nature portfolio

Peer Review File

Sono-mechanical nanostructures-enabled sustained precise ultrasound brain stimulation

Corresponding Author: Professor Lei Sun

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Non-invasive neuromodulation holds great promise for brain function mapping and the treatment of neurological disorders. Among various approaches, focused ultrasound stands out due to its clinical safety and spatiotemporal precision. Techniques such as sonogenetics, sono-chemogenetics, and sono-optogenetics have expanded the landscape of ultrasound-mediated neural control, offering non-invasive strategies for therapeutic interventions. In this work, Hou et al. present an acoustically active hollow silica nanotransducer that serves as an amplifier for focused ultrasound, enabling non-invasive deep brain stimulation without the need for genetic modification. The authors demonstrate the application of this platform for neuronal activation and Parkinson′s disease (PD) treatment. The study advances the field of sonogenetics, and I appreciate the novel approach presented here. However, I have several major concerns regarding the mechanistic basis and experimental validation of the technology.
Major Concerns:
1. The study reports sustained neuromodulation effects over two months following the introduction of non-biodegradable inorganic silica nanoparticles. Although in vitro and in vivo assessments suggest no obvious inflammation or tissue damage, the long-term biosafety and clearance of these particles remain highly uncertain. Given their potential accumulation and the unknown risks associated with chronic exposure, further discussion is needed on how to balance prolonged neuromodulation with clinical safety requirements.
2. The in vitro neuronal activation studies were performed using heterogeneous neurons from the whole rat brain. Mechanosensitive ion channels, such as PIEZO and TRP family members, are known to be expressed in specific neuronal subtypes but are absent or rare in others. Quantifying the proportion of neurons responsive to ultrasound stimulation would provide important insight into the efficacy and specificity of the proposed system.
3. The motor cortex is known to be highly sensitive to mechanical stimulation and can respond to low peak pressures (as low as ~80 kPa), as reported in prior work (Nat Commun 12, 2519 (2021)). However, no EMG or c-Fos activation was observed in the motor cortex in this study (Fig. 3a, 3e). This apparent contradiction warrants further investigation or explanation.
4. In contrast to the motor cortex, fiber photometry was used to assess neural activation in the VTA. However, the signal changes ($\Delta F/F0 \approx 1\%$) were extremely low and may reflect baseline responses to ultrasound alone, as previously documented (Research (Wash. D.C.) 2021, 2674692; Nat Metab 5, 789–803 (2023); Nature 638, 401–410 (2025)). Additionally, the immunostaining showed only sparse c-Fos activation. I recommend that the authors provide expanded c-Fos staining images across broader brain regions to better validate their findings and give further explanation for the fiber photometry signal.
5. The subthalamic nucleus (STN) was selected as the stimulation target for PD treatment. However, the manuscript lacks evidence that STN neurons express mechanosensitive ion channels or are responsive to mechanical stimulation. No supporting data from transcriptomic analysis, immunostaining, or electrophysiology are provided. The authors should include relevant evidence or references to justify the selection of STN as a sonogenetic target.
Minor Concerns:
1. The manuscript lacks detailed information on the preparation and gas-loading procedure for the hollow silica nanotransducers.
2. The FTIR spectrum (Fig. 1e) does not add critical information and may be better suited for the supplementary section.
3. The y-axis in Fig. 1g should be presented with continuous scale rather than broken segments for clarity.
4. In Fig. 6l, a two-way ANOVA analysis is more appropriate and should be used instead of the current statistical test.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
This manuscript presents a highly technically robust study on sono-mechanical neuromodulation using engineered hollow silica nanostructures (HSN) to achieve precise, sustained, and non-genetic ultrasound (US) brain stimulation. The authors demonstrate that HSN significantly amplifies US effects by activating mechanosensitive ion channels, enabling targeted neuromodulation in deep brain regions (M1, striatum, VTA, STN) for >9 weeks in mice. Key applications include alleviating Parkinson's disease (PD) motor symptoms without detectable toxicity. The work addresses several limitations of existing methods (e.g., short stability of nanobubbles, low spatial resolution of conventional US) and offers a transformative platform for chronic neuromodulation. I think the current manuscript can reach the threshold of Nature communications after major revision.
Comments and suggestions for this manuscript.
1. In Figure 2, the authors claim that HSN+US primarily acts on neurons through the activation of mechanosensitive ion channels. However, they provide no detailed description of the specific mechanosensitive ion channels involved—was it Piezo, for example? Additionally, the rationale behind using primary cortical neurons as the experimental model is not clearly explained. Furthermore, the number of replicates for Figure 2f is not specified.
2. In Figure 3e, how did the authors determine that the detected regions were located at 200 μm and 600 μm, respectively? I noticed that the neurons with c-fos fluorensence in this figure are not the neurons where the MSN particles are located. This implies that the effect is not due to the particles themselves, but rather due to the ultrasound?
3. The authors claimed that "Due to the robust nature of HSN's inorganic nanomaterial composition and their resistant shells, exceptional stability was anticipated owing to their inherent resistance to enzymatic degradation." and "HSN gradually diminished with increasing post-injection time, indicating their biodegradability", These two statements are completely contradictory. The authors should provide evidence on whether the particles are degraded or taken up by other macrophages.
4.The authors injected the HSN into the tested area, did the authors evaluate the safety of this injection method? Although this stereotactic brain injection is a transient method, it can also pose safety risks to surrounding cells.
Figure 5b, it was confused that the regions generating calcium signals do not show complete colocalization with the regions where HSNs are present.
5. Figure 6, the authors tested the ability of HSN alleviating symptoms of MPTP-induced PD mice, it would be important to include another important model of PFF-induced PD model in this work.
6. Discussion, the authors should compare the advantages and disadvantages of using ultrasound, optogenetics, and magnetic stimulation for deep brain stimulation in treating brain disorders.
7, Update the cited refs.

(Remarks on code availability)
The authors provide a custom code for neuronal calcium imaging, it is reproducible for other researchers.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Thank you for the authors' thoughtful efforts in revising the manuscript. I believe they have addressed all of my comments, and the manuscript is now well prepared for publication in Nature Communications.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
The authors have included new data to address my concerns, I suggest to accept it for publication in Nature Communications.

(Remarks on code availability)

A point-by-point response to the reviewers' comments

We thank the reviewers for their thoughtful evaluation and valuable guidance, which have greatly improved our manuscript. We appreciate the opportunity to submit a revised version of our work to ***Nature Communications***. In this revision, we have carefully addressed all points raised by the reviewers, including **conducting additional experiments and revising the manuscript to clarify our results, discussion, methods, and interpretations**. Below, we provide a detailed point-by-point response to each of the reviewers' comments.

Reviewer #1 (Remarks to the Author):

Non-invasive neuromodulation holds great promise for brain function mapping and the treatment of neurological disorders. Among various approaches, focused ultrasound stands out due to its clinical safety and spatiotemporal precision. Techniques such as sonogenetics, sono-chemogenetics, and sono-optogenetics have expanded the landscape of ultrasound-mediated neural control, offering non-invasive strategies for therapeutic interventions. In this work, Hou et al. present an acoustically active hollow silica nanotransducer that serves as an amplifier for focused ultrasound, enabling non-invasive deep brain stimulation without the need for genetic modification. The authors demonstrate the application of this platform for neuronal activation and Parkinson′s disease (PD) treatment. The study advances the field of sonogenetics, and I appreciate the novel approach presented here. However, I have several major concerns regarding the mechanistic basis and experimental validation of the technology.

Reply: We are grateful for the reviewer's positive comments regarding the novelty and potential impact of our work.

Major Concerns:

1. The study reports sustained neuromodulation effects over two months following the introduction of non-biodegradable inorganic silica nanoparticles. Although in vitro and in vivo assessments suggest no obvious inflammation or tissue damage, the long-term biosafety and clearance of these particles remain highly uncertain. Given their potential accumulation and the unknown risks

associated with chronic exposure, further discussion is needed on how to balance prolonged neuromodulation with clinical safety requirements.

Reply: We appreciate the reviewer's valuable suggestion. In response, we have substantially expanded the Discussion section to address issues related to prolonged neuromodulation and clinical safety requirements. In the current manuscript, we systematically evaluated biosafety up to 11 weeks post-injection through body weight monitoring, behavioral assessments, and detailed histological analyses (Manuscript Fig. 7, Supplementary Fig. 14 and 15), all of which indicate long-term biosafety with negligible systemic toxicity.

To further address particle clearance, we **performed additional histological staining up to 12 weeks post-injection, now presented in Supplementary Fig. 4. These data show that most nanoparticles remained in the extracellular space, with partial uptake by astrocytes and neurons and pronounced microglial internalization (Fig. R1, below)**, suggesting a potential mechanism for microglia-mediated clearance. All relevant revisions are highlighted in yellow in the manuscript. We hope these modifications satisfactorily address the reviewer's concerns.

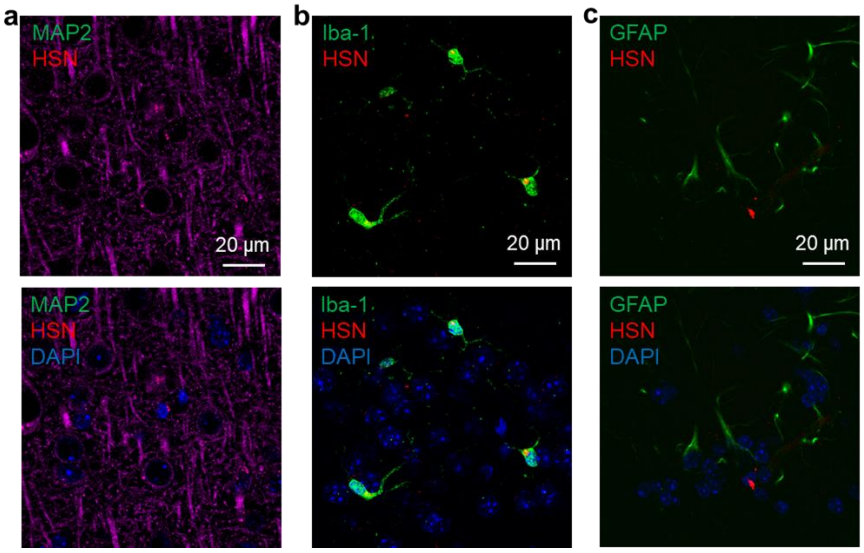

Figure R1. Representative confocal images of HSN (red) distribution in the striatum 12 weeks post-injection. (a) HSN uptake within neurons labeled with MAP2, (b) within microglia labeled with Iba-1, and (c) within astrocytes labeled with GFAP. Images are representative of six independent experiments.

2. The in vitro neuronal activation studies were performed using heterogeneous neurons from the whole rat brain. Mechanosensitive ion channels, such as PIEZO and TRP family members, are known to be expressed in specific neuronal subtypes but are absent or rare in others. Quantifying

the proportion of neurons responsive to ultrasound stimulation would provide important insight into the efficacy and specificity of the proposed system.

Reply: We apologize for any confusion and would like to clarify that the *in vitro* cultured neurons used in our study were derived from the cortex, not the whole brain. While we acknowledge that PIEZO and TRP family ion channels are expressed in specific neuronal subtypes, the primary aim of our current study was to demonstrate the overall efficacy of our strategy in activating mechanosensitive ion channels, rather than targeting specific neuronal subpopulations.

In response to the reviewer's suggestion, we have re-analyzed our *in vitro* calcium imaging data to quantify the proportion of cortical neurons responding to ultrasound stimulation in the presence of HSN (Fig. R2, below). With a 0.32 MPa stimulus, approximately 83.2% of neurons exhibited robust calcium transients under HSN+US, demonstrating a high degree of mechanosensitivity within this population. These new data and analyses have been incorporated into the Results section and are illustrated in Supplementary Fig. 3b. We appreciate the reviewer's advice, which has helped us to further strengthen and clarify our neuromodulation strategy.

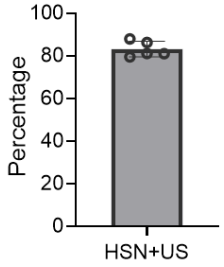

Figure R2. Quantification of the proportion of neurons responding to HSN+US stimulation (n = 5). Data are presented as mean ± SD.

3. The motor cortex is known to be highly sensitive to mechanical stimulation and can respond to low peak pressures (as low as ~80 kPa), as reported in prior work (Nat Commun 12, 2519 (2021)). However, no EMG or c-Fos activation was observed in the motor cortex in this study (Fig. 3a, 3e). This apparent contradiction warrants further investigation or explanation.

Reply: Thank you for raising this point, and we apologize for any confusion. We would like to clarify that the EMG results presented in Fig. 3a were obtained from the motor cortex, while the c-Fos activation data in Fig. 3e correspond to the striatum.

Upon careful review, we considered several possible factors that could account for differences in activation, including **ultrasound frequency attenuation by the skull, differences in targeting precision (the referenced study utilized focused ultrasound stimulation, whereas we employed a flat ultrasound transducer), and variations in ultrasound pulse repetition frequency and duty cycle (their study used a higher duty cycle of 60%, while our maximum was 40%)**[1]. Additionally, differences in experimental setup may also contribute, as our EMG results obtained using our laboratory's ultrasound stimulation system are consistent with our previous publication[2]. We hope these explanations address the reviewer's concern. Thank you for your feedback, which has helped us to further clarify our study.

4. In contrast to the motor cortex, fiber photometry was used to assess neural activation in the VTA. However, the signal changes (ΔF/F0≈1%) were extremely low and may reflect baseline responses to ultrasound alone, as previously documented (Research (Wash. D.C.) 2021, 2674692; Nat Metab 5, 789–803 (2023); Nature 638, 401–410 (2025)). Additionally, the immunostaining showed only sparse c-Fos activation. I recommend that the authors provide expanded c-Fos staining images across broader brain regions to better validate their findings and give further explanation for the fiber photometry signal.

Reply: As recommended by the reviewer, we carefully reviewed the cited publications. We found that Bian et al. did not present fiber photometry data[3], while Yang et al. measured calcium activity in POA neurons - not the VTA - and reported their results as z-score rather than percentage changes in $\Delta F/F_0$ on the y-axis[4]. Wang et al. utilized higher ultrasound energy for sono-chemogenetic stimulation in the VTA[5], a method that relies on a different mechanism than our approach, our study is based on direct ultrasound mechanical effects; therefore, we do not consider a direct comparison appropriate. Additionally, the amplitude of calcium responses observed in our current study is consistent with our previous work[6]. Importantly, our control groups exhibit negligible changes in $\Delta F/F_0$, further supporting the efficiency and specificity of our approach. We sincerely hope these explanations adequately address the reviewer's concerns.

Furthermore, we have **expanded our c-Fos immunostaining analyses to include a broader set of regions, encompassing the VTA and adjacent structures (IPN, IF, rust, ml, SNc and SNr; Fig. R3, below)**. The corresponding images are now provided in Supplementary Fig. 9. These results confirm that HSN+US leads to localized c-Fos activation in the targeted brain region. We

appreciate the reviewer's recommendations, which have enhanced the rigor and clarity of our validation experiments.

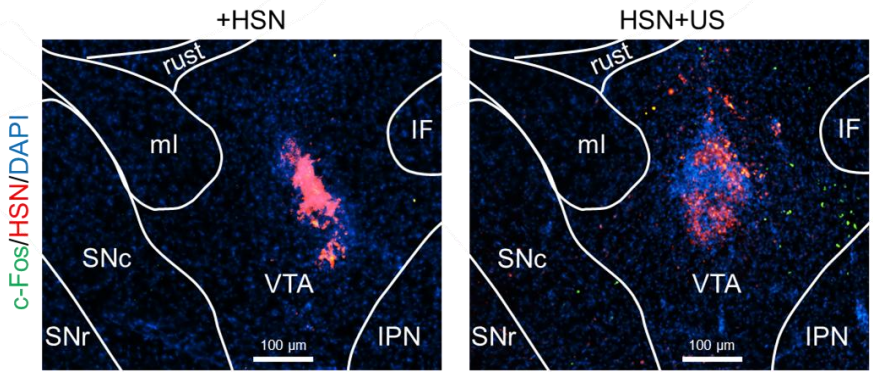

Figure R3. Representative immunofluorescence images of brain sections after treatment. c-Fos expression is predominantly localized to the VTA following HSN+US stimulation, whereas minimal c-Fos expression is observed in the HSN-only group. Abbreviations: VTA, ventral tegmental area; ml, medial lemniscus; rust, rubrospinal tract; SNr, substantia nigra reticular part; SNc, substantia nigra pars compacta; IF, interfascicular nucleus raphe; IPN, interpeduncular nucleus.

5. The subthalamic nucleus (STN) was selected as the stimulation target for PD treatment. However, the manuscript lacks evidence that STN neurons express mechanosensitive ion channels or are responsive to mechanical stimulation. No supporting data from transcriptomic analysis, immunostaining, or electrophysiology are provided. The authors should include relevant evidence or references to justify the selection of STN as a sonogenetic target.

Reply: We thank the reviewer for the insightful comment and apologize for any confusion. We would like to clarify that the **subthalamic nucleus (STN) responds to our mechanical stimulation via HSN+US, as shown in Fig. 6h-j (below) of the current manuscript, which may have been overlooked**. Moreover, both our group and other teams have reported that ultrasound stimulation of the STN can alleviate symptoms in Parkinson's disease mouse models[7-9], underscoring its established role as a therapeutic target in PD. In accordance with the reviewer's suggestion, we have added citations to these recent studies (*PNAS 120 (22) e2220575120; Research (Wash. D.C.) 2019:1748489; TNSRE.2020.2978865*) in the revised manuscript to strengthen the justification for selecting the STN as the target for ultrasound stimulation. We appreciate the reviewer's valuable suggestion, which has enhanced the scientific rationale and clarity of our work.

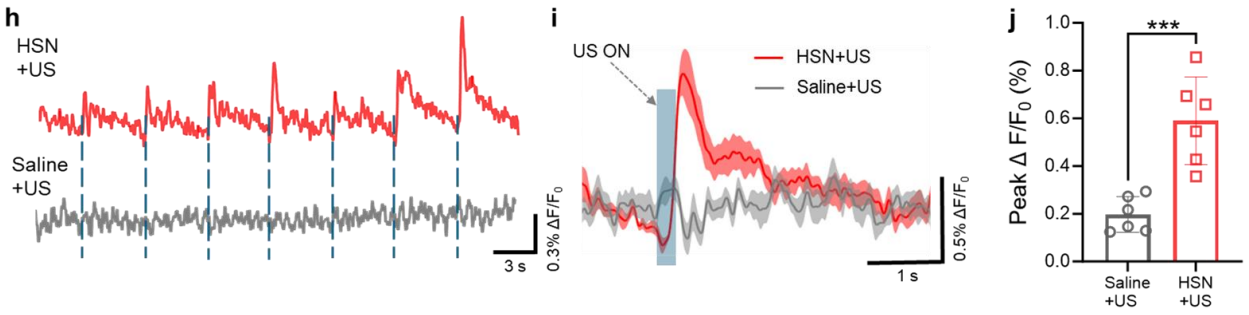

Figure 6h-j (Manuscript). (**h**) Representative dopamine fluorescence traces in the striatum of mice stimulated by multiple pulse trains of US. The blue dotted lines represent the US pulse. (**i**) Mean dopamine fluorescence traces from the striatum. The light-blue box represents the US pulse. (**j**) Mean peak of dopamine release (n = 6). Data are presented as mean ± SD. ***p < 0.001, unpaired two-tailed t-test (**j**).

Minor Concerns:

1. The manuscript lacks detailed information on the preparation and gas-loading procedure for the hollow silica nanotransducers.

Reply: Thank you for highlighting this omission. In response, we have included a description of the preparation and gas-loading procedure for the hollow silica nanotransducers. These changes are highlighted in yellow in the revised manuscript.

2. The FTIR spectrum (Fig. 1e) does not add critical information and may be better suited for the supplementary section.

Reply: The FT-IR spectrum (Fig. 1e) has been relocated to the supplementary section (Supplementary Fig. 1d), as recommended.

3. The y-axis in Fig. 1g should be presented with continuous scale rather than broken segments for clarity.

Reply: Thank you for your suggestion. We have revised Fig. 1g (now Fig. 1f) to present the y-axis with a continuous scale, which improves clarity and data interpretation.

4. In Fig. 6l, a two-way ANOVA analysis is more appropriate and should be used instead of the current statistical test.

Reply: As recommended by the reviewer, we have re-analyzed the data in Figure 6l using a two-way ANOVA in the revised manuscript. We appreciate the reviewer's valuable suggestion regarding the statistical analysis.

Reviewer #2 (Remarks to the Author):

This manuscript presents a highly technically robust study on sono-mechanical neuromodulation using engineered hollow silica nanostructures (HSN) to achieve precise, sustained, and non-genetic ultrasound (US) brain stimulation. The authors demonstrate that HSN significantly amplifies US effects by activating mechanosensitive ion channels, enabling targeted neuromodulation in deep brain regions (M1, striatum, VTA, STN) for >9 weeks in mice. Key applications include alleviating Parkinson's disease (PD) motor symptoms without detectable toxicity. The work addresses several limitations of existing methods (e.g., short stability of nanobubbles, low spatial resolution of conventional US) and offers a transformative platform for chronic neuromodulation. I think the current manuscript can reach the threshold of Nature communications after major revision.

Reply: We sincerely appreciate your thoughtful review and for the opportunity to further improve our manuscript for consideration by Nature Communications.

Comments and suggestions for this manuscript.

1. In Figure 2, the authors claim that HSN+US primarily acts on neurons through the activation of mechanosensitive ion channels. However, they provide no detailed description of the specific mechanosensitive ion channels involved—was it Piezo, for example? Additionally, the rationale behind using primary cortical neurons as the experimental model is not clearly explained. Furthermore, the number of replicates for Figure 2f is not specified.

Reply: We apologize for the lack of detail in our initial submission. In response to the reviewer's comments, we have expanded the manuscript to address each of your points:

First, neurons have been reported to express multiple mechanosensitive ion channels -including TRPV1, TRPV2, TRPV4, Piezo1, TRPC1, TRPM7, and the TRPP1/2 complex -which can mediate ultrasound neuromodulation effects[10]. While there is currently no consensus regarding the relative contribution of each channel, it is generally accepted that their importance varies with differing expression levels and activation thresholds. At the neuronal level, we have no reason to believe that HSN injection would alter the endogenous expression or dynamic properties of these channels, nor the mechanisms by which neurons respond to ultrasound stimulation. Therefore, we do not believe that HSN+US selectively targets specific channels over others. However, we acknowledge

that this possibility cannot be entirely excluded and warrants further investigation. Notably, our previous work demonstrated that Piezo1 - given its high sensitivity and expression - plays a major role in neuronal response to ultrasound stimulation[11].

Second, we have clarified our rationale for using primary cortical neurons in the revised Methods. We selected this model because primary cortical cultures provide a heterogeneous neuronal population, expressing a broad array of mechanosensitive channels. This enables a robust and generalizable assessment of HSN+US neuromodulation across multiple neuronal subtypes.

Finally, we have addressed the earlier omission by clearly indicating the number of replicates for Figure 2f in the figure legend; specifically, the analysis was performed on n = 5 independent experiments. We appreciate the reviewer's insightful feedback, which has helped us improve the rigor and clarity of our experimental approach and reporting.

2. In Figure 3e, how did the authors determine that the detected regions were located at 200 μm and 600 μm, respectively? I noticed that the neurons with c-fos fluorensence in this figure are not the neurons where the MSN particles are located. This implies that the effect is not due to the particles themselves, but rather due to the ultrasound?

Reply: We appreciate the reviewer's thoughtful observations and questions regarding Figure 3e. For spatial localization, the regions at 200 μm and 600 μm relative to the stereotaxic injection center were deliberately selected as targeted areas for analysis. Regarding the reviewer's observation that c-Fos-positive cells do not perfectly colocalize with HSN particle locations, we believe this apparent lack of colocalization may be due to a focus on c-Fos signals, which might not always be present in the same confocal imaging plane as the HSN particles. As a result, c-Fos expression may not precisely overlap with HSN localization in confocal images. We have elaborated on this explanation in the revised Results section.

Importantly, we observed no significant increase in c-Fos signals at ~600 μm - a region exposed to ultrasound stimulation but with negligible HSN presence (Manuscript Fig. 3e). This result supports the conclusion that neuronal activation depends on HSN-actuated ultrasound rather than ultrasound alone. Thank you for your valuable comments, which have helped us improve both the methodological clarity and interpretation of our findings.

3. The authors claimed that "Due to the robust nature of HSN's inorganic nanomaterial composition and their resistant shells, exceptional stability was anticipated owing to their inherent resistance to enzymatic degradation." and "HSN gradually diminished with increasing post-injection time, indicating their biodegradability", These two statements are completely contradictory. The authors should provide evidence on whether the particles are degraded or taken up by other macrophages.

Reply: We appreciate the reviewer's helpful comments and apologize for the earlier ambiguity. Our longitudinal imaging demonstrated a progressive decline in HSN signals at the injection site over time. To prevent further confusion, we have removed the previous statements regarding "resistant shells". To elucidate the mechanism underlying this decline, we performed extended histological analyses at 12 weeks post-injection with immunostaining for neuronal (MAP2), microglial (Iba1), and astrocytic (GFAP) markers. These data show that HSN nanoparticles are predominantly localized to the extracellular space, with partial uptake by astrocytes and neurons and pronounced microglial internalization (Fig. R1, above), indicating a potential mechanism for microglia-mediated clearance rather than rapid chemical or enzymatic degradation.

We have incorporated these findings and the revised interpretation into the Results and Discussion, and added the supporting images in the revised manuscript (Supplementary Fig. 4). We thank the reviewer for raising this important point, which has improved the accuracy and clarity of our work.

4.The authors injected the HSN into the tested area, did the authors evaluate the safety of this injection method? Although this stereotactic brain injection is a transient method, it can also pose safety risks to surrounding cells. Figure 5b, it was confused that the regions generating calcium signals do not show complete colocalization with the regions where HSNs are present.

Reply: Thank you for raising this important point. We would like to clarify that the safety of the injection method has been thoroughly evaluated, as shown in Fig. 7 (below) of the manuscript. Stereotactic brain injection is a widely used, minimally invasive technique for the delivery of viruses or nanoparticles, and its transient nature is generally associated with limited tissue damage. To further address the reviewer's concerns, we monitored body weight, assessed neurological function, and performed extensive histological analyses (NeuN for neurons, GFAP for astrocytes, Iba1 for microglia, and Caspase-3 for apoptosis) at multiple time points following injection. Our results revealed **no significant systemic toxicity, local inflammation, or tissue disruption**

attributable to the HSN injection procedure. If further clarification or additional experiments are required, we are happy to include them in the revised manuscript.

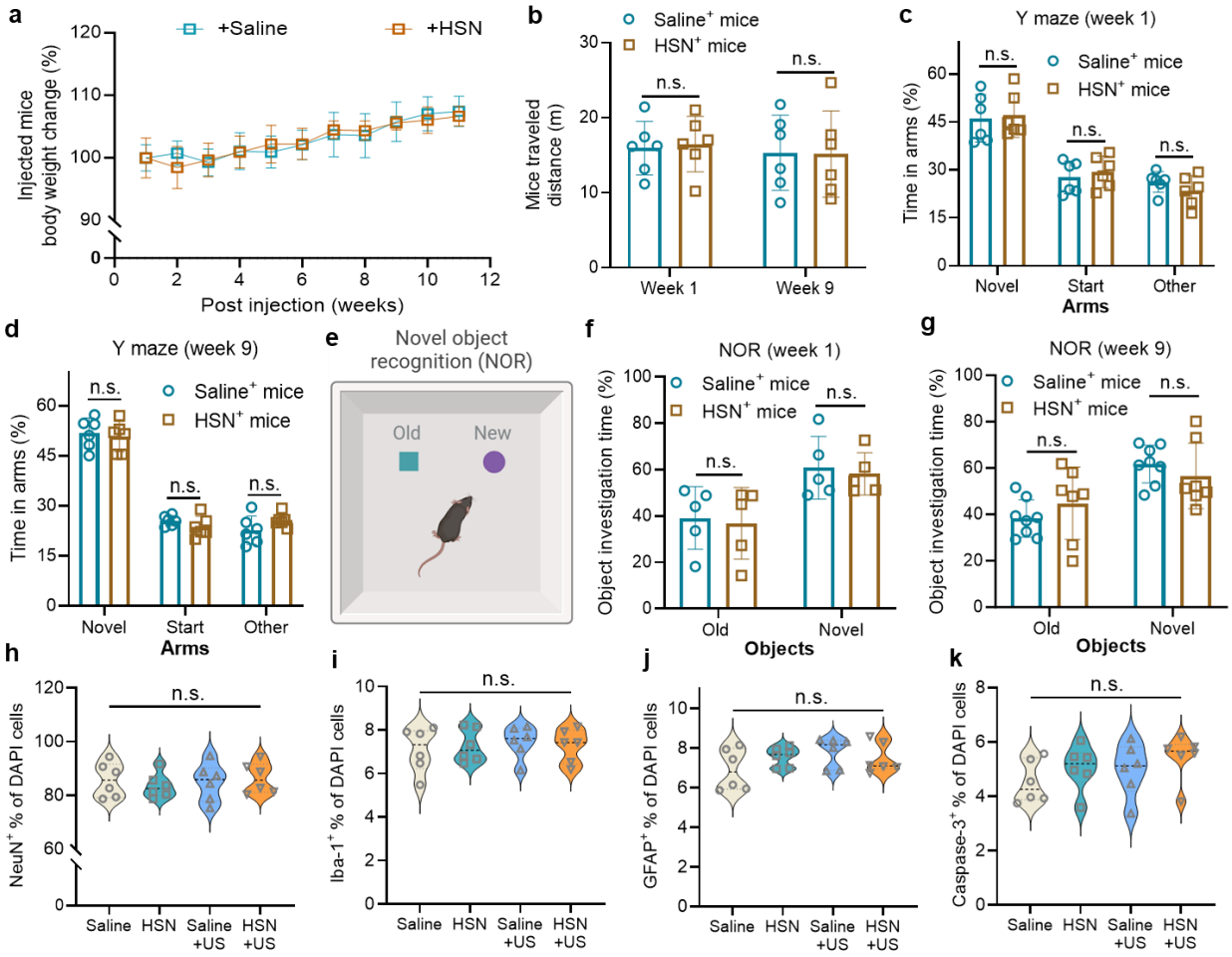

Figure 7 (Manuscript). Biosafety test *in vivo*. (**a**) Body weight measurement of mice during the 11-week evaluation period. (**b**) Total distance traveled in open field test (OFT) 1 or 9 weeks after injection of HSN or control mice. Calculated percentage of time spent by mice on the three arms (start, other, novel areas) in Saline$^+$ and HSN$^+$ mice after 1 week (**c**) or 9 weeks (**d**) of injection. (**e**) Schematic showing the novel object recognition (NOR) test. Calculated percentage of time spent by mice on the old or novel objects after Saline or HSN injection at week 1 (**f**) or week 9 (**g**). (**h-k**) Percentages of NeuN (**h**), Iba-1 (**i**), GFAP (**j**) Caspase-3 (**k**) positively stained cells to DAPI-stained cells within the 450 × 450 μm area per imaged slice in Saline$^+$ or HSN$^+$ mice with or without US stimulation. Data represent the mean ± SD. No significant differences (n.s.), ****$p < 0.0001$ were found using two-way ANOVA for (**b**), (**c**), (**d**), (**f**), (**g**), or one-way ANOVA for panels (**h-k**). Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz

Regarding Figure 5b, we apologize for the confusion. Based on the reviewer's comment, we re-examined the two enlarged panels and identified a slight misregistration of the DAPI channel, which led to an apparent mismatch in colocalization. In the revised manuscript, we have **corrected the channel alignment and updated the fluorescence images to improve clarity (Fig. R4,**

below). We appreciate the reviewer's careful observation, which helped improve the accuracy and presentation of the manuscript. We hope this revision satisfactorily addresses the concern.

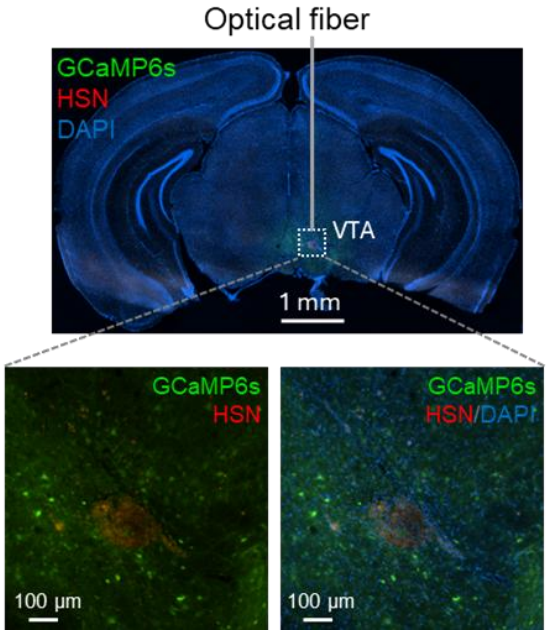

Figure R4. Representative images showing GCaMP6s expression in HSN⁺ mouse VTA neurons.

5. Figure 6, the authors tested the ability of HSN alleviating symptoms of MPTP-induced PD mice, it would be important to include another important model of PFF-induced PD model in this work.

Reply: We appreciate the reviewer's valuable suggestion. In response, we have conducted additional experiments using the α-synuclein pre-formed fibril (PFF)-induced Parkinson's disease (PD) mouse model (Fig. R5a-d, below). Our results demonstrate that HSN-mediated ultrasound stimulation significantly alleviates motor symptoms, as evidenced by improvements in both rotarod and open-field tests, in PFF-induced PD mice (Fig. R5e-h, below), which are consistent with our findings in the MPTP-induced PD model. These new data have been included in the revised manuscript (Supplementary Fig. 13), with further details provided in the Results and Methods sections. We thank the reviewer for this recommendation, which has helped to strengthen the generalizability and impact of our study by including an additional PD model.

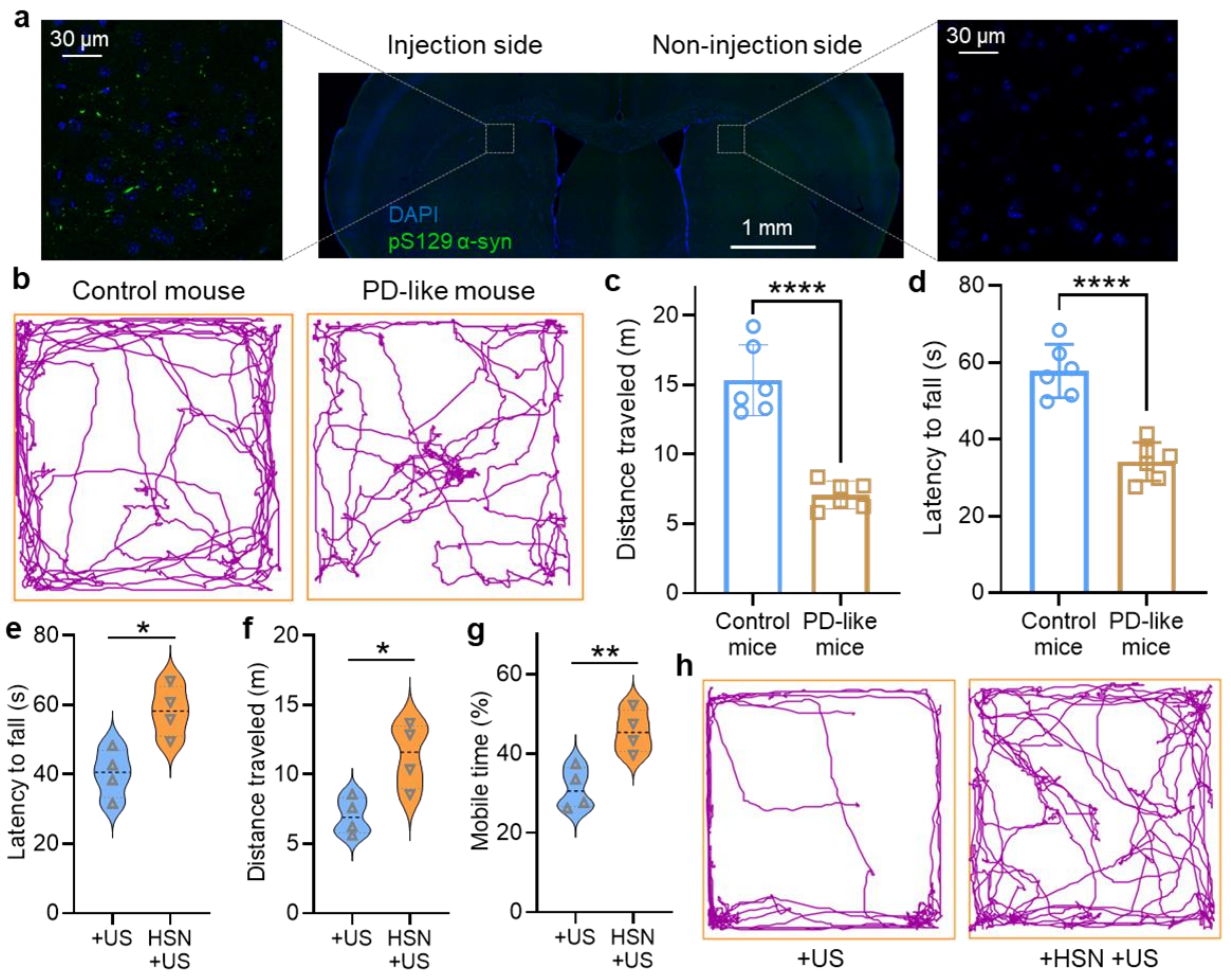

Figure R5. Characterization of the PFF-induced PD-like mouse model and ultrasound treatment. (**a**) Representative immunofluorescence images of mouse brain sections after injection PFF. (**b**) Open-field trajectories from a control mouse and a PFF-induced PD-like mouse (each trace represents 5 min). (**c**) Total distance traveled in the open-field test (n = 6). (**d**) Rotarod latency to fall (n = 6). (**e**) Rotarod retention time in PFF-induced PD-like mice after one week of ultrasound treatment (n = 4). Total distance traveled (**f**) and time spent mobile (**g**) in the open-field arena after one week of ultrasound treatment (n = 4). (**h**) Representative open-field trajectories of PD-like mice after treatment. Data are presented as mean ± SD in panels (**c**) and (**d**). No significant differences (n.s.), *p < 0.05, **p < 0.01, ****p < 0.0001 were found using unpaired t-test for (**c**-**g**).

6. Discussion, the authors should compare the advantages and disadvantages of using ultrasound, optogenetics, and magnetic stimulation for deep brain stimulation in treating brain disorders.

Reply: We appreciate the reviewer's valuable recommendation. In response, we have expanded the Discussion section to include a detailed comparison of the advantages and disadvantages of ultrasound, optogenetics, and magnetic stimulation for deep brain stimulation in the treatment of brain disorders. The new content has been highlighted in yellow in the revised manuscript for ease of reference.

7, Update the cited refs.

Reply: Thank you for your suggestion. We have carefully reviewed and updated all cited references in the manuscript to ensure they are current, relevant, and properly formatted.

Reviewer #2 (Remarks on code availability):

The authors provide a custom code for neuronal calcium imaging, it is reproducible for other researchers.

Reply: Thank you for your positive feedback regarding our code. We remain committed to open science and reproducibility, and the code will continue to be available in supplementary materials.

References:

1 Yu, K., Niu, X., Krook-Magnuson, E. & He, B. Intrinsic functional neuron-type selectivity of transcranial focused ultrasound neuromodulation. *Nature communications* **12**, 2519 (2021).

2 Qiu, Z. *et al*. Targeted neurostimulation in mouse brains with non-invasive ultrasound. *Cell reports* **32** (2020).

3 Bian, T. *et al*. Noninvasive ultrasound stimulation of ventral tegmental area induces reanimation from general anaesthesia in mice. *Research* (2021).

4 Yang, Y. *et al*. Induction of a torpor-like hypothermic and hypometabolic state in rodents by ultrasound. *Nature Metabolism* **5**, 789-803 (2023).

5 Wang, W. *et al*. H-bonded organic frameworks as ultrasound-programmable delivery platform. *Nature*, 1-10 (2025).

6 Hou, X. *et al*. Precise ultrasound neuromodulation in a deep brain region using nano gas vesicles as actuators. *Advanced Science* **8**, 2101934 (2021).

7 Xian, Q. *et al*. Modulation of deep neural circuits with sonogenetics. *Proceedings of the National Academy of Sciences* **120**, e2220575120 (2023).

8 Zhou, H. *et al*. Noninvasive ultrasound deep brain stimulation for the treatment of Parkinson's disease model mouse. *Research* (2019).

9 Yuan, Y. *et al*. The effect of low-intensity transcranial ultrasound stimulation on behavior in a mouse model of Parkinson's disease induced by MPTP. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* **28**, 1017-1021 (2020).

10 Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J. & Shapiro, M. G. Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nature communications* **13**, 493 (2022).

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A point-by-point response to the reviewers' comments

Reviewer #1 (Remarks to the Author):

Thank you for the authors' thoughtful efforts in revising the manuscript. I believe they have addressed all of my comments, and the manuscript is now well prepared for publication in Nature Communications.

Reply: We thank the reviewer for the constructive input and are pleased that our revisions have addressed the comments and strengthened the manuscript.

Reviewer #2 (Remarks to the Author):

The authors have included new data to address my concerns, I suggest to accept it for publication in Nature Communications.

Reply: Thank you for your positive assessment and recommendation for acceptance. We appreciate the reviewer's constructive feedback, which has further strengthened the manuscript.