

1 **Frequency-and circuit-specific effects of septohippocampal deep brain stimulation in mice**
2 **as measured by functional ultrasound imaging.**

3

4 Authors

5 Lindsey M. Crown, PhD^{*2}, Kofi Agyeman, MS^{*5,7}, Wooseong Choi, BS^{*1}, Nancy Zepeda, BS¹,
6 Ege Iseri MS⁵, Pooyan Pahlavan MS⁵, Steven J. Siegel, MD, PhD², Charles Liu MD, PhD^{\$1,3,4,6},
7 Vasileios Christopoulos, PhD^{\$3,5,7}, Darrin J. Lee, MD, PhD^{\$1,2,3,4,6}

8

9 Affiliations

10 ¹Department of Neurological Surgery, Keck School of Medicine, University of Southern
11 California, Los Angeles, CA, USA

12 ²Department of Psychiatry and Behavioral Sciences, Keck School of Medicine, University of
13 Southern California, Los Angeles, CA, 90033, USA

14 ³Neurorestoration Center, Keck School of Medicine, University of Southern California, Los
15 Angeles, CA, USA

16 ⁴Viterbi School of Engineering, University of Southern California, Los Angeles, CA, USA

17 ⁵Department of Bioengineering, University of California Riverside, Riverside, CA, USA

18 ⁶Rancho Los Amigos National Rehabilitation Center, Downey, CA, USA

19 ⁷Neuroscience Graduate Program, University of California Riverside, Riverside, CA, USA

20 * These authors contributed equally

21 § These authors jointly supervised this work (Correspondence)

22

23 Please send correspondence to: Dr. Darrin J. Lee, MD, PhD (Darrin.Lee@med.usc.edu)

24

25 **Abstract**

26 Background

27 Deep brain stimulation (DBS) has shown remarkable success in treating neurological and
28 psychiatric disorders such as Parkinson's disease, dystonia, epilepsy, and obsessive-compulsive
29 disorder. Despite this success, the underlying mechanism of action remains unknown. DBS is
30 now being explored to improve functional outcomes in other psychiatric conditions, such as
31 those characterized by reduced N-methyl-D-aspartate (NMDA) function (i.e. schizophrenia).
32 While DBS for movement disorders requires high-frequency continuous stimulation, there is
33 evidence that intermittent low-frequency stimulation in neuropsychiatric conditions may have
34 persisting cognitive benefits, necessitating a broader exploration of how DBS alters brain
35 networks.

36 Objective

37 We characterize the effects of pharmacologic NMDA antagonism on the septohippocampal
38 network and the impact of high- and low-frequency MSN DBS on cerebral blood volume (CBV)
39 in brain structures within and outside of the septohippocampal network.

40 Methods

41 In this study, we utilize a novel technology, functional ultrasound imaging (fUSI), to characterize
42 the cerebrovascular impact of medial septal nucleus (MSN) DBS under conditions of NMDA
43 antagonism (pharmacologically using Dizocilpine [MK-801]) in anesthetized male mice.

44 Results

45 Imaging from a sagittal plane across a variety of brain regions, we find that MSN theta-
46 frequency (7.7Hz) DBS has a larger effect on hippocampal CBV after stimulation offset. This is
47 observed following an intraperitoneal (i.p.) injection of either saline vehicle or MK-801 (1

48 mg/kg). This effect is not present using standard high-frequency DBS stimulation parameters
49 (i.e. gamma [100Hz]).

50 Conclusion

51 These results indicate the MSN DBS increases circuit-specific hippocampal neurovascular
52 activity in a frequency-dependent manner that continues beyond the period of electrical
53 stimulation.

54

55 **Introduction**

56 There is growing interest in utilizing neuromodulation to treat cognitive impairment
57 associated with neurological and psychiatric disorders. Many of these disorders involve aberrant
58 electrophysiology and cerebral blood perfusion [1,2]. Recent evidence demonstrates that
59 electrical neuromodulation can improve functional outcomes [3,4]. Particularly, deep brain
60 stimulation (DBS) has shown remarkable success in treating neurological diseases such as
61 movement disorders and epilepsy, and there is increasing evidence for its efficacy in cognitive
62 and psychiatric conditions.

63 Despite this growing utility, the underlying mechanism of DBS for cognitive outcomes
64 remains largely unknown. Pre-clinical studies have been hindered by technological limitations,
65 such as the inability to record electrical brain activity during stimulation and the low spatial
66 resolution of electrographic measures. Functional ultrasound imaging (fUSI) is a relatively new
67 technology that enables large-scale estimates of neural activity through measures of cerebral
68 blood volume (CBV). fUSI provides a unique combination of high spatiotemporal resolution
69 ($\sim 100 \mu\text{m}^3$, up to 10 ms) and high sensitivity to slow blood flow ($\sim 1 \text{ mm/s}$ velocity) across a
70 large field of view. In fact, fUSI has already been proven to be an effective tool for imaging
71 large-scale brain activity and pharmacodynamics [5–8]. As such, it is well-positioned to
72 improve our understanding of the impact of DBS on large-scale brain networks during and
73 immediately following stimulation.

74 In many disorders, cognitive dysfunction is accompanied by altered septohippocampal
75 network activity. For instance, neural oscillatory patterns within the septohippocampal network,
76 such as gamma- and theta-band activity, are characteristically altered in disorders such as
77 schizophrenia and Alzheimer's disease and are often correlated with impaired memory [1,9–11].

78 Alterations in specific neurotransmitter signal transduction pathways also play an important role
79 in modulating neural oscillatory activity. In particular, the glutamatergic *N*-methyl-D-aspartate
80 (NMDA) receptor is crucial to regulating hippocampal theta and gamma oscillations and is the
81 predominant molecular control for synaptic plasticity and memory function [12–14]. As such,
82 pharmacologic NMDA receptor antagonism (i.e. via MK-801) results in characteristic changes to
83 neural oscillatory patterns and memory dysfunction [15–19].

84 The medial septal nucleus (MSN) is a key structure in the septohippocampal network that
85 modulates sensory-motor processing and acts as a “pacemaker” for hippocampal theta
86 oscillations via dense glutamatergic, cholinergic, and GABAergic projections to the
87 hippocampus [20,21]. This makes the MSN a promising target for DBS in cognitive disorders
88 involving memory impairments [22]. Our recent work as well as that of others suggest that
89 modulating the septohippocampal network via MSN theta frequency-specific (7.7 Hz) DBS can
90 restore cognitive impairment and memory dysfunction in preclinical models of epilepsy,
91 traumatic brain injury, Alzheimer’s disease, and schizophrenia [22–27].

92 In the current study, we utilize fUSI to characterize the effects of MK-801 on CBV in the
93 septohippocampal network (Fig. 1A) including the hippocampus, and medial prefrontal cortex
94 (mPFC). Within the same sagittal plane, we also image CBV changes (Δ CBV) to regions of
95 interest (ROIs) outside this network including the striatum, thalamus, hypothalamus, and
96 pallidum. Note that other regions that are connected with the MSN, such as amygdala, habenula,
97 or raphe nucleus were not recorded, since they were not accessible from the selected 2D image
98 plane. Additionally, we determine the effect of direct MSN stimulation on blood flow in these
99 areas using two distinct frequencies, theta (7.7Hz) and gamma (100Hz). Finally, we test the

100 hypothesis that MSN theta-frequency-specific stimulation can improve blood flow under
101 conditions of reduced NMDA function.

102

103 **Materials and Methods**

104 *Animals and surgical procedures*

105 82 male 8–12-week-old C57BL/6 mice (Charles River Laboratories; Hollister, CA) were
106 used in this study. fUSI data from two animals were excluded due to extreme values (Grubbs
107 test for outliers, 98th percentile of all maximum change in pD intensities) that did not appear
108 physiological [28,29]. The animals were divided into two main groups: saline vehicle control
109 (n=46) and MK-801 drug-administered (n=34). Each group was then sub-divided into three
110 categories: no stimulation (saline: n=14; MK-801: n=10), theta stimulation (saline: n=16, MK-
111 801: n=12), and gamma stimulation (saline: n=14, MK-801: n=14).

112 Mice were anaesthetized with 5% isoflurane in O₂/N₂O (1:2) carrier gas and then
113 maintained at a constant rate (1.5- 2%) through surgery and data acquisition. Body temperature
114 was kept constant throughout recordings by placing animals on an electric heating pad. Hair was
115 removed from the mouse's head for fUSI using a commercially available depilatory cream (Nair,
116 Pharmapacks).

117 To implant DBS electrodes, mice were head-fixed in a stereotaxic frame (David Kopf
118 instruments, Tujunga, CA) and a midline incision of the scalp was made to expose the skull. A
119 2mm burr hole was then drilled to implant bipolar stimulating electrodes (E363T/2/SPC ELEC
120 .008"/.2MM, Plastics One Inc., Roanoke, VA) targeting the midline MSN (AP: +0.7mm, ML: -
121 0.9mm, from bregma. Z: -4.39mm at 11.8 degrees) from the left hemisphere. Prior to
122 implantation, the electrodes were connected to an electronic interface board (Neuralynx Inc.,

123 Bozeman, MT) and bent at 4.5mm from the tip to maximize the proximity of the fUSI probe to
124 the skull (Fig. 1B). Recordings took place over one hour, animals were injected after 5 minutes
125 with saline vehicle or MK-801 (1mg/kg) and stimulation or sham stimulation was delivered for 5
126 minutes 45 minutes into the recording period (Fig. 1C). All procedures were approved by the
127 University of Southern California, Institutional Animal Care and Use Committee (IACUC
128 #21006).

129

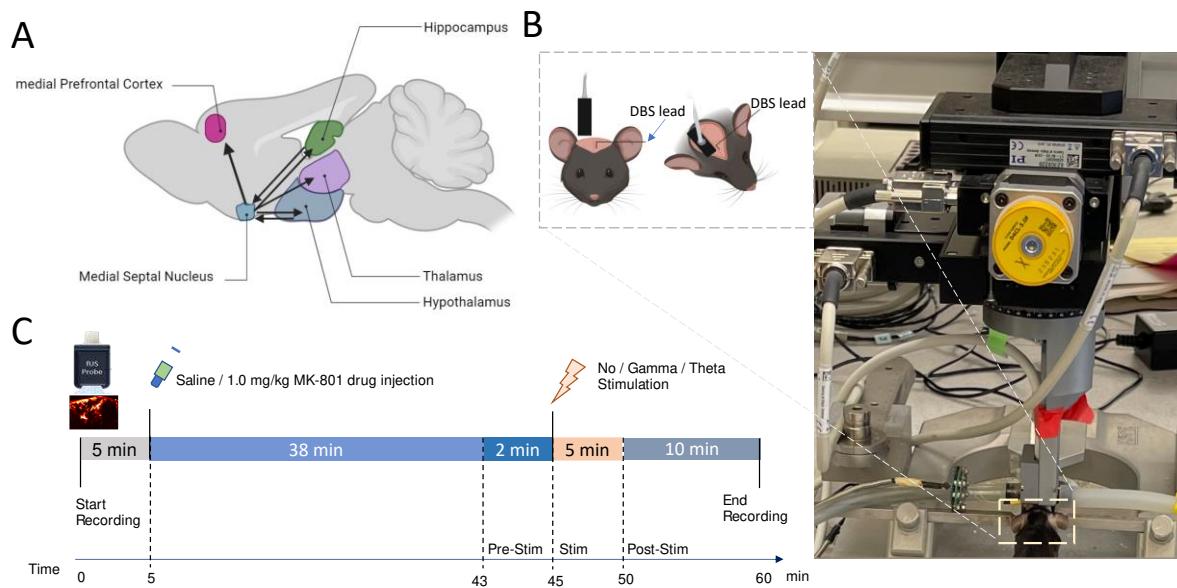


Figure 1. Experimental setup and fUSI recording protocol. A) Schematic illustration of connectivity between the MSN and ROIs. Arrowheads represent axonal projections to and/or from MSN. **B)** Experimental set-up showing the anesthetized mouse in a stereotaxic frame under the Iconeous One motorized probe mount. DBS stimulating electrodes were implanted on the left hemisphere and a sagittal plane of the right hemisphere was imaged. **C)** Diagram of the protocol for 60 minutes of continuous fUSI acquisition consisting of saline or 1.0 mg/kg MK-

801 drug injection (at the 5-minute mark) and 5 minutes of gamma- or theta-frequency DBS (at the 45-minute mark).

130

131

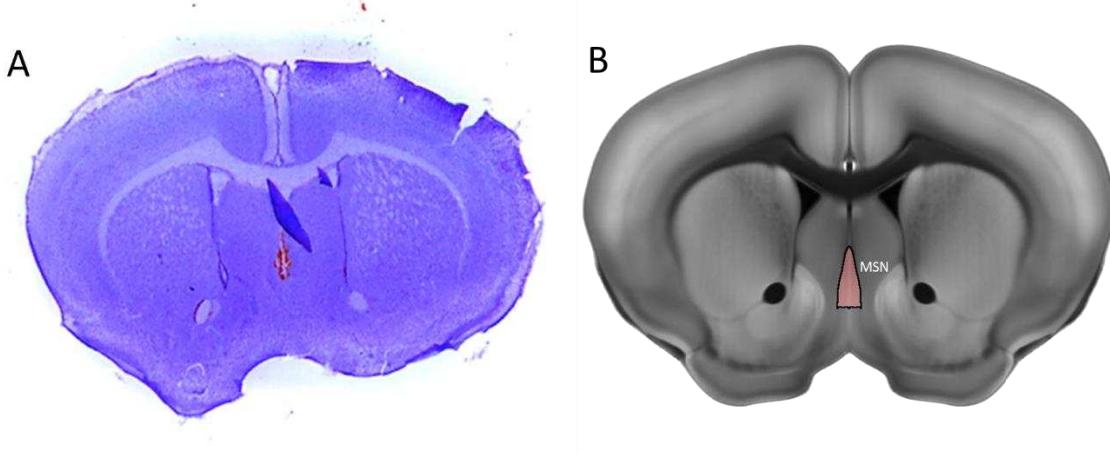


Figure 2. Histological mapping of electrode placement in the MSN. A) Representative Nissl stain of the mouse brain with a blood mark indicating the electrode placement B) Annotation of the MSN from the Allen Reference Atlas – Mouse Brain in the same slice position as A.

132

133

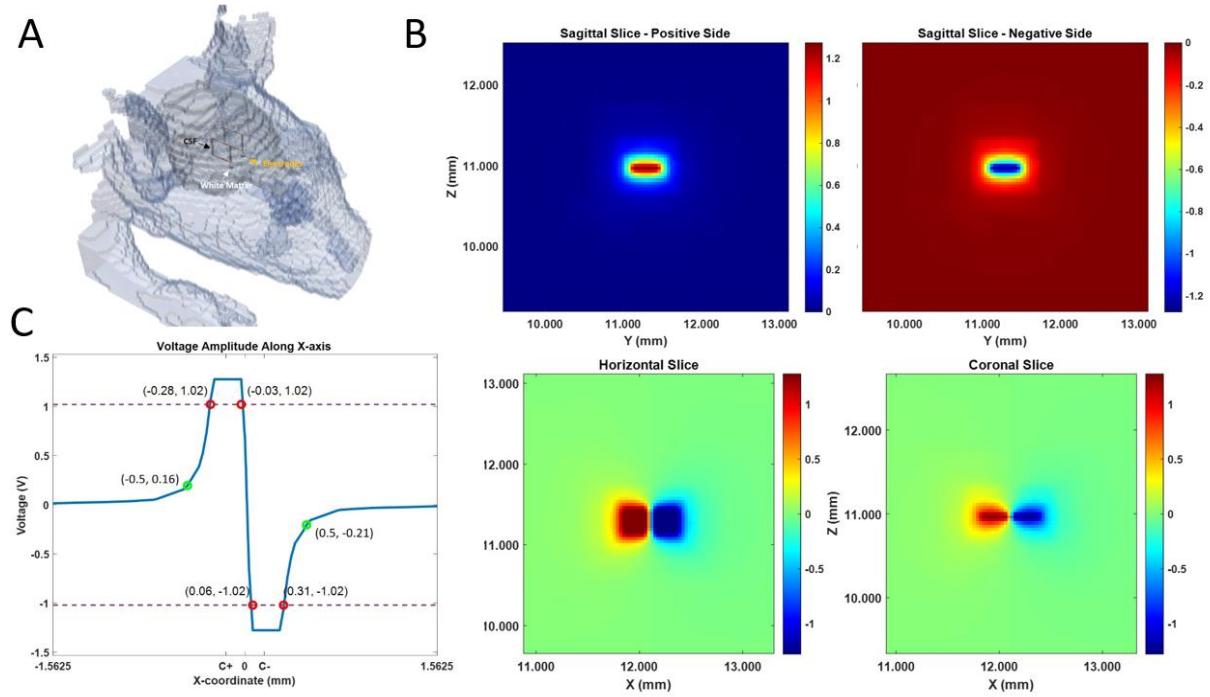
134 *Histology*

135 Mice were euthanized immediately after the fUSI recording by isoflurane overdose followed by
136 transcardial perfusion using 50 mL of 0.1M sodium phosphate buffer saline (PBS) and 50 mL of
137 4% paraformaldehyde. Brains were harvested and stored in phosphate buffered saline at 4°C. To
138 confirm electrode positioning within the MSN, coronal sections were cut at 100µm thickness
139 with a vibratome (Leica VT 1200; Leica Biosystems, Buffalo Grove, IL) and then Nissl stained
140 with Cresyl Violet (Fig. 2A,B).

141 *Computational Modeling of the Induced Voltages: Volume of Tissue Activation*

142 Our group has developed a computational analysis method for applications in electrophysiology
143 and bioelectromagnetic interactions, namely the Admittance Method (AM)[32]. AM discretizes a
144 bulk tissue model into cuboid voxels, where each voxel is represented by an equivalent circuit of
145 lumped passive elements. The admittance value at each voxel is calculated using the material
146 properties such as conductivity and permittivity. A set of linear equations using iterative methods
147 are used to calculate the induced voltage at each node of the voxelized network. Here, a 3-D bulk
148 tissue model with the desired electrode configuration was constructed, a current-controlled
149 stimulation pulse was applied through the electrodes and the induced voltages at each voxel were
150 calculated. A mouse head model (Fig. 3A) was used for this study where three types of tissue were
151 considered (grey matter, white matter (WM) and cerebrospinal fluid (CSF). Electrodes were set as
152 platinum and the medium surrounding the mouse head was set as air. The locations and geometries
153 of the WM and CSF with respect to the septal medial nucleus were approximated using an
154 interactive online mouse brain atlas (<http://labs.gaidi.ca/mouse-brain-atlas/>). The output from AM
155 was then processed using a MATLAB script to produce an illustration of the voltage mapping in
156 the mouse head (Fig. 3B). A one-dimensional voltage profile in the proximity of the electrodes
157 was plotted to estimate the volume of tissue that was electrically affected/activated (Fig. 3C).

158



159

Figure 3. Volume of Tissue Activation **A)** 3-D illustration of the discretized mouse head model.

The bulk tissue model is divided into voxels at a resolution of $31.25 \mu\text{m}$. Each voxel represents an equivalent electrical circuit according to its material type. The brain model is mostly uniform as grey matter. The proximity of the medial septal nucleus, where the electrodes are placed, also contains areas of white matter and CSF modeled as basic geometries. **B)** The voltage mapping near the medial septal nucleus when stimulated with a bipolar electrode configuration. Top row: two sides of the sagittal slice, looking into both the positively and negatively polarized electrodes. Bottom row: the horizontal and coronal slices capturing the voltage distribution around both electrodes. The absolute voltage decays to zero rapidly moving away from the electrodes, demonstrating that the volume of activation is confined within a diameter of about 1 millimeter. **C)** The voltage amplitude plotted along a single axis. The voltage decay profile moving away from the electrode is very similar along all three axes and the volume of activation

can be modeled as a sphere. The “equivalent diameter” for the volume affected by stimulation is approximated by choosing points where the slope is approaching zero (green circles), which determines the current density generated at that location. Thresholding can also be used to visualize the points where the target voltage amplitude is maintained. The red circles label the coordinates at which an amplitude of 1 Volt or more is observed. C+ and C- labels mark the centers of the positive and negative electrodes, respectively.

160

161 *Data analysis*

162 *Data pre-processing*

163 The Iconeus One acquisition system generates power Doppler (pD) images pre-processed
164 with built-in phase-correlation based sub-pixel motion registration and singular-value-
165 decomposition (SVD) based clutter filtering algorithms [33]. These algorithms were used to
166 separate tissue signal from blood signal to obtain pD images (Fig. 4A/B). To correct potential
167 physiological and motion artifacts, we adopted rigid motion correction techniques that have
168 successfully been used in fUSI and other neuroimaging studies [34–36]. These were combined
169 with high frequency filtering algorithms to eliminate noise artifacts. We employed a low-pass
170 filter with normalized passband frequency of 0.02 Hz, with a stopband attenuation of 60 dB that
171 compensates for delay introduced by the filter, to remove high-frequency fluctuations in the pD
172 signals.

173

174 *Effects of MK-801 on CBV*

175 We investigated the temporal effects of intraperitoneal MK-801 administration on the
176 septohippocampal circuit (hippocampus, mPFC) and surrounding regions (striatum, pallidum,

177 thalamus, hypothalamus). To do so, we generated event-related average (ERA) time course
178 curves of the CBV changes (Δ CBV) as a percentage change of the pD signal from baseline
179 activity for the selected ROIs. The average pD signal from 2 minutes prior to the saline or drug
180 injection was used as the baseline. We utilized a repeated measures analysis of variance
181 (rmANOVA) to assess the effects and interactions between drug (saline and MK801) and ROI
182 factors over time. We fitted a repeated measures ‘*within-design*’ model to the CBV percentage
183 change signals over 42-min interval (including 2 minutes just before the drug injection and the
184 40 minutes after injection) for each mouse and ROI for the rmANOVA analysis. To further
185 quantify the relative differences in Δ CBV between saline-vehicle and MK-801-treated mice in
186 various ROIs, we used the last 2 minutes of recordings to compute the mean effects-size
187 differences in Δ CBVs and the 95% confidence interval of the effect size (if 95% confidence
188 interval contains zero, then the effect is not significant at the 0.05 level) in each ROI. We also
189 computed the Cohen’s d value in each ROI as a measure of the drug effect size that describes the
190 standardized difference between the means of Δ CBVs in the two groups of animals [37]. A
191 Cohen’s d value of 0.2 represents a small effect size, 0.5 represents a moderate effect size, 0.8
192 represents a large size and greater than 0.8 represents a very large size.

193 Effects of MSN DBS during stimulation on brain hemodynamics

194 We computed the Δ CBV for each ROI to investigate effects of MSN theta- and gamma-
195 frequency stimulation on the cerebral hemodynamics following saline control or MK-801
196 injection. We observed ERA time-series of the Δ CBV of each selected ROI relative to the
197 average pD signal acquired 2 minutes prior to stimulation onset to visualize the temporal
198 dynamics of DBS effects to CBVs of the ROIs (Fig. 1C). We utilized a three-way rmANOVA
199 to assess the effects and interactions between drug (saline and MK801), stimulation (gamma,

200 theta, no-stimulation) and ROI factors across time during the stimulation process. Here, we fit a
201 repeated measures ‘*within-design*’ model to the Δ CBV signals over the 7-min period (including 2
202 minutes prior to stimulation, 5 minutes during stimulation) for each animal and ROI.
203 Subsequently, we computed the mean effects-size differences in Δ CBVs, the 95% confidence
204 interval of the effect size, and the Cohen’s *d* value in each ROI as a measure of the stimulation
205 effect size. We measured the mean Δ CBVs during stimulation in theta [saline: n=16, MK-801:
206 n=12], gamma [saline: n=14, MK-801: n=14], and no-stimulation [saline: n=14; MK-801: n=10]
207 animal groups. The mean Δ CBVs were calculated utilizing the last 2 minutes of pD signal during
208 stimulation across animals in each of the three stimulation categories.

209

210 Effects of MSN DBS after stimulation offset on brain hemodynamics

211 We are also interested in assessing the effects of MSN DBS after stimulation offset. To do
212 so, we repeated the same analysis described above, but we used the 10 minutes of pD signal after
213 stimulation offset, plus 2 minutes prior to stimulation onset as a baseline. The mean Δ CBVs were
214 calculated utilizing the last 2 minutes of pD signal after stimulation offset across animals in each
215 of the three stimulation categories. Our goal is to identify whether MSN DBS causes persistent
216 and delayed changes in CBVs within and/or outside the septohippocampal network.

217

218 Statistical analysis of drug and stimulation effects on Δ CBVs

219 All the analysis was performed using Matlab Version 9.13.0.2193358 (R2022b). We
220 assessed the drug and stimulation effects and interactions utilizing the Matlab functions ‘*fitrm*’
221 and ‘*ranova*’ for the repeated measures model fitting and the rmANOVA respectively. We
222 utilized the Greenhouse-Geisser approximation to correct for the possibility of non-compound

223 symmetry (same variance in means and shared common correlation in paired responses) in the
224 ROI-time series assessed. The mean effect-size differences and the *Cohen's d* values for the
225 mean difference in Δ CBVs between saline and MK801, as well as the between no-stimulation-
226 gamma, no-stimulation-theta, and gamma-theta were computed with the '*meanEffectSize*' Matlab
227 function.

228

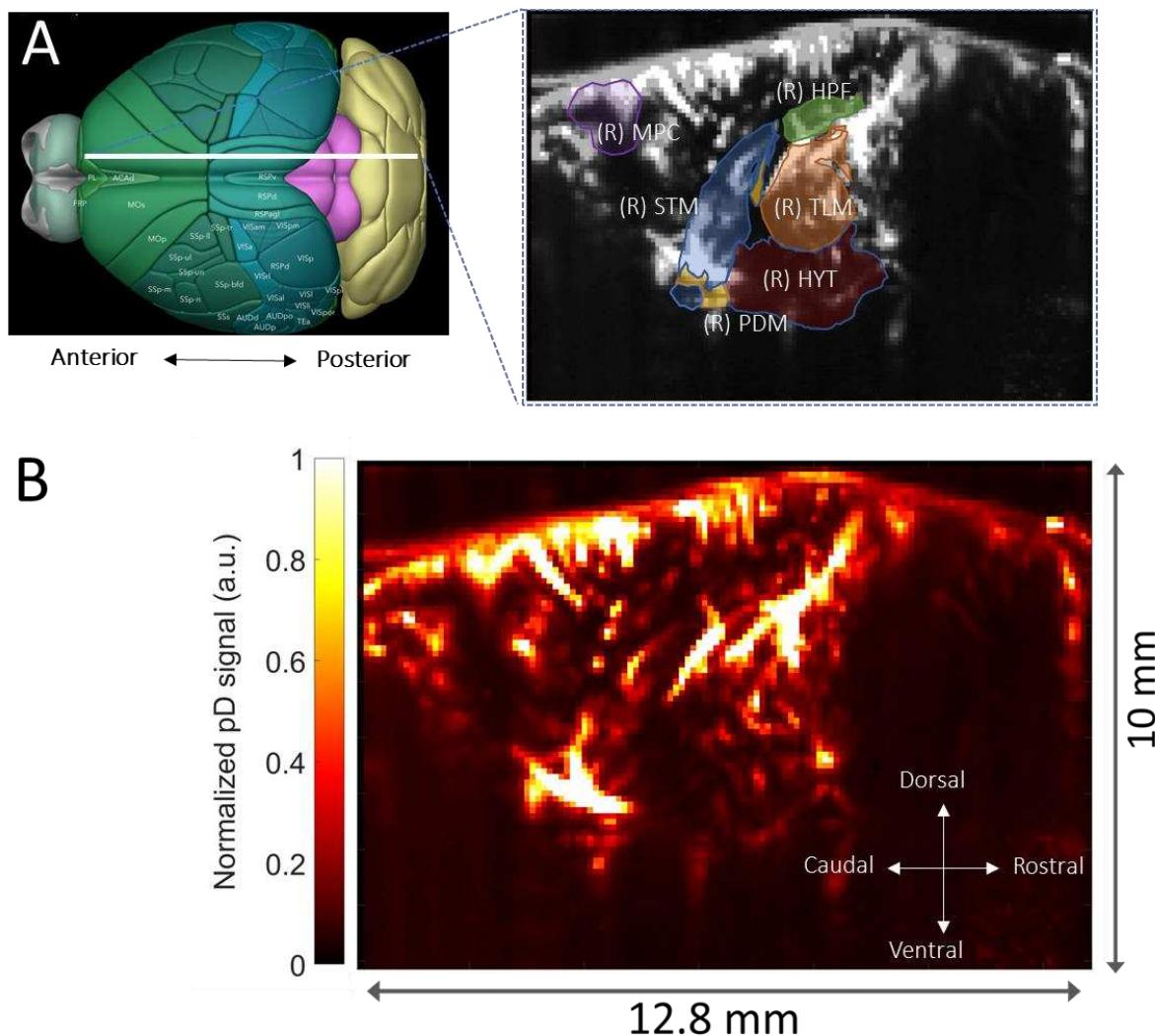


Figure 4. Functional ultrasound imaging (fUSI) of the mouse brain. A) 3D mouse brain model with fUSI probe positioning (white bar) and ROIs – hippocampus (HPF), medial

prefrontal cortex (mPFC), hypothalamus (HYT), thalamus (TLM), pallidum (PDM), and striatum (STM), superimposed onto a mean grayscale fUSI vascular map of the sagittal mouse brain. B) pD image of cerebral blood volume (CBV) in a sagittal plane (max-min normalized relative scale).

229

230 **Results**

231 NMDA antagonist MK-801 reduced blood perfusion

232 We analyzed 40 minutes of pD signal from the septohippocampal circuit (hippocampus, 233 mPFC) as well as surrounding structures (hypothalamus, thalamus, pallidum, and striatum) to 234 assess the effects of MK-801 on cerebral hemodynamics (Fig. 4A). We quantified changes in 235 CBV (Δ CBV) as a percent change relative to baseline activity (average of 2 minutes pD signal 236 acquired prior to drug injection). A repeated measures ANOVA (factors treatment \times ROI; where 237 treatment is saline vs. MK-801 and ROI is the 6 recorded brain areas) revealed that there was a 238 statistically significant effect over time ($F(2519, 56406) = 8.76, p = 6.3 \text{ e-5}$) after Greenhouse- 239 Geiser approximation correction. To quantify the effect of MK-801 on CBV, we computed the 240 percentage change (i.e., Δ CBV) relative to baseline using the last two minutes before stimulation 241 onset (38-40 minutes after drug injection) and found a reduction of Δ CBV (mean \pm SEM) in the 242 hippocampus ($-3.6 \pm 1.1\%$), mPFC ($-4.1 \pm 0.61\%$), hypothalamus ($-1.0 \pm 0.2\%$), pallidum (-1.5 243 $\pm 0.4\%$), striatum ($-1.7 \pm 0.3\%$), and thalamus ($-3.0 \pm 0.5\%$). For saline-treated animals these 244 values were: hippocampus ($-0.7 \pm 0.6\%$), mPFC ($-0.1 \pm 0.2\%$), hypothalamus ($-0.7 \pm 0.6\%$), 245 pallidum ($0.9 \pm 0.4\%$), striatum ($-0.3 \pm 0.4\%$), and thalamus ($0.2 \pm 0.4\%$) (Fig. 5A-F, radar 246 chart insert). The mean effect-size difference in Δ CBVs and Cohen's d analysis comparing mean

247 Δ CBV induced by saline and MK-801 over a 2-minute interval (38 – 40 minutes post-drug
248 injection), revealed that MK-801 induces greater decreases in CBV than saline control in all
249 ROIs investigated (Fig. 5A-F, radar chart insert) - i.e., mPFC (Δ CBV mean difference between
250 saline and MK-801 animals \pm confidence, Cohen's d; $3.96 \pm 0.38\%$, d= 0.42), thalamus ($3.17 \pm$
251 0.23% , d= 0.55), hippocampus ($2.82 \pm 0.42\%$, d= 0.27), pallidum ($2.42 \pm 0.20\%$, d= 0.49),
252 hypothalamus ($1.72 \pm 0.12\%$, d= 0.59) and striatum ($1.33 \pm 0.18\%$, d= 0.29). Together, these
253 results indicate that MK-801 reduces CBV both within and outside of the septohippocampal
254 network.

255

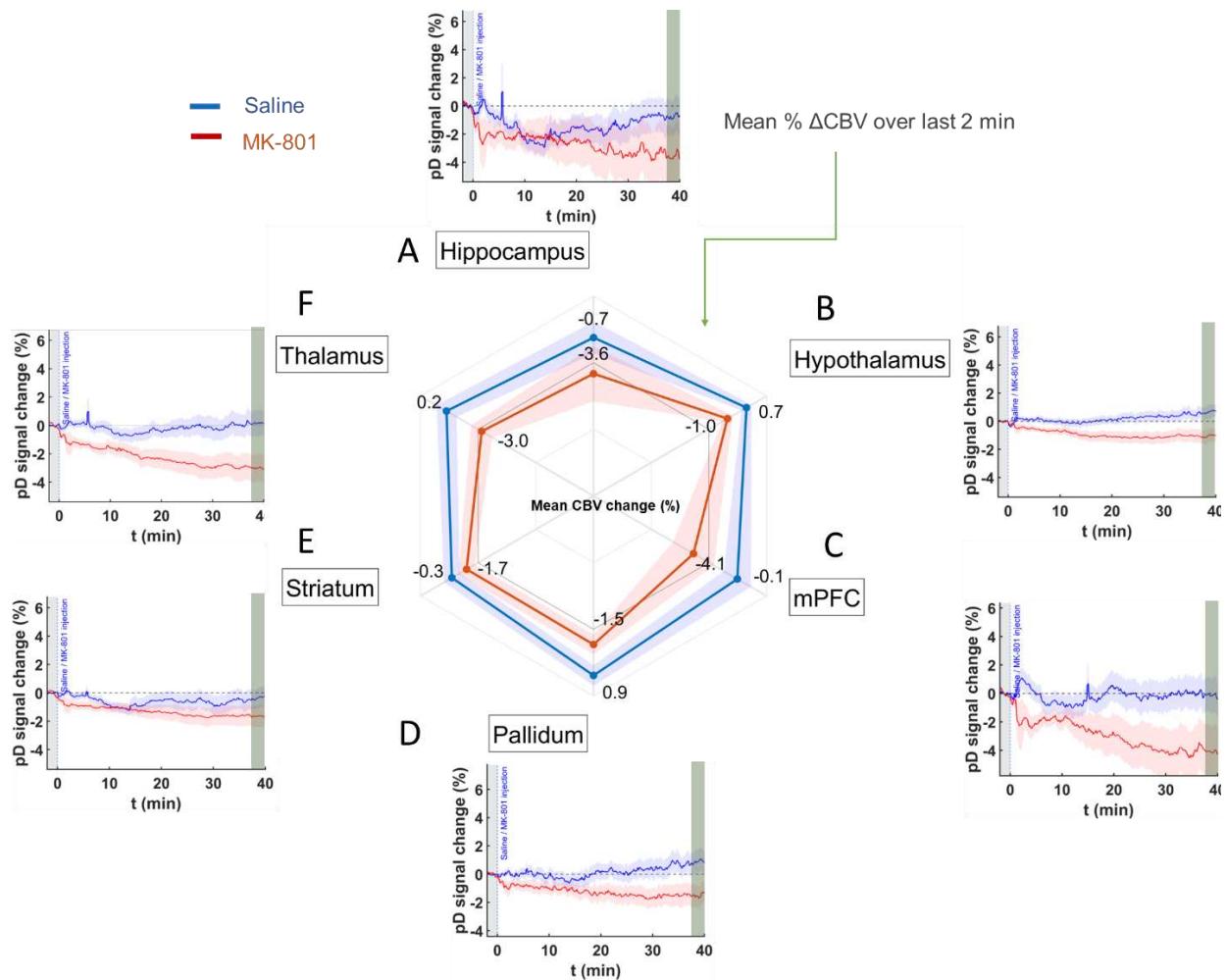


Figure 5. Event related average (ERA) temporal course curves prior to stimulation onset and mean Δ CBVs during the last 2 minutes in saline and MK-801 treated animals. A – F)

Temporal course (42 minutes) of Δ CBV relative to baseline (2-minute average pD signal before saline or MK-801 drug injection) in the **A**) hippocampus, **B**) hypothalamus, **C**) mPFC, **D**) pallidum, **E**) striatum, and **F**) thalamus after saline [blue] and 1.0 mg/kg MK-801 [1] injection. The radar chart insert shows MK-801-induced decreases in CBV in all ROIs compared to saline over the last 2 minutes interval from 38-40 minutes post injection.

256 MSN stimulation increases CBV in saline control animals in a frequency- and region-dependent
257 manner.

258 We assessed whether theta- and gamma-frequency MSN stimulation has disparate
259 impacts on CBV measures in saline-treated control mice. ERAs of Δ CBV reflect the temporal
260 responses to theta, gamma, and no stimulation during and post stimulation time periods (Fig. 6,
261 7). A three-way repeated measures ANOVA (factors, treatment \times ROI \times DBS; where treatment
262 is saline vs. MK-801, ROI is the 6 recorded brain areas, and DBS is theta frequency vs. gamma
263 frequency vs no DBS) was utilized to examine the effects and interactions of drug, stimulation,
264 and ROIs during the 5 minutes period after onset of MSN stimulation. The two-minute baseline
265 period pre-stimulation was included in the analysis. We found significant effects of drug over
266 time ($F(419, 186036) = 7.35, p = 1.85 \text{ e-8}$), stimulation over time ($F(823, 186036) = 2.71, p =$
267 7.20 e-4), as well as, interaction of drug and stimulation over time ($F(838, 186036) = 1.96, p =$
268 1.95 e-2) during the 5 minutes stimulation interval, after Greenhouse-Geisser approximation
269 correction. To further quantify the effects of DBS, we computed the mean Δ CBV in the last 2
270 minutes during stimulation across animals in each stimulation category and compared the mean-
271 effect size differences in Δ CBVs between no stimulation and stimulation in each ROI. We found
272 that MSN theta stimulation increased CBV compared to no-stimulation only in the mPFC (mean
273 Δ CBV difference between theta- and no-stimulation \pm confidence, Cohen's d ; $0.82 \pm 0.12 \%$, $d=$
274 0.45) and hippocampus ($0.39 \pm 0.12 \%$, $d= 0.21$). For the rest on ROIs the effect size magnitude
275 was either very small (i.e., Cohen's $d < 0.08$) or theta-frequency stimulation caused further
276 reduction in CBVs compared to no-stimulation. On the other hand, MSN gamma stimulation
277 caused increases in CBV compared to no-stimulation in the mPFC ($0.60 \pm 0.11 \%$, $d= 0.36$),
278 pallidum ($0.38 \pm 0.09 \%$, $d= 0.30$) and striatum ($0.22 \pm 0.06 \%$, $d= 0.26$). For the rest of the ROIs

279 the effect size magnitude was either very small (i.e., Cohen's $d < 0.095$) or gamma-frequency
280 stimulation resulted in further reduction in CBVs compared to no-stimulation. When comparing
281 the Δ CBV induced by the theta and gamma stimulation in mPFC – the only ROI that exhibited
282 moderate effect on both types of stimulations – we found very small effect size on Δ CBV
283 between the two types of stimulations (mean Δ CBV differences between theta- and gamma-
284 stimulation \pm confidence, Cohen's d ; 0.22 ± 0.10 , $d= 0.14$).

285

286 The next step was to assess the effects of DBS after stimulation offset (i.e., post-
287 stimulation). To do so, we conducted a three-way repeated measures ANOVA (factors, treatment
288 \times ROI \times DBS; where treatment is saline vs. MK-801, ROI is the 6 recorded brain areas, and DBS
289 is theta frequency vs. gamma frequency vs no DBS) over the 10 minutes period after the offset of
290 MSN stimulation. We found significant effects of drug over time ($F(1019, 452436) = 5.28$, $p =$
291 1.27 e-4), stimulation over time ($F(2038, 452436) = 3.67$, $p = 1.17 \text{ e-4}$), as well as, interaction of
292 drug and stimulation over time ($F(2038, 186036) = 3.09$, $p = 9.40 \text{ e-4}$), after Greenhouse-Geisser
293 approximation correction, across the 10 minutes post-stimulation interval. We further quantified
294 the post-effects of DBS by computing the mean Δ CBV in the last 2 minutes of the acquisition –
295 i.e., 8-10 minutes post-stimulation and comparing the mean-effect size differences on Δ CBV
296 between no stimulation and stimulation in each ROI. The results showed that MSN theta
297 stimulation causes increases in CBV compared to no-stimulation in the hippocampus (mean
298 Δ CBV differences between theta- and no-stimulation \pm confidence, Cohen's d ; 1.30 ± 0.21 , $d=$
299 0.42), mPFC ($1.20 \pm 0.22 \%$, $d= 0.37$) and thalamus ($0.97 \pm 0.11 \%$, $d= 0.58$) (Fig. 7A, F – radar
300 chart). On the other hand, MSN gamma stimulation resulted in an increase in CBV compared to
301 no stimulation in the mPFC ($2.01 \pm 0.22 \%$, $d= 0.60$), striatum ($0.77 \pm 0.14 \%$, $d= 0.37$) and

302 pallidum ($0.61 \pm 0.16 \%$, $d= 0.25$). When comparing the differences in Δ CBVs induced by theta
303 and gamma stimulation in the mPFC – the only ROI that exhibited medium to moderate effect on
304 both types of stimulations – we found that gamma induces higher Δ CBV than theta stimulation
305 with medium effect size difference (mean Δ CBV differences between gamma- and theta-
306 stimulation \pm confidence, Cohen's d ; 0.81 ± 0.17 , $d= 0.31$).

307

308

309 Theta-frequency stimulation elicits stronger CBV increases than gamma-frequency stimulation
310 in MK-801 treated animals.

311 Recently, our group showed that theta, but not gamma frequency DBS of the MSN
312 improves spatial memory in MK-801 treated rats [27]. Therefore, we sought to determine if
313 MSN theta and gamma frequency stimulation had differing impacts on neurovascular activity
314 measures within memory-associated regions including the mPFC and hippocampus as well as
315 neighboring regions outside the septohippocampal network (striatum, pallidum, thalamus,
316 hypothalamus) following MK-801 drug-administration. Again, we assessed the mean effect-size
317 differences in Δ CBV between MSN theta-, gamma-, and no-stimulation in each of the selected
318 ROIs, relative to 2 minutes of pD signal recordings just prior to stimulation onset. The analysis
319 was performed after repeated measures ANOVA over the stimulation and post-stimulation time
320 intervals to examine the effects and interactions of drug, stimulation, and ROI (results presented
321 in previous section). Fig. 8 and 9 display the ERA curves for ROIs in response to theta, gamma,
322 and no DBS during and post stimulation periods in the MK-801 group. We found that MSN
323 theta stimulation in the MK-801 treated group caused increased Δ CBV with respect to no-
324 stimulation group with medium to large effect size in all ROIs except mPFC – i.e., hippocampus

325 (mean Δ CBV differences between theta- and no-stimulation \pm confidence, Cohen's d; $2.01 \pm$
326 0.20% , d= 0.78), thalamus ($0.49 \pm 0.07\%$, d= 0.54), pallidum ($0.36 \pm 0.07\%$, d= 0.43), striatum
327 ($0.18 \pm 0.04\%$, d= 0.31) and hypothalamus ($0.14 \pm 0.04\%$, d= 0.30) (Fig. 8). Intriguingly, we
328 only found a medium effect-size increase in Δ CBV for the pallidum ($0.60 \pm 0.08\%$, d= 0.53) and
329 striatum ($0.23 \pm 0.05\%$, d= 0.38) between MSN gamma- stimulation and no-stimulation groups,
330 during the stimulation period. For the rest of the ROIs, the effect-size was very small.

331 Importantly, we found that Δ CBV increased in theta- relative to no-stimulation animals
332 after stimulation offset with medium to larger effects in the pallidum ($1.26 \pm 0.15\%$, d=0.66),
333 hippocampus ($2.8 \pm 0.37\%$, d=0.60) and thalamus ($1.24 \pm 0.17\%$, d= 0.57) and small to
334 medium effects in the striatum ($0.50 \pm 0.13\%$, d= 0.31), hypothalamus ($0.21 \pm 0.06\%$, d= 0.28)
335 and mPFC ($0.64 \pm 0.22\%$, d= 0.22) (Fig. 9). Importantly, we found only small to medium effects
336 of gamma MSN stimulation on Δ CBV in the pallidum, relative to stimulation animals, and after
337 stimulation offset (mean Δ CBV differences between gamma- and no-stimulation \pm confidence,
338 Cohen's d; $0.66 \pm 0.13\%$, d=0.39) (Fig. 9). For the rest of the ROIs, the effect-size was either
339 very small (i.e., Cohen's d < 0.1 in hippocampus, striatum and thalamus) or gamma-frequency
340 stimulation resulted in further CBV reduction (i.e., in hypothalamus and mPFC) compared to no-
341 stimulation.

342 Additionally, our results showed medium or large mean effect-size differences in
343 Δ CBVs between the theta and gamma stimulated MK801 groups after stimulation offset in the
344 hippocampus ($2.67 \pm 0.28\%$, d= 0.70), mPFC ($1.83 \pm 0.23\%$, d= 0.59), and thalamus ($1.15 \pm$
345 0.14% , d= 0.56) (Fig. 9). Together these results demonstrate that theta-frequency stimulation
346 elicits the strongest CBV response in MK-801-treated mice in the hippocampus and pallidum,

347 while gamma stimulation had almost no effect on hippocampal CBV (Cohen's $d = 0.03$) and
348 decreased CBV in the mPFC compared to no-stimulation mice.

349

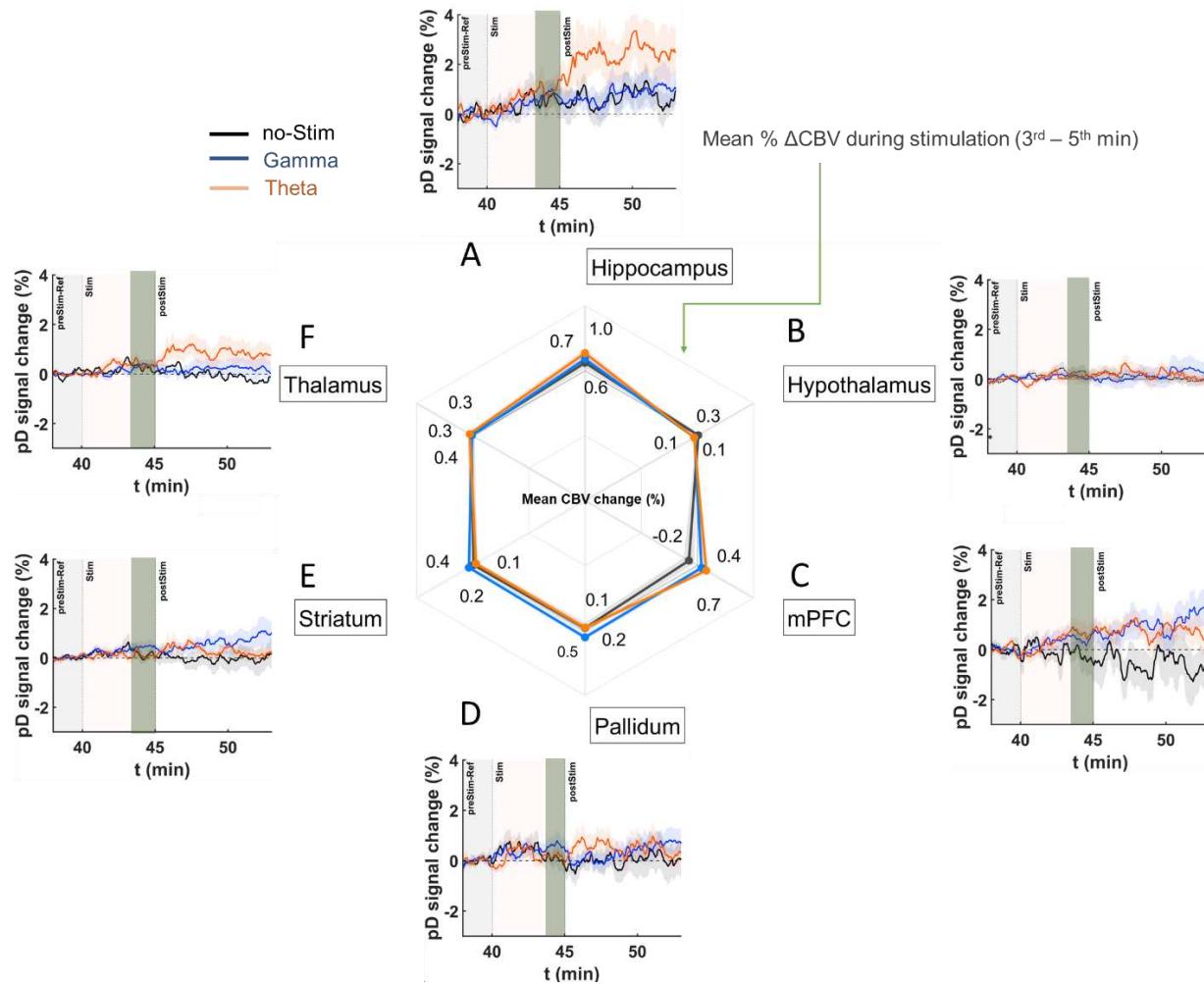


Figure 6. DBS ERA curves after stimulation onset and mean Δ CBVs in saline mice during the last 2 minutes of stimulation period. A–F) Temporal course (theta [orange], gamma [blue], no-stimulation [black]) of mean Δ CBV relative to baseline (2 minutes average pD signal prior to DBS) for the A) hippocampus, B) hypothalamus, C) mPFC, D) pallidum, E) striatum, and F) thalamus regions in the saline-treated animals. Radar chart insert gives the

mean percentage Δ CBVs during stimulation for theta [orange], gamma [blue], and no-stimulation [dark gray] animals in the ROIs investigated. Means were calculated utilizing the last 2 minutes of pD signals acquired during stimulation (3rd – 5th minute after stimulation onset) across animals in each stimulation category.

350

351

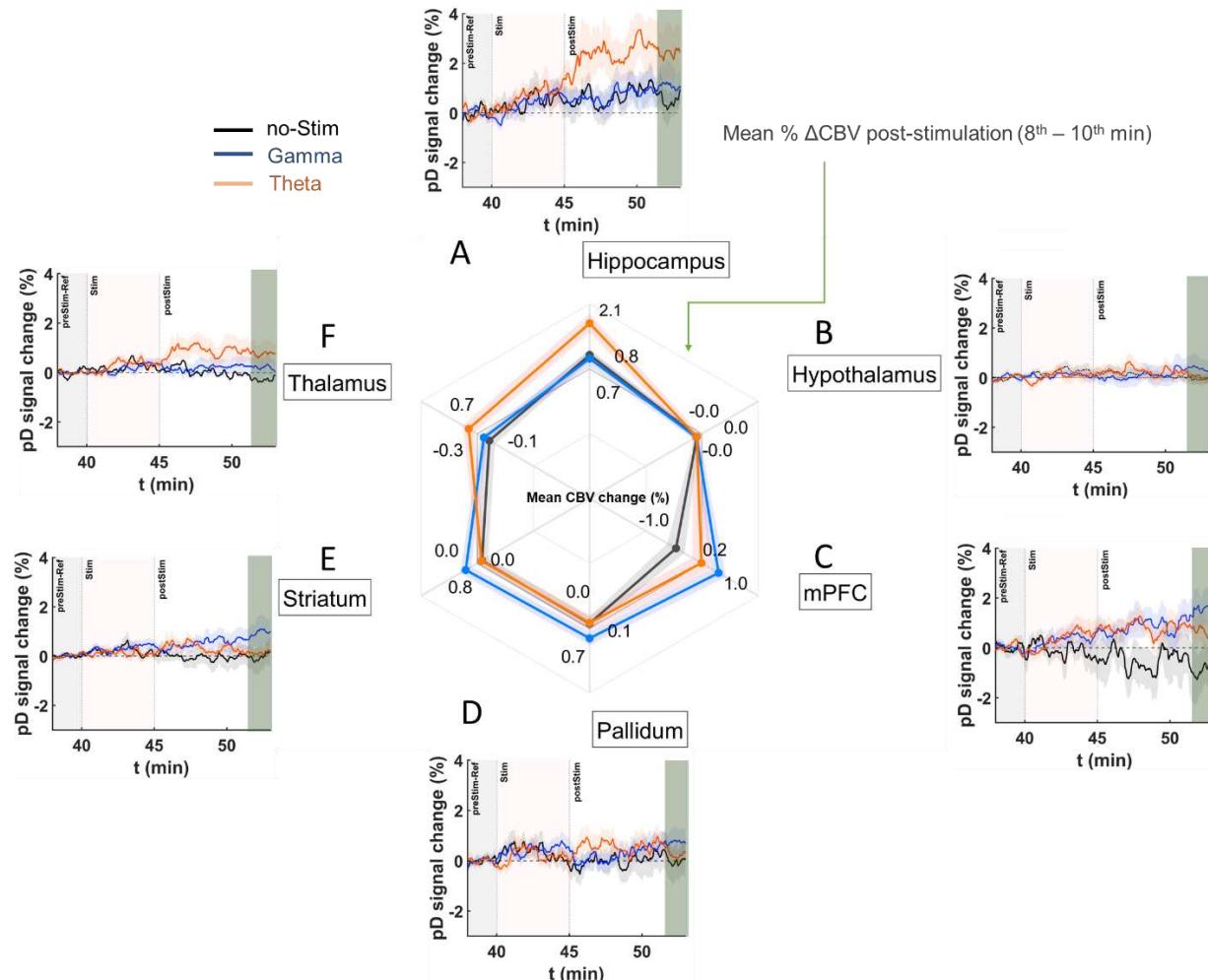


Figure 7. DBS ERA curves after stimulation onset and mean Δ CBVs in saline mice during the last 2 minutes of recordings in the post-stimulation period in 6 ROIs. Similar to Figure 6, but the radar chart gives the mean percentage Δ CBVs during post-stimulation for theta

[orange], gamma [blue], and no-stimulation [dark gray] for the **A**) hippocampus, **B**) hypothalamus, **C**) mPFC, **D**) pallidum, **E**) striatum, and **F**) thalamus regions in the saline-treated animals. Means were calculated utilizing the last 2 minutes of pD signals acquired post stimulation (8th – 10th minute after stimulation offset) across animals in each stimulation category.

352

353

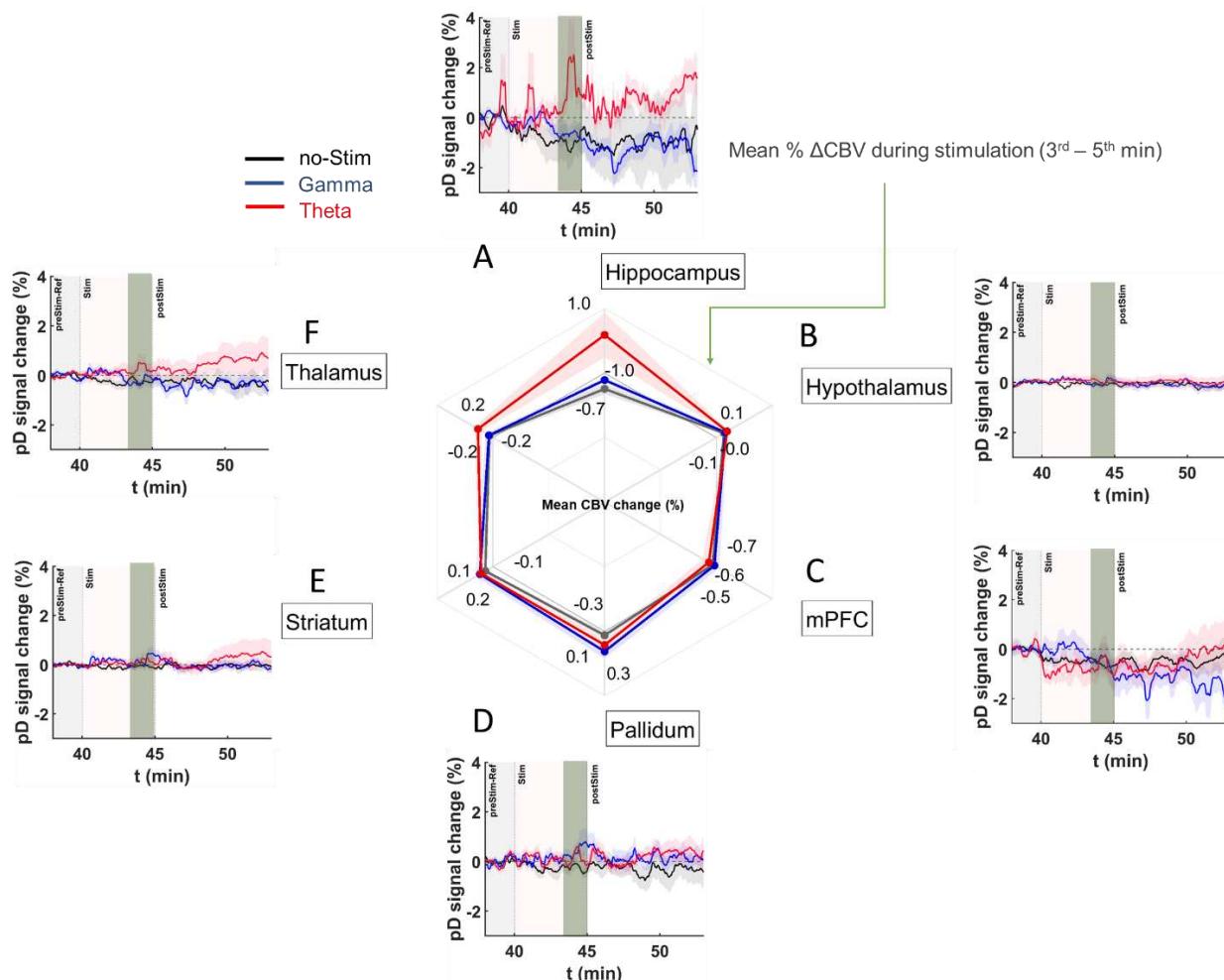


Figure 8. DBS ERA curves after stimulation onset and mean Δ CBVs in MK-801 treated mice during the last 2 minutes of stimulation period A – F) Temporal course (theta [1],

*gamma [blue], no-stimulation [black]) of mean Δ CBV relative to baseline (2 minutes average pD signal prior to DBS) for **A**) hippocampus, **B**) hypothalamus, **C**) mPFC, **D**) pallidum, **E**) striatum, and **F**) thalamus regions in the MK-801 drug injected mice. Radar chart insert gives the mean percentage Δ CBVs during stimulation for theta [1], gamma [blue], and no-stimulation [dark gray] animals in the ROIs investigated. Means were calculated utilizing the last 2 minutes of pD signals acquired during stimulation (3rd – 5th minute after stimulation onset) across animals in each stimulation category.*

354

355

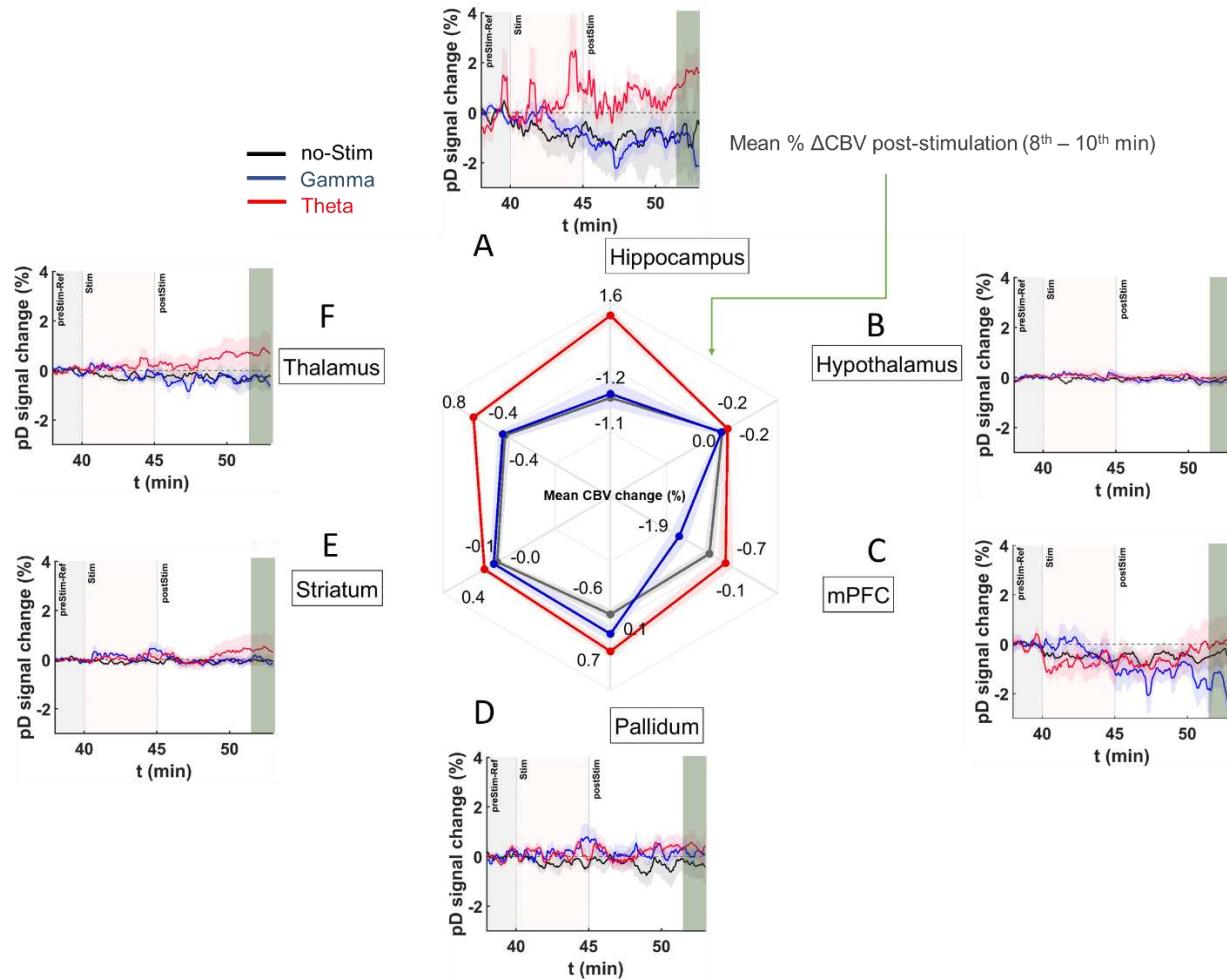


Figure 9. DBS ERA curves after stimulation onset and mean Δ CBVs in MK-801 treated mice during the last 2 minutes of recordings in the post-stimulation period. Similar to Figure 8, but the radar chart gives the mean percentage Δ CBVs in post-stimulation period for theta [1], gamma [blue], and no-stimulation [dark gray] for the **A) hippocampus, **B**) hypothalamus, **C**) mPFC, **D**) pallidum, **E**) striatum, and **F**) thalamus regions in the MK-801 treated animals. Means were calculated utilizing the last 2 minutes of pD signals acquired post stimulation (8th – 10th minute after stimulation offset) across animals in each stimulation category.**

357

358 **Discussion**

359 The present study utilized the high spatiotemporal resolution and sensitivity of fUSI to
360 demonstrate that acute administration of MK-801 causes a significant reduction in CBV across
361 all ROIs. Furthermore, we demonstrated that theta frequency MSN DBS alters regions within the
362 septohippocampal network, with the strongest effect on the hippocampus. Intriguingly, the
363 observed increase in hippocampal CBV remain even after cessation of DBS. On the other hand,
364 structures outside the septohippocampal network, such as the hypothalamus and striatum, show
365 less of a response to DBS. These effects were less pronounced with gamma frequency
366 stimulation with very small effects on the hippocampus. These findings suggest that MSN theta
367 frequency DBS precisely modulates neurovascular activity in cognitive networks [27].

368

369 MK-801 reduced CBV in all ROIs

370 MK-801 and other NMDA antagonists have been widely used in preclinical models to
371 mimic the behavioral and electrophysiological deficits associated with schizophrenia
372 [16,18,38,39]. However, the regionally specific effects of MK-801 on CBV in such models is
373 not well known. We observed that MK-801 reduced CBV across all ROIs. Importantly,
374 previous fMRI studies have observed reduced BOLD signals in hippocampal and prefrontal areas
375 in schizophrenia patients [40–42]. In this context, our findings support the use of MK-801 as a
376 neurovascular model of schizophrenia. Furthermore, our study demonstrates the feasibility of
377 using fUSI to identify network-specific hemodynamic changes as an additional modality for
378 studying neurocognitive disorders.

379

380 MSN theta stimulation was relatively specific to cognitive networks.

381 We observed that theta frequency MSN DBS resulted in an increase to CBV in the
382 hippocampus in both the saline- and MK-801-treated animals. Importantly, this effect was
383 greatest in the hippocampus, which receives direct projections from the MSN, and is a primary
384 target for neuromodulatory interventions to treat memory dysfunction [23–25,27]. In saline
385 control animals, significant increases to CBV were also observed in the mPFC (during and after
386 stimulation) and thalamus (after stimulation), both of which play important roles in memory
387 function and receive direct projections from the MSN (Fig. 2A) [22]. Interestingly, gamma
388 stimulation did not alter hippocampal CBV, but did increase regions of the brain that were
389 anatomically closer to the MSN. Specifically, mPFC CBV was increased in saline- treated
390 animals, while the pallidum and striatum were increased to a lesser degree in MK-801-treated
391 animals. This suggests that MSN gamma stimulation may have a local response to stimulation
392 but is less specific to the neural circuitry being stimulated.

393

394 MSN theta stimulation increased hippocampal CBV during and after stimulation despite NMDA
395 antagonism.

396 A leading hypothesis is that reduced N-methyl-D-aspartate (NMDA) receptor-mediated
397 glutamatergic transmission underlies psychiatric conditions involving cognitive and memory
398 dysfunction [16,18,38,39,43,44]. Reductions to NMDA activity either pharmacologically or
399 through genetic manipulation have been shown to decrease theta activity, increase gamma
400 activity and lead to deficits in spatial navigation and memory [16,17,27,45,46]. Research by our
401 group has found that acute (<5 minutes) theta frequency (7.7 Hz), but not gamma frequency (100
402 Hz) stimulation of the MSN during the Barnes maze task improves spatial memory in rodents

403 following pharmacological NMDA antagonism [27]. Further, we have also demonstrated that
404 MSN theta stimulation prior to the task can also improve spatial memory [23]. Interestingly,
405 hippocampal theta oscillations return to baseline after cessation of MSN DBS. Therefore, the
406 question remains open as to how MSN DBS mediates sustained improvements. In this current
407 study, we demonstrate that hippocampal CBV remains elevated after cessation of MSN
408 stimulation.

409

410 MSN theta stimulation may drive high frequency or spiking activity via hippocampal
411 interneurons.

412 A number of studies utilizing various modalities in combination with fUSI have found a
413 strong relationship between pD signal and neuronal activity [47–49]. This relationship was also
414 true for high frequency oscillatory activity (~100Hz) but was much weaker for lower frequency
415 oscillations [49]. This suggests that the theta-induced increases to hippocampal CBV may reflect
416 increased hippocampal gamma or spiking activity, rather than increases to theta oscillatory
417 activity itself. However, given that gamma band activity is often correlated with spiking activity,
418 differentiating between changes in oscillatory dynamics and spiking activity is not possible in the
419 present study [50].

420 GABAergic interneurons in the hippocampus play an important role in synchronizing
421 hippocampal oscillatory activity. Indeed, inhibitory neurons are hypothesized to be a primary
422 source of dysfunction in pathologies involving NMDA dysfunction (for review see [51]) and are
423 inhibited by NMDA-antagonists [52,53]. One possibility is that theta frequency MSN
424 stimulation may, by briefly stimulating afferent populations at theta-frequency, act as a ‘reset’
425 and allow synchronous innervation of hippocampal interneurons that regulate the activity of

426 glutamatergic pyramidal cells. Indeed, it has been previously shown that optogenetic stimulation
427 of GABAergic neurons decreases spontaneous neural activity and leads to an increase in local
428 blood flow [54]. This hypothesis is further supported by a recent study by Nunez-Elizadle and
429 colleagues who observed that the relationship between the fUSI signal and firing rates were
430 greatest for putative interneurons [49]. Future studies combining single unit activity and fUSI
431 could test this hypothesis.

432

433 MSN gamma stimulation did not affect hippocampal CBV.

434 Our previous work suggests that MSN theta, but not gamma stimulation can improve
435 spatial memory in MK-801-treated rodents [27]. While MSN theta stimulation increased
436 hippocampal CBV during and after stimulation in MK-801-treated animals, this was not true of
437 MSN gamma stimulation. Gamma stimulation resulted in delayed increases to mPFC CBV in
438 saline-treated animals and had no effect on MK-801-treated animals in any of the ROIs. These
439 results suggest that MSN gamma stimulation is not sufficient to engage hippocampal activity and
440 highlights the importance of frequency parameters in DBS paradigms for spatial memory. This is
441 further supported by our previous study demonstrating no improvement to spatial memory in
442 MK-801 treated animals with MSN gamma stimulation [27].

443

444 Implications for neuromodulation

445 We observed that theta-frequency stimulation of the MSN increased blood perfusion in
446 the hippocampus following cessation of the stimulus. These effects were not observed using
447 gamma stimulation and were still present even under conditions of pharmacologic NMDA
448 antagonism. It is worth noting that arguably the most effective form of neuromodulation is still

449 electroconvulsive therapy (ECT), which is performed under anesthesia and also results in
450 increases to cerebral blood flow [55,56]. However, ECT is very non-specific, this lack of
451 specificity may well contribute to its detrimental effect on memory [57]. Alternatively,
452 transcranial magnetic stimulation (TMS) is most effective when applied focally to awake, alert
453 patients [58,59]. Because DBS can combine a relatively high degree of modulation in deep
454 structures with greater spatial and temporal specificity than ECT or TMS, it is plausible that DBS
455 may have more benefits beyond that of ECT or TMS in treating disorders of cognitive function.

456

457 Limitations and future directions

458 While the current study was performed in anesthetized animals, future studies will
459 investigate the effects of reduced NMDA function and MSN DBS in awake, behaving animals
460 during memory-associated behavioral tasks (e.g., novel object recognition and Barnes Maze).
461 The goal will be to determine if the observed Δ CBV within the septo-hippocampal network
462 following theta- frequency MSN DBS is also associated with improved memory function,
463 linking the present study with our previous study demonstrating improved memory following
464 MSN DBS in MK-801 treated animals [27]. Another limitation of our study is that fUSI
465 recordings performed using the conventional 1-dimensional linear ultrasound transducer array
466 necessarily generates 2-dimensional pD vascular maps of the animals' CBV. As a result, other
467 regions that are connected with the MSN besides the hippocampus and mPFC (e.g., amygdala,
468 habenula, raphe nucleus) were not accessible from the selected sagittal 2-dimensional image
469 plane. Recent studies are tackling this challenge using whole-brain 3-dimensional fUSI with
470 either moving linear arrays (similar to the array used in our study), matrix arrays or raw column
471 arrays (RCAs) [60–62]. Future studies can use these probes to cover volumes rather slices of the

472 mouse brain providing access to all areas of the septohippocampal network. Overall and
473 regardless of these limitations, our findings demonstrate the feasibility of using fUSI to
474 characterize network-specific neurovascular changes in disease models as well understand what
475 parameters in neuromodulatory techniques most impact cerebral perfusion dynamics.

476

477

478

479

480

481

482

483 **References:**

484 [1] Ranasinghe KG, Petersen C, Kudo K, Mizuiri D, Rankin KP, Rabinovici GD, et al.
485 Reduced synchrony in alpha oscillations during life predicts post mortem neurofibrillary
486 tangle density in early-onset and atypical Alzheimer's disease. *Alzheimer's & Dementia*
487 2021;17:2009–19. <https://doi.org/10.1002/alz.12349>.

488 [2] Tu M-C, Chung H-W, Hsu Y-H, Yang J-J, Wu W-C. Stage-Dependent Cerebral Blood
489 Flow and Leukoaraiosis Couplings in Subcortical Ischemic Vascular Disease and
490 Alzheimer's Disease. *J Alzheimers Dis* 2022;86:729–39. <https://doi.org/10.3233/JAD-215405>.

491 [3] Dougherty DD, Chou T, Corse AK, Arulpragasam AR, Widge AS, Cusin C, et al. Acute
492 deep brain stimulation changes in regional cerebral blood flow in obsessive-compulsive
493 disorder. *J Neurosurg* 2016;125:1087–93. <https://doi.org/10.3171/2015.9.JNS151387>.

494 [4] Laxton AW, Tang-Wai DF, McAndrews MP, Zumsteg D, Wennberg R, Keren R, et al. A
495 phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. *Ann
496 Neurol* 2010;68:521–34. <https://doi.org/10.1002/ana.22089>.

497 [5] Macé E, Montaldo G, Cohen I, Baulac M, Fink M, Tanter M. Functional ultrasound
498 imaging of the brain. *Nat Methods* 2011;8:662–4. <https://doi.org/10.1038/nmeth.1641>.

499 [6] Mace E, Montaldo G, Osmanski B-F, Cohen I, Fink M, Tanter M. Functional ultrasound
500 imaging of the brain: theory and basic principles. *IEEE Transactions on Ultrasonics,
501 Ferroelectrics, and Frequency Control* 2013;60:492–506.
502 <https://doi.org/10.1109/TUFFC.2013.2592>.

503 [7] Rabut C, Ferrier J, Bertolo A, Osmanski B, Mousset X, Pezet S, et al. Pharmaco-fUS:
504 Quantification of pharmacologically-induced dynamic changes in brain perfusion and
505 connectivity by functional ultrasound imaging in awake mice. *NeuroImage*
506 2020;222:117231. <https://doi.org/10.1016/j.neuroimage.2020.117231>.

507 [8] Norman SL, Maresca D, Christopoulos VN, Griggs WS, Demene C, Tanter M, et al.
508 Single-trial decoding of movement intentions using functional ultrasound neuroimaging.
509 *Neuron* 2021;109:1554–1566.e4. <https://doi.org/10.1016/j.neuron.2021.03.003>.

510 [9] Uhlhaas PJ, Singer W. Abnormal neural oscillations and synchrony in schizophrenia.
511 *Nature Reviews Neuroscience* 2010;11:100–13. <https://doi.org/10.1038/nrn2774>.

512 [10] Barr MS, Rajji TK, Zomorodi R, Radhu N, George TP, Blumberger DM, et al. Impaired
513 theta-gamma coupling during working memory performance in schizophrenia.
514 *Schizophrenia Research* 2017;189:104–10. <https://doi.org/10.1016/j.schres.2017.01.044>.

515 [11] Jafari Z, Kolb BE, Mohajerani MH. Neural oscillations and brain stimulation in
516 Alzheimer's disease. *Prog Neurobiol* 2020;194:101878.
517 <https://doi.org/10.1016/j.pneurobio.2020.101878>.

518 [12] Gonzalez-Burgos G, Lewis DA. NMDA Receptor Hypofunction, Parvalbumin-Positive
519 Neurons, and Cortical Gamma Oscillations in Schizophrenia. *Schizophrenia Bulletin*
520 2012;38:950–7. <https://doi.org/10.1093/schbul/sbs010>.

521 [13] Gu Z, Alexander GM, Dudek SM, Yakel JL. Hippocampus and Entorhinal Cortex Recruit
522 Cholinergic and NMDA Receptors Separately to Generate Hippocampal Theta Oscillations.
523 *Cell Reports* 2017;21:3585–95. <https://doi.org/10.1016/j.celrep.2017.11.080>.

524 [14] Collingridge G. The role of NMDA receptors in learning and memory. *Nature*
525 1987;330:604–5. <https://doi.org/10.1038/330604a0>.

527 [15] Spangler EL, Bresnahan EL, Garofalo P, Muth NJ, Heller B, Ingram DK. NMDA receptor
528 channel antagonism by dizocilpine (MK-801) impairs performance of rats in aversively
529 motivated complex maze tasks. *Pharmacol Biochem Behav* 1991;40:949–58.
530 [https://doi.org/10.1016/0091-3057\(91\)90111-e](https://doi.org/10.1016/0091-3057(91)90111-e).

531 [16] Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, et al.
532 Ketamine-Induced NMDA Receptor Hypofunction as a Model of Memory Impairment and
533 Psychosis. *Neuropsychopharmacology* 1999;20:106–18. [https://doi.org/10.1016/S0893-133X\(98\)00067-0](https://doi.org/10.1016/S0893-133X(98)00067-0).

535 [17] Korotkova T, Fuchs EC, Ponomarenko A, von Engelhardt J, Monyer H. NMDA receptor
536 ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial
537 representations, and working memory. *Neuron* 2010;68:557–69.
538 <https://doi.org/10.1016/j.neuron.2010.09.017>.

539 [18] Saunders JA, Gandal MJ, Siegel SJ. NMDA antagonists recreate signal-to-noise ratio and
540 timing perturbations present in schizophrenia. *Neurobiology of Disease* 2012;46:93–100.
541 <https://doi.org/10.1016/J.NBD.2011.12.049>.

542 [19] Billingslea EN, Tatard-Leitman VM, Anguiano J, Jutzeler CR, Suh J, Saunders JA, et al.
543 Parvalbumin Cell Ablation of NMDA-R1 Causes Increased Resting Network Excitability
544 with Associated Social and Self-Care Deficits. *Neuropsychopharmacol* 2014;39:1603–13.
545 <https://doi.org/10.1038/npp.2014.7>.

546 [20] Fuhrmann F, Justus D, Sosulina L, Kaneko H, Beutel T, Friedrichs D, et al. Locomotion,
547 Theta Oscillations, and the Speed-Correlated Firing of Hippocampal Neurons Are
548 Controlled by a Medial Septal Glutamatergic Circuit. *Neuron* 2015;86:1253–64.
549 <https://doi.org/10.1016/j.neuron.2015.05.001>.

550 [21] Leão RN, Targino ZH, Colom LV, Fisahn A. Interconnection and synchronization of
551 neuronal populations in the mouse medial septum/diagonal band of Broca. *Journal of
552 Neurophysiology* 2015;113:971–80. <https://doi.org/10.1152/jn.00367.2014>.

553 [22] Takeuchi Y, Nagy AJ, Barcsai L, Li Q, Ohsawa M, Mizuseki K, et al. The Medial Septum
554 as a Potential Target for Treating Brain Disorders Associated With Oscillopathies. *Frontiers
555 in Neural Circuits* 2021;15.

556 [23] Lee DJ, Gurkoff GG, Izadi A, Berman RF, Ekstrom AD, Muizelaar JP, et al. Medial Septal
557 Nucleus Theta Frequency Deep Brain Stimulation Improves Spatial Working Memory after
558 Traumatic Brain Injury. *Journal of Neurotrauma* 2013;30:131–9.
559 <https://doi.org/10.1089/neu.2012.2646>.

560 [24] Lee DJ, Gurkoff GG, Izadi A, Seidl SE, Echeverri A, Melnik M, et al. Septohippocampal
561 Neuromodulation Improves Cognition after Traumatic Brain Injury. *Journal of
562 Neurotrauma* 2015;32:1822–32. <https://doi.org/10.1089/neu.2014.3744>.

563 [25] Lee DJ, Izadi A, Melnik M, Seidl S, Echeverri A, Shahlaie K, et al. Stimulation of the
564 medial septum improves performance in spatial learning following pilocarpine-induced
565 status epilepticus. *Epilepsy Research* 2017;130:53–63.
566 <https://doi.org/10.1016/j.eplepsyres.2017.01.005>.

567 [26] Cole ER, Grogan DP, Laxpati NG, Fernandez AM, Skelton HM, Isbaine F, et al. Evidence
568 supporting deep brain stimulation of the medial septum in the treatment of temporal lobe
569 epilepsy. *Epilepsia* 2022;63:2192–213. <https://doi.org/10.1111/epi.17326>.

570 [27] Zepeda NC, Crown LM, Medvidovic S, Choi W, Sheth M, Bergosh M, et al. Frequency-
571 specific medial septal nucleus deep brain stimulation improves spatial memory in MK-801-

572 treated male rats. *Neurobiology of Disease* 2022;170:105756.
573 <https://doi.org/10.1016/j.nbd.2022.105756>.

574 [28] Grubbs FE. Procedures for Detecting Outlying Observations in Samples. *Technometrics*
575 1969;11:1–21. <https://doi.org/10.1080/00401706.1969.10490657>.

576 [29] Stefansky W. Rejecting Outliers in Factorial Designs. *Technometrics* 1972;14:469–79.
577 <https://doi.org/10.2307/1267436>.

578 [30] Wegener N, Nagel J, Gross R, Chambon C, Greco S, Pietraszek M, et al. Evaluation of
579 brain pharmacokinetics of (+)MK-801 in relation to behaviour. *Neurosci Lett* 2011;503:68–
580 72. <https://doi.org/10.1016/j.neulet.2011.08.012>.

581 [31] Wang Q, Ding S-L, Li Y, Royall J, Feng D, Lesnar P, et al. The Allen Mouse Brain
582 Common Coordinate Framework: A 3D Reference Atlas. *Cell* 2020;181:936–953.e20.
583 <https://doi.org/10.1016/j.cell.2020.04.007>.

584 [32] Paknahad J, Kosta P, Iseri E, Farzad S, Bouteiller J-MC, Humayun MS, et al. Modeling ON
585 Cone Bipolar Cells for Electrical Stimulation. *Annu Int Conf IEEE Eng Med Biol Soc*
586 2021;2021:6547–50. <https://doi.org/10.1109/EMBC46164.2021.9629884>.

587 [33] Ledoux LA, Brands PJ, Hoeks AP. Reduction of the clutter component in Doppler
588 ultrasound signals based on singular value decomposition: a simulation study. *Ultrason Imaging*
589 1997;19:1–18. <https://doi.org/10.1177/016173469701900101>.

590 [34] Stringer C, Pachitariu M. Computational processing of neural recordings from calcium
591 imaging data. *Current Opinion in Neurobiology* 2019;55:22–31.
592 <https://doi.org/10.1016/j.conb.2018.11.005>.

593 [35] Friedrich J, Giovannucci A, Pnevmatikakis EA. Online analysis of microendoscopic 1-
594 photon calcium imaging data streams. *PLOS Computational Biology* 2021;17:e1008565.
595 <https://doi.org/10.1371/journal.pcbi.1008565>.

596 [36] Pnevmatikakis EA, Giovannucci A. NoRMCorre: An online algorithm for piecewise rigid
597 motion correction of calcium imaging data. *Journal of Neuroscience Methods* 2017;291:83–
598 94. <https://doi.org/10.1016/j.jneumeth.2017.07.031>.

599 [37] Liu XS. *Statistical Power Analysis for the Social and Behavioral Sciences: Basic and*
600 *Advanced Techniques*. Routledge; 2013.

601 [38] Balu DT. The NMDA Receptor and Schizophrenia: From Pathophysiology to Treatment.
602 *Adv Pharmacol* 2016;76:351–82. <https://doi.org/10.1016/bs.apha.2016.01.006>.

603 [39] Farber NB. The NMDA Receptor Hypofunction Model of Psychosis. *Annals of the New*
604 *York Academy of Sciences* 2003;1003:119–30. <https://doi.org/10.1196/annals.1300.008>.

605 [40] MacDonald SWS, Nyberg L, Bäckman L. Intra-individual variability in behavior: links to
606 brain structure, neurotransmission and neuronal activity. *Trends in Neurosciences*
607 2006;29:474–80. <https://doi.org/10.1016/J.TINS.2006.06.011>.

608 [41] Gur RE, Gur RC. Functional magnetic resonance imaging in schizophrenia. *Dialogues in*
609 *Clinical Neuroscience* 2010;12:333–43. <https://doi.org/10.31887/DCNS.2010.12.3/rgur>.

610 [42] Bedford NJ, Surguladze S, Giampietro V, Brammer MJ, David AS. Self-evaluation in
611 schizophrenia: an fMRI study with implications for the understanding of insight. *BMC*
612 *Psychiatry* 2012;12:106. <https://doi.org/10.1186/1471-244X-12-106>.

613 [43] Moghaddam B, Javitt D. From Revolution to Evolution: The Glutamate Hypothesis of
614 Schizophrenia and its Implication for Treatment. *Neuropsychopharmacol* 2012;37:4–15.
615 <https://doi.org/10.1038/npp.2011.181>.

616 [44] Lin C-H, Huang Y-J, Lin C-J, Lane H-Y, E. Tsai G. NMDA Neurotransmission
617 Dysfunction in Mild Cognitive Impairment and Alzheimer's Disease. *Current*
618 *Pharmaceutical Design* 2014;20:5169–79.

619 [45] Stark E, Eichler R, Roux L, Fujisawa S, Rotstein HG, Buzsáki G. Inhibition-induced theta
620 resonance in cortical circuits. *Neuron* 2013;80.
621 <https://doi.org/10.1016/j.neuron.2013.09.033>.

622 [46] Ward KR, Featherstone RE, Naschek MJ, Melnychenko O, Banerjee A, Yi J, et al. Src
623 deficient mice demonstrate behavioral and electrophysiological alterations relevant to
624 psychiatric and developmental disease. *Progress in Neuro-Psychopharmacology and*
625 *Biological Psychiatry* 2019;93:84–92. <https://doi.org/10.1016/j.pnpbp.2019.02.017>.

626 [47] Boido D, Rungta RL, Osmanski B-F, Roche M, Tsurugizawa T, Le Bihan D, et al.
627 Mesoscopic and microscopic imaging of sensory responses in the same animal. *Nat*
628 *Commun* 2019;10:1110. <https://doi.org/10.1038/s41467-019-09082-4>.

629 [48] Aydin A-K, Haselden WD, Goulam Houssen Y, Pouzat C, Rungta RL, Demené C, et al.
630 Transfer functions linking neural calcium to single voxel functional ultrasound signal. *Nat*
631 *Commun* 2020;11:2954. <https://doi.org/10.1038/s41467-020-16774-9>.

632 [49] Nunez-Elizalde AO, Krumin M, Reddy CB, Montaldo G, Urban A, Harris KD, et al. Neural
633 correlates of blood flow measured by ultrasound. *Neuron* 2022;110:1631–1640.e4.
634 <https://doi.org/10.1016/j.neuron.2022.02.012>.

635 [50] Buzsáki G, Schomburg EW. What does gamma coherence tell us about inter-regional
636 neural communication? *Nat Neurosci* 2015;18:484–9. <https://doi.org/10.1038/nn.3952>.

637 [51] Marín O. Interneuron dysfunction in psychiatric disorders. *Nat Rev Neurosci* 2012;13:107–
638 20. <https://doi.org/10.1038/nrn3155>.

639 [52] Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on
640 prefrontal cortex interneurons and pyramidal neurons. *J Neurosci* 2007;27:11496–500.
641 <https://doi.org/10.1523/JNEUROSCI.2213-07.2007>.

642 [53] Cohen SM, Tsien RW, Goff DC, Halassa MM. The impact of NMDA Receptor
643 hypofunction on GABAergic interneurons in the pathophysiology of schizophrenia.
644 *Schizophr Res* 2015;167:98–107. <https://doi.org/10.1016/j.schres.2014.12.026>.

645 [54] Anenberg E, Chan AW, Xie Y, LeDue JM, Murphy TH. Optogenetic Stimulation of GABA
646 Neurons can Decrease Local Neuronal Activity While Increasing Cortical Blood Flow. *J*
647 *Cereb Blood Flow Metab* 2015;35:1579–86. <https://doi.org/10.1038/jcbfm.2015.140>.

648 [55] Espinoza RT, Kellner CH. Electroconvulsive Therapy. *New England Journal of Medicine*
649 2022;386:667–72. <https://doi.org/10.1056/NEJMra2034954>.

650 [56] Singh A, Kar SK. How Electroconvulsive Therapy Works?: Understanding the
651 Neurobiological Mechanisms. *Clinical Psychopharmacology and Neuroscience*
652 2017;15:210–21. <https://doi.org/10.9758/cpn.2017.15.3.210>.

653 [57] Andrade C, Arumugham SS, Thirthalli J. Adverse Effects of Electroconvulsive Therapy.
654 *Psychiatric Clinics* 2016;39:513–30. <https://doi.org/10.1016/j.psc.2016.04.004>.

655 [58] Gersner R, Kravetz E, Feil J, Pell G, Zangen A. Long-Term Effects of Repetitive
656 Transcranial Magnetic Stimulation on Markers for Neuroplasticity: Differential Outcomes
657 in Anesthetized and Awake Animals. *J Neurosci* 2011;31:7521–6.
658 <https://doi.org/10.1523/JNEUROSCI.6751-10.2011>.

659 [59] Xu T, Sturgeon D, Ramirez JSB, Froudist-Walsh S, Margulies DS, Schroeder CE, et al.
660 Interindividual Variability of Functional Connectivity in Awake and Anesthetized Rhesus

661 Macaque Monkeys. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*
662 2019;4:543–53. <https://doi.org/10.1016/j.bpsc.2019.02.005>.

663 [60] Bertolo A, Nouhoum M, Cazzanelli S, Ferrier J, Mariani J-C, Kliewer A, et al. Whole-
664 Brain 3D Activation and Functional Connectivity Mapping in Mice using Transcranial
665 Functional Ultrasound Imaging. *JoVE (Journal of Visualized Experiments)* 2021:e62267.
666 <https://doi.org/10.3791/62267>.

667 [61] Rabut C, Correia M, Finel V, Pezet S, Pernot M, Deffieux T, et al. 4D functional ultrasound
668 imaging of whole-brain activity in rodents. *Nat Methods* 2019;16:994–7.
669 <https://doi.org/10.1038/s41592-019-0572-y>.

670 [62] Sauvage J, Porée J, Rabut C, Férin G, Flesch M, Rosinski B, et al. 4D Functional Imaging
671 of the Rat Brain Using a Large Aperture Row-Column Array. *IEEE Transactions on*
672 *Medical Imaging* 2020;39:1884–93. <https://doi.org/10.1109/TMI.2019.2959833>.

673

674