Supporting Information

Title: Novel focused ultrasound gene therapy approach non-invasively restores dopaminergic neuron function in a rat Parkinson's disease model

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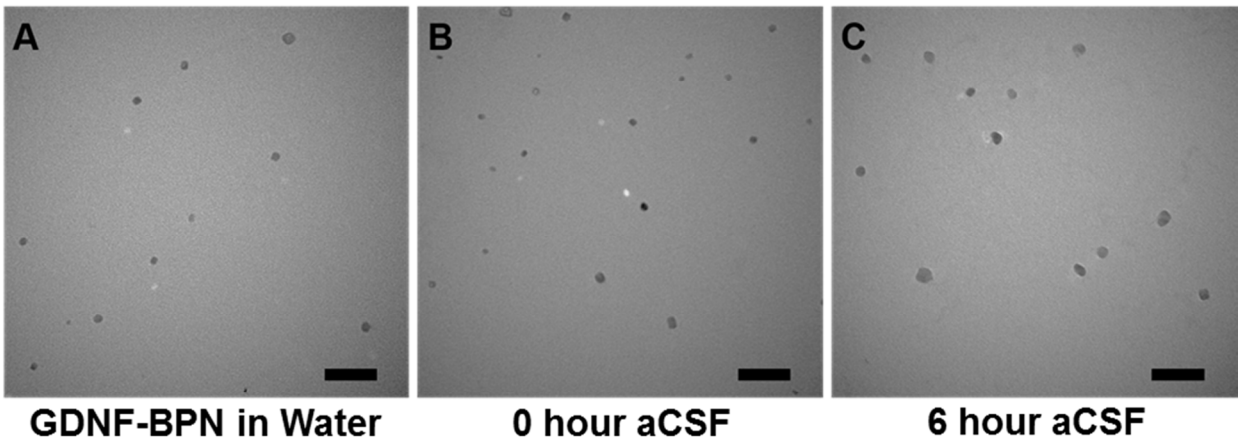

Figure S1. GDNF-BPN are colloidally stable when incubated in water or artificial cerebrospinal fluid (aCSF). Representative transmission electron microscope images of GDNF-BPN in (A) ultrapure water, (B) immediately after mixing in aCSF, or (C) after 6 hours of incubation in aCSF. Scale bar = 200 nm.

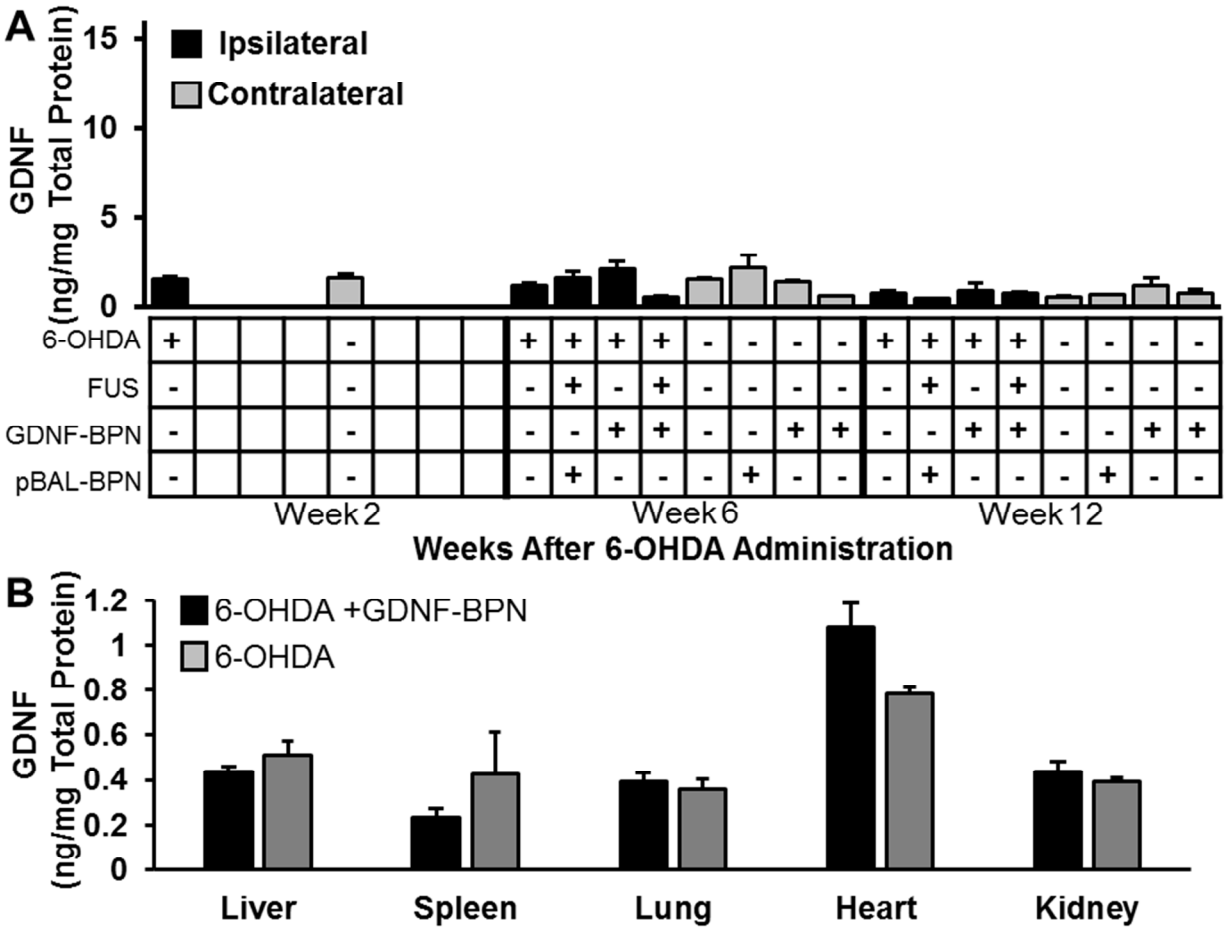

Figure S2. FUS-mediated delivery of GDNF-BPN to the striatum of PD rats does not change GDNF protein levels in the SNpc or other major organs. (A) Bar graphs show GDNF protein levels in the ipsilateral (black) and contralateral (gray) SNpc. n = 5 (6-OHDA +FUS +GDNF-BPN), n = 4 (6-OHDA only) or n = 3 in each group at each time point. (B) Bar graph shows GDNF protein levels in animals 6-OHDA treated (gray) or 6-OHDA + GDNF-BPN treated (black) animals. n = 3 (6-OHDA) or n = 5 (6-OHDA + GDNF-BPN).

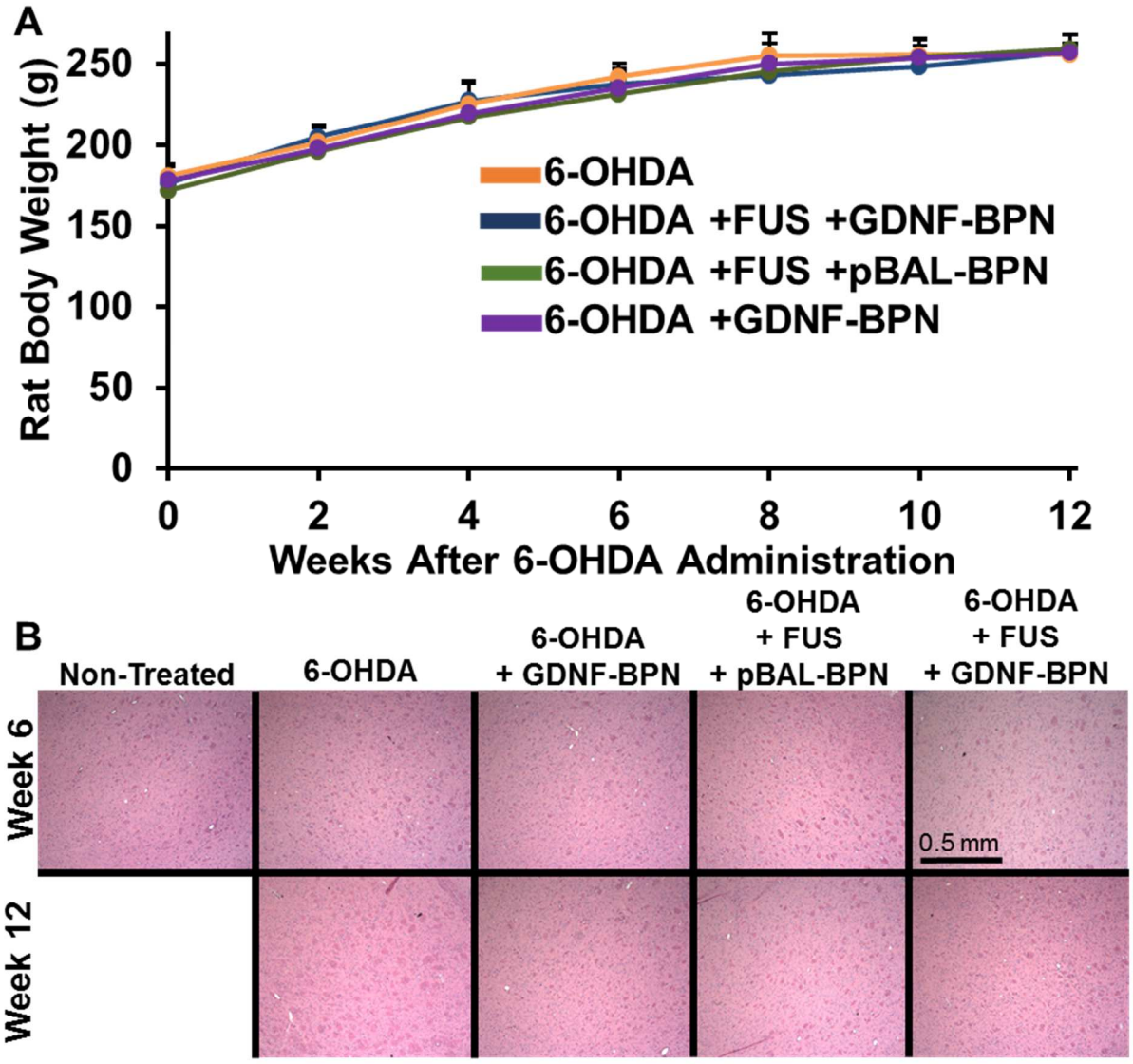

Figure S3. FUS-mediated delivery of GDNF-BPN to the striatum of PD rats does not lead to systemic or local toxicity. (A) Line graph shows animal weights for all animals used in the study. n >14 at each group at Week 0 through 6; n > 7 at each group at Week 8 through 12. (B) Representative images from H&E stained sections through the striatum 6 or 12 weeks after 6-OHDA administration. No signs of toxicity beyond the needle injection tract were found.

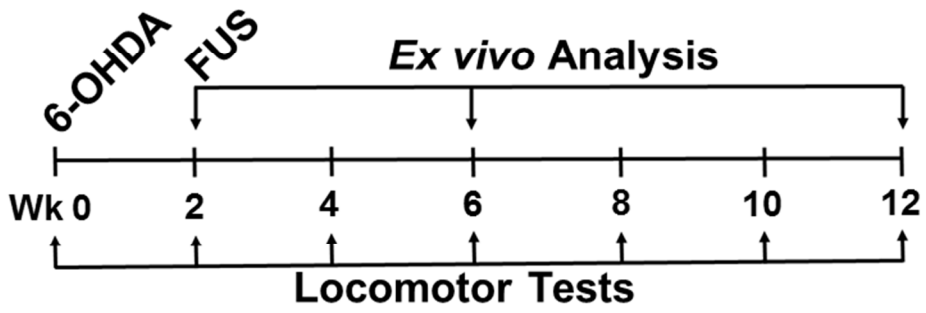

Figure S4. Time schedule of study. Rats received intrastriatal 6-OHDA at week 0 and locomotor function was examined 1-3 days prior to 6-OHDA administration and biweekly with apomorphine-induced rotational behavior and cylinder forepaw use bias tests. Animals were sacrificed at week 2, 6 or 12 and brains were either homogenized or fixed/sectioned for further *ex vivo* analysis.

Tables:

Table S1. Dopamine Metabolites in the Ipsilateral Striatum

DOPAC (ng/mg tissue)	6-OHDA	6-OHDA + GDNF-BPN	6-OHDA + FUS + pBAL-BPN	6-OHDA + FUS + GDNF-BPN
Week 2	Not Detected			
Week 6	0.194 ± 0.083	0.109 ± 0.047	0.309 ± 0.128	2.140 ± 0.200*
Week 12	0.887 ± 0.107	0.272 ± 0.088	0.393 ± 0.043	3.343 ± 0.657*

HVA (ng/mg tissue)	6-OHDA	6-OHDA + GDNF-BPN	6-OHDA + FUS + pBAL-BPN	6-OHDA + FUS + GDNF-BPN
Week 2	0.071 ± 0.011			
Week 6	0.349 ± 0.072	0.080 ± 0.015	0.087 ± 0.034	0.375 ± 0.023‡
Week 12	0.289 ± 0.050	0.132 ± 0.028	0.104 ± 0.019	1.24 ± 0.351*

* $P < 0.05$ vs all other groups at the same time point. ‡ $p < 0.05$ vs 6-OHDA + FUS + pBAL-BPN and 6-OHDA + GDNF-BPN groups at the same time point.