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Supplementary Materials for

BBB opening with focused ultrasound in nonhuman primates and Parkinson's disease patients: Targeted AAV vector delivery and PET imaging

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This PDF file includes:

SUPPLEMENTARY MATERIALS

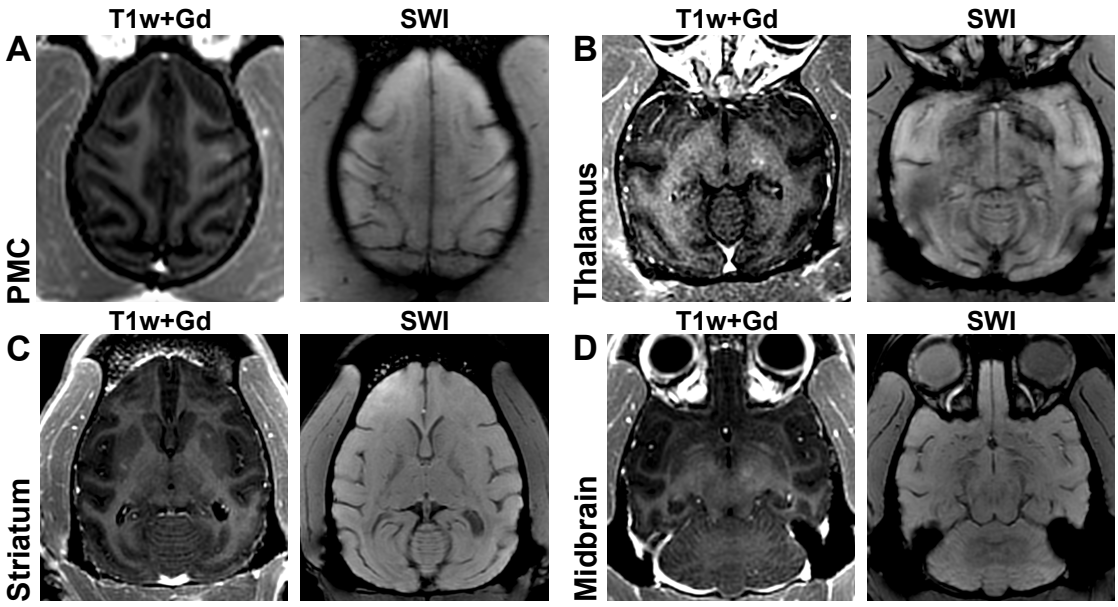

Fig. S1. Safety assessment based on MRI following BBB openings in NHPs.

Gadolinium (Gd) enhanced T1-weighted and susceptibility-weighted (SWI) images acquired immediately after BBB openings in the PMC and thalamus (M1) **(A, B)** and in the striatum and midbrain (M2) **(C, D)**. All images are shown in axial orientation in a single slice corresponding to the area of the largest BBB disruption as demonstrated by Gd extravasation.

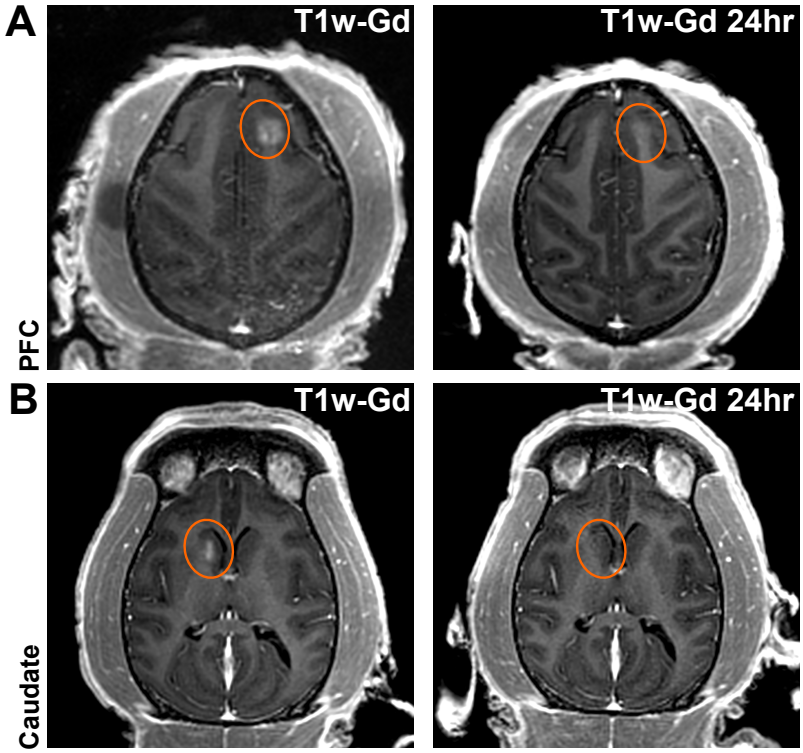

Fig. S2. BBB reversibility after sonication in NHPs.

Gadolinium (Gd) enhanced T1-weighted images immediately (**left column**) and 24 hr (**right column**) after LIFU BBB openings in the prefrontal cortex (PFC) **(A)** and caudate nucleus **(B)** of M1 (indicated by red circles). All images are shown in axial orientation in a single slice corresponding to the area of the largest BBB disruption as demonstrated by Gd extravasation.

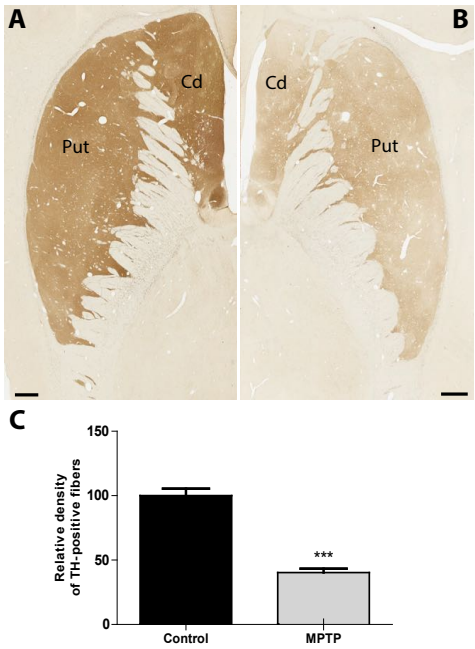

Fig. S3. MPTP treatment.

Representative axial images showing TH immunostaining in the striatum of a control monkey **(A)** and parkinsonian monkey M5 after mild MPTP treatment **(B)**. **(C)** The density of TH^+ fibers of the putamen of the MPTP-treated monkey relative to that on a control monkey. Cd, caudate nucleus; Put, putamen. Student *t*-test. ***, $P < 0.001$. Scale bars, 1 mm.

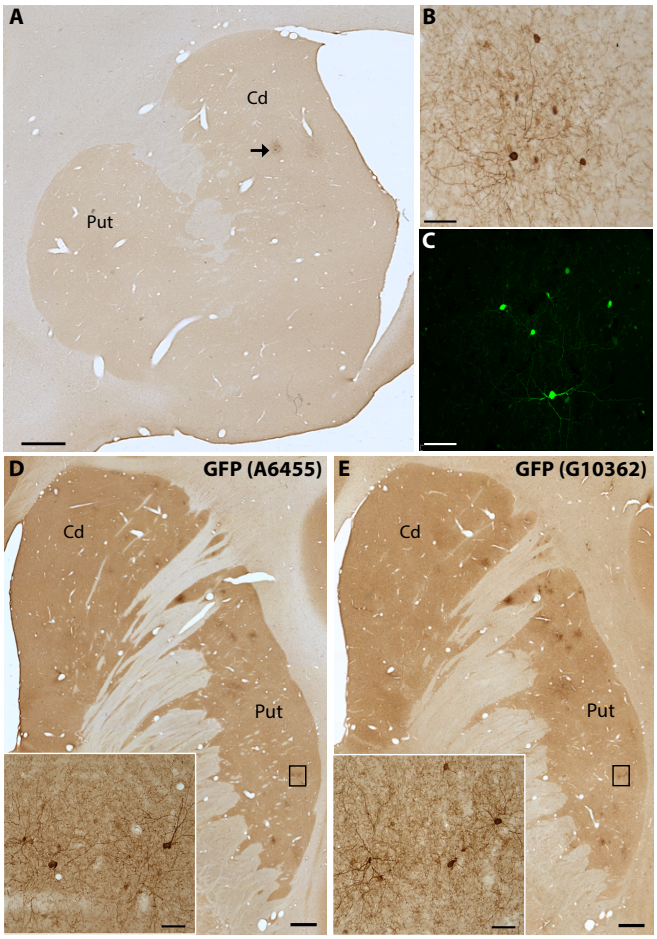

Fig. S4. GFP staining.

Indirect immunohistochemistry shows low- **(A)** and higher-magnification **(B)** photomicrographs of the striatum in M1. A focus of GFP^+ neurons (indicated by the arrow) is observed in the caudate nucleus. **(C)** Intrinsic fluorescence of GFP^+ neurons from the same striatal region. **(D, E)** Adjacent sections immunostained for GFP with two different antibodies (A6455 and G10362). These findings revealed the same localization of GFP^+ staining in the putamen. Cd, caudate nucleus; Put, putamen. Scale bars, 1 mm (A, D, E), 50 μm (B, C, insets in D and E).

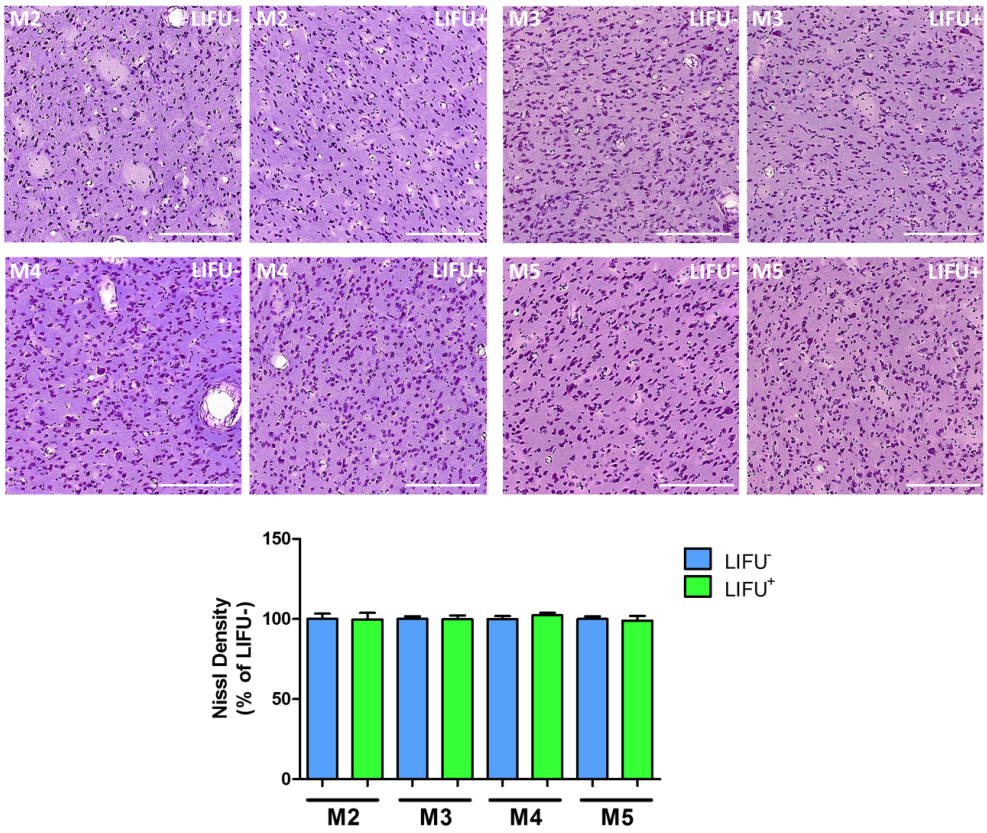

Fig. S5. Nissl staining and counting.

Representative 10x-magnified images of BBB-opened putamen (LIFU+) and their contralateral non-opened putamen (LIFU-) for each monkey (**upper**) and graph showing absence of changes in Nissl-stained cell density in the putamen of M2, M3, M4, and M5 (**lower**). Scale bars, 200 μm.

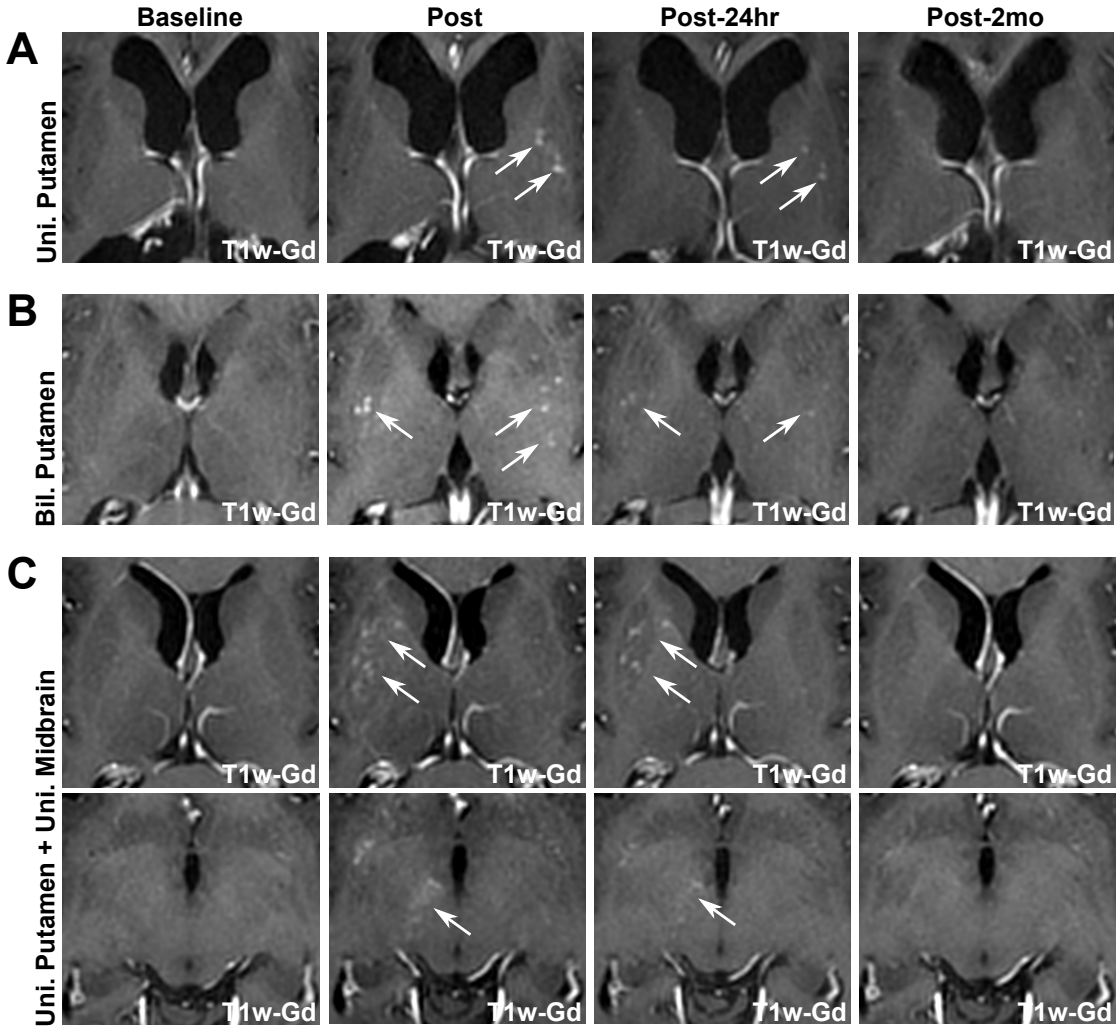

Fig. S6. BBB reversibility after sonication in PD patients.

Gadolinium (Gd) enhancement on T1w-Gd MRI after BBB opening with LIFU in the unilateral putamen **(A)**, in the bilateral putamen **(B),** and in both the putamen and the midbrain ipsilaterally **(C)**. Sample images for each patient are axial T1w-Gd MRIs at the baseline (**Baseline**) and follow-up sessions immediately (**Post**), 24 hours (**Post-24hr**), and 2 months (**Post-2mo**) after sonication.

Table S1. Sonication parameters in NHP LIFU treatments.

Summary of all parameters relevant to LIFU BBB openings. Including (from left to right) animal identifier; target identifier (R., right; L., left); effective power delivered in watts; sonication duration in seconds; number of spots and spatial spacing in millimeters between, and average cavitation dose achieved for the target. **This represents four sonications in which real spacing between spots was 2 mm. We combined such sonications by shifting our target in space to achieve an effective grid spacing of 1 mm.*

NHP ID	Target	Power (W)	Sonication time (sec)	Spots (#)	Spacing (mm)	Dose (a.u.)
M1	1 (R. premotor cortex)	1.2	150	4	2	0.02
	2 (L. thalamus)	1	150	4	2	0
M2	1 (L. posterior putamen)	1	150	2	2	0
	2 (L. caudate head)	1	150	2	2	0
	3 (R. anterior putamen)	1.2	150	2	2	0
	4 (R. midbrain)	1	150	2	2	0.01
M3	1 (R. putamen dorsal) *	1	150	33	1	0
	2 (R. putamen ventral) *	1	150	59	1	0
	3 (R. putamen intermediate) *	1	150	50	1	0
M4	1 (R. putamen dorsal) *	1	150	33	1	0
	2 (R. putamen ventral) *	1	150	59	1	0
	3 (R. putamen intermediate) *	1	150	50	1	0
M5	1 (L. putamen dorsal) *	1	60	42	1	0
	2 (L. putamen ventral) *	1	60	50	1	0
	3 (L. putamen intermediate) *	1	60	61	1	0
M6	1 (L. putamen dorsal) *	1	150	47	1	0
	2 (L. putamen ventral) *	1	150	43	1	0
	3 (L. putamen intermediate) *	1	150	62	1	0
	4 (L. midbrain) *	1	150	17	1	0

Table S2. Percentages of ^{18}F-Choline uptake increases after BBB openings.

	Target 1	^{18}F-Choline increase	Target 2	^{18}F-Choline increase
M6	Right Posterior Putamen	90.1%	Right Substantia Nigra	61.3%
HP01	Right Posterior Putamen	50.9%	-	-
HP02	Right Posterior Putamen	57.0%	Left Posterior Putamen	62.3%
HP03	Left Posterior Putamen	24.2%	Left Substantia Nigra	39.6%

Table S3. Principal demographic characteristics of PD patients.

ID	Gender	Age (y)	Disease duration (y)	MoCA	MDS-UPDRS III (OFF)	Hoehn & Yahr	LEDD (mg)	Target
HP01	Male	75	8	10	41	2	940	Right putamen
HP02	Male	78	6	26	30	2	950	Bilateral putamen
HP03	Male	63	5	23	31	2	455	Left putamen - left midbrain

MoCA: Montreal Cognitive Assessment; MDS-UPDRS: Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale; LEDD: Levodopa equivalent daily doses; y: years

Table S4. Microbubble infusion parameters LIFU treatments in PD patients.

All parameters describing microbubble infusion procedure including volume of microbubble contrast agent, total volume of saline and rate of infusion in ml/min

Patient ID	Microbubble (MB) (ml)	Saline (ml)	Infusion rate (ml/min)	MB Concentration (%)	MB Infusion rate (10^{-3} ml/min)
HP01	1.3	50	2.5	2.53%	63
HP02	1.5	50	3	2.91%	87
HP03	1.3	50	2.5	2.53%	63

Table S5. Sonication parameters in LIFU treatments in PD patients.

Summary of all parameters relevant to LIFU BBB openings. Including (from left to right) patient identifier; target identifier (R., right; L., left; post., post-commissural); effective power delivered in watts; sonication duration in seconds; number of spots and spatial spacing in millimeters between, and average cavitation dose achieved for the target.

ID	Target	Power (W)	Sonication time (sec)	Spots (#)	Spacing (mm)	Dose (a.u.)
HP01	1 (R. post. putamen dorsal)	4.8	100	21	2.5	0.62
	2 (R. post. putamen ventral)	5.5	100	18	2.5	0.62
HP02	1 (L. post. putamen dorsal anterior)	2.6	150	12	2.5	0.59
	2 (L. post. putamen dorsal posterior)	2.1	150	9	2.5	0.59
	3 (L. post. putamen ventral anterior)	2.5	150	9	2.5	0.39
	4 (L. post. putamen ventral posterior)	2.3	150	12	2.5	0.39
	5 (R. post. putamen dorsal anterior)	2.6	150	12	2.5	0.48
	6 (R. post. putamen dorsal posterior)	3	150	8	2.5	0.49
	7 (R. post. putamen ventral anterior)	3	150	16	2.5	0.48
	8 (R. post. putamen ventral posterior)	3.5	150	16	2.5	0.48
HP03	1 (L. post. putamen dorsal)	11.7	100	17	2.2	0.73
	2 (L. post. putamen intermediate)	9.2	100	32	2.2	0.75
	3 (L. post. putamen ventral)	8.7	100	32	2.2	0.75
	4 (L. substantia nigra)	4.3	100	17	2.2	0.62