Mesotrode: chronic simultaneous mesoscale cortical imaging and subcortical or peripheral nerve spiking activity recording in mice.

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Abstract

Brain function originates from hierarchical spatial-temporal neural dynamics distributed across cortical and subcortical networks. However, techniques available to assess large-scale brain network activity with single-neuron resolution in behaving animals remain limited. Here we present Mesotrode that integrates chronic wide-field mesoscale cortical imaging and compact multi-site cortical/subcortical cellular electrophysiology in head-fixed mice that undergo self-initiated running or orofacial movements. Specifically, we harnessed the flexibility of chronic multi-site tetrode recordings to monitor single-neuron activity in multiple subcortical structures while simultaneously imaging the mesoscale activity of the entire dorsal cortex. A mesoscale spike-triggered averaging procedure allowed the identification of cortical activity motifs preferentially associated with single-neuron spiking. Using this approach, we were able to characterize chronic single-neuron-related functional connectivity maps for up to 60 days post-implantation. Neurons recorded from distinct subcortical structures display diverse but segregated cortical maps, suggesting that neurons of different origins participate in distinct cortico-subcortical pathways. We extended the capability of Mesotrode by implanting the micro-electrode at the facial motor nerve and found that facial nerve spiking is functionally associated with the PTA, RSP, and M2 network, and optogenetic inhibition of the PTA area significantly reduced the facial movement of the mice. These findings demonstrate that Mesotrode can be used to sample different combinations of cortico-subcortical networks over prolonged periods, generating multimodal and multi-scale network activity from a single implant, offering new insights into the neural mechanisms underlying specific behaviors.

Introduction

System-level mechanisms of cognition and action across networks of single neurons in awake, behaving mice remain largely elusive (Buzsaki, 2006; Aru, Suzuki and Larkum, 2020; Roth and Ding, 2020). The difficulty comes from recording neural activity over large spatial scales but with single neuron resolution during sensory and motor processes (Alivisatos *et al.*, 2012). In the mammalian brain, large-scale cortical network activity is dynamically sculpted by local or long-range inputs from individual neurons in various cortical or subcortical structures (Koch *et al.*, 2016). Decoding the principles of neuronal network activity is essential for understanding brain function (Oh *et al.*, 2014; Jiang *et al.*, 2015). One important aspect of such understanding is mapping the functional connectivity of single neurons in relation to cortical networks. Emerging studies using *in vivo* electrophysiological and imaging techniques have revealed that activities of single neurons are functionally coupled to those of local microcircuits or the global cortical networks, and such connectivity is dynamically regulated depending on the behavioral state of the animal (Barson *et al.*, no date; J. L. Chen *et al.*, 2013; Xiao *et al.*, 2017; Clancy, Orsolic and Mrsic-Flogel, 2019). While most of the current studies only provide a snapshot of the connectivity map of a single neuron, the development of procedures where both single-unit activity and large-scale cortical network dynamics of awake-behaving mice can be chronically recorded is desirable.

Previously, chronic extracellular recordings in rodent brains were achieved most widely using bundled microwires called tetrodes, and more recently extended to the use of high-throughput recording devices such as silicon probes including Neuropixels (Bragin *et al.*, 2000; Nguyen *et al.*, 2009; Vandecasteele *et al.*, 2012; Voigts *et al.*, 2013; Delcasso *et al.*, 2018; Juavinett,

73   Bekheet and Churchland, 2019; Steinmetz *et al.*, 2021). In the case of conventional chronic

74   tetrode recording setups, a common feature is the inclusion of a microdrive that provides axial

75   control over the positioning of the electrodes which allows the electrode position to be adjusted

76   for better recording qualities throughout the experimental periods. However, this microdrive

77   hinders simultaneous wide-field optical imaging of the cortex, making it difficult to investigate

78   the correlation between subcortical single-unit activation and cortical network activity. On the

79   other hand, chronic silicon probe recordings rely on a skull-mounted probe and a large

80   headstage, also have the same limitation of not allowing for simultaneous wide-field imaging.

81

82   To overcome these challenges, we designed the Mesotrode system where we combine *in vivo*

83   electrophysiology via multi-site tetrode implants and mesoscale brain imaging to characterize the

84   functional connectivity of individual cortical or subcortical neurons chronically. We show that

85   these tetrode preparations can obtain high-fidelity, single-unit activity in any cortical or

86   subcortical structure. More importantly, this setup preserves optical access to the entire cranial

87   window of the animal which permits simultaneous functional brain imaging as well as

88   optogenetic manipulation of neuronal activity across the whole dorsal cortex. To obtain high-

89   resolution wide-field imaging of mouse brain activity, we chose to utilize GCaMP6, a genetically

90   encoded Ca^{2+} indicator (GECI) widely used to optically record suprathreshold neuronal

91   activation due to its high sensitivity and optimal signal-to-noise ratio (T.-W. Chen *et al.*, 2013).

92   Furthermore, it has been shown that GECIs can stably report neuronal activity over several

93   months(Huber *et al.*, 2012; Margolis *et al.*, 2012; Silasi *et al.*, 2016), making them ideal for

94   chronic studies of cortical network dynamics. We found that neurons from various subcortical

95   structures, including the hippocampus, thalamus, striatum, and other midbrain areas, display

96   distinct functional connectivity patterns with the cortex. More importantly, we show that the

97   spike-triggered maps (STMs) of recorded neurons can be stable for up to two months. Moreover,

98   we extended Mesotrode to record facial nerve spiking activity and identified a novel cortical

99   network that is causally involved in controlling facial movement, further highlighting the wide

100 applicability of this technique. These results indicate that our Mesotrode can be widely exploited

101 to investigate multiscale functional connectivity within the central nervous system of mice over

102 an extended timescale.

103

104 **Results**

105 **Chronic single-unit recording with wide-field transcranial imaging window.**

106 We developed the Mesotrode implantation procedure with the aim to make them low profile,

107 flexible in location, minimally invasive, and yet stable while maintaining optical access to the

108 entire dorsal cortex such that we can record chronic single neuron activity and mesoscale cortical

109 dynamics simultaneously. Briefly, we fabricated tetrodes from tungsten wires which were then

110 soldered to a miniature connector that was cemented onto the back of the mouse skull during

111 surgery (see Methods). The single tetrode was stiff enough to be straightly inserted into the deep

112 brain areas through a small burr hole (<1mm) (**Figure 1A**). The body of the tetrode that

113 remained outside of the brain was bent parallel to the skull and the minimal size helped preserve

114 optical transparency during imaging sessions (**Figure 1B**). As the miniature connectors are low-

115 profile, mice were group-housed after the surgery (**Figure 1C**), and we did not observe

116 significant damage to the window or the connectors after long-term monitoring. The tetrodes can

117 be flexibly implanted in almost any brain structure of interest, either cortical or subcortical. We

118 have successfully obtained high-quality recordings in the mouse cortex, hippocampus, thalamus,

119   striatum, and midbrain. The recording site of each tetrode is confirmed using *post-hoc*

120   histological analysis (**Figure 1D**). We found that the single unit activity was relatively stable

121   during one week, two weeks, and two months of recordings after implantation (**Figure 1F, G**),

122   which is consistent with previously reported chronic tetrode recordings in mice (Tolias *et al.*,

123   2007; Hong and Lieber, 2019; Kim, Brünner and Carlén, 2020; Voigts *et al.*, 2020).

124

125   We implanted a total of 29 tetrodes in the midbrain, hippocampus, thalamus, striatum, and cortex

126   of 14 mice, and recorded the activity of 110 neurons with simultaneous mesoscale cortical

127   imaging for up to 2 months after implantation (**Figure 2**). We registered all the putative positions

128   of the recorded neurons as well as the tetrode tracks to a 3D mouse brain model, which was

129   reconstructed from an MRI scan (Egan *et al.*, 2015) (**Figure 2A, B**). First, we compared the

130   firing profiles of the neurons from different brain regions. On average, we obtained 3-5 neurons

131   per tetrode implanted, and this yield was consistent across regions (**Figure 2C**). The inter-spike

132   interval (ISI) of neurons recorded from most brain regions ranged between 0.2-0.7s, except for

133   neurons of the striatum, which fires significantly less compared to neurons in other brain regions

134   (**Figure 2D**, $p < 0.05$). The coefficient of variation (CV) of ISI, a metric that indicates the

135   consistency of the firing rate, was similar across brain regions (**Figure 2E**).

136

137   **Spike-triggered average mapping of the thalamus, striatum, hippocampus, and midbrain**

138   **neuron-defined mesoscale cortical networks.**

139   Spike-triggered average mapping (STM) has been previously used to investigate functional

140   connectivity between single spiking neurons and cortical networks (Barson *et al.*, no date; Xiao

141   *et al.*, 2017; Clancy, Orsolic and Mrsic-Flogel, 2019; Liu *et al.*, 2021). However, this has only

142 been achieved using acute preparation. To assess the functional connectivity of the chronically

143 recorded neurons, we combined electrophysiological recordings with simultaneous mesoscale

144 cortical imaging through a bilateral window that encompassed the entire dorsal cortex in head-

145 fixed, awake mice (**Figure 3A, B, Video 1, 2**). To obtain STMs of the recorded neuron, we

146 calculated the peak response of normalized Ca^{2+} activity ($\Delta F/F$) of each pixel averaged between

147 3s before and after ($\pm$3s) the onset of each spike, which gave us a wide-field mapping of the

148 cortical areas that were associated with spiking activity of the recorded neuron (Xiao *et al.*,

149 2017). Green reflectance signals, which were recorded with the same frequency as the GCaMP6

150 signal, were used to correct hemodynamic artifacts (**Figure 3C, D**).

151

152 We found that single neuron defined functional maps were stable across recording sessions and

153 days. For example, the STM of a hippocampus neuron was associated with the RSP, BCS, and

154 M2 region for 10 recording sessions on different days (**Figure 4A, B**). To better visualize the

155 stability of STMs across recording, we used Mesonet, a machine-learning based toolbox for

156 parcellating brain regions, to accurately align our mesoscale images to a common brain

157 atlas(Xiao *et al.*, 2021). We show that the STMs of a midbrain neuron were relatively stable over

158 2 months (**Figure 4C, D**). The spiking activity of the neuron on different days was highly

159 correlated with the lower limb, upper limb, and trunk sensorimotor areas on both hemispheres of

160 the cortex. To examine the neural correlates of different behaviors, we also incorporated a

161 Raspberry pi camera to monitor the spontaneous behavior of the mice during brain activity

162 recordings (**Figure 4C, E**). In this example, we show that the instantaneous firing rate and

163 patterns of this midbrain neuron were highly correlated with the paw movement of the mouse,

164 consistent with our finding that this neuron is functionally associated with the limb sensorimotor

165 areas. These results highlight the power of this multimodal approach, in that it can link high-

166 resolution single neuron activity with network dynamics of the entire cortex and concurrent

167 animal behavior, which greatly improves our ability to dissect the functional role of individual

168 neurons of any brain region.

169

170 To quantify the distribution of distinctively patterned STMs of neurons across brain regions, we

171 applied a graph-based clustering algorithm, Phenograph, on z-scored STMs (Levine *et al.*, 2015)

172 (**Figure 5**). In total, we included 1146 STMs of 110 neurons recorded during multiple sessions.

173 This resulted in 10 clusters with each cluster representing a distinct STM pattern evidenced by

174 their within-cluster averaged map (**Figure 5A**). Un-matched STMs that were below the

175 similarity threshold (Pearson's correlation < 0.3) compared to any of the 10 cluster average maps

176 were excluded (94 out of 1146 STMs). For all clusters, individual STMs within the clusters

177 showed high similarity to the cluster average maps (**Figure 5B**). In order to validate the optimal

178 number of clusters for partitioning our dataset, we evaluated the clustering effectiveness of a

179 varied number of clusters when applying K-means clustering algorithm using Silhouette score

180 (Rousseeuw, 1987) and confirmed that 10 clusters are most appropriate (**Figure 5C**). Degrees of

181 separation between clusters were visually inspected by projecting dimensionally reduced STMs

182 onto 3D space using t-distributed stochastic neighbor embedding (t-SNE), a commonly used

183 statistical method for plotting high-dimensional data points onto 3D space (**Figure 5D**).

184

185 **Figure 5E** shows the proportions of STMs of neurons in different brain regions that were

186 assigned to each cluster. We found that STMs in each region exhibited a stereotypical

187 distribution pattern. For example, STMs of cortical neurons were primarily assigned to clusters

188   #1-3 and #5-7, corresponding to motor, barrel, and primary somatosensory cortex activation

189   patterns. These activation areas also matched the locations of the recorded cortical neurons (e.g.

190   motor cortical neurons showed motor area activated STMs), suggesting that the functional

191   connectivity patterns of cortical neurons were localized to cortical areas surrounding the

192   recorded neuron, which is consistent with previous reports (Xiao *et al.*, 2017; Clancy, Orsolic

193   and Mrsic-Flogel, 2019). Hippocampal neurons primarily displayed STMs with retrosplenial

194   cortex activation (cluster#10, 59.6% of all hippocampal neuron STMs), reaffirming the dense

195   functional connectivity between these two regions (Karimi Abadchi *et al.*, 2020). STMs of

196   striatal neurons were dominated by two opposing patterns, where 47.7% of them showed a global

197   activation except for the body (limb and trunk) somatosensory regions (cluster#4) and 28.8% of

198   them showed only activation in the body sensory regions (cluster#9). Thalamic neurons

199   exhibited diverse STM patterns with the primary activated regions in lateral anterior cortical

200   regions (cluster#5, 5.6%, cluster#6, 23.3%, cluster#7 26.3% of all thalamic STMs) and medial

201   cortical regions (cluster#8, 6.5%, cluster#9, 11.6%, cluster#10, 9.9% of all thalamic STMs). This

202   suggested that thalamic neurons had diverse functional connectivity patterns in relation to the

203   dorsal cortex, consistent with previous studies (Xiao *et al.*, 2017). STMs of midbrain neurons

204   were most assigned to cluster#8 with medial frontal and somatosensory cortex activation

205   (cluster#8, 44.6% of all midbrain STMs), but also included functional connectivity patterns with

206   various other cortical regions (cluster#6, 10.5%, cluster#9, 12.6%, cluster#10, 11.2% of all

207   midbrain STMs). Interestingly, we found that neurons of different subcortical origins sometimes

208   have overlapping STMs (e.g. cluster#9, and #10 exhibited by neurons from all subcortical

209   regions), which suggests that they may be involved in the same subcortico-cortical functional

210   pathway (**Figure 5E**). Taken together, these findings highlighted the capability of Mesotrode to

9

211   capture the diverse cortical functional connectivity patterns of neurons across brain regions using

212   chronic electrodes.

213

214   **Linkage of spiking peripheral nerve to specific mesoscopic cortical maps**

215   One key advantage of using our minimally invasive micro-electrode setup is the flexibility of the

216   location where they can be implanted and the relatively small footprint at the insertion site. This

217   allows us to explore the functional connectivity of neurons that are otherwise difficult to study.

218   For example, it's difficult to simultaneously record the neural activity of the facial motor nucleus

219   and cortex in order to investigate the cortico-brainstem motor pathways that directly innervate

220   facial muscles that control whisker movement and facial expressions in mice (Petersen, 2014;

221   Sreenivasan *et al.*, 2016; Mercer Lindsay *et al.*, 2019). Here, we implanted micro-wires on the

222   facial motor nerve to record the spiking activity of the axonal projections from the facial motor

223   neurons (**Figure 6A, B).** We obtained the STMs of facial motor nerves by simultaneous wide-

224   field imaging of GCaMP6 mice (n = 5 mice). Surprisingly, we found a distinct cortical pattern,

225   activation in RSP, M2, and PTA areas (**Table 1)**, associated with facial motor nerve spiking

226   activity (**Figure 6C, D, and E, Figure 6—figure supplement 1, Video 3**) in awake mice

227   showing spontaneous movements. Using automated light-based mapping technology (Ayling *et*

228   *al.*, 2009), we found a broad area of the dorsal cortex, including the PTA region could evoke

229   facial motor nerve spiking activity when optogenetically stimulated (**Figure 6F, G, and H,**

230   **Figure 6—figure supplement 2-7, Video 4**) within Thy1-ChR2 mice (n = 6 mice). To further

231   investigate causal relationships, we directly inhibited the PTA by optogenetically stimulating

232   inhibitory neurons of the PTA region of VGAT-ChR2 mice (n = 3 mice). Echoing our previous

233   finding, inhibiting the PTA not only stopped the PTA neurons from firing but also prevented the

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234   facial movements of these mice when the blue light stimulation was on (**Figure 7, Video 5**). To

235   eliminate the possibility that this observation is due to indirect inhibition of other brain areas, we

236   simultaneously recorded neuronal activities in M1 and the barrel cortex (BCS). We confirmed

237   that the neuron firing in these brain areas was not decreased by PTA inhibition (**Figure 7A, B**).

238   More interestingly, we saw a "rebound effect", a sudden increase in PTA neuron firing and facial

239   movement, right after the stimulation is turned off (**Figure 7C, D**). This "off response" supports

240   the causal relationships between PTA and facial movement. These findings are consistent with

241   the previous report that the PTA is involved in controlling active movements (Chapman *et al.*,

242   2002; Cohen and Andersen, 2002; Rathelot, Dum and Strick, 2017; Auffret *et al.*, 2018; Lyamzin

243   and Benucci, 2019; Lee *et al.*, 2022). It also underscores that our chronic tetrode implants in

244   combination with optogenetics and optical imaging can be a powerful tool in mapping and

245   identifying novel functional pathways.

246

247   **Discussion**

248   How activities of individual neurons contribute to brain-wide population dynamics remains

249   poorly understood(Brecht *et al.*, 2004; Rancz *et al.*, 2007; Houweling and Brecht, 2008; Packer

250   *et al.*, 2015). It is additionally challenging to study long-range connectivities (e.g. those between

251   subcortical and cortical structures) given the extraordinary complexity of neuronal wiring

252   between brain regions. Non-invasive methods such as fMRI have generated important

253   connectivity models, such as the default mode network (DMN) model, which advanced our

254   understanding of how distinct brain regions communicate with each other during different

255   behavioral contexts(White *et al.*, 2011; Gutierrez-Barragan *et al.*, 2022). However, these

256   methods typically lack the spatial and temporal resolution to understand the connectivity patterns

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257   of individual neurons. On the other hand, for more invasive techniques such as viral tracing,

258   although they often provide detailed characterizations of the anatomical patterns between brain

259   areas, the nature of the preparation for these techniques precludes gaining insights into the

260   dynamic nature of the connectivity rules. In this study, we developed the Mesotrode that

261   combined chronic tetrode recording with mesoscale Ca^{2+} imaging to characterize mesoscale

262   connectivity patterns of individual neurons. We showed that the functional connectivity patterns

263   of individual neurons are relatively stable in the same behavioral state, and neurons from

264   different brain regions display distinct distributions of these patterns. We further showcased the

265   utility of our procedure by recording from the facial motor nerve and found a distinct cortical

266   activation associated with facial nerve activity. Additionally, Mesotrode provides a wide-field

267   access window for optogenetic stimulations. We showed a causal link between PTA activity and

268   facial movement in mice. These findings established that our multimodal recordings combining

269   chronic electrophysiology, mesoscale imaging, optogenetics, and behavioral characterization can

270   be widely applied to further our understanding of neuronal connectivity and behaviors.

271

272   **Compared to other chronic recording setups**

273   Our approach adds to the plethora of techniques previously described to characterize long-range

274   functional connectivity by combining single-neuron activity recording and mesoscale cortical

275   imaging. These techniques typically differ in how and where the individual neurons' activities

276   are recorded. For instance, Barson et.al used two-photon imaging to record individual cortical

277   neurons' activities, which allows differentiation of different neuronal types through genetic

278   manipulation (Barson *et al.*, no date), but it has lower temporal resolution and is restricted to

279   recording cortical neurons. Xiao et.al, Clancy et.al, Peters et.al, and Liu et.al all used either

280   silicon probes or neuropixel probes to record large populations of cortical or subcortical neurons

281   (Xiao *et al.*, 2017; Clancy, Orsolic and Mrsic-Flogel, 2019; Liu *et al.*, 2021; Peters *et al.*, 2021),

282   but it remains difficult to use these setups to track activities of the same neurons over days. In

283   addition to these approaches, we can use our chronic tetrode implantation to track neuronal

284   activities at multiple brain regions over long periods of time to better describe the dynamic

285   nature of global neuronal affiliations; however, the number of neurons that can be recorded per

286   tetrode is limited which does reduce our data acquisition throughput.

287

288   Compared to most of the previously reported chronic tetrodes setups with the inclusion of a

289   microdrive, our approach has the unique advantages that setting up the apparatus and implanting

290   the tetrodes is a lot more straightforward, mini-connector enables group housing and it is

291   compatible with overhead cameras which permits simultaneous optical interrogation of neuronal

292   circuits (Battaglia *et al.*, 2009; Nguyen *et al.*, 2009; Voigts *et al.*, 2013; Billard *et al.*, 2018;

293   Delcasso *et al.*, 2018; Voigts and Harnett, 2020). However, it does come with the price that the

294   tetrode cannot be moved after it is implanted, which means that the implant would fail if the

295   health of the neuron deteriorates too much over time. Albeit, since our goal is to record the

296   activity of the same neurons, we deemed it a worthy tradeoff for our purpose.

297

298   **Variability of spike-triggered average maps in different brain regions**

299   Activities of cortical pyramidal neurons are known to be coupled to local network activity; as

300   such, STMs of these neurons often display activity motifs localized to the cortical regions they

301   reside, indicating that their functional connections are mostly involved in local networks (Xiao *et*

302   *al.*, 2017; Clancy, Orsolic and Mrsic-Flogel, 2019). On the other hand, subcortical neurons (i.e.

13

303   thalamic neurons) can display more diverse and distal connectivity patterns with cortical

304   networks that are less confined by their locations (Xiao *et al.*, 2017). Here, we extended these

305   characterizations to more subcortical regions (i.e. striatum, hippocampus, and midbrain), and

306   found that these neurons, similar to thalamic neurons, generate functionally diverse STMs. This

307   can be attributed to the diverse cell types within these structures (Phillips and Irvine, 1979;

308   Cembrowski *et al.*, 2016). Furthermore, by classifying these STMs, we found that the

309   connectivity patterns of neurons in different subcortical structures are varied. For instance, STMs

310   of midbrain neurons can be primarily categorized into 4 classes, with each class having distinct

311   activity motifs, which is in contrast to the striatum neurons, the STMs of which are grouped into

312   only two main classes. More interestingly, STMs of different subcortical regions display low

313   levels of overlap, suggesting that neurons of different subcortical origins form their own distinct

314   connectivity pattern to the cortex.

315

316   **The spike-triggered average map associated with specific brain function**

317   The single neuron defined functional maps (STMs) can reveal functional cortical architecture

318   related to the activity of individual cortical and subcortical-cortical neurons (Xiao *et al.*, 2017).

319   The distinct STM patterns indicate that single neuron embedded large-scale subcortico-cortical

320   networks may be associated with specific brain functions (Pessoa, 2014; Liu, Leopold and Yang,

321   2021; Amunts *et al.*, 2022). To explore this possibility, for the first time, we simultaneously

322   record the output spiking activity of the facial motor nucleus and wide-field calcium dynamics of

323   the cortex, which enable us to investigate the cortico-brainstem motor pathways that directly

324   control facial movements in mice. Interestingly, we found that facial nerve spike-triggered

325   average map functionally associated with active facial movements, and the causal relationship

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326 can be tested by optogenetic stimulation or inhibition of the PTA area. Previous studies using

327 anterograde virus tracing have revealed that the motor cortex sends few direct projections to the

328 facial nucleus while the most prominent projections from the motor, somatosensory, and

329 association cortex are polysynaptic, presumably through nuclei in the brainstem (Grinevich,

330 Brecht and Osten, 2005; Matyas *et al.*, 2010; Rathelot, Dum and Strick, 2017). Studies in

331 humans and monkeys have identified parietal-eye-field and reach-specific areas that involve

332 voluntary control of eye or hand movement (Lynch, 1980; Andersen, 1989; Sakata *et al.*, 1997;

333 Chapman *et al.*, 2002; Konen and Kastner, 2008; Archambault, Caminiti and Battaglia-Mayer,

334 2009; Pouget, 2015; Rathelot, Dum and Strick, 2017). Our findings suggest that the PTA region

335 is also involved in facial movements in mice, and supplement the current understanding of the

336 functional connectivity between cortex and facial nucleus motor neurons.

337

Conclusion

339 Our results demonstrated that our minimally invasive, flexible, low profile, low cost, chronically

340 implanted Mesotrode can be readily applied to interrogating the functional connectivity between

341 single neurons and large-scale cortical networks, and the causal relationship can be tested by

342 optogenetic stimulation.

343

Materials and methods

Mice

346 Animal protocols (A13-0336 and A14-0266) were approved by the University of British

347 Columbia Animal Care Committee and conformed to the Canadian Council on Animal Care and

348 Use guidelines and animals were housed in a vivarium on a 12 h daylight cycle (7 AM lights on).

15

349   Most experiments were performed toward the end of the mouse light cycle. Transgenic

350   GCaMP6s mice (males, 2–4 months of age, weighing 20–30g; n=19) were produced by crossing

351   Emx1-cre (B6.129S2-*Emx1$^{tm1(cre)Krj}$*/J, Jax #005628), CaMK2-tTA (B6.Cg-Tg(Camk2a-

352   tTA)1Mmay/DboJ, Jax #007004) and TITL-GCaMP6s (Ai94;B6.*Cg-Igs7$^{tm94.1(tetO-GCaMP6s)Hze}$*/J,

353   Jax #024104) strain. The presence of GCaMP expression was determined by genotyping each

354   animal before each surgical procedure with PCR amplification. These crossings are expected to

355   produce a stable expression of the calcium indicator (T.-W. Chen *et al.*, 2013) specifically within

356   all excitatory neurons across all layers of the cortex (Vanni and Murphy, 2014).

357   Channelrhodopsin-2 transgenic mice (Arenkiel *et al.*, 2007) (n = 6) were obtained from the

358   Jackson Labs (line 18, stock 007612, strain B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J). Neuronal

359   activity was driven by optically activating inhibitory neurons targeted in VGAT-ChR2-YFP

360   expressing mice (Zhao *et al.*, 2011) (n = 3). No method of randomization was used since all mice

361   belonged to the same sample group. Samples sizes were chosen based on previous studies using

362   similar approaches (Mohajerani *et al.*, 2013; Vanni and Murphy, 2014). Given the use of

363   automated acquisition and analysis procedures, we did not employ blinding.

364

365   **Tetrode fabrication**

366   Polyimide insulated, tungsten wire (40 cm long, diameter 25 μm, Stablohm 650, California Fine

367   Wire, USA) was folded into four wires and clamped together with the modified alligator clip.

368   The loop of wires was hung over the horizontal bar. The alligator clip was placed into the

369   motorized stage. Eighty clockwise twists were applied and followed by forty counter clock-wise

370   twists to the wire bundle over the course of approximately three minutes. After tetrode twisting

371   was completed, the wires were fused together by heating from three different angles with a heat

372   gun (420°C or 790°F), using medium-low flow. For each angle, begin 1-2 cm below where the

373   wire bundle splits, the heat gun was held 2 cm away from the wire and moved periodically over

374   the length of the wire for about 5 s. The tetrode was removed from the twisting apparatus by

375   gently lifting the alligator clip to relieve tension on the tetrode, and cutting the tetrode near the

376   alligator clip. At the other end, the loop was cut off such that there were four non-bonded strands

377   of wire of equal length. The tetrode was then soldered to a miniature connector using a fine-tip

378   soldering iron (Thermaltronics M8MF375 Micro Fine 0.25 mm).

379

380   **Surgical procedures**

381   Mice were anesthetized with isoflurane (4% for induction and 1.5% for maintenance in air). The

382   eyes were covered with eye lubricant (Lacrilube; www.well.ca) and body temperature was

383   maintained at 37 °C with a heating pad with feedback regulation. Mice were then placed in a

384   stereotaxic frame and received an injection of Lidocaine (0.1ml, 0.2%) under the scalp. Mice

385   also received a 0.5 ml subcutaneous injection of a saline solution containing buprenorphine (2

386   mg/ml), atropine (3μg/ml), and glucose (20 mM). Respiration rate and response to toe pinch

387   were checked every 10-15 min to maintain the surgical anesthetic plane. A skin flap extending

388   over both hemispheres approximately 8 mm in diameter (3 mm anterior to bregma to the

389   posterior end of the skull and down lateral to eye level) was cut and removed. Fascia or

390   connective tissue is lightly scraped away from the skull and small (< 1 mm diameter) holes are

391   drilled through the skull, using a high-speed dental drill with a sterile bit, over the cortex.

392   Tetrode was directed toward the center of the hole and placed in the target using a motorized

393   micromanipulator (MP-225, Sutter Instrument Company). Miniature connectors (2 x 2 x 2 mm)

394   are cemented to the skull (with dental adhesive) around the imaging window (the total weight is

395   < 1 gm). Ground and reference electrodes are fixed into place on the surface of the posterior

396   skull.

397

398   After tetrode implantation, the chronic transcranial window was implanted as previously

399   described (Silasi *et al.*, 2016). Briefly, the skin between the ears and the eyes was properly

400   cleaned with Betadine dissolved in water and ethanol. The skin covering the occipital, parietal

401   and frontal bones was cut away. The fascia and any connective tissue on the skull surface were

402   gently wiped off. C&B-Metabond transparent dental cement (Parkell, Edgewood NY, USA;

403   Product: C&B Metabond) was prepared in a ceramic bowl and used to glue a head-fixing

404   titanium bar to the cerebellar plate or a 4/40 stainless steel setscrew slightly angled posteriorly

405   (~120° relative to skull). With the bar or the setscrew in place, a thick layer of dental adhesive

406   was applied to the skull. A coverglass (Marien-feld, Lauda-Konigshofen, Germany; Cat n:

407   0111520) previously cut to the size of the final cranial window (~8 mm diameter), was placed on

408   top of the dental cement before it solidified, preventing the formation of bubbles. The cement

409   remains transparent after it solidifies and the surface vasculature should be readily seen through

410   the final result.

411

412   **Recovery and post-operative monitoring**

413   At the end of the surgical procedures, mice received a subcutaneous injection of saline (0.5 ml)

414   with 20 mM of glucose and were allowed to recover in their home cages with an overhead heat

415   lamp. The activity level of mice that underwent the procedure was monitored hourly for the first

416   4 h and every 4-8 h thereafter. Mice are allowed to recover for 7 days from the window

417   implantation before performing electrophysiology recording.

418

Electrophysiology data acquisition

For Electrophysiology recordings, a custom adapter was connected to the miniature connector. The tetrode signals were amplified using a 16-channel data acquisition system (20 kHz, USB-ME16-FAI-System, Multi-Channel Systems) and recorded for at least 30 min every day.

Raw extracellular traces were imported into Spike2 (Cambridge Electronic Design, Cambridge, UK) or SpikeSorter software (Swindale and Spacek, 2014; Swindale *et al.*, 2021) for spike sorting and analysis. Briefly, data were high pass-filtered at 1kHz, and excitatory spikes were detected using a threshold of 4.5 times the median of the standard deviation over 0.675. Sorting was carried out by an automated method previously described (Swindale and Spacek, 2014) and followed by manual visual inspection of units.

Wide-field calcium imaging

All mice were habituated for 1 week prior to data collection. Awake mice were head-fixed and placed on a wheel in a dark imaging chamber for data collection. A behavioral camera (Raspberry Pi camera) and an infrared light were placed inside the imaging chamber to monitor active behaviors, such as running or whisking. A Pantera 1M60 CCD camera (Dalsa) was equipped with two front-to-front lenses (50 mm, f ¼ 1.4:35 mm, f ¼ 2; Nikon Nikkor) and a bandpass emission filter (525/36 nm, Chroma). The 12-bit images were captured at a frame rate of 120 Hz (exposure time of 7 ms) with 8×8 on-chip spatial binning using EPIX XCAP v3.8 imaging software. The cortex was sequentially illuminated with alternating blue and green LEDs (Thorlabs). Blue light (473 nm) with a bandpass filter (467 to 499 nm) was used to excite

19

441 calcium indicators and green light (525 nm) with a bandpass filter (525/50 nm) was used to

442 observe changes in cerebral blood volume. The blue and green LEDs were sequentially activated

443 and synchronized to the start of each frame's exposure period with transistor-transistor logic

444 such that each frame collected only fluorescence or reflectance signals at 60 Hz each. This LED

445 strobe frequency of 60 Hz exceeded the critical flicker fusion frequency for mice, which marks

446 the highest temporal frequency that an observer can resolve flicker before it becomes

447 indistinguishable from constant light and was likely imperceptible to the mice. Reflectance

448 signals were subtracted from fluorescence signals to mitigate the contribution of hemodynamics

449 to fluorescence signals.

450

451 **Optogenetic stimulation protocols**

452 Inhibitory neurons in the cortex were optogenetically manipulated separately in VGAT-ChR2

453 mice. A 473nm laser (Crystal Laser BCL-473-050, Reno, NV, USA) was connected to a fiber

454 optic patch cable (0.22 NA, 200 µm gauge; Thorlabs FG200UCC, USA). During the experiment,

455 1 mW (measured from the end of the cable) of optical stimulation was delivered for an overall

456 time of 10 s. For automated Thy1-ChR2-based facial motor mapping, we chose a relatively

457 collimated 473 nm laser targeted through a simple microscope. The laser was moved in random

458 order to each of the predefined stimulation locations (8x8 grid) and delivered a flash of laser

459 light (10 ms, 2.0 mW) to each point while collecting facial motor nerve response.

460

461 **Spike-triggered average maps (STM) Clustering**

462 We employed an unsupervised clustering algorithm, PhenoGraph (Levine *et al.*, 2015), to

463 categorize STMs. The graph was built in 2 steps: 1) it finds k nearest neighbors for N input

20

464   vectors (4096 dimensions for 64x64 STMs) using Euclidean distance, resulting in N sets of k

465   nearest neighbors, 2) a weighted graph is built where the edge weight between pairs of nodes

466   depends on the number of neighbors they share. We then perform Louvain community detection

467   (Blondel *et al.*, 2008) on this graph to partition the graph that maximizes modularity. This

468   algorithm only requires one input parameter which is k, the number of nearest neighbors to be

469   found for each input vector, and the resulting number of clusters (10) is relatively insensitive to

470   the k chosen over the range of typically chosen values. We also performed k-means clustering

471   using the same input and compared clustering effectiveness using the Silhouette score and found

472   that 10 clusters were optimal. We then performed a cluster identity refinement step, where an

473   individual STM whose similarity (Pearson correlation value) with the cluster average STM of the

474   assigned cluster was lower than 0.3 was manually reassigned if its similarity with group average

475   STMs of any other clusters was higher than 0.3; otherwise, that STM was excluded. A total of 94

476   out of 1146 STMs (8.2%) were excluded.

477

478   **Histology**

479   At the end of each experiment, animals were killed with an intraperitoneal injection of

480   pentobarbital (24 mg). Mice were transcardially perfused with PBS followed by chilled 4% PFA

481   in PBS. Coronal or sagittal brain sections (50 µm thickness) were cut on a vibratome (Leica

482   VT1000S). Images of brain tissues with counter-stained DAPI were acquired using confocal

483   microscopy (Zeiss LSM510) to reveal the electrode track and help identify the approximate

484   cortical/subcortical location of recorded cells.

485

486   **Statistical analysis**

487 Statistical analyses were performed using GraphPad Prism, Version 9. Statistical tests and results

488 are reported in the figure legends. More than two groups were compared using one-way ANOVA

489 followed by Dunnett's test comparing experimental flies to each control. All graphs represent

490 mean ± SEM. Sample sizes are listed in the figure legends. No explicit power analyses were used

491 to determine sample sizes prior to experimentation. Minimum sample sizes were decided prior to

492 experimentation based on previous experience or pilot experiments revealing how many samples

493 are typically sufficient to detect reasonable effect sizes.

494

495 **Data and code availability**

496 All code used for data analysis and sample data generated in this study have been deposited in

497 the Open Science Framework repository (https://osf.io/67p3s/).

498

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511

Competing interests

513 The authors declare no competing interests.

514

Author contributions

516 Dongsheng Xiao, Conceptualization, Data curation, Software, Formal analysis, Visualization,

517 Methodology, Writing – original draft, Writing – review and editing; Yuhao Yan, Formal

518 analysis, Visualization, Methodology, Writing – original draft, Writing – review and editing;

519 Timothy H Murphy, Conceptualization, Supervision, Funding acquisition, Investigation,

520 Visualization, Methodology, Project administration, Writing – review and editing.

521

Figure legends

523 **Figure 1. Chronic tetrodes recording compatible with mesoscale transcranial Imaging.** (**A**)
524 Images of skull surface during tetrode implantation. Panels depict the location of 4 tetrodes
525 placed in skull burr holes using a micromanipulator. (**B**) Image showing transcranial window
526 with tetrodes placed in the striatum, thalamus, and midbrain. (**C**) Chronic tetrode recording is
527 compatible with group-housed mice. (**D**) Post-mortem histology is performed to confirm the
528 tetrode location. (**E**) Cartoon of tetrode and local neurons. (**F**) Example of long-term recording
529 using transcranial imaging window plus tetrodes. (**G**) Sorted spikes from (**F**) (brown, blue, and
530 gray indicate 1 week, 2 weeks, and 2 months after tetrode implantation).

531

532 **Figure 2. Group statistics on tetrode implantation across mice.** (**A**) Labels corresponding to
533 mouse IDs (n = 14 mice) were registered to a 3D mouse brain model based on tetrode locations.
534 (**B**) Side view of the 3D reconstruction of 29 tetrode tracks implanted in 14 mice registered to the
535 3D mouse brain model. (**C**) The mean number of well-isolated neurons recorded per tetrode
536 implanted in different brain regions. (**D**) Mean inter-spike interval (ISI) of neurons recorded in
537 different brain regions (Dunn's Multiple Comparison Test, striatum vs midbrain, $P < 0.01$,
538 thalamus vs midbrain, $P < 0.05$, n = 14 mice). (**E**) Mean coefficient of variation of ISIs of
539 neurons across brain regions (Dunn's Multiple Comparison Test, thalamus vs hippocampus, $P <$
540 0.05, n = 14 mice).

541

Figure 3. Recording setup and example spike-triggered average maps. (**A**) Illustration of experiment setup incorporating wide-field imaging, and tetrode recording with simultaneous behavioral monitoring. (**B**) Example tetrodes implantation in the thalamus, BCS1, and striatum. The middle and bottom panel shows the tetrode location registered in a 3D model of the mouse brain. (**C**) Spike-triggered average of GCaMP6s fluorescence (left), hemodynamic signal (middle, green reflectance), and hemodynamic-subtracted cortical maps (right) of thalamic, BCS1, and striatal neurons. (**D**) Average traces of GCaMP6s epi-fluorescence (blue), hemodynamic signal (green reflectance), and hemodynamic-subtracted signal (red) surrounding spikes of single neurons, and random trigger average traces (black).

Figure 4. Chronic spike-triggered average maps of hippocampus and midbrain neurons. (**A**) Unfiltered brain RGB image showing the position of the tetrode (left), GCaMP6 fluorescence image (middle), and histology showing the recording site of the tetrode (right). (**B**) Example raw and high-pass filtered (> 300 Hz) electrophysiological traces of hippocampal recording 7 days after tetrode implantation and STMs of the hippocampal neuron across recording sessions(top). Another day of hippocampal recording and STMs of the hippocampal neuron across recording sessions(bottom). (**C**) Unfiltered brain RGB image showing the position of the tetrode (top) and behavior video recording (bottom). (**D**) Top: Ca^{2+} images recorded 1 week (left), 2 weeks (middle), and 2 months (right) after tetrode implanted in the midbrain overlaid with automatically registered brain atlas using Mesonet. Bottom: STMs of the midbrain neuron recorded at the same time points as Top. (**D**) Synchronized traces of paw movements, instantaneous firing rate, and electrophysiological signals of the midbrain neuron are shown in (**C**).

Figure 5. Clustering STMs of cortical/subcortical neurons. (**A**) Average z-scored STM of each cluster. Each cluster represents a unique cortical functional network that individual neurons were associated with. STMs of the same neurons recorded during different sessions were included (1146 total STMs from 110 neurons of 14 mice). (**B**) Box plot showing the correlation between individual STM and average STM of its assigned cluster. (**C**) Silhouette coefficient of varying numbers of clusters to validate the optimal number of clusters. (**D**) 3D-t-SNE plot showing dimensionality reduced representation of clustered individual STMs. (**E**) The proportion of neurons in each cortical and subcortical region assigned to each STM cluster.

Figure 6. Spike-triggered and optogenetics mapping facial nerve spiking associated cortical maps. (**A**) Mouse anatomy illustrates facial motor nucleus axons (facial nerve) that innervate facial muscles. Micro-wire was implanted in the facial nerve. (**B**) Example spiking activity recorded from facial motor nerve using the micro-wire. (**C**) Example calcium imaging during facial nerve recording. (**D**) Facial nerve spike-triggered average map temporal dynamics surrounding facial nerve spikes (t = 0ms) of a single mouse (top row) or the average of 5 mice (bottom row). (**D**) Reference atlas (white outlines; ©2004 Allen Institute for Brain Science. Allen Mouse Brain Atlas. Available from: http://mouse.brain-map.org/) (**F**) Illustration of automated light-based mapping of the dorsal cortex (8x8 grid) by photoactivation of Thy1-ChR2 mice. (**G**) Example single trial facial nerve response after light stimulation. (**H**) Average change of facial nerve responses of 6 mice represented on a 2D color-coded map corresponding to each stimulation coordinate overlayed on an example cortical image of Thy1-ChR2 mouse. The

587   amplitude of the average change for each stimulation coordinate corresponds to the total variance
588   of all the trials (>5 trials, within 200 ms after stimulus) where there is no facial nerve spiking
589   before the stimulus.
590
591   **Figure 7. Optogenetic inhibition of the PTA area significantly reduced the facial movement**
592   **of the mouse.** (**A**) Unfiltered brain RGB image showing the position of the tetrodes. (**B**)
593   Simultaneous cellular electrophysiological recording in the cortical regions of M1, BCS, and
594   PTA with optogenetic inhibition on the PTA region. (**C**) Behavioral image showing masks used
595   for facial movement detection (top). Brain image of a VGAT-ChR2 mouse (ChR2 expressed in
596   cortical inhibitory neurons) with laser targeting the PTA area (bottom). (**D**) Synchronized traces
597   of optogenetic activation of PTA inhibitory neurons, facial movement, and LFP of the PTA
598   region. Inhibiting the PTA region strongly inhibited facial nerve activity. Cessation of PTA
599   inhibition reliably induced rebound PTA neuron firing and facial movements. (**E**) The neuronal
600   firing of 12 trials of optogenetic inhibition in PTA. (**F**) Facial movements ((gradient-
601   mean)/mean) 3s before, 3s during, and 3s immediately after optogenetic inhibition in PTA for 29
602   trials (Dunn's Multiple Comparison Test, $p < 0.001$, n = 3 mice).
603

604   **Table 1. Abbreviation used to define different cortical/sub-cortical areas**

Mop (M1)	primary motor area
Mos (M2)	secondary motor area
SSp-m	primary somatosensory area, mouse
SSp-ul	primary somatosensory area, upper limb
SSp-ll	primary somatosensory area, lower limb
SSp-n	primary somatosensory area, nose
SSp-bfd	primary somatosensory area, barrel field
SSp-tr	primary somatosensory area, trunk
VISp	primary visual area
VISa	anterior visual area
VISam	anteromedial visual area
VISpm	posteromedial visual area
VISrl	rostrolateral visual area
VISal	anterolateral visual area
VISl	lateral visual area
RSP	retrosplenial area
AUD	auditory areas
PTA	parietal association area
HPF	hippocampal formation
VTA	ventral tegmental area

605

Video 1. Video of the 4D spatiotemporal whole-brain dynamics of mesoscale cortical calcium imaging and subcortical neuronal firing. Brain activity data was registered in a 3D mouse brain model. Calcium dynamic intensity and neuronal firing rate of subcortical neurons (Top) were color-coded in the ROIs in the 3D mouse brain model (bottom left). The calcium dynamic was overlaid on a cortical atlas (bottom right).

Video 2. Simultaneous recording of behavior video (left), L-VTA neuronal firing (middle), and wide-field calcium imaging (right) in a behaving mouse.

Video 3. Simultaneous recording of behavior video (left), facial nerve spiking activity (middle), and wide-field calcium imaging (right) during mouse whiskering.

Video 4. Video of the facial response after optogenetic stimulation (blue laser, 10 ms) of PTA in a Thy1-ChR2 mouse.

Video 5. Video of the facial response after optogenetic inhibition (blue laser, 10 s) of PTA in a VGAT-ChR2 mouse.

Supplemental Figure legends

Figure 6—figure supplement 1. Facial nerve spike-triggered average maps of 5 mice.

Figure 6—figure supplement 2. Optogenetic mapping of facial nerve responses (blue laser, 10 ms, 2.0 mW) in a Thy1-ChR2 mouse. Averaged responses (> 5 trials) were normalized to the range of 0 and 1 (right).

Figure 6—figure supplement 3. Optogenetic mapping of facial nerve responses (blue laser, 10 ms, 2.0 mW) in a Thy1-ChR2 mouse. Averaged responses (> 5 trials) were normalized to the range of 0 and 1 (right).

Figure 6—figure supplement 4. Optogenetic mapping of facial nerve responses (blue laser, 10 ms, 2.0 mW) in a Thy1-ChR2 mouse. Averaged responses (> 5 trials) were normalized to the range of 0 and 1 (right).

Figure 6—figure supplement 5. Optogenetic mapping of facial nerve responses (blue laser, 10 ms, 2.0 mW) in a Thy1-ChR2 mouse. Averaged responses (> 5 trials) were nomalized to the range of 0 and 1 (right).

Figure 6—figure supplement 6. Optogenetic mapping of facial nerve responses (blue laser, 10 ms, 2.0 mW) in a Thy1-ChR2 mouse. Averaged responses (> 5 trials) were normalized to the range of 0 and 1 (right).

Figure 6—figure supplement 7. Optogenetic mapping of facial nerve responses (blue laser, 10 ms, 2.0 mW) in a Thy1-ChR2 mouse. Averaged responses (> 5 trials) were normalized to the range of 0 and 1 (right).

References

Alivisatos, A.P. *et al.* (2012) 'The brain activity map project and the challenge of functional connectomics', *Neuron*, 74(6), pp. 970–974.

Amunts, K. *et al.* (2022) 'Linking Brain Structure, Activity, and Cognitive Function through Computation', *eNeuro*, 9(2). Available at: https://doi.org/10.1523/ENEURO.0316-21.2022.

Andersen, R.A. (1989) 'Visual and eye movement functions of the posterior parietal cortex', *Annual review of neuroscience*, 12, pp. 377–403.

Archambault, P.S., Caminiti, R. and Battaglia-Mayer, A. (2009) 'Cortical mechanisms for online control of hand movement trajectory: the role of the posterior parietal cortex', *Cerebral cortex* , 19(12), pp. 2848–2864.

Arenkiel, B.R. *et al.* (2007) 'In Vivo Light-Induced Activation of Neural Circuitry in Transgenic Mice Expressing Channelrhodopsin-2', *Neuron*, pp. 205–218. Available at: https://doi.org/10.1016/j.neuron.2007.03.005.

Aru, J., Suzuki, M. and Larkum, M.E. (2020) 'Cellular Mechanisms of Conscious Processing', *Trends in cognitive sciences*, 24(10), pp. 814–825.

Auffret, M. *et al.* (2018) 'Optogenetic stimulation of cortex to map evoked whisker movements in awake head-restrained mice', *Neuroscience*, 368, pp. 199–213.

Ayling, O.G.S. *et al.* (2009) 'Automated light-based mapping of motor cortex by photoactivation of channelrhodopsin-2 transgenic mice', *Nature methods*, 6(3), pp. 219–224.

Barson, D. *et al.* (no date) 'Simultaneous mesoscopic and two-photon imaging of neuronal activity in cortical circuits'. Available at: https://doi.org/10.1101/468348.

Battaglia, F.P. *et al.* (2009) 'The Lantern: an ultra-light micro-drive for multi-tetrode recordings in mice and other small animals', *Journal of neuroscience methods*, 178(2), pp. 291–300.

Billard, M.W. *et al.* (2018) 'The systemDrive: a Multisite, Multiregion Microdrive with Independent Drive Axis Angling for Chronic Multimodal Systems Neuroscience Recordings in Freely Behaving Animals', *eNeuro*, 5(6). Available at: https://doi.org/10.1523/ENEURO.0261-18.2018.

Blondel, V.D. *et al.* (2008) 'Fast unfolding of communities in large networks', *Journal of statistical mechanics* , 2008(10), p. P10008.

Bragin, A. *et al.* (2000) 'Multiple site silicon-based probes for chronic recordings in freely moving rats: implantation, recording and histological verification', *Journal of neuroscience methods*, 98(1), pp. 77–82.

Brecht, M. *et al.* (2004) 'Whisker movements evoked by stimulation of single pyramidal cells in rat motor cortex', *Nature*, 427(6976), pp. 704–710.

686 Buzsaki, G. (2006) *Rhythms of the Brain*. Oxford University Press.

687 Cembrowski, M.S. *et al.* (2016) 'Hipposeq: a comprehensive RNA-seq database of gene
688 expression in hippocampal principal neurons', *eLife*, 5, p. e14997.

689 Chapman, H. *et al.* (2002) 'Posterior parietal cortex control of reach-to-grasp movements in
690 humans', *The European journal of neuroscience*, 15(12), pp. 2037–2042.

691 Chen, J.L. *et al.* (2013) 'Behaviour-dependent recruitment of long-range projection neurons in
692 somatosensory cortex', *Nature*, 499(7458), pp. 336–340.

693 Chen, T.-W. *et al.* (2013) 'Ultrasensitive fluorescent proteins for imaging neuronal activity',
694 *Nature*, 499(7458), pp. 295–300.

695 Clancy, K.B., Orsolic, I. and Mrsic-Flogel, T.D. (2019) 'Locomotion-dependent remapping of
696 distributed cortical networks', *Nature neuroscience*, 22(5), pp. 778–786.

697 Cohen, Y.E. and Andersen, R.A. (2002) 'A common reference frame for movement plans in the
698 posterior parietal cortex', *Nature reviews. Neuroscience*, 3(7), pp. 553–562.

699 Delcasso, S. *et al.* (2018) 'HOPE: Hybrid-Drive Combining Optogenetics, Pharmacology and
700 Electrophysiology', *Frontiers in neural circuits*, 12, p. 41.

701 Egan, G.F. *et al.* (2015) 'Average wild-type C57BL/6J mouse 3D MRI brain image'. Available
702 at: https://espace.library.uq.edu.au/view/UQ:370299.

703 Grinevich, V., Brecht, M. and Osten, P. (2005) 'Monosynaptic pathway from rat vibrissa motor
704 cortex to facial motor neurons revealed by lentivirus-based axonal tracing', *The Journal of
705 neuroscience: the official journal of the Society for Neuroscience*, 25(36), pp. 8250–8258.

706 Gutierrez-Barragan, D. *et al.* (2022) 'Unique spatiotemporal fMRI dynamics in the awake mouse
707 brain', *Current biology: CB*, 32(3), pp. 631–644.e6.

708 Hong, G. and Lieber, C.M. (2019) 'Novel electrode technologies for neural recordings', *Nature
709 Reviews Neuroscience*, pp. 330–345. Available at: https://doi.org/10.1038/s41583-019-0140-6.

710 Houweling, A.R. and Brecht, M. (2008) 'Behavioural report of single neuron stimulation in
711 somatosensory cortex', *Nature*, 451(7174), pp. 65–68.

712 Huber, D. *et al.* (2012) 'Multiple dynamic representations in the motor cortex during
713 sensorimotor learning', *Nature*, 484(7395), pp. 473–478.

714 Jiang, X. *et al.* (2015) 'Principles of connectivity among morphologically defined cell types in
715 adult neocortex', *Science*, 350(6264), p. aac9462.

716 Juavinett, A.L., Bekheet, G. and Churchland, A.K. (2019) 'Chronically implanted Neuropixels
717 probes enable high-yield recordings in freely moving mice', *eLife*, 8. Available at:
718 https://doi.org/10.7554/eLife.47188.

719   Karimi Abadchi, J. *et al.* (2020) 'Spatiotemporal patterns of neocortical activity around
720   hippocampal sharp-wave ripples', *eLife*, 9. Available at: https://doi.org/10.7554/eLife.51972.

721   Kim, H., Brünner, H.S. and Carlén, M. (2020) 'The DMCdrive: practical 3D-printable micro-
722   drive system for reliable chronic multi-tetrode recording and optogenetic application in freely
723   behaving rodents', *Scientific reports*, 10(1), p. 11838.

724   Koch, C. *et al.* (2016) 'Neural correlates of consciousness: progress and problems', *Nature*
725   *reviews. Neuroscience*, 17(5), pp. 307–321.

726   Konen, C.S. and Kastner, S. (2008) 'Representation of eye movements and stimulus motion in
727   topographically organized areas of human posterior parietal cortex', *The Journal of*
728   *neuroscience: the official journal of the Society for Neuroscience*, 28(33), pp. 8361–8375.

729   Lee, J.J. *et al.* (2022) 'Task specificity in mouse parietal cortex', *Neuron*, 110(18), pp. 2961–
730   2969.e5.

731   Levine, J.H. *et al.* (2015) 'Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like
732   Cells that Correlate with Prognosis', *Cell*, 162(1), pp. 184–197.

733   Liu, X. *et al.* (2021) 'Multimodal neural recordings with Neuro-FITM uncover diverse patterns
734   of cortical–hippocampal interactions', *Nature neuroscience*, 24(6), pp. 886–896.

735   Liu, X., Leopold, D.A. and Yang, Y. (2021) 'Single-neuron firing cascades underlie global
736   spontaneous brain events', *Proceedings of the National Academy of Sciences of the United States*
737   *of America*, 118(47). Available at: https://doi.org/10.1073/pnas.2105395118.

738   Lyamzin, D. and Benucci, A. (2019) 'The mouse posterior parietal cortex: Anatomy and
739   functions', *Neuroscience research*, 140, pp. 14–22.

740   Lynch, J.C. (1980) 'The functional organization of posterior parietal association cortex', *The*
741   *Behavioral and brain sciences*, 3(4), pp. 485–499.

742   Margolis, D.J. *et al.* (2012) 'Reorganization of cortical population activity imaged throughout
743   long-term sensory deprivation', *Nature neuroscience*, 15(11), pp. 1539–1546.

744   Matyas, F. *et al.* (2010) 'Motor control by sensory cortex', *Science*, 330(6008), pp. 1240–1243.

745   Mercer Lindsay, N. *et al.* (2019) 'Orofacial Movements Involve Parallel Corticobulbar
746   Projections from Motor Cortex to Trigeminal Premotor Nuclei', *Neuron*, 104(4), pp. 765–780.e3.

747   Mohajerani, M.H. *et al.* (2013) 'Spontaneous cortical activity alternates between motifs defined
748   by regional axonal projections', *Nature neuroscience*, 16(10), pp. 1426–1435.

749   Nguyen, D.P. *et al.* (2009) 'Micro-drive array for chronic in vivo recording: tetrode assembly',
750   *Journal of Visualized Experiments* [Preprint]. Available at: https://doi.org/10.3791/1098.

751   Oh, S.W. *et al.* (2014) 'A mesoscale connectome of the mouse brain', *Nature*, 508(7495), pp.

752   207–214.

753   Packer, A.M. *et al.* (2015) 'Simultaneous all-optical manipulation and recording of neural circuit
754   activity with cellular resolution in vivo', *Nature methods*, 12(2), pp. 140–146.

755   Pessoa, L. (2014) 'Understanding brain networks and brain organization', *Physics of life reviews*,
756   11(3), pp. 400–435.

757   Peters, A.J. *et al.* (2021) 'Striatal activity topographically reflects cortical activity', *Nature*,
758   591(7850), pp. 420–425.

759   Petersen, C.C.H. (2014) 'Cortical control of whisker movement', *Annual review of neuroscience*,
760   37, pp. 183–203.

761   Phillips, D.P. and Irvine, D.R. (1979) 'Acoustic input to single neurons in pulvinar-posterior
762   complex of cat thalamus', *Journal of neurophysiology*, 42(1 Pt 1), pp. 123–136.

763   Pouget, P. (2015) 'The cortex is in overall control of "voluntary" eye movement', *Eye* , 29(2),
764   pp. 241–245.

765   Rancz, E.A. *et al.* (2007) 'High-fidelity transmission of sensory information by single cerebellar
766   mossy fibre boutons', *Nature*, 450(7173), pp. 1245–1248.

767   Rathelot, J.-A., Dum, R.P. and Strick, P.L. (2017) 'Posterior parietal cortex contains a command
768   apparatus for hand movements', *Proceedings of the National Academy of Sciences of the United
769   States of America*, 114(16), pp. 4255–4260.

770   Roth, R.H. and Ding, J.B. (2020) 'From neurons to cognition: Technologies for precise recording
771   of neural activity underlying behavior', *BME Frontiers*, 2020, pp. 1–20.

772   Rousseeuw, P.J. (1987) 'Silhouettes: A graphical aid to the interpretation and validation of
773   cluster analysis', *Journal of computational and applied mathematics*, 20, pp. 53–65.

774   Sakata, H. *et al.* (1997) 'The TINS Lecture. The parietal association cortex in depth perception
775   and visual control of hand action', *Trends in neurosciences*, 20(8), pp. 350–357.

776   Silasi, G. *et al.* (2016) 'Intact skull chronic windows for mesoscopic wide-field imaging in
777   awake mice', *Journal of neuroscience methods*, 267, pp. 141–149.

778   Sreenivasan, V. *et al.* (2016) 'Movement Initiation Signals in Mouse Whisker Motor Cortex',
779   *Neuron*, 92(6), pp. 1368–1382.

780   Steinmetz, N.A. *et al.* (2021) 'Neuropixels 2.0: A miniaturized high-density probe for stable,
781   long-term brain recordings', *Science*, 372(6539). Available at:
782   https://doi.org/10.1126/science.abf4588.

783   Swindale, N.V. *et al.* (2021) 'Voltage distributions in extracellular brain recordings', *Journal of
784   neurophysiology*, 125(4), pp. 1408–1424.

785 Swindale, N.V. and Spacek, M.A. (2014) 'Spike sorting for polytrodes: a divide and conquer
786 approach', *Frontiers in systems neuroscience*, 8, p. 6.

787 Tolias, A.S. *et al.* (2007) 'Recording chronically from the same neurons in awake, behaving
788 primates', *Journal of neurophysiology*, 98(6), pp. 3780–3790.

789 Vandecasteele, M. *et al.* (2012) 'Large-scale Recording of Neurons by Movable Silicon Probes
790 in Behaving Rodents', *Journal of Visualized Experiments* [Preprint]. Available at:
791 https://doi.org/10.3791/3568.

792 Vanni, M.P. and Murphy, T.H. (2014) 'Mesoscale transcranial spontaneous activity mapping in
793 GCaMP3 transgenic mice reveals extensive reciprocal connections between areas of
794 somatomotor cortex', *The Journal of neuroscience: the official journal of the Society for
795 Neuroscience*, 34(48), pp. 15931–15946.

796 Voigts, J. *et al.* (2013) 'The flexDrive: an ultra-light implant for optical control and highly
797 parallel chronic recording of neuronal ensembles in freely moving mice', *Frontiers in systems
798 neuroscience*, 7, p. 8.

799 Voigts, J. *et al.* (2020) 'An easy-to-assemble, robust, and lightweight drive implant for chronic
800 tetrode recordings in freely moving animals', *Journal of neural engineering*, 17(2), p. 026044.

801 Voigts, J. and Harnett, M.T. (2020) 'Somatic and Dendritic Encoding of Spatial Variables in
802 Retrosplenial Cortex Differs during 2D Navigation', *Neuron*, 105(2), pp. 237–245.e4.

803 White, B.R. *et al.* (2011) 'Imaging of functional connectivity in the mouse brain', *PloS one*, 6(1),
804 p. e16322.

805 Xiao, D. *et al.* (2017) 'Mapping cortical mesoscopic networks of single spiking cortical or sub-
806 cortical neurons', *eLife*. Available at: https://doi.org/10.7554/elife.19976.

807 Xiao, D. *et al.* (2021) 'MesoNet allows automated scaling and segmentation of mouse mesoscale
808 cortical maps using machine learning', *Nature communications*, 12, p. 5992.

809 Zhao, S. *et al.* (2011) 'Cell type–specific channelrhodopsin-2 transgenic mice for optogenetic
810 dissection of neural circuitry function', *Nature methods*, 8(9), pp. 745–752.

A Implantation of tetrodes to multiple targets

B Transparent window

To striatum
To thalamus
To midbrain

1 mm
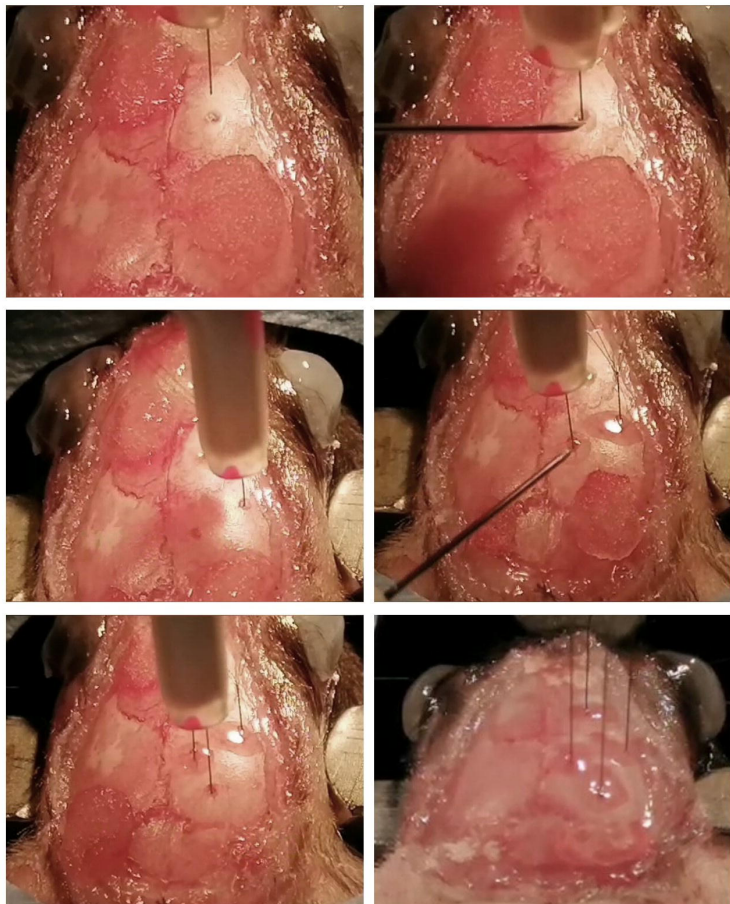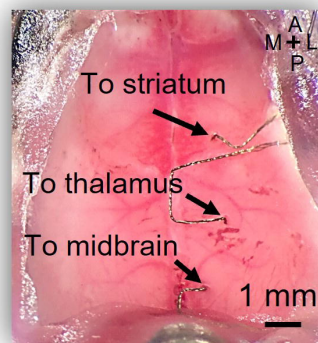
C Group housing

D Histology

Tetrode track to midbrain

E Tetrode

F 1 week

0.2 mV
0.2 s

2 weeks

0.3 mV
0.2 s

2 months

0.2 mV
0.2 s

G Single unit

0.25 mV
0.2 ms
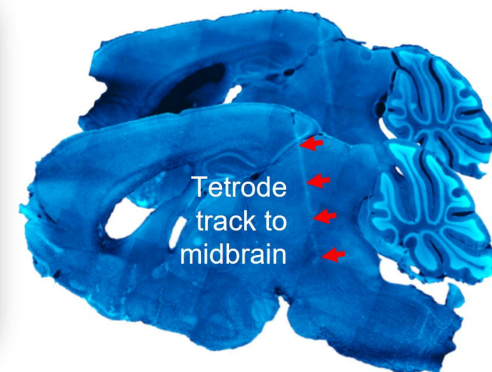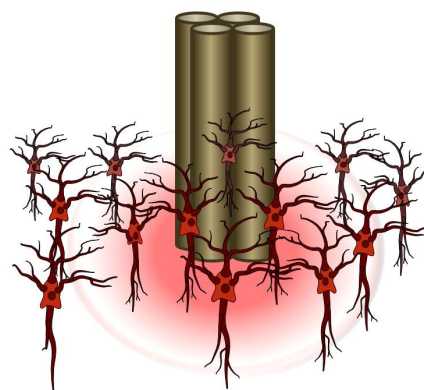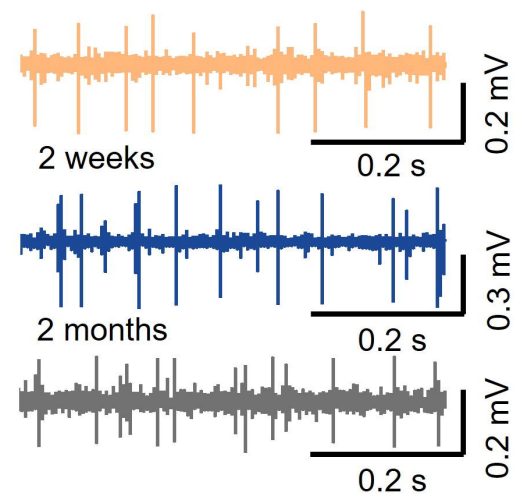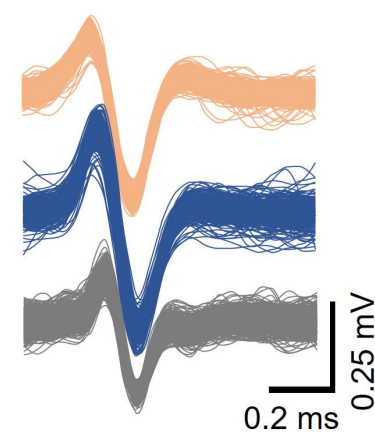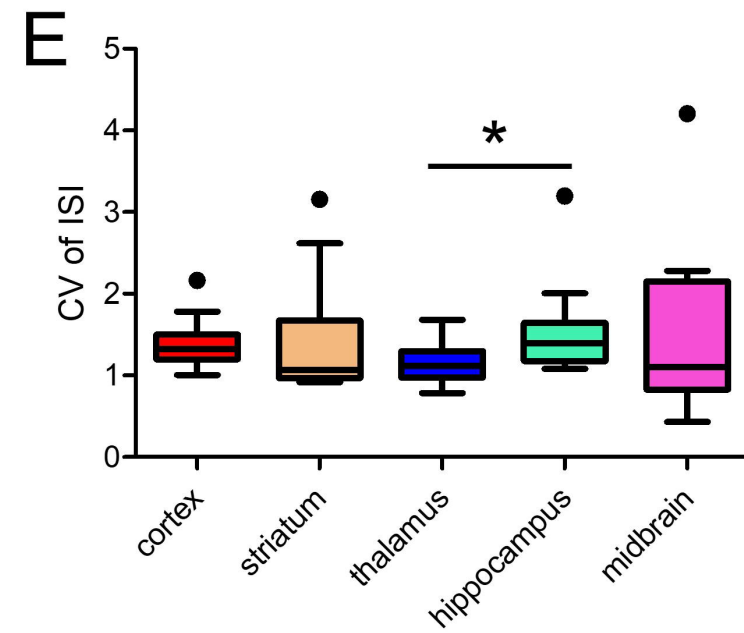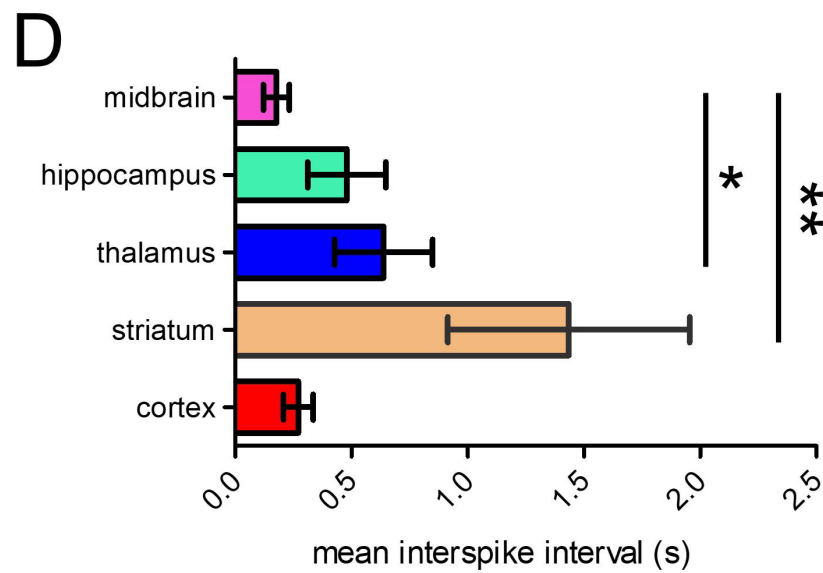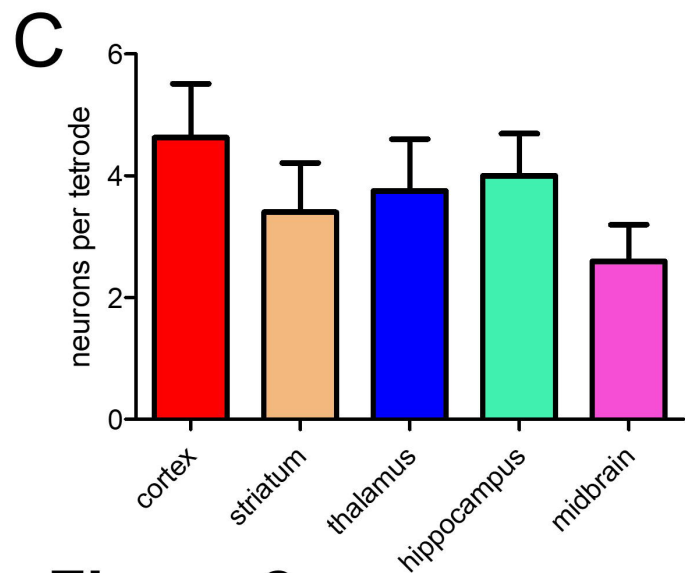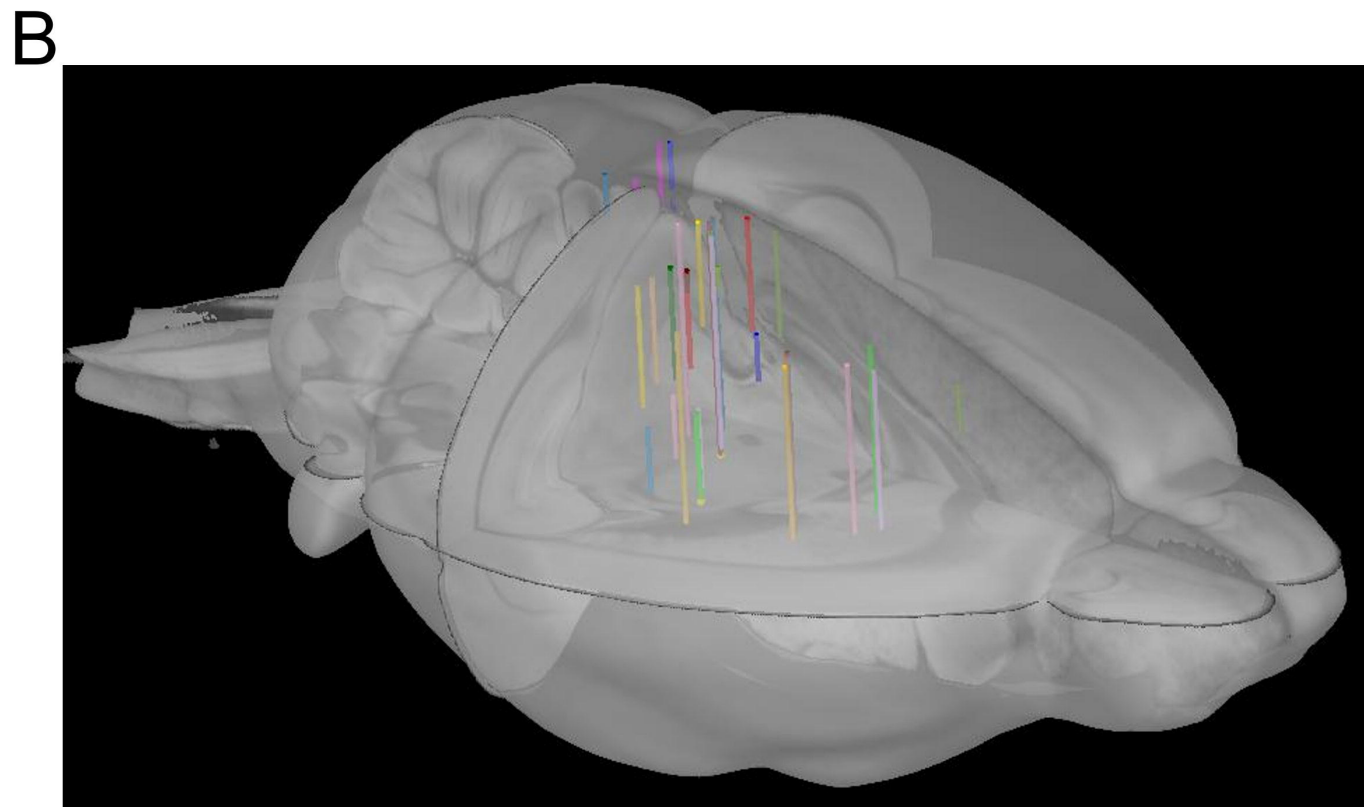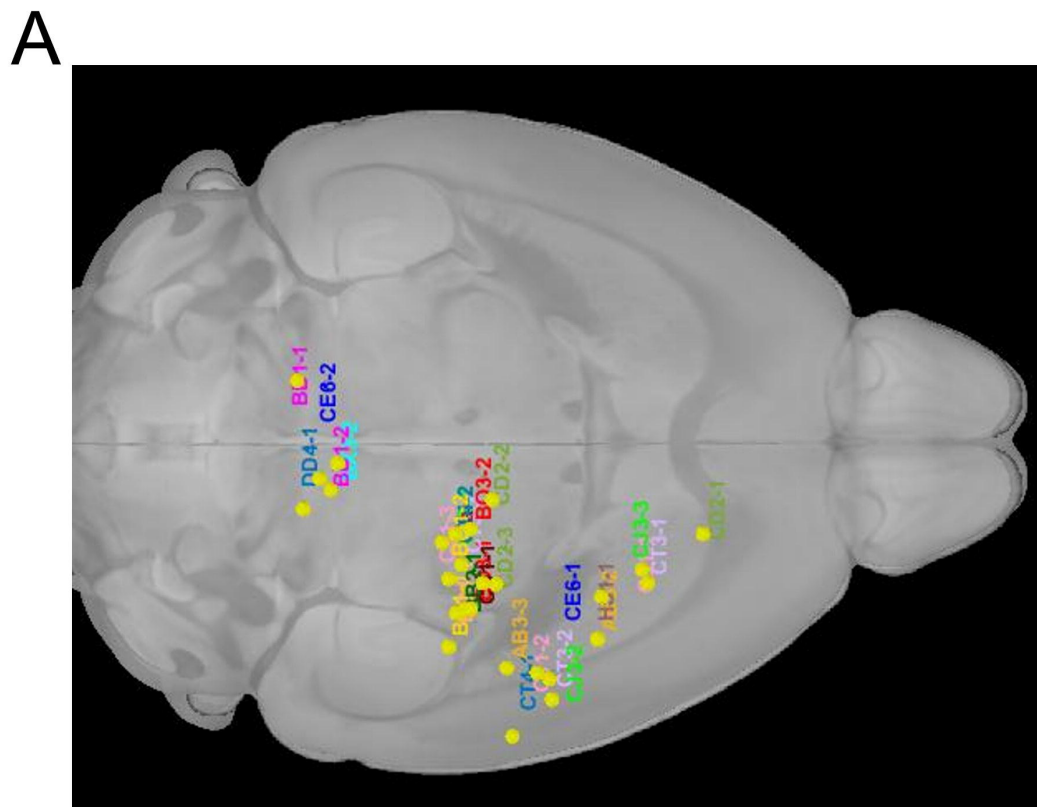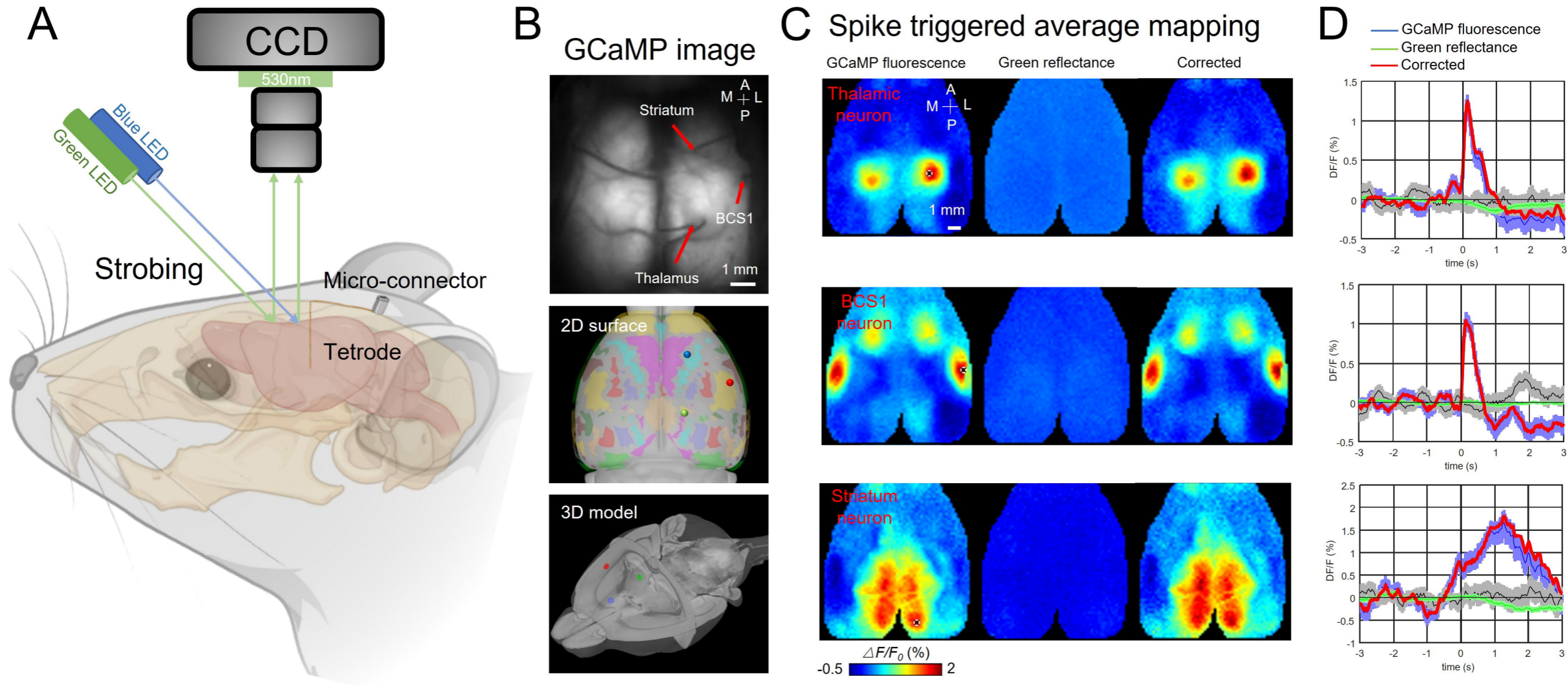
Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

A Facial motor nucleus
Facial nerve

B Facial motor nerve spiking activity

0.5 mV

0.5 ms

Facial nerve electrode recording sites

C Transcranial window

A
M ┼ L
P

Bregma

1 mm

E

MOs
MOp
SSp-m
SSp-ul SSp-n
SSp-ll SSp-bfd
VISa
RSP VISam VISrl AUD
VISpm VISal
VISp VISl

Allen Mouse Brain Atlas

STM

1 mm

D Facial nerve spike triggered average map temporal dynamic

-0.02s | 0ms | 0.02s | 0.05s | 0.07s | 0.1s | 0.12s | 0.15s | 0.17s | 0.2s | 0.22s | 0.25s | 0.27s | 0.3s | 0.32s | 0.35s | 0.37s | 0.4s | 0.42s | 0.45s

Single mouse

A
M ┼ L
P
1 mm

$\Delta F/F_0$ (%)
1
-0.5

Averaged (5 mice)

F Blue laser 473 nm

G Laser flash: 10 ms, power 2.0 mW

Raw response of facial nerve

0.2 mV
100 ms

Filtered (>300 HZ)

H Average facial responses of 6 Thy1-ChR2 mice

×10⁻³

Bregma
*

Normalized total variance

1 mm

Figure 6.

Figure 7.

Facial nerve spike triggered average map temporal dynamic

Mouse 1#
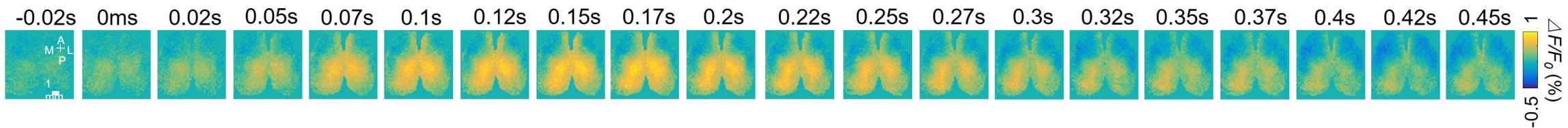
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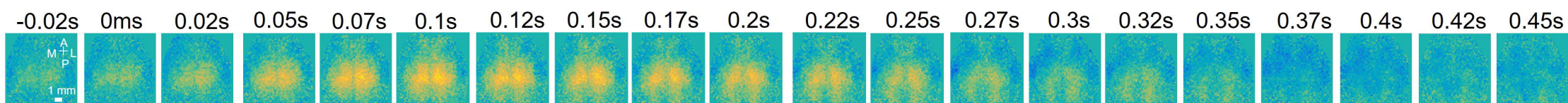
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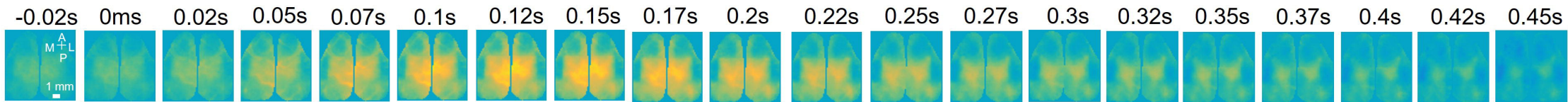
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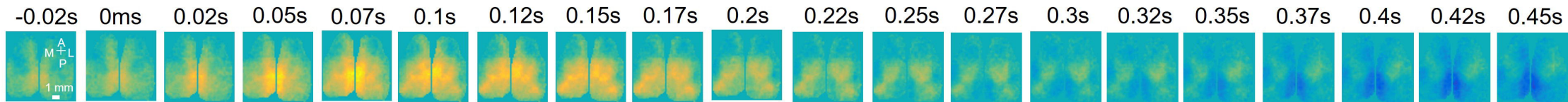
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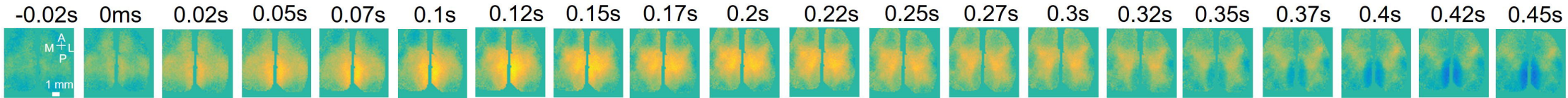
Figure 6—figure supplement 1.

MouseQE1

400 ms
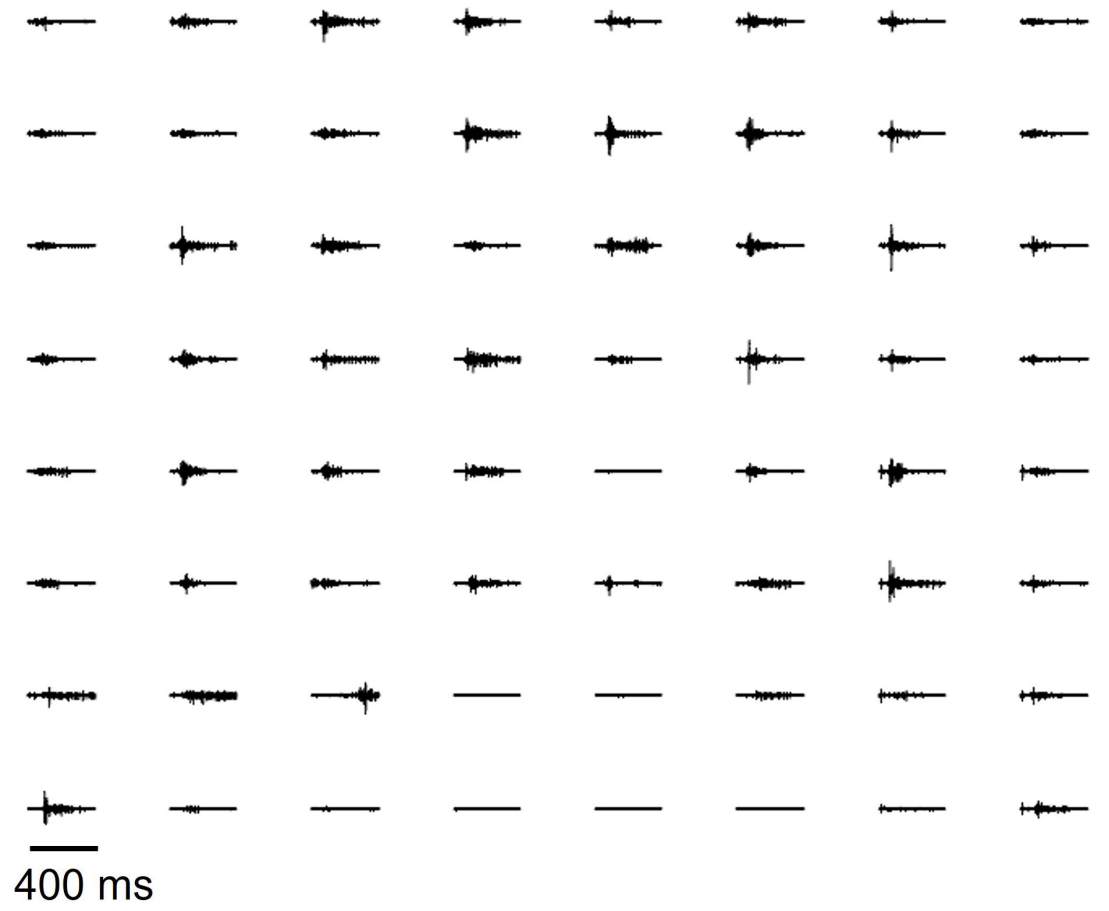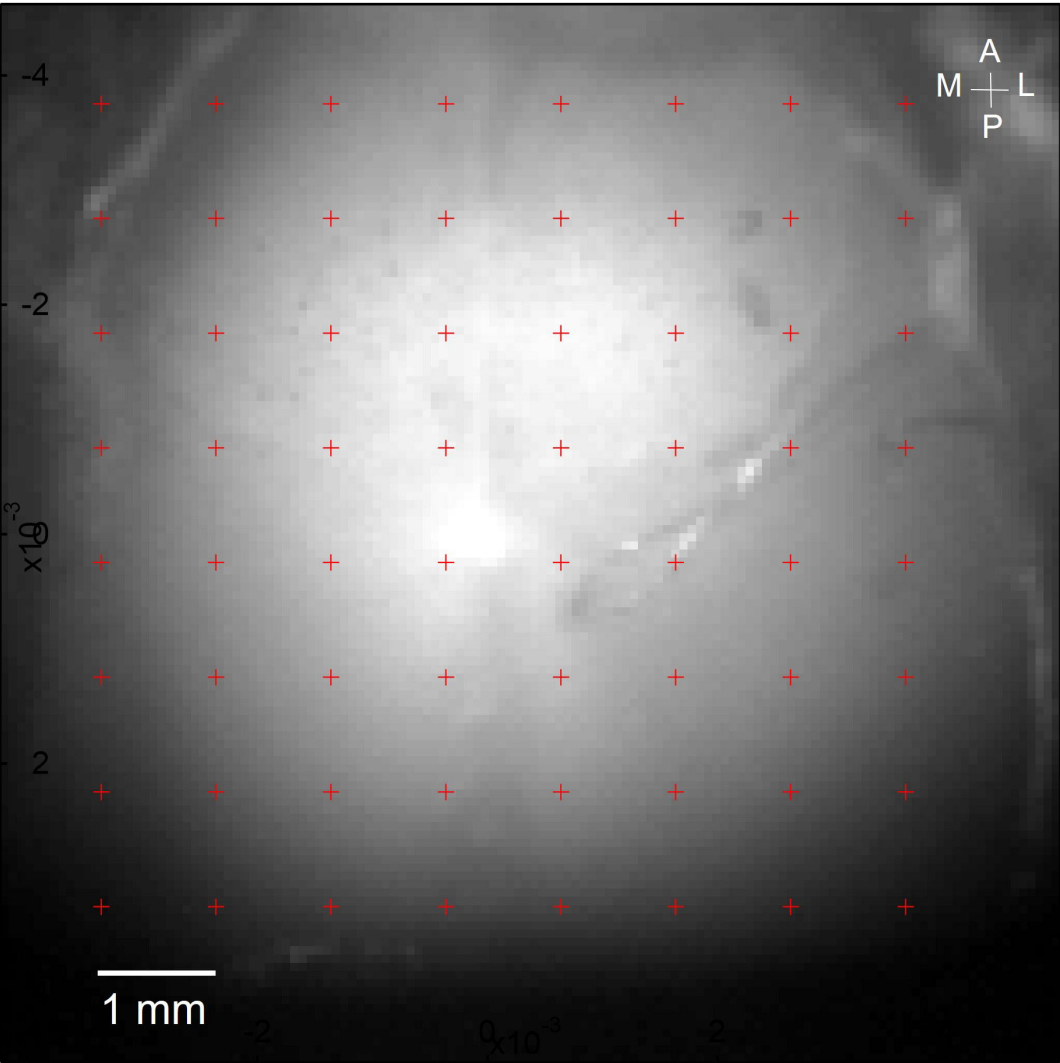
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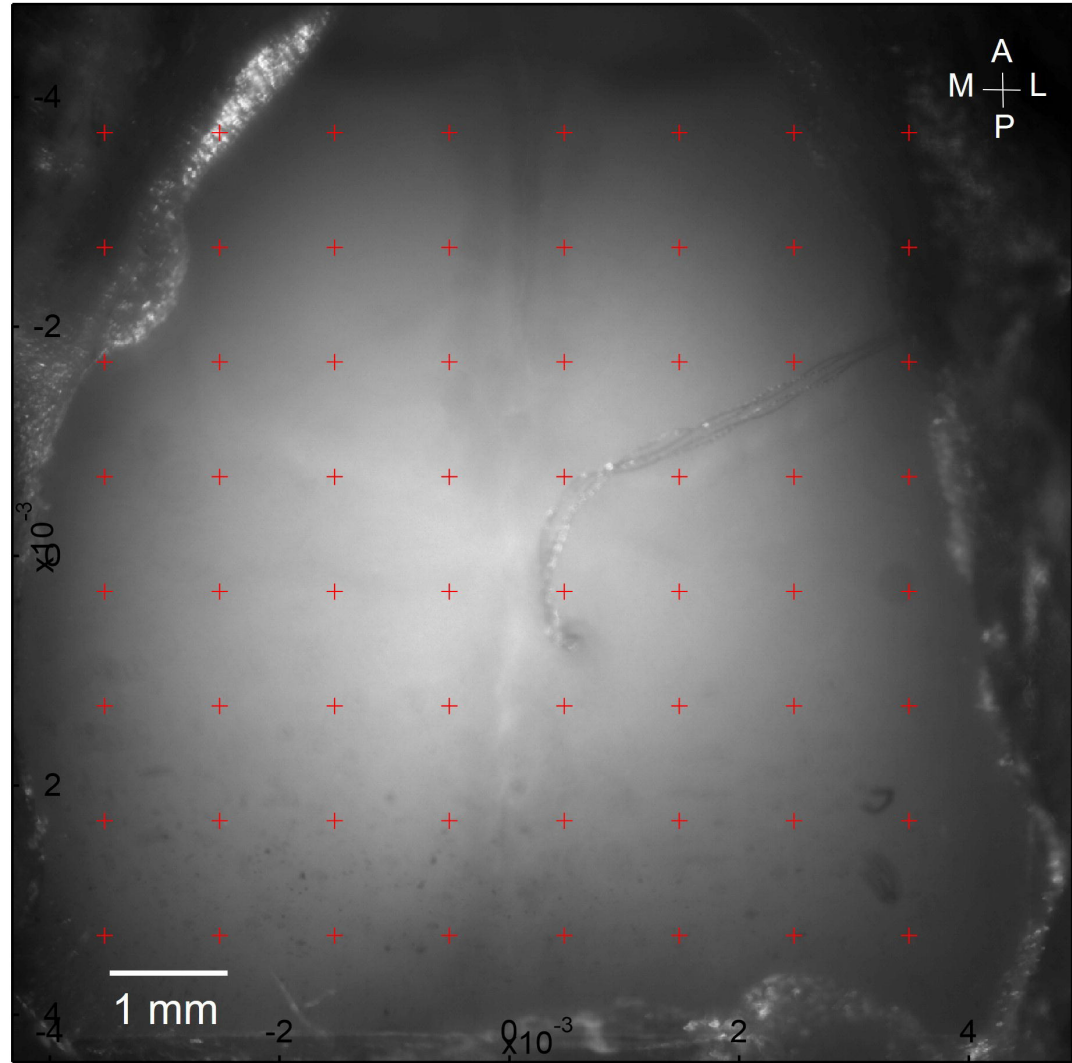
MouseQE2
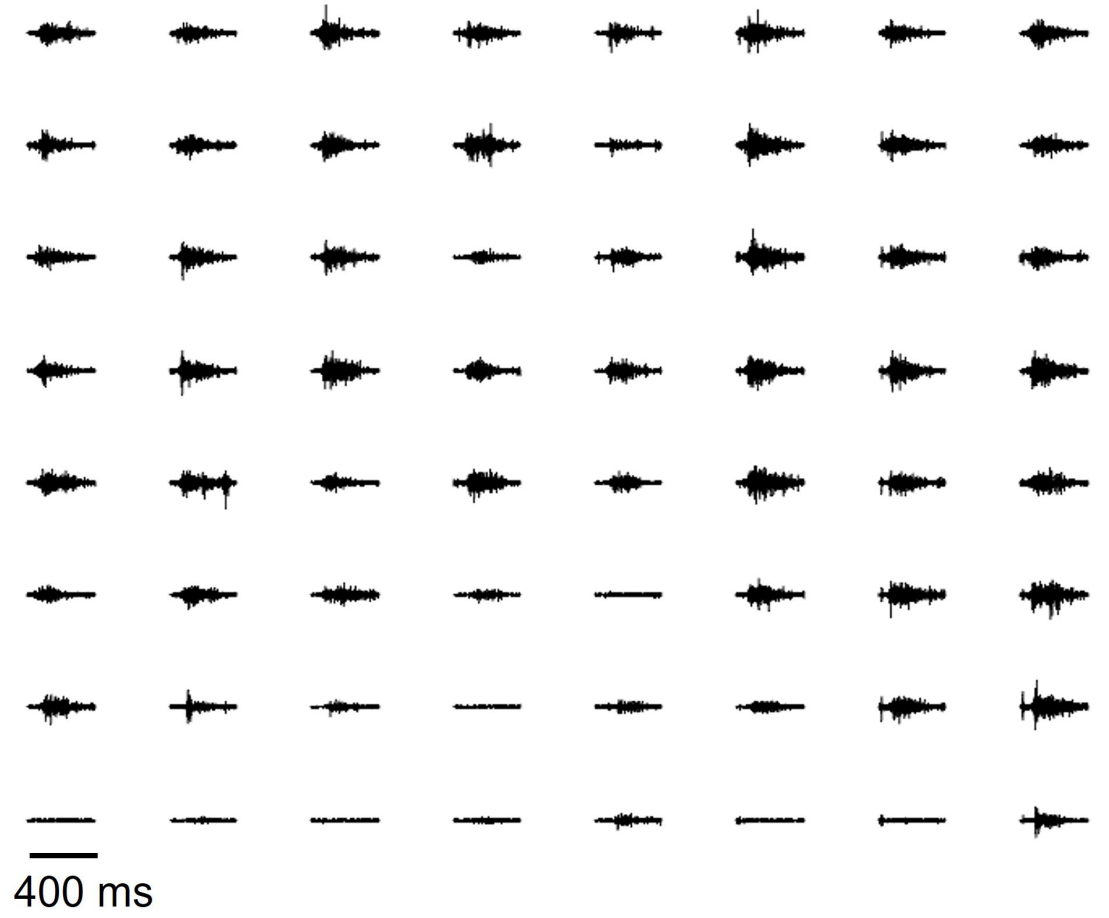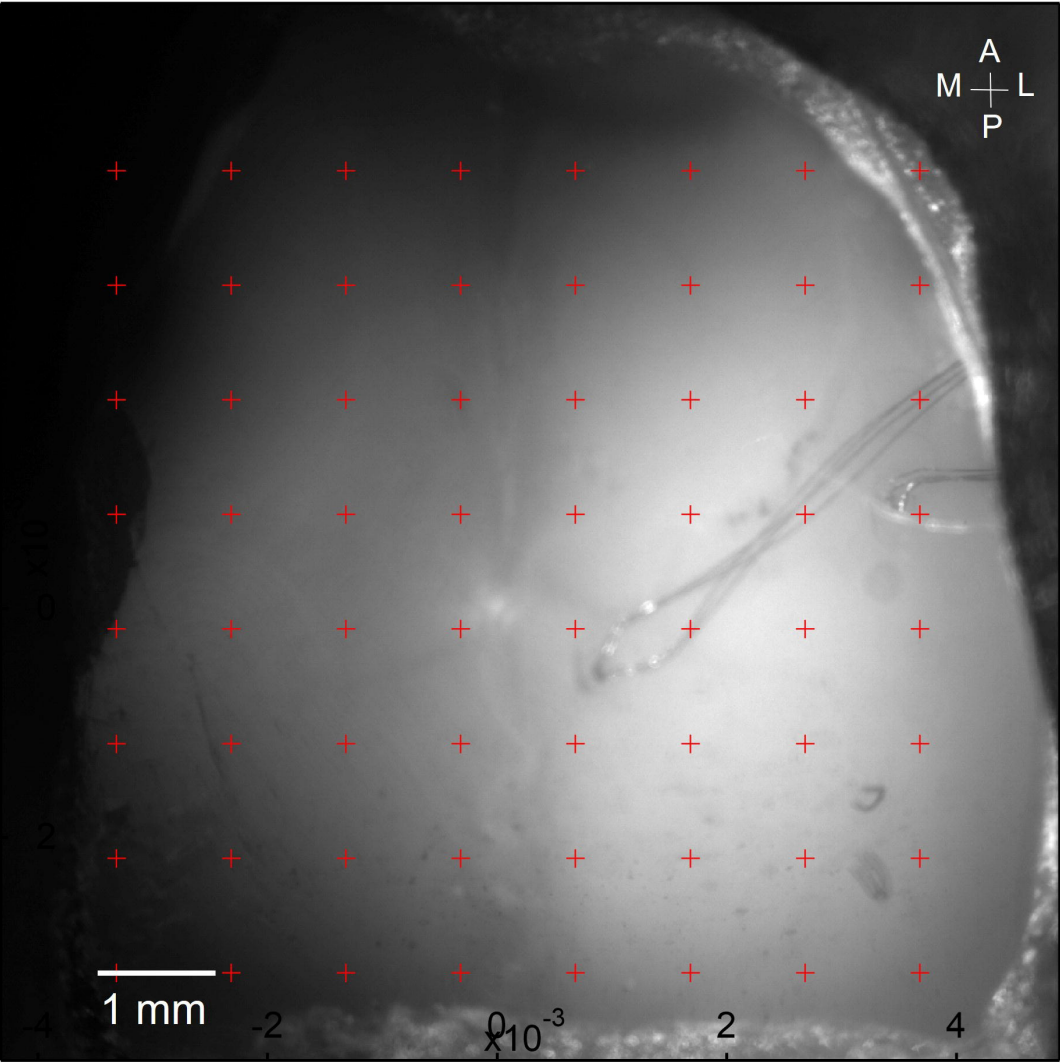
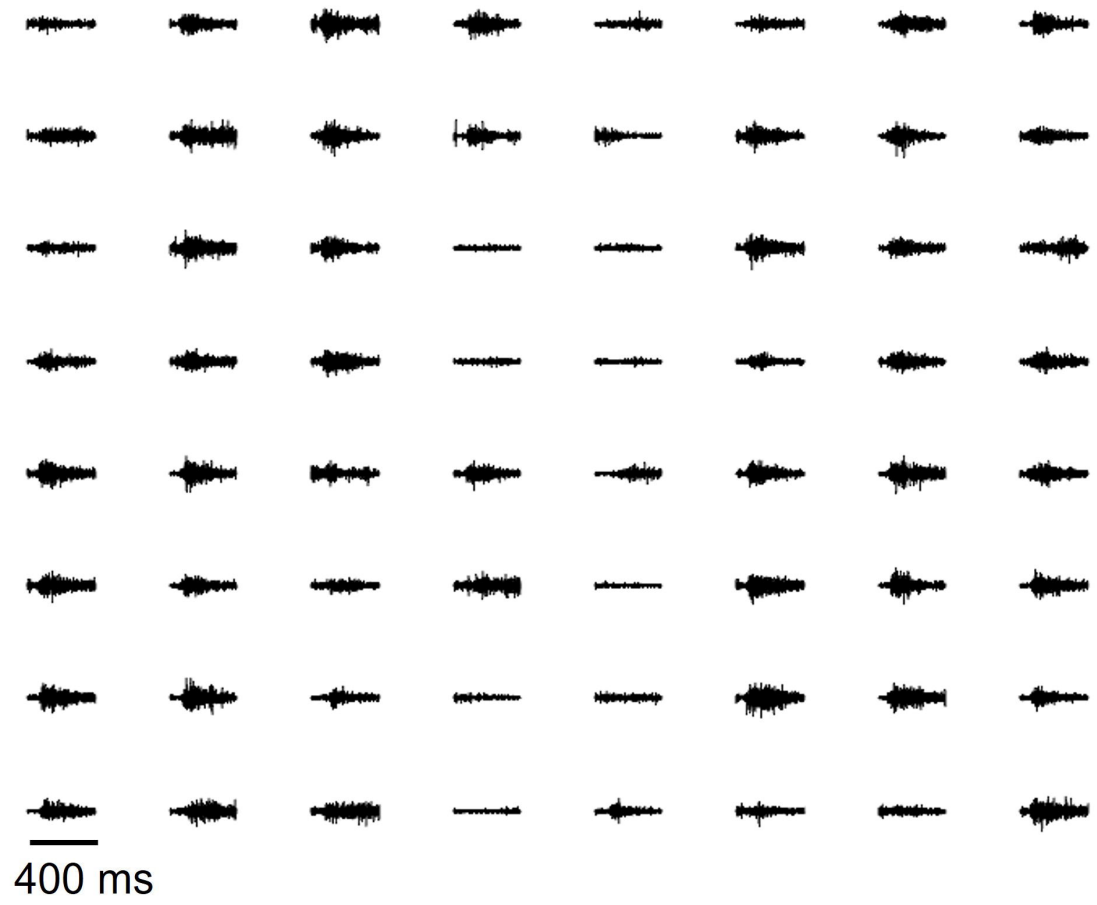**Figure 6—figure supplement 3.**
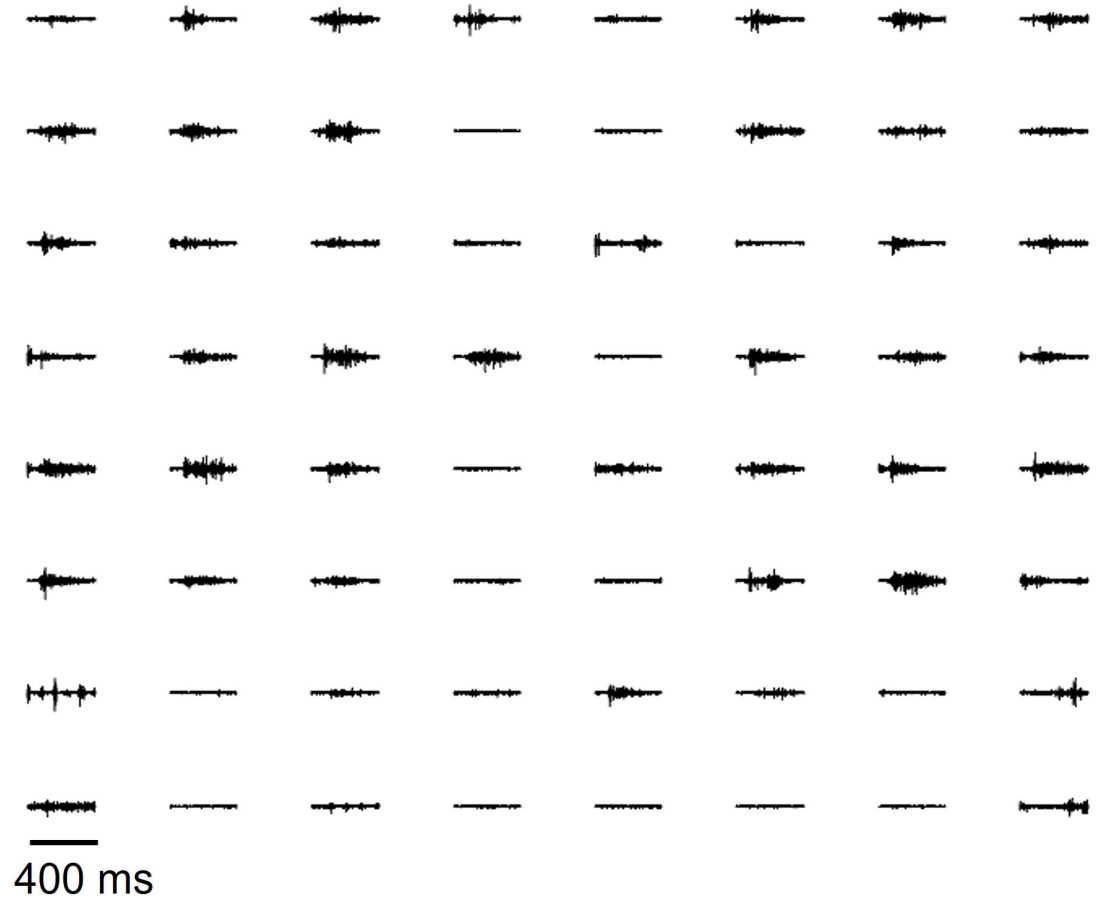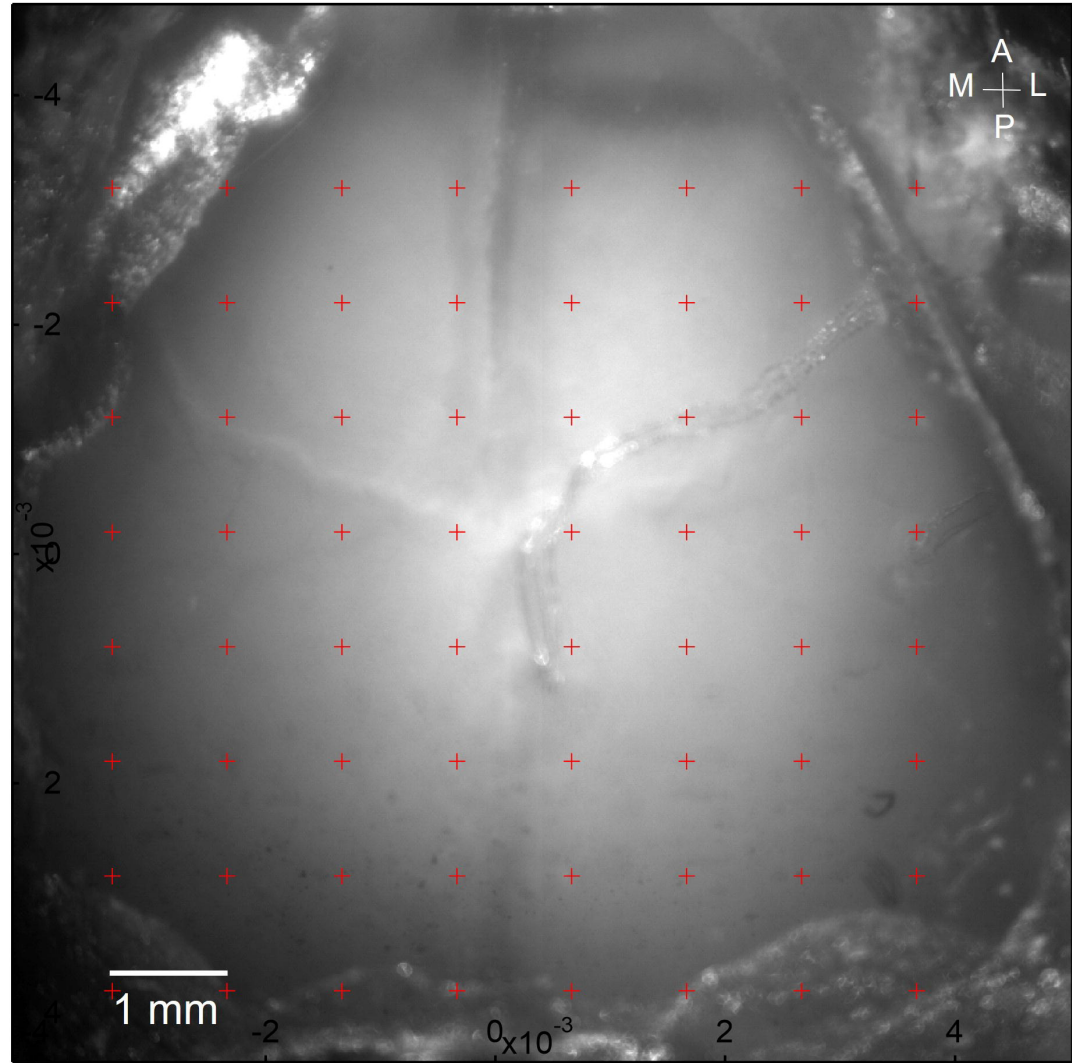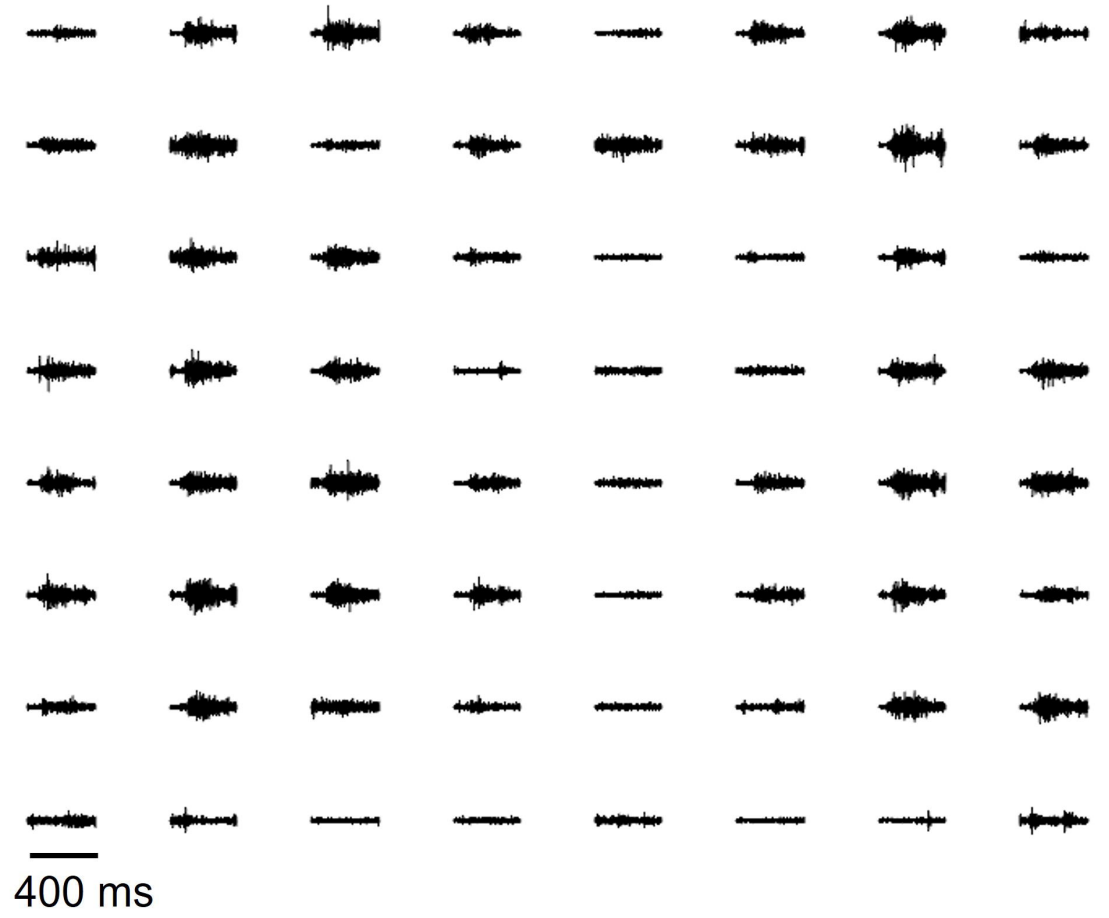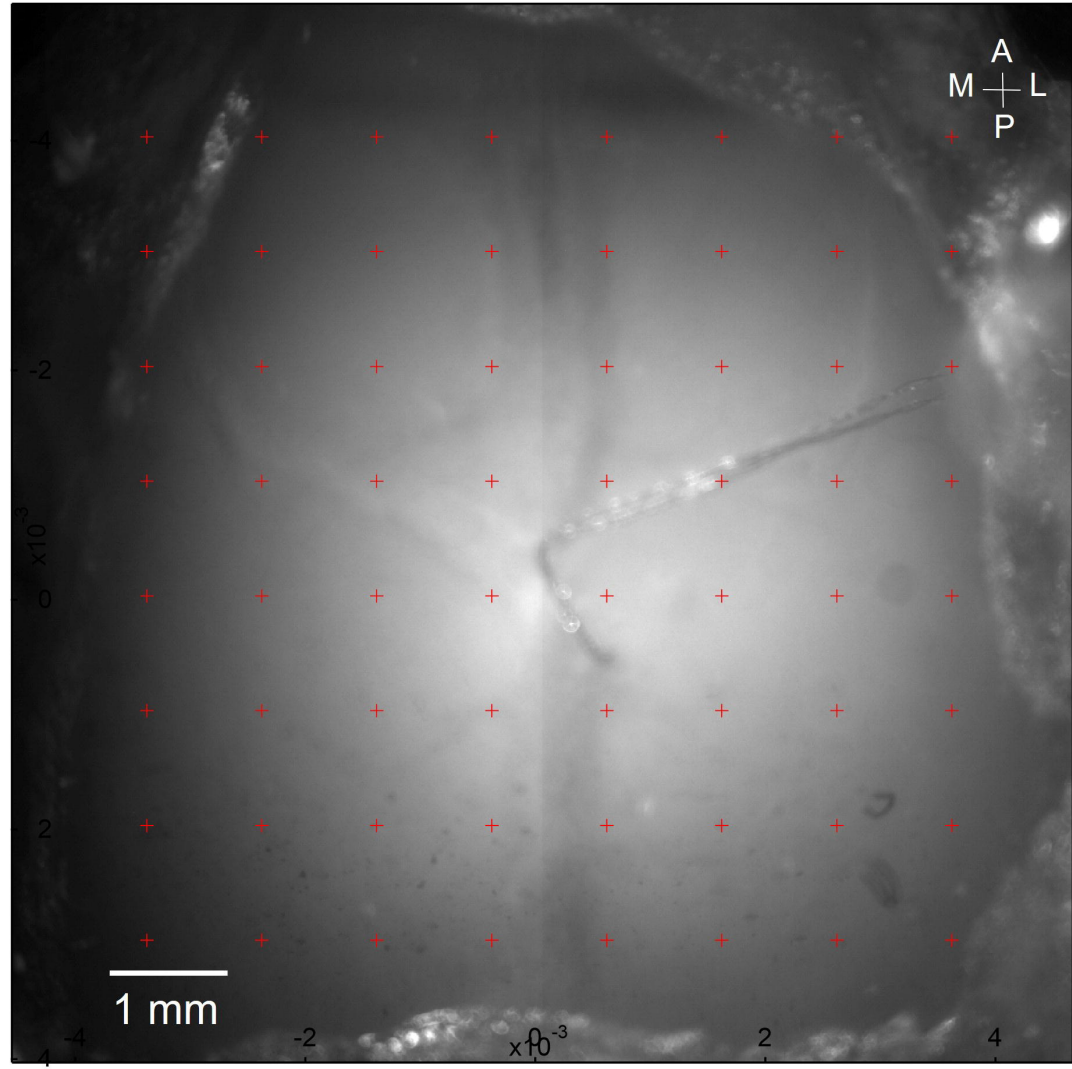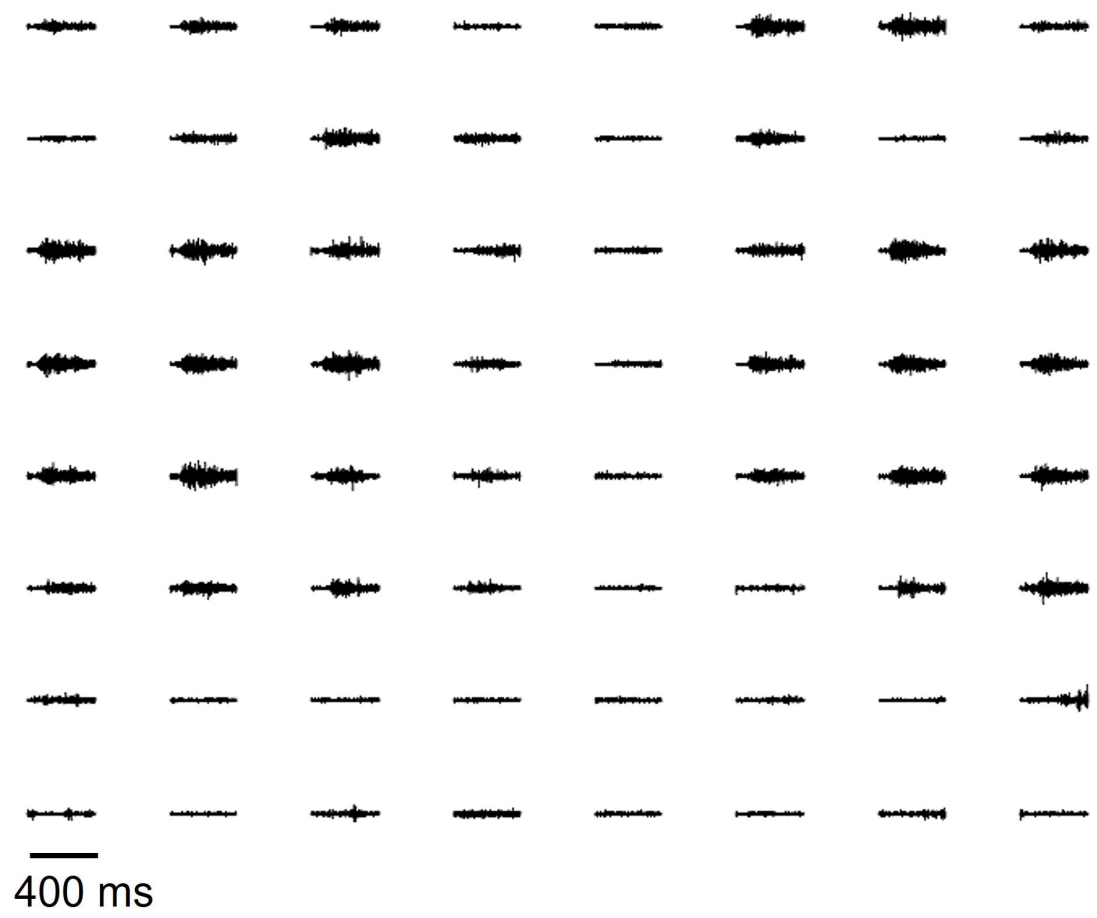
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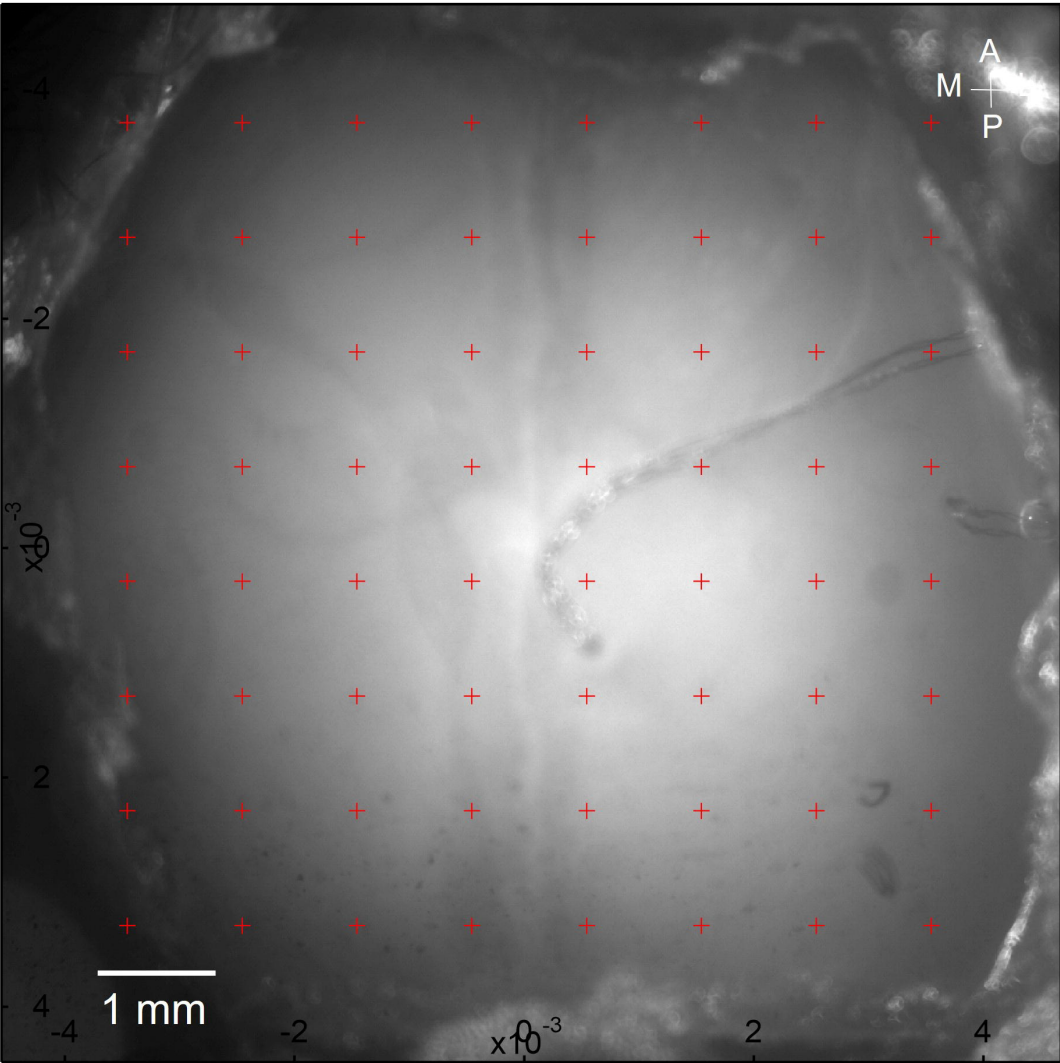
MouseQN2

Figure 6—figure supplement 5.

MouseQN3

Figure 6—figure supplement 6.

Figure 6—figure supplement 7.