicated if $p < 0.05$.

3.0 RESULTS

The frequency distribution data for our Species groups is presented in Figure 3, showing Human, Cynomolgus, and Rhesus data respectively. Age data was missing on one of our Human subjects, so only twenty ages were available for this plot. A relatively normal distribution is presented around a mean age of 69 or 70, except for the outlier (age 88). The ages of the 38 Cynomolgus monkeys (2 animal ages were not available) also appear normally distributed. Our 32 Rhesus monkeys, however, were distributed into two age groups; a younger group (the majority of which had received MPTP), and an older group. Due to the limited number of subjects available in each group, further age analysis within each Species group, based on sex or other factors, was not formally carried out. As defined in the Methods, however, our PD patients' mean age was 7 years younger than the control patients.

Descriptive statistics for our three subject groups are shown in Table 2. Analysis of intra-observer and inter-observer correlation coefficients did not show statistically significant differences between our two observers or their three individual observations, with all correlations > 0.99 . AC-PC distance means were calculated from the total number of observations per Species group, initially separated by observer and finally presented with the combined observer's data in the last two columns of Table 2. Combined AC-PC line measures in our Species groups had mean values (mm, $\pm$ SD) of 28.3 ± 1.6 , 12.4 ± 0.8 , and 13.8 ± 0.7 for Humans, Cynomolgus, and Rhesus, respectively.

A scatterplot of subject age with MRI-defined AC-PC line length observations, for our three Species groups, is shown in Figure 4. For each Species group, there was poor correlation, r (see dotted line), and the coefficient of determination, r^2 , between age and AC-PC length measures obtained via MRI. The Cynomolgus group, being primarily younger animals, showed a slight trend towards increasing AC-PC length with age. Of interest, the more varied aged rhesus monkeys and humans trended (but not significantly) toward lower AC-PC length with increasing age. While both of our NHP groups tended to show increasing AC-PC length measures with increased weight and the male sex, these factors did not achieve statistical significance in our analysis.

We examined which common independent factors (Species, age, sex, Observer [A or B], Observation number [1, 2, or 3], PD [yes or no], MPTP [yes or no]) were statistically significant in predicting AC-PC length in our subject groups using linear regression analysis (see Table 3). Since we did not have weight data on our human subjects, weight was not evaluated in the regression models for our three Species. A series of factor models were analyzed to determine which variables most importantly affected the AC-PC distance prediction. Observer and Observation number did not prove to be statistically significant in determining AC-PC length in this analysis. Our well-trained observers' measurements had very high inter- and intra-observer correlations in this study. The most statistically significant factors included Species and Sex, as seen in Models 1–4, where stepwise removal of other factors did not significantly change the predictive ability, r^2 -value (Albright et al., 1999), of the subsequent Model. AC-PC measures predicted with Model 4 had an r -value of

0.988, and an r^2 -value of 0.976, indicating that Species and Sex alone predicted greater than 97% of the variability in the AC-PC measure in our subjects. The Coefficient analysis of Model 5, which uses Species only, and has a similar r^2 -value (see Table 3, **Regression Coefficients**), yields the following linear regression formula for the mean AC-PC distance:

$$\text{AC-PC (mm)} = 28.3 (\text{constant, Human}) - 15.9 (\text{if Cynomolgus}) - 14.5 (\text{if Rhesus}).$$

This formula accurately predicts the mean values of AC-PC obtained from our measurement data, as noted in Table 2 (Combi AC-PC). The limited number of subjects in our primate groups does not allow us to fully predict the importance of factors such as age and sex in predicting AC-PC distance in larger populations.

4.0 DISCUSSION

Translating stereotactic targeting information from NHP studies into humans is not always straightforward. In the treatment of PD with gene therapy (Fiandaca and Bankiewicz, 2010) two major sites of vector delivery have been effectively utilized, the STN and putamen. The results of target transduction in these clinical trials have so far been most effective when covering a small, discrete structure, such as the STN (Kaplitt et al., 2007), that is easily seen on MRI and can be confirmed with electrophysiological testing. When targeting the larger putamen, however, protocols that provide a more extensive volumetric distribution by the vector (Valles et al., 2010) appear more clinically effective (Christine et al., 2009; Eberling et al., 2008) than those methods (Marks et al., 2010; Marks et al., 2008) that provide a more limited volume of gene expression (Ceregene, 2009, <http://videocast.nih.gov/ram/rac061709.ram>). In attempts to maximize gene therapeutic delivery to larger brain targets, such as putamen, our group has tried to better understand the volumetric differences between the human and NHP brains (Yin et al., 2009b), to take advantage of the superior distribution characteristics afforded by CED (Bobo et al., 1994), and to define the optimal putamenal site for stereotactic catheter placement (Yin et al., 2009a). The optimal putamenal target site, however, is only definable by stereotactic coordinates based on the AC-PC line, since it lacks a discrete anatomic correlate on neuroimaging studies, and does not have a neurophysiological signature.

Modern stereotactic targeting often features high resolution MR imaging for direct target visualization, with or without image fusion techniques (O'Gorman et al., 2009). The latter allow the MRI to be overlaid on/with a CT scan, from which direct measurements can be obtained. Image fusion of CT and MRI, therefore, enhances measurement precision compared to MRI alone, due to the measurement artifacts associated with MRI (Pan et al., 2007; Snell et al., 2006; Sumanaweera et al., 1994). Despite the advancements afforded by imaging technology, there remain some discrepancies regarding the AC-PC distance in humans, and thereby the accuracy of targeting using AC-PC line measures alone. The average AC-PC distance in humans has been cited as 24 mm (range of 20 mm to 30 mm) (Spiegelmann, 1996). In a historical, small comparative study looking at human AC-PC measures with ventriculography or CT, mean values were 25.3 mm (range 23 mm to 28.5 mm, SD = 1.5), and 25.2 mm (range 22 mm to 28 mm, SD = 1.7), respectively (Hariz et al., 1993). A more recent MRI-based analysis of AC-PC length in humans with advanced PD resulted in mean values of 26.3 (range 22.9 to 29.9, SD = 1.8) (Daniluk et al., 2010). Despite these discrepancies in mean AC-PC measures, early functional neurosurgeons were quite effective in targeting paraventricular targets (Rezai et al., 2008). To further improve localization for many deep brain targets, such as thalamus, globus pallidus, and STN, microelectrode recording/stimulation (MER) are often used to help confirm final target

location prior to permanent placement of an electrode or generation of a lesion (Ondo and Bronte-Steward, 2005).

Within the putamen to date, however, electrophysiologic target localization has not been clinically applicable. The most precise way of reproducibly targeting a specified locus within the putamen, therefore, remains via stereotactic guidance. Such precision is essential to the proper volumetric delivery of a gene therapy product. The goal of this study was to better understand the scale differences in measures of the AC-PC line between three primate species (human and two NHP) using 1.5T MRI. This scale information may be effective in translating stereotactic targeting data from preclinical NHP studies into human clinical trials. We postulated that understanding the relative differences in the AC-PC distance measurement, based on MRI alone, through the Human/NHP AC-PC ratio, would provide an important translational tool for future stereotactic guidance in PD and other neurological applications. We also postulated that although the relatively limited number of subjects analyzed might limit the power of our analysis and our ability to estimate the true mean AC-PC distance value for each Species group, the ratio between Human/NHP AC-PC distances would be less likely affected, especially if the AC-PC distance variations within each Species group were small. Additionally, any MRI artifacts present in the measurement data for each Species group would be cancelled out in the ratio.

Based on this study, we determined the Human/NHP AC-PC ratio to be 2.3 with our Cynomolgus monkeys and 2.1 with our Rhesus group. While the measured mean AC-PC distances ($\pm$ SD) for this study's Species groups are 28.3 ± 1.6 mm, 12.3 ± 0.8 mm, and 13.8 ± 0.7 mm, for the Human, Cynomolgus, and Rhesus groups, respectively, these Species-specific data would clearly be stronger with a larger number of subjects in each group, more diverse ages and weights represented, and equal numbers from each sex. Although the reliability of our measurements within and between observers increases the confidence that our analysis truly represents the included subjects in this study, we understand the limitations of our relatively small dataset in representing the Human and NHP populations. We find comfort in the small variation in AC-PC distance measures between our two NHP groups, despite significant age and weight ranges in the two species evaluated. Although the true Human mean AC-PC measurement varies somewhere between 20 mm and 30 mm, and in our small group of human subjects was approximately 28 mm, the Humans/NHP AC-PC ratio can be safely approximated at 2.0. We believe this ratio will be useful to investigators translating NHP distance measurements, such as stereotactic coordinates, for planning future human clinical trials the call for specific, reproducible localization within the putamen or other brain structures.

We propose using stereotactic targeting data from NHP putamen (Yin et al., 2009a) that has been adjusted for scale differences using the Human/NHP AC-PC ratio, in future human clinical trials targeting putamen for therapeutic infusions using CED. Preliminary stereotactic (X, Y, and Z) coordinates for targeting in humans would be approximately twice (2 $\times$) the stereotactic coordinates obtained in the NHPs. Targeting other brain structures, such as thalamus and brainstem (Yin et al., 2010), would require a similar multiplication scale factor based on the Human/NHP AC-PC ratio that we have defined. It is this definition of the scale differences in linear brain measurements between human and NHP that may enhance targeting accuracy and reproducible target coverage in future clinical trials employing CED for the treatment of PD.

5.0 CONCLUSIONS

The Human/NHP AC-PC ratio is approximately 2. The definition of the scale difference between AC-PC measures of these human and NHP species improves the accuracy of

translation of preclinical stereotactic targeting data in planning human clinical trials that require precise stereotactic guidance to subcortical loci within relatively large structures such as the putamen.

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Figure 1. Hybrid schematic representation of mid-sagittal brain MRI slice

Depicted are the anterior commissure (AC), posterior commissure (PC), the AC-PC line [small dotted white line along Antero-Posterior (y) axis], the mid-commissural point (MCP), and the three major orthogonal axes: the Medial/Lateral (x) axis; the Antero-Posterior (y) axis; and the Dorso-Ventral (z) axis. The AC-PC coordinate system is established by drawing the AC-PC line from the midpoint of the two commissures on mid-sagittal MRI scans. The MCP establishes the origin (0,0,0) of the 3D Cartesian coordinate system used to spatially localize structures within the brain. By definition, positive coordinates are anterior, to the right, and above the MCP, while negative coordinates are in the opposite directions. Coronal images are reconstructed in planes parallel to those defined by the x-z axes, along the Antero-Posterior (y) direction. Similarly, axial image reconstructions are parallel to the plane of the x-y axes, along the Dorso-Ventral (z) direction. Sagittal image reconstructions are parallel to the plane of the y-z axes, along the Medial/Lateral (x) direction.

Figure 2. Examples of sagittal MR image AC-PC measurements from the three Species groups Mid-sagittal T1-wighted MRI examples used for measuring AC-PC in (A) Cynomolgus, (B) Rhesus, and (C) Human. A larger field of view of the brain above a magnified image, showing labeled AC and PC (in Cynomolgus), and dotted white AC-PC line, indicating typical measure obtained in each Species group.

Figure 3. Age frequency distribution of study subjects

Plotted frequency distribution of ages for subjects from the three Species groups in this analysis. (A) Human (n=20, one PD patient did not have age data). (B) Cynomolgus (n=38, the age of two animals was not available). (C) Rhesus (n=32). Note the relatively normal distribution for the Human and Cynomolgus age data (with a single human outlier), and the bimodal distribution for the Rhesus ages.

Figure 4. Scatter plots of Species age vs. AC-PC distance

(A) Human, (B) Cynomolgus, and (C) Rhesus. Data points plotted include six measures of AC-PC distance per subject, three measures each by the two observers. Note the apparent lack of close correlation between age and AC-PC measure, with the coefficient of determination (r^2), $r^2 < 0.1$ in all three Species groups.

Table 1

Subject Information for MRI Analysis of AC-PC Measures

Species	Number	Male/Female	Age Range (yrs)	Mean Age (yrs)	Weight Range (kg)	Mean Weight (kg)
Normal Human	10	4/7	64–88	73	-	-
PD Human	11	4/6	61–71 ^a	66 ^a	-	-
All Humans	21	8/13	61–88 ^b	70 ^b	-	-
All Cynomolgus	40	28/12	5–12 ^c	8 ^c	3.1–11.5	7.0
Normal Rhesus	24	18/6	5–25	18.5	4.0–14.5	10.2
MPTP Rhesus	8	8/0	5–8	6.9	7.4–16.8	12.3
All Rhesus	32	26/6	5–25	16	4.0–16.8	10.7

^a Age data from 10 of 11 PD Human subjects.

^b Age data from 20 of 21 Human subjects.

^c Age data from 38 of 40 Cynomolgus subjects

Table 2

Descriptive Statistics for AC-PC line Measures in Humans and NHP

Species	Number of subjects	Total # Measures/Obs	Observer 1				Observer 2				Combi AC-PC ^a	Combi SEM
			Mean AC-PC ^a	SEM	Range	SD/Var	Mean AC-PC ^a	SEM	Range	SD/Var		
Human	21	63	28.3	0.2	25.4–31.9	1.6/2.7	28.3	0.2	24.9–31.7	1.6–2.5	28.3	0.1
Cynomolgus	40	120	12.3	0.1	10.1–14.6	0.8/0.7	12.4	0.1	10.1–14.1	0.9/0.8	12.4	0.1
Rhesus	32	96	13.8	0.1	12.1–15.2	0.7/0.6	13.9	0.1	12.9–15.2	0.6/0.4	13.8	0.1

Obs = Observer.

^a AC-PC measures in millimeters (mm), SEM = Standard Error of the Mean, SD = Standard Deviation, Var = Variance, Combi = Mean when data from both Observers is combined.

Table 3
Regression Analysis of Factors determining AC-PC length in humans and non-human primates

Model	Predictors (Factors)	R	R Square	Adjusted R Square	Standard error of estimate
1	(Constant), Age, Species Rhesus, Species Cyno, Sex Female, Observer-A, Observation-2, Observation-3, No MPTP, No PD.	0.989	0.977	0.977	0.9799
2	(Constant), Age, Species Rhesus, Species Cyno, Sex Female, No MPTP, No PD.	0.989	0.977	0.977	0.9781
3	(Constant), Age, Species Rhesus, Species Cyno, Sex Female.	0.988	0.976	0.976	1.0046
4	(Constant), Species Rhesus, Species Cyno, Sex Female.	0.988	0.976	0.976	1.0081
5	(Constant), Species Rhesus, Species Cyno.	0.988	0.976	0.976	1.0179
6	(Constant), Sex Female.	0.290	0.084	0.083	6.2343

	Regression Coefficients					
Model 5	Unstandardized		Standardized		t	Sig.
	B	Std. Error	Beta			
Constant	28.311	0.091			312.188	0.000
Species Cyno	-15.934	0.112	-1.213		-142.281	0.000
Species Rhesus	-14.480	0.117	-1.058		-124.073	0.000