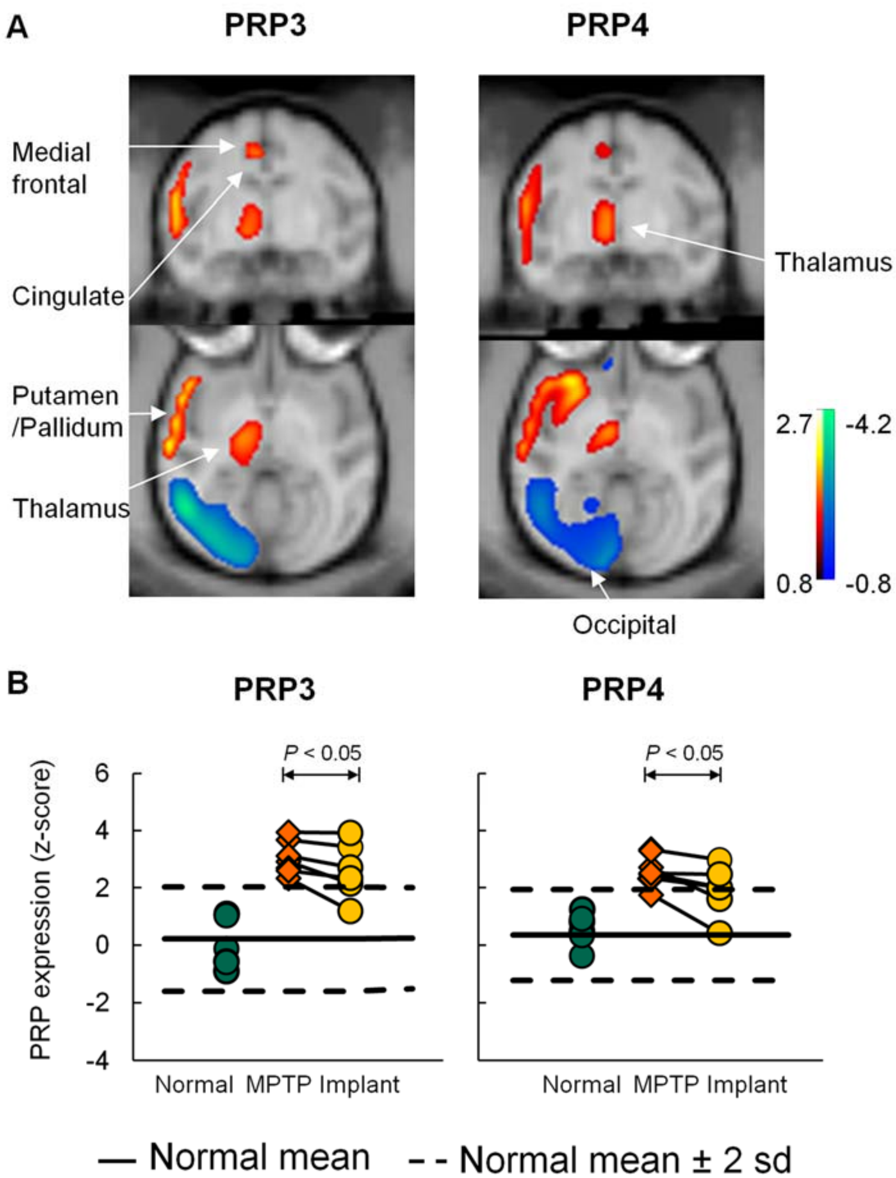
We have previously derived five PRPs by using high resolution FDG PET images in MPTP-induced parkinsonian and healthy macaques (*1*). The voxel-based spatial covariance analysis was performed by utilizing a novel multivariate brain mapping method based on principal component analysis. Derivation of PRPs and computation of network scores in individual subjects were obtained on a hemispheric (PRPs 1-4) and whole-brain (PRP 5) basis respectively. Here was a summary of sample sizes used for the derivation of each PRP. PRP1: 5 MPTP (10 hemispheres) and 6 normal (12 hemispheres) animals. PRP2: 5 MPTP (10 hemispheres) and 5 different normal (10 hemispheres) animals. PRP5: 5 MPTP and 6 normal animals (whole brains) as for PRP1. PRP3/PRP4: 6 MPTP (7 hemispheres: 5 unilateral + 1 bilateral) and 6 normal (two different groups each having 12 hemispheres) animals. Patterns (PRPs 1, 2 and 5) were derived in parkinsonian animals with more severe motor symptoms and exhibited greater extent of brain regions and higher subject scores than those (PRPs 3 - 4) in animals with less severe motor symptoms. All five PRPs showed an excellent degree of comparability in terms of topographies, group discriminations and network modulation (Suppl. Fig. 1; c.f., Fig. 3 in the main text).

The characteristics and experimental conditions of all monkeys participated in this study were provided in Table 1 and Suppl. Tables 1-2. The left and right hemispheres were averaged (after flipping the right to the left) before computing PRP network activity for images that were symmetrical - healthy and bilaterally-lesioned MPTP monkeys. This made the number of data points (i.e., hemispheres) equal to that of animals in all graphs (e.g. Suppl. Figs. 1-3). The primary analyses in the manuscript included hemispheric images from 8 normal, 7 untreated MPTP, and 6 hRPE-implanted scans after averaging both hemispheres in some animals. The changes in network activity remained significant between the normal, MPTP and implant hemispheres when using data from either hemisphere instead of the averaged hemisphere.

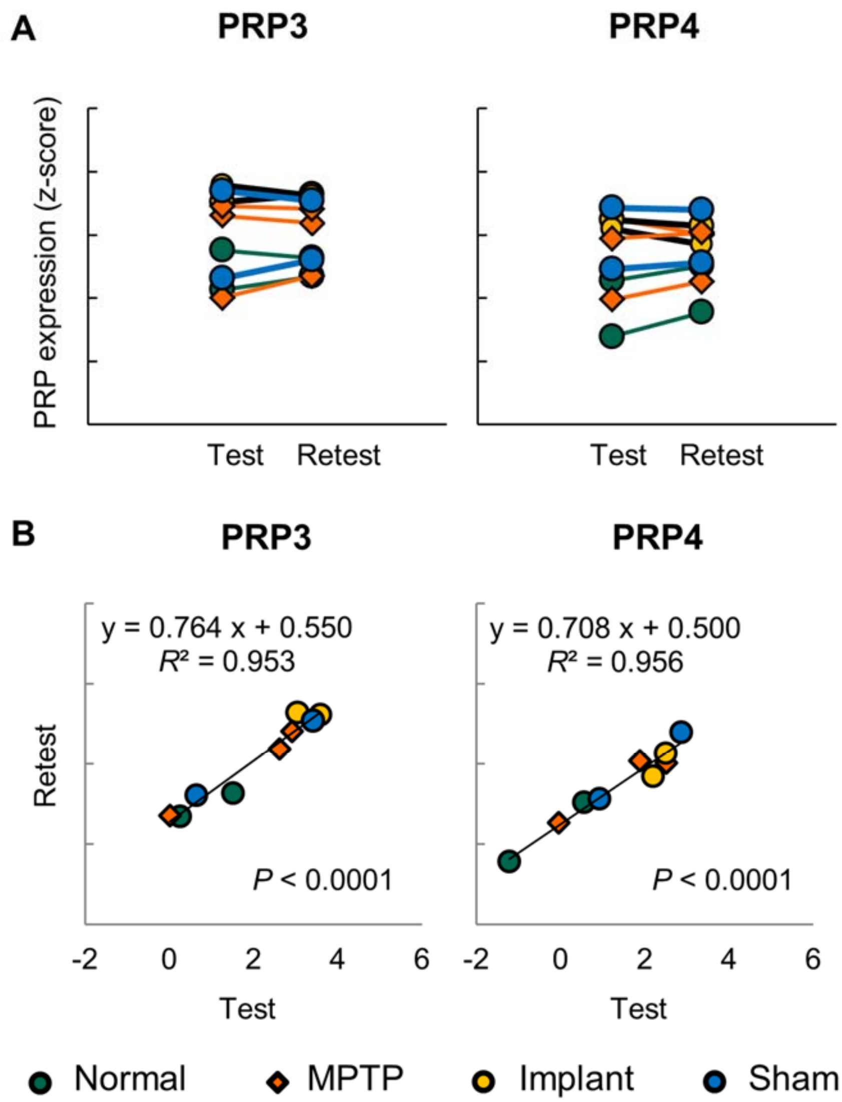
Seven animals were involved in the test-retest study as shown in Suppl. Table 2. There were 12 hemispheres owe to unilateral implantation of hRPE or GM: 2 hRPE-implanted hemispheres (2 animals), 2 GM-implanted + 2 MPTP hemispheres (2 animals), 2 MPTP hemispheres (1 animal) and 4 normal hemispheres (2 animals). This became 9 hemispheres in the same 7 animals after averaging the left/right hemispheres for 1 MPTP and 2 healthy monkeys. Network scores were computed in the test and retest scans on a hemispheric or whole-brain basis. There was a consistent level of stability in network activity across all animals for each of the five PRPs (Suppl. Fig. 2; c.f., Fig. 5 in the main text).

Animals 2-3 received hRPE implants in the other (i.e., right) hemispheres before the 2nd scan. The test-retest analysis used the first and second hRPE hemispheres on the left side while the analysis of implant effect used the first MPTP hemisphere and the second implant hemispheres on the right side. The effect of the first or the second implant would remain predominately in the ipsilateral hemisphere as discussed previously (see Items 2-3 in Response 2 above). This was also supported by comparable PRP scores between the left and right hemispheres in the second scans of these two animals. For instance, the mean network activity values in the left/right hemispheres were 3.95/3.78, 4.65/4.38 and 3.86/3.62 giving rise to the left-right increases of 4.3, 6.6 and 6.5 % or the right-left decreases of 4.2, 5.6 and 6.1 % for PRPs 1, 2 and 5. The mean test-retest values in the left hemisphere declined by 10.8, 4.1 and 5.0 % in the corresponding PRP activity. These changes were much smaller than the corresponding magnitudes of implant effects in Table 2. This provided further evidence in support of the key hypothesis that the hRPE implants induce mainly a unilateral effect on PRP brain network without causing marked network changes in the contralateral hemisphere or clinical improvement in the limbs ipsilateral to the implantation hemisphere. Suitable imaging and lateralized clinical data before and after implant will be needed to confirm this hypothesis with certainty.

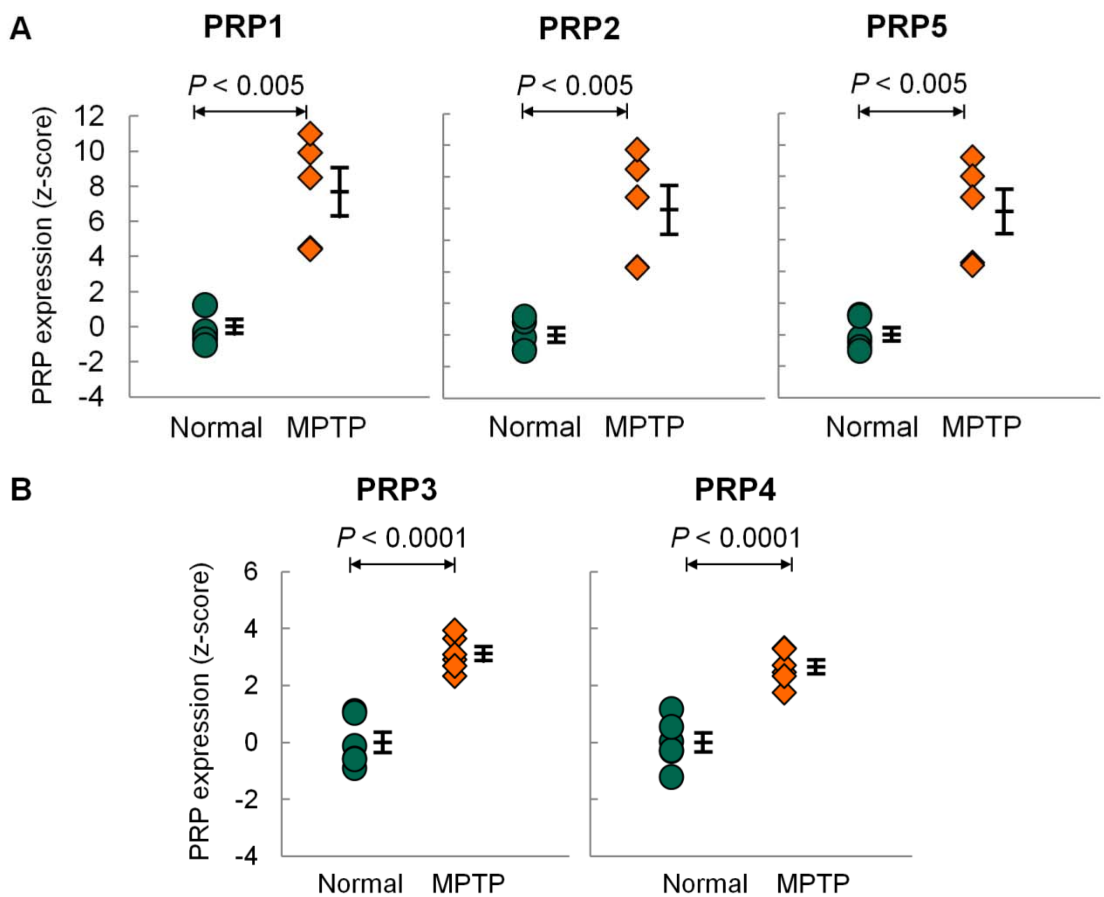
Animal 5 was scanned three times resulting in two test-retest hemispheres: MPTP on the left and right sides (scans 1 and 2) – both were averaged as one hemisphere. The analysis of implant effect used the data from the test MPTP (left/right average) hemisphere and only hPRE hemisphere (scans 1 and 3) on the right side. The data from the third MPTP hemisphere on the left side was excluded to avoid redundancy. Animal 6 was also scanned three times resulting in two test-retest hemispheres: MPTP on the left side (scans 1 and 2) and sham (scans 2 and 3) on the right side. The analysis of implant effect used the data from the test MPTP (left/right average) hemisphere and only hPRE hemisphere (scans 1 and 3) on the left side.



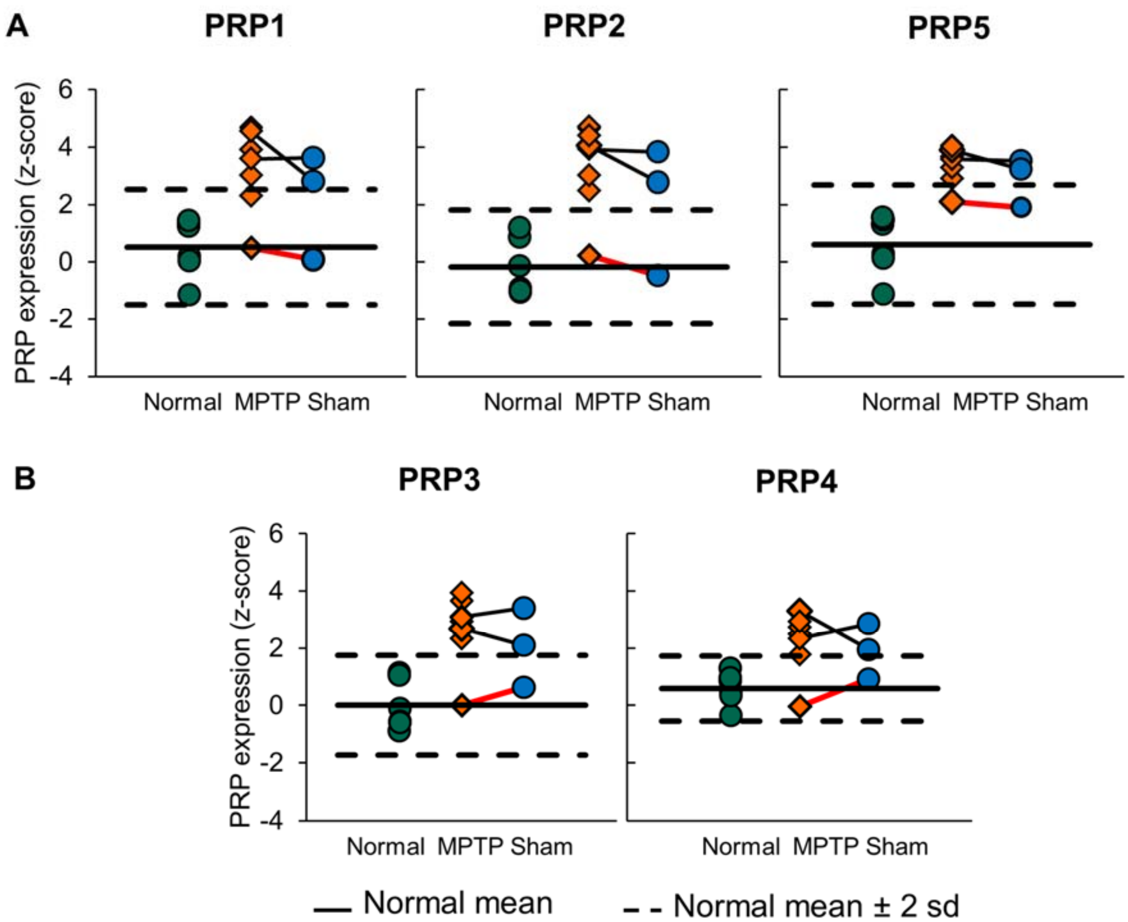
Supplemental Fig. 1: Modulation of abnormal metabolic brain networks in MPTP-induced experimental parkinsonism by hPRE cell transplantation therapy. A. Parkinsonism-related patterns (PRPs) identified on a hemispheric basis using FDG PET images in parkinsonian and age-matched healthy macaques (*1*). All analyses revealed a similar metabolic topography with increased (*red to yellow*) and decreased (*blue to green*) metabolic activity in subcortical and cortical regions. B. Network activity in individual hemispheres was elevated ($P < 0.00005$) in the 7 MPTP monkeys compared to the 8 normal controls, but showed a consistent decline ($P < 0.05$) in the 6 hPRE-implanted hemispheres versus the 6 untreated MPTP hemispheres. [The patterns are displayed on a macaque MRI brain template (*2*). The solid and dash lines in each graph represent the mean and the range of two standard deviations from the mean in the normal animals].



Supplemental Fig. 2: Test-retest reproducibility of PRP network activity. A. Network activity was highly reproducible ($P > 0.50$) between the 9 test and retest scans in the 4 subgroups of 7 macaques. B: Network scores correlated significantly between the test and retest scans ($R^2 > 0.95$; $P < 0.00001$) in individual macaques.



Supplemental Fig. 3: Network activity in individual hemispheres (PRPs 1-4) or whole brains (PRP5) discriminated MPTP monkeys and normal controls in the derivation cohort ($P < 0.005$) for each PRP (*1*). PRP1/PRP5: 6 normal and 5 MPTP animals; PRP2: 5 normal and 5 MPTP animals; PRP3/PRP4: 6 normal and 6 MPTP animals (two different groups of normal controls). The left and right hemispheres were averaged before computing PRP network activity for healthy and bilaterally-lesioned MPTP animals. Error bars in the graphs represent the standard error of the mean.



Supplemental Fig. 4: Network modulation by sham (GM carriers alone) transplantation therapy. Network activity in individual hemispheres was elevated in the two MPTP monkeys compared to the normal controls, but showed a decline in one animal and no change in another animal versus the untreated MPTP hemispheres after sham implantation. Network activity did not change in the third parkinsonian animal without PRP elevation at baseline. [The solid and dash lines in each graph represent the mean and the range of two standard deviations from the mean in the normal animals].

Supplemental Table 1: Healthy Macaques Characteristics and Imaging-related Parameters

ID	**Gender**	**Age** (y)	**Weight** (kg)	**Dose** (MBq)	**Glucose** (mmol/L)
9	M	22	10	229	4.7
10	M	8	11	222	4.6
11	M	10	14	222	3.8
12	M	10	8	222	3.7
13	M	10	9	222	3.7
14	M	10	13	222	4.3
15	F	8	7	185	3.7
16	F	8	7	167	3.2

Supplemental Table 2: Macaques Characteristics and Imaging-related Parameters in the Test-retest Study

		Test					Retest							
ID	**Gender**	**Age** (y)	**Weight** (kg)	**Motor Rating**	**Dose** (MBq)	**Glucose** (mmol/L)	**Age** (y)	**Weight** (kg)	**Motor Rating**	**Dose** (MBq)	**Glucose** (mmol/L)	**Left Striatum**	**Right Striatum**	**Time Interval** (y)
2	M	13	8	8	148	3.7	15	9	4	222	2.9	RPE		2.26
3	M	13	10	8	185	3.3	15	7	7	237	2.3	RPE		1.83
5	M	8	12	13	237	4.2	9	11	12	241	3.3	MPTP	MPTP	0.54
6	M	12	8	15	252	4.0	13	7	14	222	3.9	MPTP		0.68
6	M	13	7	14	222	3.9	14	7	8	185	4.1		GM	0.91
8	M	9	10	14	222	4.5	10	9	15	185	5.6	MPTP	GM	0.72
15	F	8	7		185	3.7	8	7		185	3.4	Normal	Normal	0.27
16	F	8	7		167	3.2	8	7		189	2.4	Normal	Normal	0.27

Animal 5 was scanned three times, twice with bilateral MPTP lesions and once with hRPE in the right striatum. Animal 6 was also scanned three times with bilateral MPTP lesions, GM in the right striatum and hRPE in the left striatum. There were total nine hemispheres available for the test-retest study after averaging both hemispheres in one animal (No. 5) with bilateral MPTP and two normal animals (Nos. 15 and 16).

References:

1. Ma Y, Peng S, Spetsieris PG, Sossi V, Eidelberg D, Doudet DJ. Abnormal metabolic brain networks in a nonhuman primate model of parkinsonism. *J Cereb Blood Flow Metab.* 2012;32:633-642.

2. Black KJ, Koller JM, Snyder AZ, Perlmutter JS. Template images for nonhuman primate neuroimaging: 2. Macaque. *Neuroimage.* 2001;14:744-748.