Supplementary information

Sono-mechanical nanostructures-enabled sustained precise ultrasound brain stimulation

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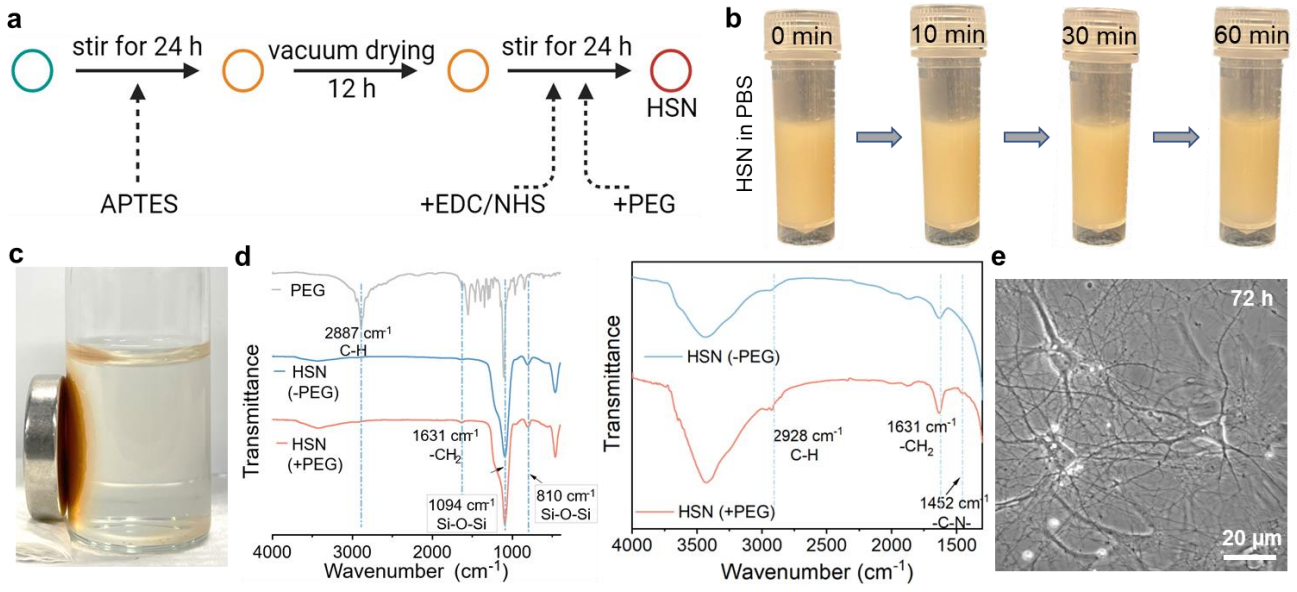
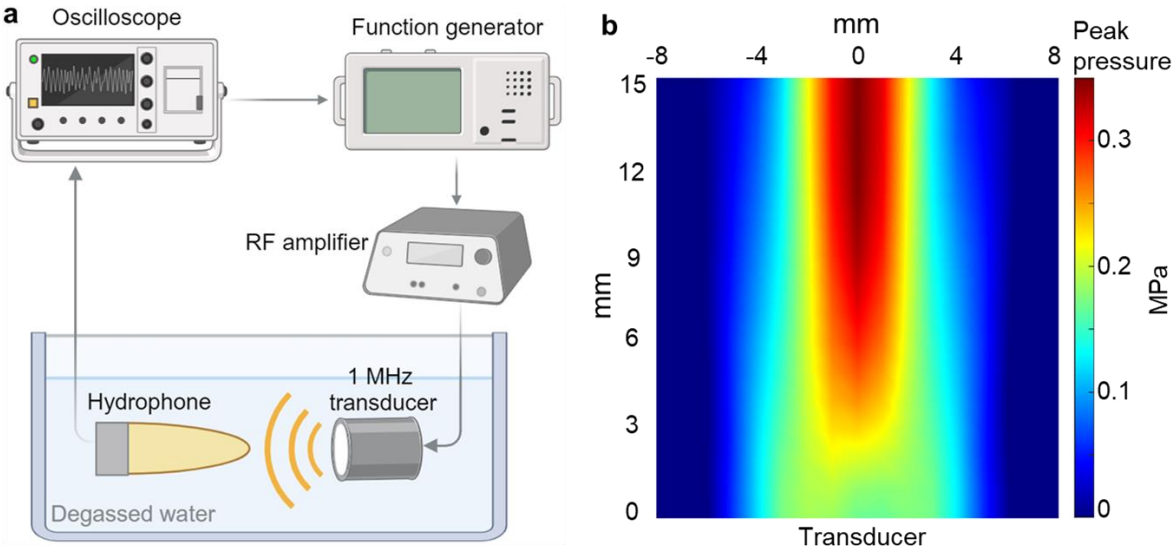
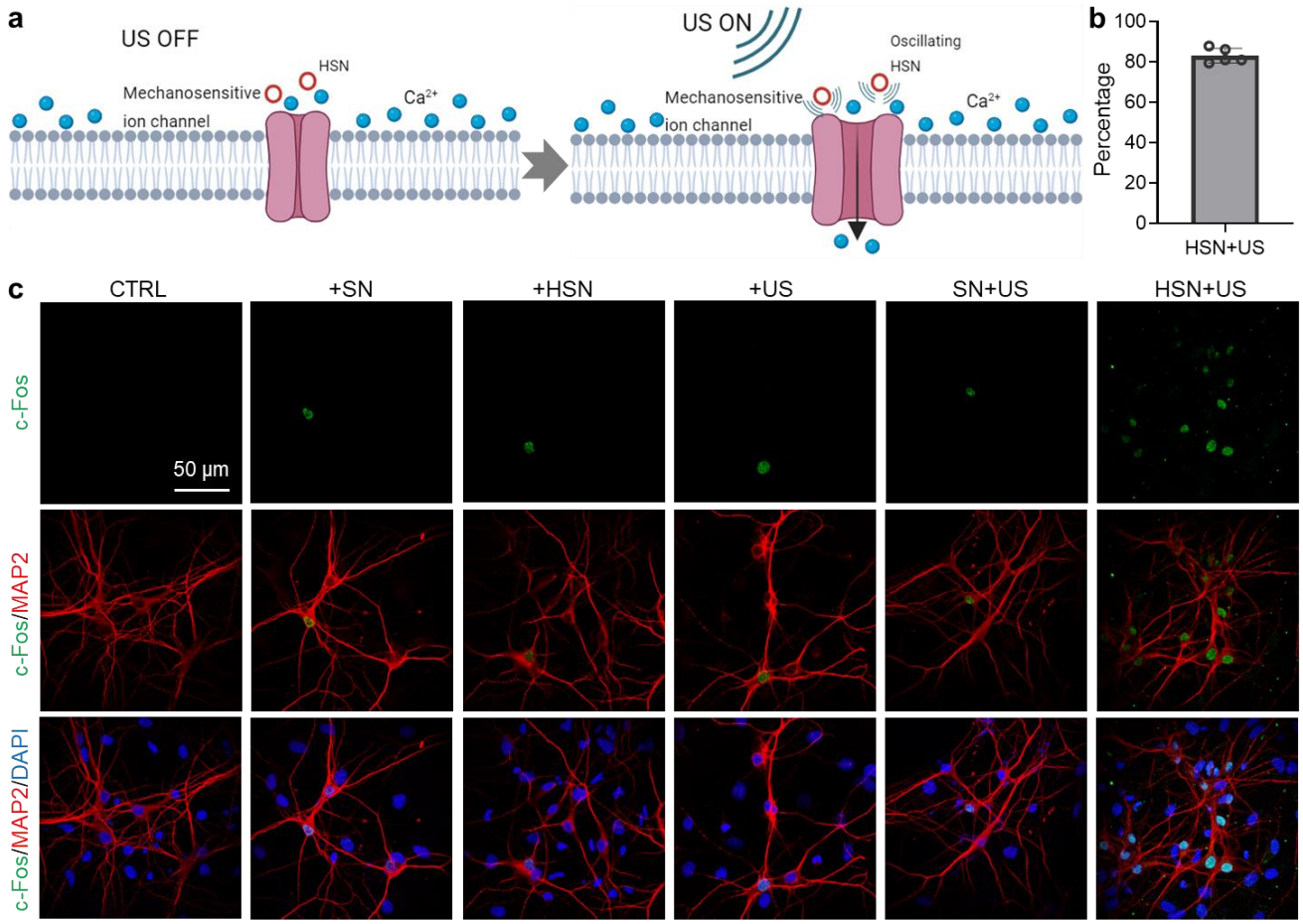
Supplementary Figures 1-15, Custom code, Movies 1-4



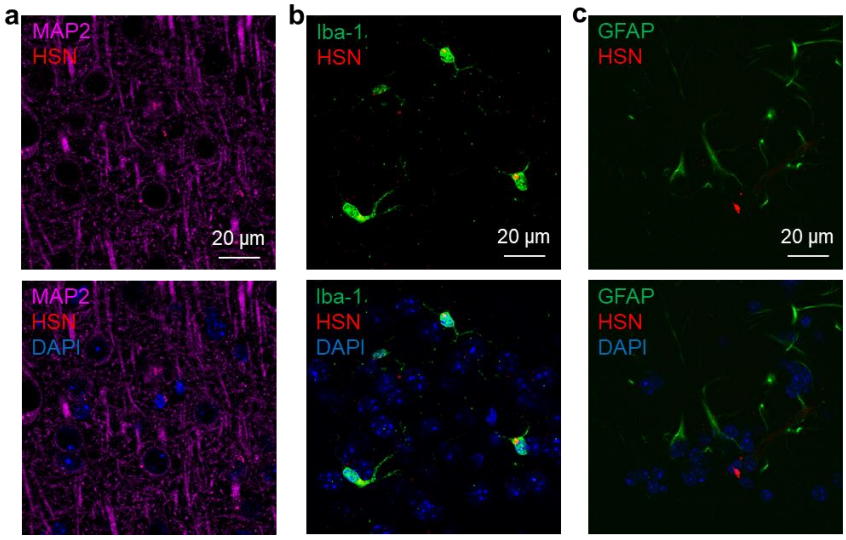
Supplementary Figure 1. Characterization of generated nanoparticles. **a**) Schematic representation illustrating the surface modification of hollow silica nanostructures (HSN) using PEG. **b**) Photographs showing the suspension of HSN at different time points. **c**) Photograph of iron-doped HSN responding to an external magnetic field, collecting on the magnet-adjacent side of the bottle. **d**) Comparison of Fourier-transform infrared spectroscopy (FT-IR) of HSN before and after PEG modification. Results are representative of 3 independent experiments. **e**) Phase contrast photograph of neurons cocultured with HSN. Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.



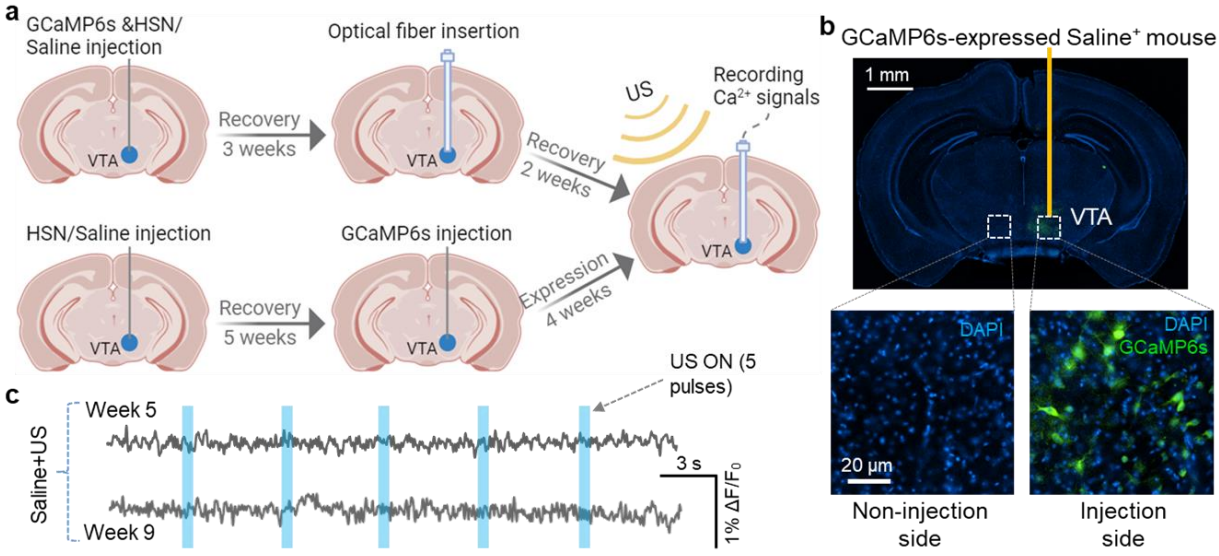
Supplementary Figure 2. Acoustic field test. **a**) Experimental setup for acoustic field measurement using a hydrophone (1.0 MHz transducer). **b**) Colormap illustrating the distribution of acoustic energy, with the planar transducer located at the bottom of the colormap. Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.



Supplementary Figure 3. HSN-enabled ultrasound neuromodulation through the activation of mechanosensitive ion channels. a) Illustration depicting the ultrasonic activation of mechanosensitive ion channels in the plasma membrane of cells facilitated by HSN. **b**) Quantification of neuronal proportion response to HSN+US stimulation. Data are presented as mean ± SD (n = 5 independent experiments). **c**) Representative images displaying c-Fos expression under various treatment conditions (n = 4 independent experiments). Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.

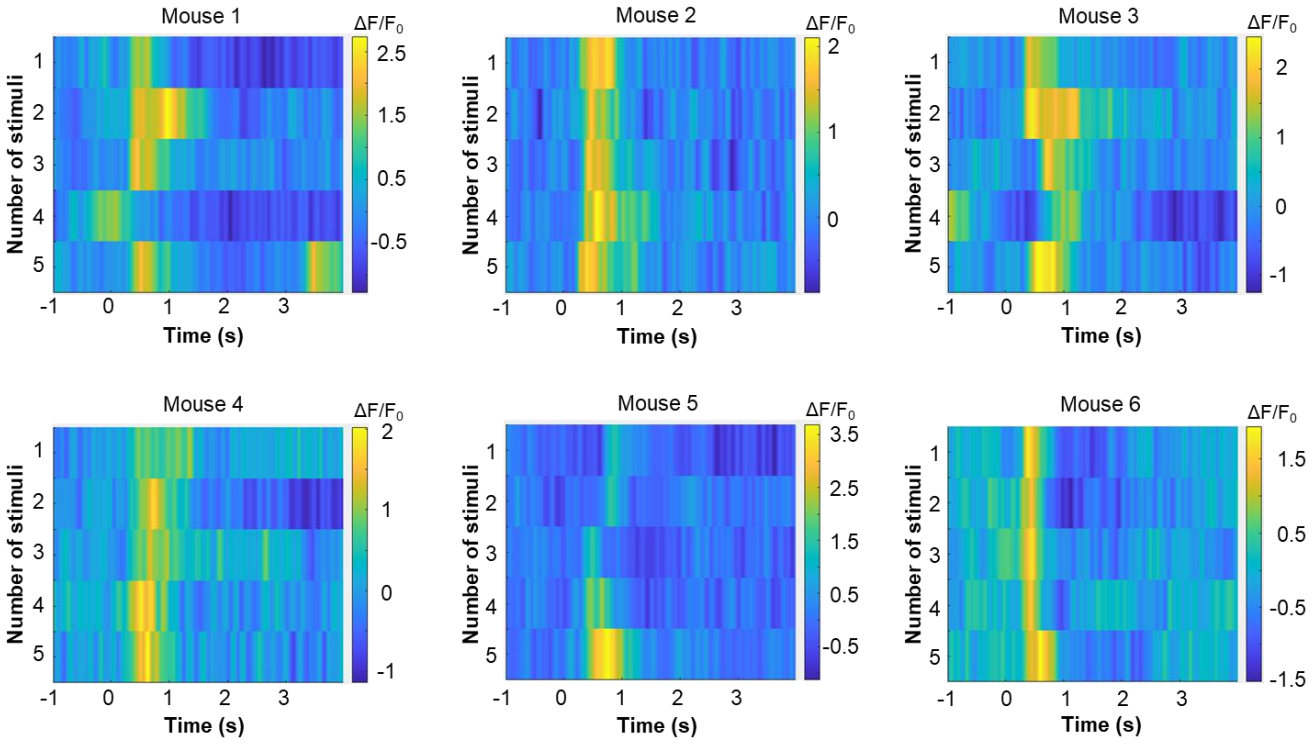


Supplementary Figure 4. Representative confocal images of HSN (red) distribution in the striatum 12 weeks post-injection. a) HSN uptake within neurons labeled with MAP2, (**b**) within microglia labeled with Iba-1, and (**c**) within astrocytes labeled with GFAP. Images are representative of six independent experiments.

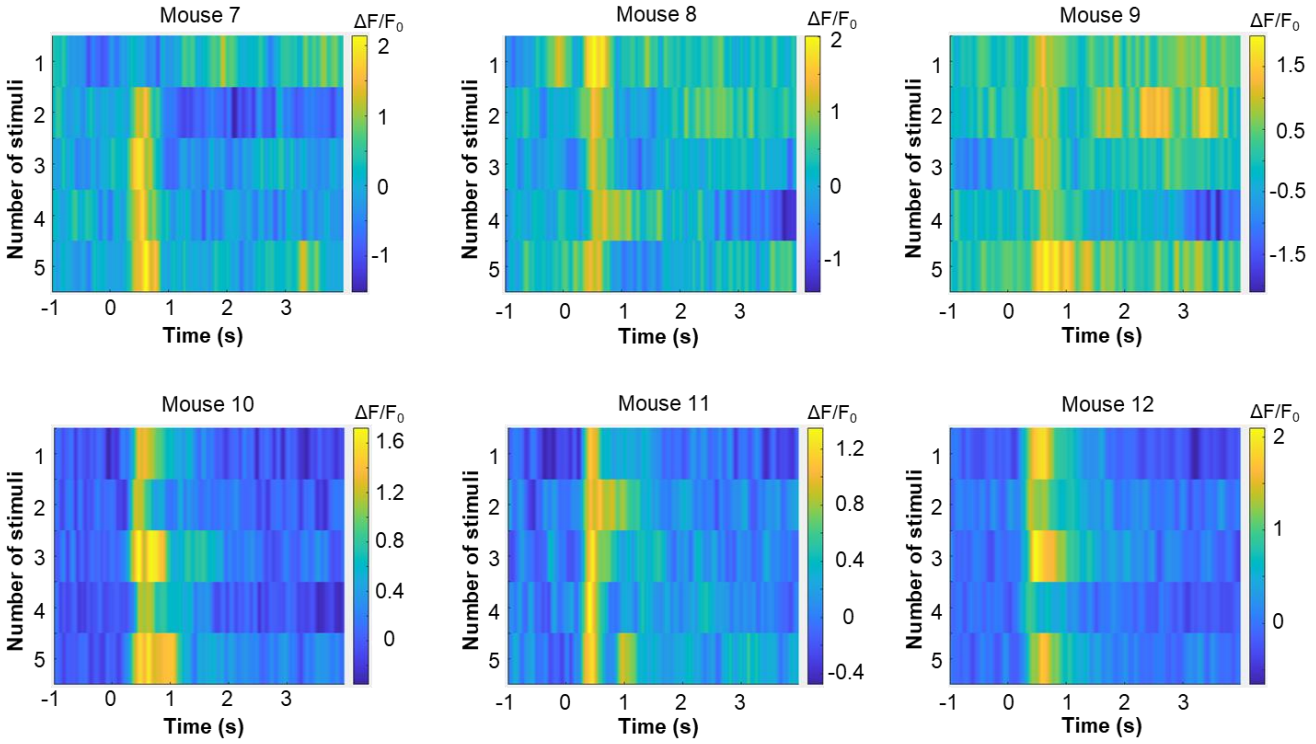


Supplementary Figure 5. Fiber photometry monitoring neural activity in VTA mouse brain. a) Schematic illustration depicting the experimental process for fiber photometry. **b**) Representative images displaying the

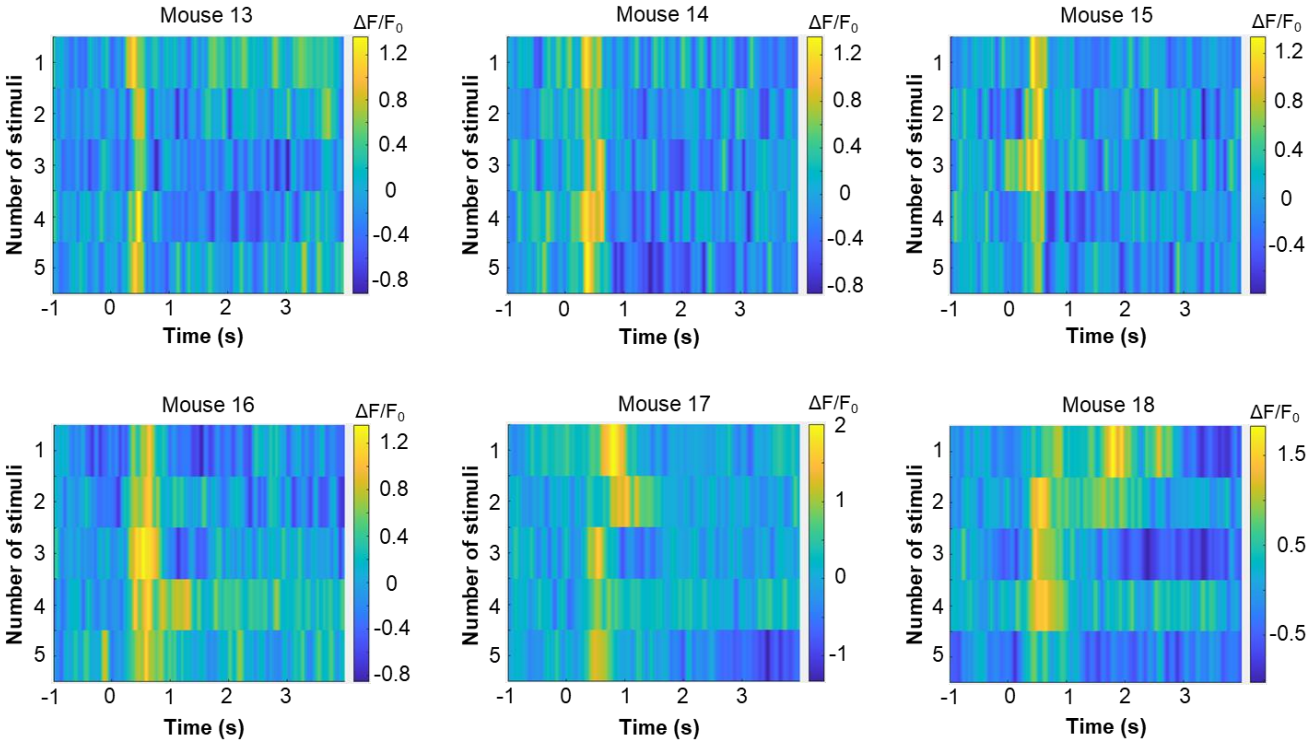
expression of GCaMP6s in a Saline^{+} mouse. **c**) Representative fluorescent traces of GCaMP6s obtained from the VTA of mice treated with Saline (week 5 / 9) under US stimulation. The light blue lines indicate the US pulses. Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.



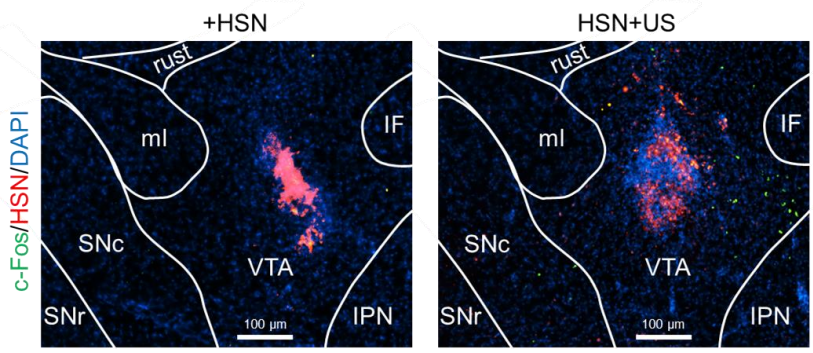
Supplementary Figure 6. Neural calcium responses were monitored by fiber photometry in week 1. Representative heatmaps displaying GCaMP6s fluorescence changes under 5 pulses of ultrasound stimulation for individual HSN^{+} mouse (n = 6 mice).



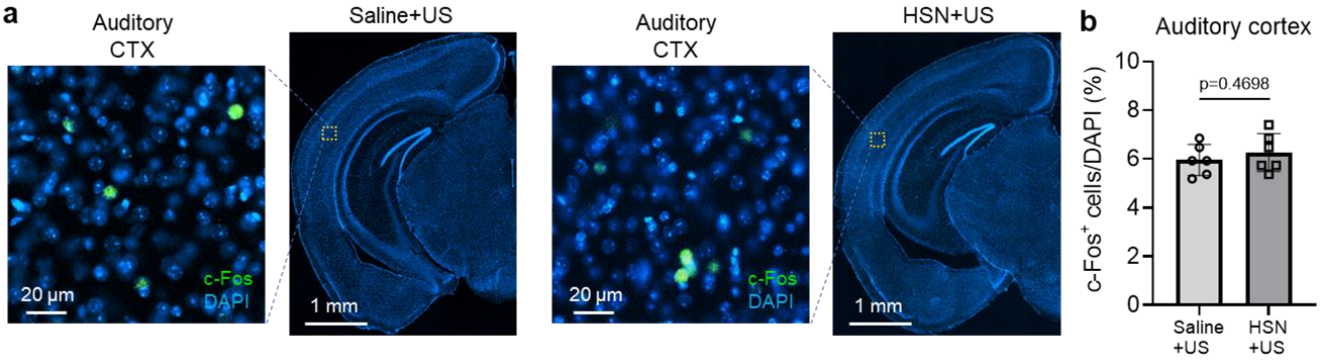
Supplementary Figure 7. Fiber photometry recorded GCaMP6s fluorescence changes in week 5. Representative heatmaps illustrating VTA neural Ca^{2+} responses to ultrasound stimulation (5 pulses) for each HSN^{+} mouse (n = 6 mice).



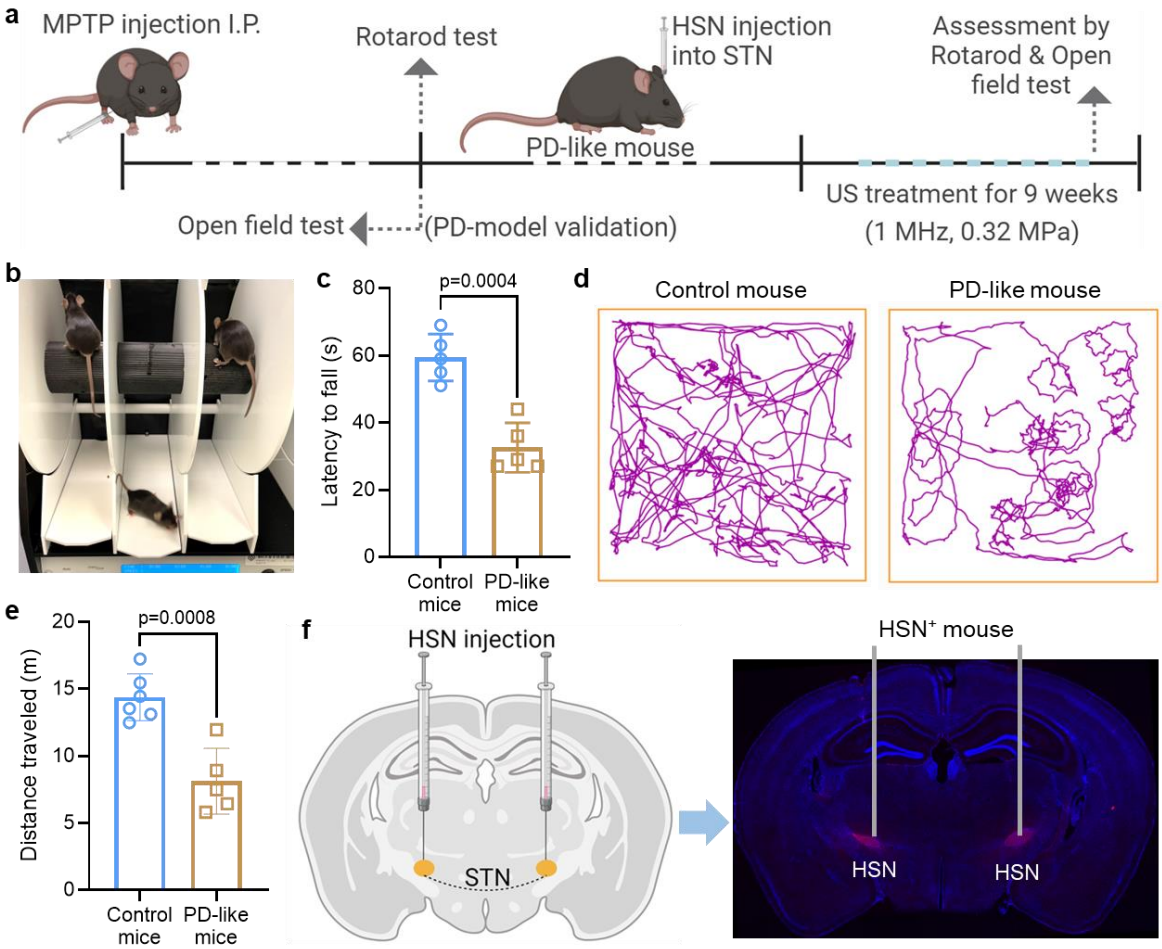
Supplementary Figure 8. Fiber photometry captured GCaMP6s fluorescence changes in week 9. Heatmaps depict calcium responses evoked by five ultrasound pulses in individual HSN^{+} mice (n = 6 mice).



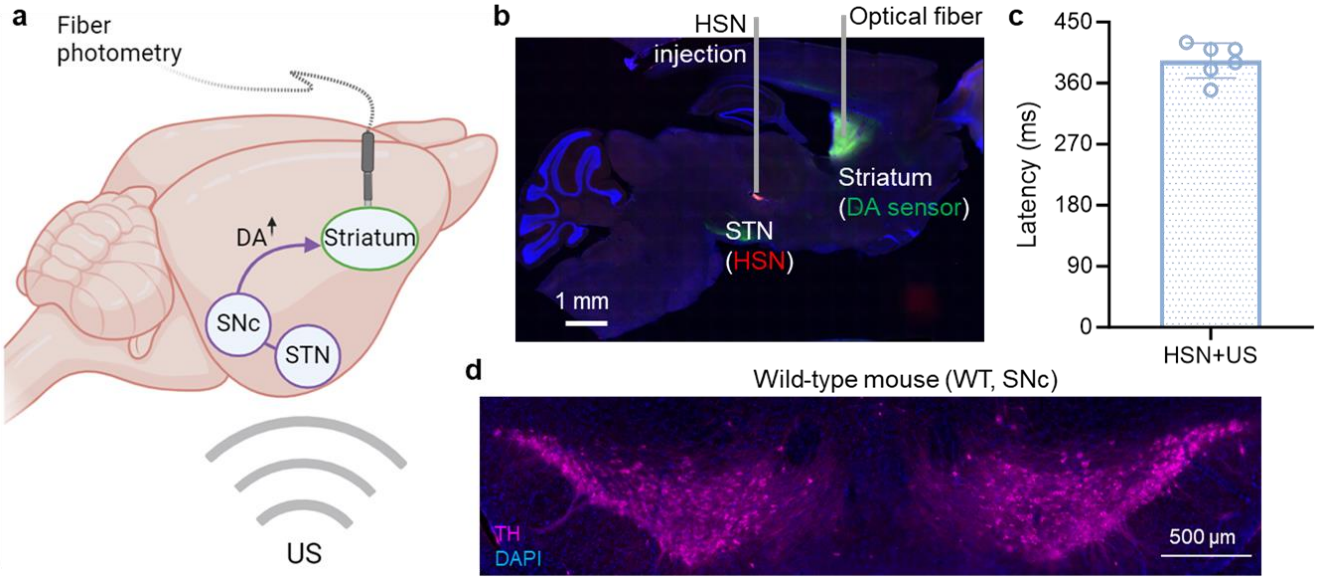
Supplementary Figure 9. Representative immunofluorescence images of brain sections after treatment. c-Fos expression was predominantly localized to the VTA following HSN+US stimulation, whereas minimal c-Fos expression was observed in the HSN-only group. Abbreviations: VTA, ventral tegmental area; ml, medial lemniscus; rust, rubrospinal tract; SNr, substantia nigra reticular part; SNc, substantia nigra pars compacta; IF, interfascicular nucleus raphe; IPN, interpeduncular nucleus.



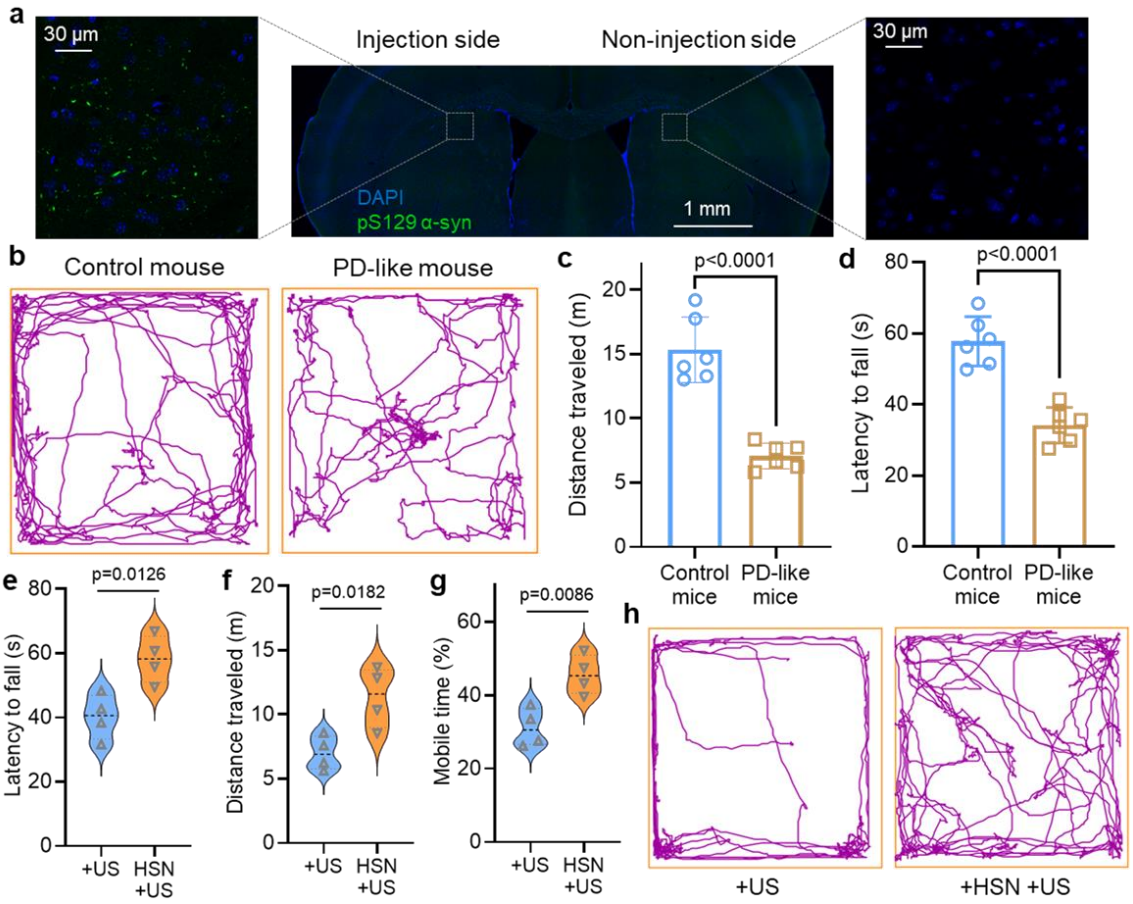
Supplementary Figure 10. c-Fos expression in mouse auditory cortex (CTX) upon US stimulation. a) Representative images illustrating c-Fos staining in the auditory cortex of Saline+US-treated mouse (left) and HSN+US-treated mouse (right). **b**) Quantification of c-Fos counts in slices (n = 6 mice per group). Data are presented as mean ± SD. No significant differences (n.s.) were found using a two-tailed unpaired t-test.



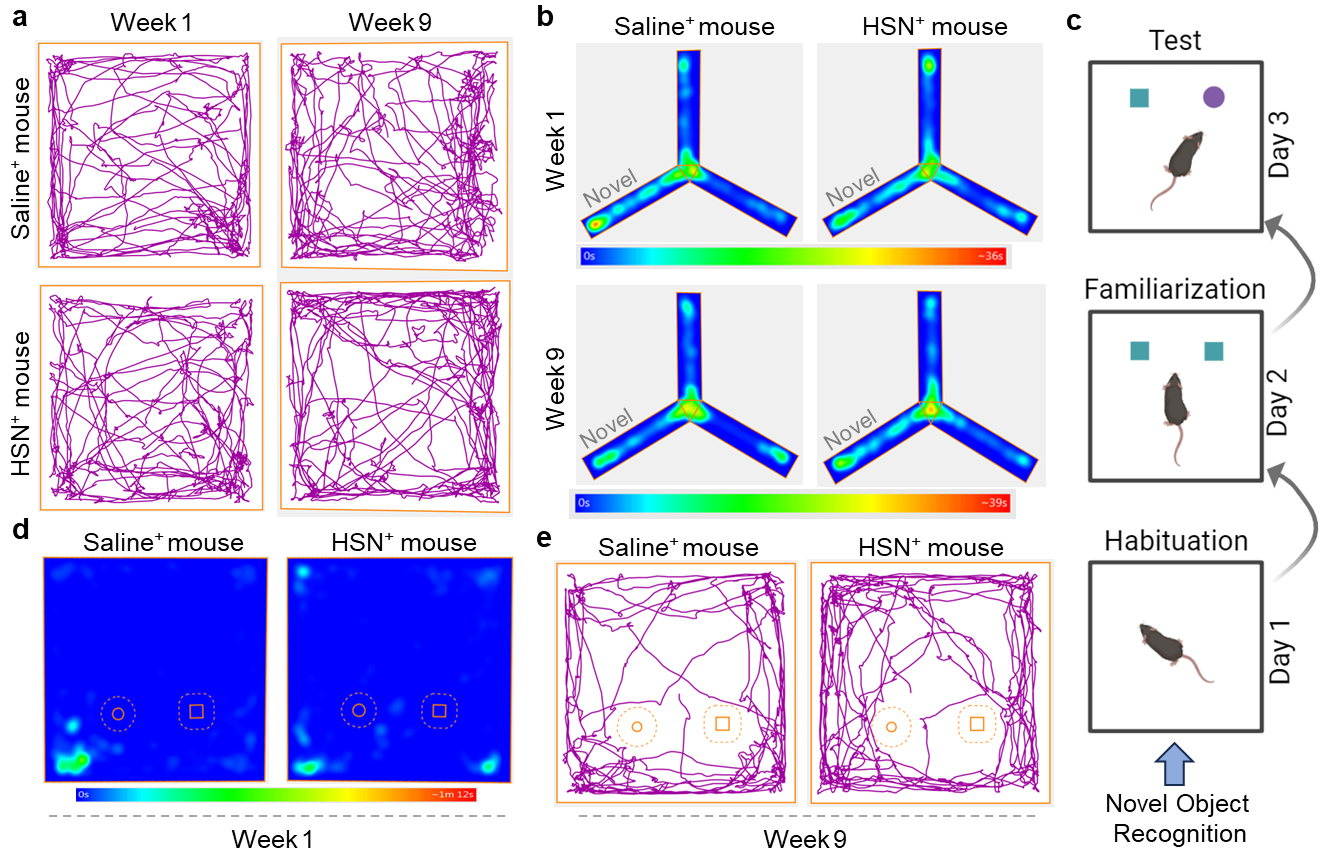
Supplementary Figure 11. Assessment of PD mouse model establishment using MPTP and HSN+US treatment. **a**) Overview of the experimental timeline for the in vivo study incorporating the PD model, HSN+US stimulation, and treatment assessment. **b**) Image showing mice on the rotarod apparatus for assessing motor function. **c**) Fall latency time on the rotarod for control mice and PD model mice. Mice n = 5 for each group. **d**) Representative open field trajectories recorded from a control mouse and a PD-like mouse (each trace is 5 minutes long). **e**) Total distance traveled by control mice (n = 6) and PD model mice (n = 5) during the open field test. **f**) Illustrative representation of a brain slice from a mouse following HSN delivery into the STN. Statistical analyses were performed using a two-tailed unpaired t-test for (**c**) and (**e**). Data are presented as mean ± SD. Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.



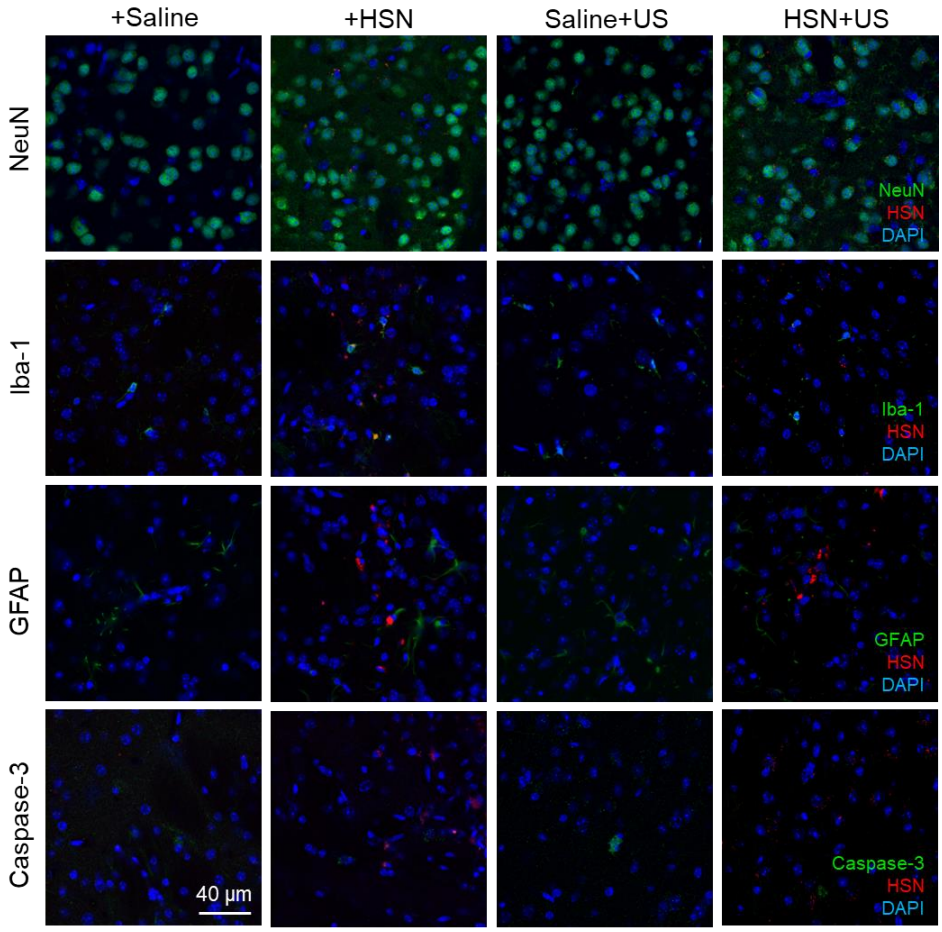
Supplementary Figure 12. HSN+US treatment and assessment. a) Schematic illustration of fiber photometry recording of dopamine (DA) transients in the striatum during ultrasound irradiation on the STN. **b**) Representative fluorescence image of a brain slice from a mouse injected with HSN and DA sensor. HSN (red) are in the STN region, and the DA sensor (green) is expressed in the striatum. **c**) Mean latency of DA release under ultrasound stimulation in the presence of HSN (n = 6 mice). Data are presented as mean ± SD. **d**) Representative immunofluorescent staining image of TH in the SNc of a WT mouse brain section. Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.



Supplementary Figure 13. 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 mice per group). **d**) Rotarod latency to fall (n = 6 mice per group). **e**) Rotarod retention time in PFF-induced PD-like mice after one week of ultrasound treatment (n = 4 mice per group). Total distance traveled (**f**) and time spent mobile (**g**) in the open-field arena after one week of ultrasound treatment (n = 4 mice per group). **h**) Representative open-field trajectories of PD-like mice after treatment. Data are presented as mean ± SD in panels (**c**) and (**d**). Statistical analyses were performed using unpaired t-test for (**c**-**g**).



Supplementary Figure 14. Biosafety assessment through mice behavior. **a**) Representative trajectories recorded from mice injected with Saline or HSN (1, 9 weeks) into the striatum (each trace represents a 5-minute recording). **b**) Heatmap illustrating the movement patterns of mice in the Y maze, measured at week 1 or week 9 after Saline/HSN injection. **c**) Schematic diagram depicting the novel object recognition (NOR) task for mouse, involving habituation on day 1, familiarization on day 2, and the final test on day 3. Representative trajectories were recorded from Saline$^+$ mice and HSN$^+$ mice during the NOR test week 1 (**d**) and week 9 (**e**). Created in BioRender. Sunlab, P. (2025) https://BioRender.com/3b6pyaz.



Supplementary Figure 15. In vivo safety assessment. Evaluation of neural integrity, inflammation, and apoptosis in the striatum post-treatment through immunofluorescent staining of neurons (NeuN), microglia (Iba-1), astrocytes (GFAP), and Caspase-3. The red color highlights the injected HSN, while the blue color represents the DAPI-stained nuclei (n = 6 mice for each group).

Custom code for neuronal calcium imaging:

```
import matplotlib.pyplot as plt

from matplotlib.colors import LinearSegmentedColormap

import numpy as np

from PIL import Image

import os
```

```
custom_cmap = LinearSegmentedColormap.from_list('custom', ['blue', 'cyan', 'yellow', 'red'])


def convert_to_heatmap(image_path, vmin=None, vmax=None, show_colorbar=True):
    image = Image.open(image_path).convert('L')
    image_array = np.array(image)


    plt.imshow(image_array, cmap=custom_cmap, vmin=vmin, vmax=vmax)
    if show_colorbar:
        plt.colorbar()
    plt.axis('off')
    plt.grid(False)
    plt.title(os.path.basename(image_path), fontsize=10)
    plt.show()


ref_image_path = 'your_reference_image.tif'
ref_image = Image.open(ref_image_path).convert('L')
ref_array = np.array(ref_image)
vmin, vmax = ref_array.min(), ref_array.max()


image_list = ['1.tif', '2.tif', '3.tif', '4.tif', '5.tif']
for img_path in image_list:
    convert_to_heatmap(img_path, vmin=vmin, vmax=vmax)
```